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Effect of 6-BA on the Plant Regeneration *via* Organogenesis from Cotyledonary Node of Cowpea (*Vigna unguiculata* L. Walp)

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Abstract

The present study compares effects of different concentrations of 6-BA on regeneration from cotyledonary node explants of cowpea (*Vigna unguiculata* L. Walp). The seeds were inoculated on MSB_5 medium [Murashige and Skoog (1962) salts and Gamborg B_5 vitamins (1968)] containing different concentrations (0, 1, 2, 3, 4, 5 mg/L) of 6-BA for 4 days. The cotyledonary node explants with one cotyledon excised from 4-day-old seedlings, placed *in vitro* on MSB_5 medium containing 6-BA at different dose (0, 0.5, 1.0, 1.5 mg/L) for shoot induction and elongation. Best response in terms of shoot number and shoot length were obtained with explants derived from seedling preconditioning with 3mg/L 6-BA followed by the induction and elongation stage pretreated with 0.5mg/L 6-BA. The elongated shoots were rooted on MSB_5 medium without hormone.

Keywords: cowpea, plant regeneration, cotyledonary node, organogenesis, 6-BA

Abbreviations: 6-BA: 6-Benzylaminopurine

1. Introduction

Cowpea (*Vigna unguiculata* L.) is widely grown in Africa, Latin America, Southeast Asia and southwestern regions of North America, and is a major source of high-quality dietary protein and energy for local people. It plays an important role in the lives of millions of people in developing countries of Africa and Asia. In spite of the great importance of this crop, its productivity is low, which is mainly limited by the damage caused by biotic and abiotic stresses (Singh et al., 1997). In addition, limited genetic diversity in cowpea breeding programs is of special concern because cowpea appears to have lower inherent genetic diversity than other cultivated crops as a result of a hypothesized single domestication event (Fang et al., 2007). Although some resistance genes to insect pests and fungi have been identified in some IITA cowpea varieties and other closely related Vigna species (Latunde-Dada et al., 1990), the attempts using conventional breeding methods to introduce the resistance genes into the cultivated cowpea have made little progress for the strong hybrid incompatibility. Hence, genetic engineering approaches stand out as the most effective alternative strategy to overcome the production constraints (Zaidi et al., 2005). An effective and rapid regeneration protocol is essential for genetic transformation. Plant regeneration of cowpea *via* organogenesis has been achieved from epicotyls, hypocotyls, primary leaves, cotyledons, cotyledonary nodes, shoot tips, plumular apices and shoot meristem. Of these, cotyledonary node explants seemed the most responsive for the induction of multiple shoots, which was appropriate to *agrobacterium*-mediated transformation (Chaudhury et al., 2007; Raji et al., 2008; Solleti et al., 2008a, 2008b; Adesoye et al., 2010).

Previous work has studied the effect of varied hormones used together on the regeneration of cowpea. But the regeneration of cowpea via cotyledonary node uses 6-BA alone has not been explored. The aim of this paper is to explore the effect of 6-BA on different stages of regeneration of cowpea, to provide a theoretical and technical basis for rapid propagation.

2. Materials and Methods

2.1 Plant Materials and Seeds Preconditioning

Mature seeds of cv. Cheng-jiang VII of cowpea was obtained from the Research Institute of Horticulture, Academy of Chengdu Agriculture and Forestry Science, Chengdu, China. The seeds were soaked with 70% ethanol for 1 min, surface-sterilized with 0.2% (w/v) $HgCl_2$ for 5 min, followed by rinsed five times with sterile

distilled water and blotted with sterilized filter papers. Then the seeds were cultured on MSB_5 medium supplemented with 6-BA at different concentrations (0, 1, 2, 3, 4, 5 mg/L) for 4 days.

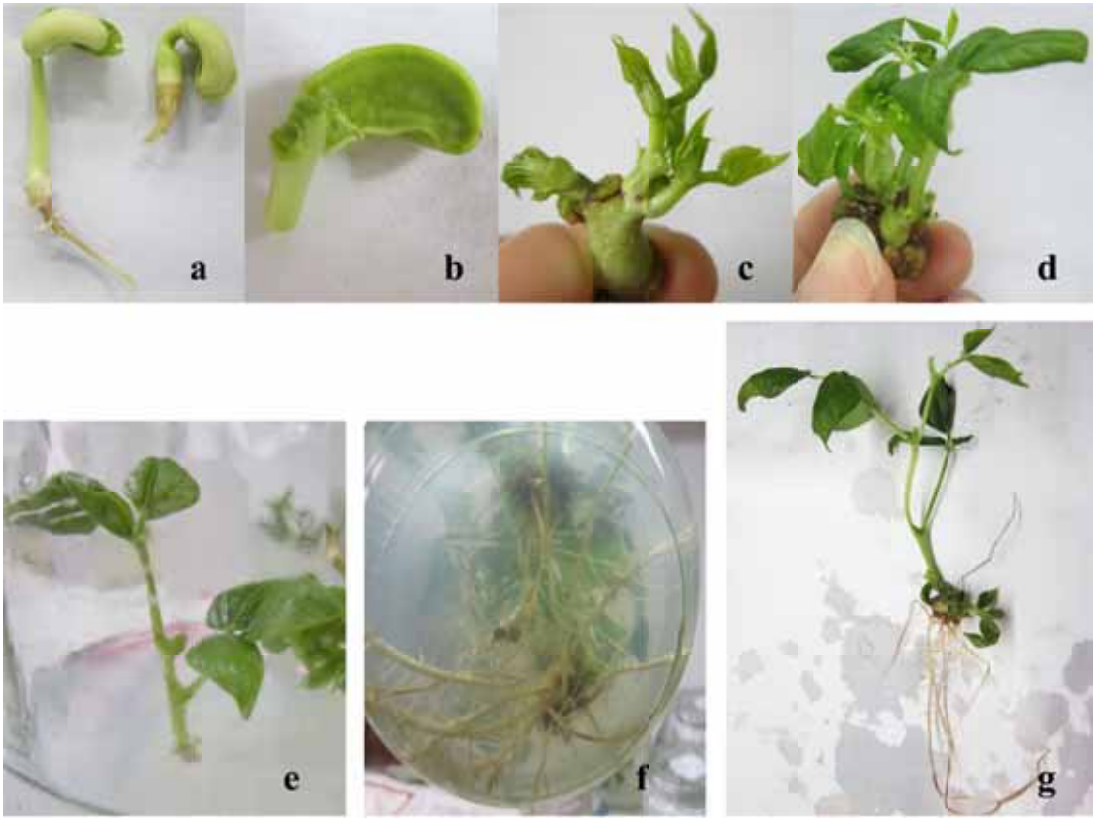

Figure 1. Regeneration system from cotyledonary node explants of cowpea. (a) Seedling preconditioning with 6-BA (right) and seedling without preconditioning (left). (b) Cotyledonary node explants removing one cotyledon and cutting both the epicotyls and hypocotyls. (c) and (d) Explants excised from seedling preconditioning with 3 mg/L 6-BA, followed by shoot induction and elongation on MSB_5 + 0.5 mg/L 6-BA for two weeks (c) and four weeks (d). (e) Elongated shoots were separated and transferred to hormone-free MSB_5 medium for rooting. (f) Elongated shoots forming roots on hormone-free MSB_5 medium. (g) Rooted plantlet

2.2 Shoot Induction and Elongation

The cotyledonary node explants excised from 4-day-old seedlings were cultured in a vertical upright position with the hypocotyls end slightly embedded in MSB_5 medium supplemented with various concentrations of 6-BA (0, 0.5, 1.0, 1.5 mg/l). The explants were excised by removing one cotyledon and cutting both the epicotyls and hypocotyls approximately 2 mm above and 3-5 mm below the nodal point (Figure 1b). And the initial axillary buds were also removed. After 2 weeks of culture (Figure 1c), the multiple shoots were removed from the explants and transferred to fresh medium with the same concentrations of 6-BA for subculture for another 2 weeks (Figure 1d).

2.3 Rooting and Acclimatization

Regenerated shoots were separated and transferred to hormone-free MSB_5 medium for rooting (Figure 1e). After 2 weeks of culture, the rooted plantlets (Figure 1g) were washed in running rap water and transferred to pods containing sterilized soil, green manure and vermiculite at 1:1:1 ratio. Each pot was covered with transparent polyethylene bags to maintain adequate humidity during the first few days. Subsequently, the bags were removed and the plants were allowed to grow at room temperature with 50% relative humidity.

2.4 Culture Medium and Conditions

MSB_5 medium [Murashige and Skoog (1962) salts and Gamborg B_5 vitamins (1968)] supplemented with 3% (w/v) sucrose and 0.6% (w/v) agar was used throughout this study. The pH of the medium was adjusted to 5.8

with 0.1 N NaOH or HCl before autoclaving at 121°C for 15 min. All the cultures were maintained at 26±2°C temperature with 16 h light photoperiod. The experiment started from mid-August and finished in mid to end of October in 2012.

2.5 Data Collection and Statistical Analysis

The length and the number of adventitious buds were recorded after 2 weeks of culture on the shoot induction and elongation medium. The date of the shoots began to take roots were also recorded. The experiments were arranged to repeat thrice with 20 replicates per treatment. The data were determined by analysis of variance and the significant difference between the means were compared using Duncan's new multiple range method with the help of statistical software DPS.

3. Result and Discussion

3.1 Effect of 6-BA Preconditioning on Seed Germinating

Cowpea is recalcitrant to regeneration from shoot proliferation and genetic manipulation (Dita et al., 2006). The regenerative competence could be increased *via* seedling preconditioning using high dose of cytokinin because of its promoting in cell division.

In this study, the seeds cultured on the medium containing 6-BA grew obviously stronger than those cultured on hormone-free medium. The differences are expressed in following aspects made up of dramatic enlarged primary leaves, stubby hypocotyls, hyperplastic region of the cotyledonary node, and thick and short roots (Figure 1a). This conclusion was similar to some other scholars (Bakshi et al., 2012; Tang et al., 2012). The effect was significantly more pronounced when the concentration of 6-BA was up to 3 mg/L. Looking just from morphological terms, the seedlings showed no obvious differences at high dose (3,4,5 mg/L) of 6-BA.

3.2 Effect of 6-BA on Shoot Induction, Elongation and Rooting

Table1. Effect of different concentration of 6-BA on shoot regeneration from cotyledonary node explants of cowpea following culture on MSB_5 medium for two weeks

Concentration of 6-BA (mg/L)		Mean number of shoots per explant	Mean shoot length (cm)
Seedling preconditioning	Shoot induction and elongation		
0	0	1.82d	2.83ab
	0.5	3.68c	2.17abcd
	1	4.59ab	1.50cde
	1.5	4.44bc	0.93e
1	0	1.90d	2.93ab
	0.5	4.93ab	2.50abc
	1	5.30ab	2.33abcd
	1.5	4.97ab	1.86bcde
2	0	2.27d	2.53abc
	0.5	5.17ab	1.97bcde
	1	4.89ab	2.17abcd
	1.5	5.32ab	1.47cde
3	0	2.23d	3.20a
	0.5	5.20ab	2.80ab
	1	5.10ab	2.40abcd
	1.5	5.13ab	1.53cde
4	0	2.00d	3.10a
	0.5	5.30ab	2.22abcd
	1	5.41ab	1.93bcde
	1.5	5.15ab	1.37de
5	0	2.13d	3.10a
	0.5	5.53a	2.49abc
	1	5.42ab	1.88bcde
	1.5	5.33ab	1.60cde

Values represent means. Means having the same letters are not significantly different according to Duncan's multiple range test at $P = 0.05$.

At the stage of shoot induction and elongation, more adventitious buds were observed on the media supplement with 6-BA compared with that of control. When the concentration of 6-BA was 0.5 mg/L, 1.0 mg/L and 1.5 mg/L, the number of shoots per explant displayed increasing but the distinction was not very significant (Table 1). This might be because higher concentration of 6-BA precondition at the stage of seedlings made a large impact on the following stage (Brar et al., 1999; Le et al., 2002; Raveendar et al., 2009). The shoot length was decreased with the concentration of 6-BA increasing, which was in agreement with the research of pioneers (Diallo et al., 2008; Aasim et al., 2009; Tang et al., 2012). Besides, it is important to note that when the concentration of 6-BA was 1.5 mg/L, abnormal morphology of shoots would be observed. The stems grew and bend downwards, the leaves were shrunken. And the abnormal shoots were difficult to elongate.

After induction and elongation, the regenerated shoots were removed on MSB_5 medium without hormone for rooting. More than 95% of the regenerative shoots could produce roots. But the time at the beginning of forming roots were different. If the concentration of 6-BA was higher at the stages of induction and elongation, it was difficult to produce roots, which manifested as it would take a longer time to start rooting. The experiment showed that the optimal concentration of 6-BA promoted the propagation of adventitious buds, but inhibit both the shoot elongation and rhizogenesis.

In the present investigation, 3 mg/L 6-BA preconditioning during the seedlings and 0.5 mg/L 6-BA at the induction and elongation stages was the best concentration to induce adventitious buds and to elongate comprehensively considered efficacy and cost.

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Impact of Communal Violent Conflict on Farmer's Livelihood Activities in Two Agro-Ecological Zones of Nigeria

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Abstract

In Nigeria there is hardly a year where there are no major violent conflicts. However, much has not been published on the quantitative impact of the conflicts on farmers' livelihood the manager of crops, domesticated and wild life animals. Hence, this study tend to provide information for understanding how conflict handling styles employed by conflicting parties made most of the communal conflicts degenerate into destruction of farmers livelihood activities. Two violent communal conflicts ridden states one in rainforest and derive savannah region of Nigeria were purposively selected to reflect discrepancy in impact of the conflict on livelihood activities the means of generating livelihood in two main agro-ecological regions of Nigeria. Based on the conflict severity the two agro-ecological zones were stratified into core and outside conflict areas. Using farmers register as sampling frame work 60 and 67 farmers were randomly selected in core and outside violent conflict areas of rainforest and savannah zones respectively. Interview schedule instrument was used to collect data while frequency count, percentage t-test and ANOVA were statistical tools used for data analysis. The findings revealed that in Core Violent Conflict Area (CVCA) of rainforest and derived savannah areas 72.1% and 23.8% of the farmers were displaced from their farms respectively. Consequently tree (cocoa) crops production level were severely affected as reflected in lower ($\bar{x}$ 295) and higher mean ($\bar{x}$ 697) cocoa production level in tons recorded in CVCA and Outside Violent Conflict Areas (OVCA) respectively in rainforest areas. The severity of conflict impact was not reflected in derived savannah area because yam production level means gap in tons between CVCA ($\bar{x}$ 423.0) and OVCA ($\bar{x}$ 629) were very close. However, the savannah area felt the impact of the conflict on sheep and goat production because CVCA recorded lower mean ($\bar{x}$ 180) numbers of sheep and goats as against higher mean ($\bar{x}$ 2007) number of sheep and goat recorded in OVCA. The decline in production of sheep and goat could be attributed to conflict because majority (78.4%) of the farmers claimed that they have lost their productive land to conflict. Farmers' means of generating livelihood activities such as crops production level, sheep and goat number produced were statistically different across conflict zones at $P < 0.05$ in rainforest and savannah zones. The conflict had severe impact on crops, sheep and goat production hence, a sustainable capacity building program, as a post conflict coping strategies should be organised for conflict victims.

Keywords: communal conflict, impact, livelihood, agro-ecological zone and production level

1. Introduction

Nigeria is a tropical country that cut across all tropical ecological zones. These include: the southern zone of Mangrove Swamp, the Tropical Rainforest stretching from the South-west to the South-east, the Guinea Savannah belt, the Sudan Savannah belt and the Sahel Savannah as shown Figure 1.

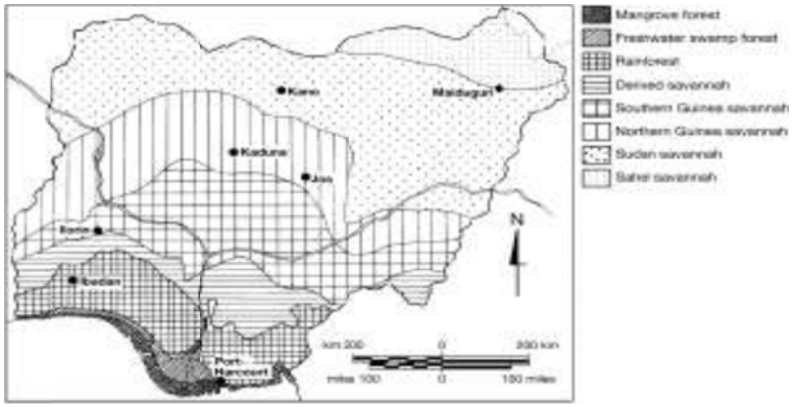

Figure 1. Agro-Ecological zones in Nigeria

These environmental regions greatly affect the cultures of the people who live there. The dry, open grasslands of the savannah make cereal farming and herding a way of life for the Hausa- Fulani (Fabusoro et al., 2007). The wet tropical forests in the south provide opportunities of planting different tree and arable crops and income generation for the Yoruba, Igbo, and other ethnic groups. Rainfall controls the livestock and crops production activities in Nigeria. Decrease in rainfall with increase in surface temperature over the years resulted to pressure on land in the Guinea Savannah zone and the rainforest belt. Most of these pressure resulted from the long range trans-humans of the Fulani cattle rearers from Sahel and Sudan savannah to the Guinea savannah and now the rainforest belt. Many cattle Fulanis with their herds are found permanently settled or roaming within the Guinea savannah and the rainforest belt. This is in contrast to what obtained in the 1960s and 1970s where they only moved down-south when grasses are no longer "green" in the Sudan and Sahel, and make the return migration with the onset of rainy season in the north. The natural result of this pressure on land is conflict, especially between the cattle Fulanis and the crop growing natives of the guinea savannah over the right to the land which in most cases result in destruction of their farmlands by the cattle (Mayowa et al., 2005). Other clashes over land include inter-community struggle for dominance and control of land resources which is common in the densely people of south east of Nigeria, and of course the case of the Niger Delta area which combines the struggle for control of land, environment and oil activities. The result of past newspaper clippings that were reviewed to generate a list of some critical communal clashes that were reported between 1991 and 2005 as shown in Table 1 indicated that 19 of the 37 cases representing about 51% were basically crisis/clashes triggered by land resources . Clashes on oil and environment were basically localized in the Niger Delta area (Mayowa et al., 2005).

Table 1. Summary of some crisis in Nigeria 1991-February 2005

S/n	Crisis/Clashes Group	No	Percent
1	Land - Agric	13	35.1
2	Land -Oil/environment	4	10.8
3	land-urban	2	5.4
4	Religious	5	13.5
5	Political	5	13.5
6	Ethnic	7	9
7	Others	1	2.7
		37	100.0

These conflicts explain noticeable distortions in farmers' livelihoods since they live and earn their living from rural areas. Livelihood is a process by which people make a living through specific capabilities, assets, and activities (Ellis, 2000). The context of farmers' livelihood comprises farming activities, natural resources, economic, cultural, social equitability, and political environment, shocks and stress maintenance. In coping with livelihood sustainability farmers' compete for resources that exist in limited quantities and scarce. Competition

creates a situation where people struggle for possession of these scarce resources, which often generate conflict. Conflict situation threaten livelihood outcomes and termination of farmers' sustainable livelihood income. Conflict within the two communities became a menance when farmer employed negative or aggressive conflict handling style. It is evident from diverse sources that negative conflict handling style employed in the affected communities in Taraba and Osun states which represent savannah and rainforest zones led to destruction of lives and properties, diversion of resources meant for development to conflict mitigation (Bolarinwa, 2007). It further imposed hardship on the citizens, worsening their social conditions and led to mass migration of farm families. In view of anecdotal account of the conflicts effect on farmers' livelihood in the conflict ridden areas and unavailability of empirical records to established the discrepancy in severity of conflict on farmers' livelihood activities in the two agro-ecological areas that this research on impact of communal violent conflict on farmer's livelihood activities in two agro-ecological zones was conducted.

2. Purpose of the Study

The purpose of the study is to establish conflict severity across tow agricultural zones in Nigeria. In order to accomplish this aim the following specific objectives were set: describe personal characteristics of farmers in the 2 agro-ecological zones, determine farmers' access to their productive activities after the conflict , ascertaining the impact of the conflict on the farmers' productive land, examining the impact of the violent conflict on livelihood activities (crops and live stocks production). The hypotheses tested were: There is no significant difference between farmers' crops production level in core conflict area and outside conflict areas. There is no significant difference between sheep and goat numbers produced in core conflict area and outside conflict area of the 2 agro-ecological zones.

3. Methodology

The study was conducted in two agro-ecological regions of rain forest and derived savannah states of middle-belt and south-western Nigeria that are agrarian states in Nigeria. Multi stage sampling procedure was used to select farmers for the study. At first stage rainforest and savannah in the 2 agro-ecological zones respectively were purposively selected based on record of incessant occurrence of violent conflicts in the zones. At the second stage, zones were stratified into core, and outside conflict areas based on conflict severity or anecdotal account of conflict in each zone. At the third stage 7 villages were randomly selected from core and outside conflicts areas of savannah and rainforest zones respectively to give 14 villages both for the rainforest and the derived savannah agro ecological zones respectively. At the last stage, 10 farmers from the list of the extension agents covering each of the selected villages in core and outside conflict areas were randomly selected to give 140 farmers for each of the zones respectively. In all, 256 farmers were interviewed for the study. Selected personal characteristics of farmers like age, years of formal education, household size, and farm size in hectare were measured by their absolute values. Furthermore, farmers were asked to supply information on their access to farm land, number of productivities land lost to conflict, crop production was measured in tons, farmers' six crops standardized mean yield weight for conflict strata was computed, while numbering was used to measure livestock production level. Data were analyzed using frequency, percentage and standard deviation and a two-sample t-test was used to compare the impact of conflict on crops and livestock production level in core and outside conflict areas of the 2 agro-ecological zones.

4. Result and Discussion

4.1 Personal Characteristics of Farmers in Rainforest and Savannah Zones

Entries in Table 2 show the personal characteristics of farmers from, conflict and non-conflict areas of rainforest and savannah. The Table indicates that in core conflict area 85. 4% and 90.2% of farmers in rainfall and savannah zones respectively fell within the age range 25-63 years. Also 95.0% and 80.4% of the farmers in outside conflict areas of rainforest and savannah respectively were in age range of 25-63 years. According to Ekong (2003) any age range between 0-14 years, is classified as children and 16-64 years is classified as adult and 65 years and above as aged person. In line with the classification the sampled farmers belong to adulthood. Adolescences is an adventurous age when young people explore new horizons for green pastures of which attempt to block this ambition may result into personal and inter-group conflict in the society, which may have negative effect on framers' livelihood. Table 2 also indicates that in core conflict areas 83.3% and 86.8% of farmers in rainforest and savannah zones respectively were males while 87.0% and 69.0% of farmers in outside conflict areas rainforest and savannah respectively were males. Table 2 further shows that in core conflict areas majority (69.0%) of farmers in rainforest zone are Christian, while 44.3% of the farmers in the core conflict area of savannah zone are Christian. However, in outside conflict area of rainforest zone majority (60.9%) of farmers are Muslims while 12.7% are Muslims in savannah zone. Table 2 shows that in core conflicts 27.9% and 24.6% of farmers in rainforest and

savannah zones respectively have no formal education. Also, in outside conflict areas 27.5% and 28.6% of farmers in rainforest and savannah zones respectively did not have formal education. The implication of these findings is that many of the farmers across the 2 conflict strata in each agro-ecological zone were educated and would be able to adopt new agricultural technologies, have access to credit facilities, which will leads to improvement in farmers' livelihood. Entries in Table 2 revealed that in core 29.5% and 50.8% of the farmers from rainforest and savannah zones respectively inherited their pieces of land. The implication of this finding is that the most common land sources to farmers in rainforest zone's core conflict area are least, purchase and gift while inheritance and purchase of land were the most common land sources for savannah. Table 2 further indicates that in core conflict area of rainforest, 57.4% of farmers had 1-5ha farmland as against 27.8% farmers in savannah. Those that had 6-10ha in rainforest and savannah accounted for 42.0% and 92.2% respectively. Those that had farm holding greater than 20 ha, accounted for 9.8% and 39.5% in core conflict area of rainforest and savannah respectively. The implication of this finding is that farmers' farm holdings are still small. This justifies the reason for combination of farming with other work in order to sustain their livelihood. This finding is in line with the submission of Okunmadewa (2002) that small scale farming largely dominates the agricultural sector in Nigeria. Also Table 2, indicates distribution of farmers by type of farming systems which provides the primary means of livelihood to farmers. The findings revealed that 60.0% of the farmers derived livelihood needs from mixed planting of permanent crops with arable crops as against majority (63.0%) of the farmers who derived their livelihood need from mixed farming of combining livestock keeping with arable crops planting activities. This finding is in line with Olawoye (2000) who postulated that with several sources of income or produce, farmers' household food security could be guaranteed, as they are likely to suffer in the event that one activity fails.

Table 2. Farmers personal characteristics across the conflict location in the two ecological zone

Personal characteristics	Rainforest		Savannah	
	Core N = 61	Outside N=69	Core = 61	Outside =71
	%	%	%	%
Age				
12-24	6.5	2.9	0.0	8.9
25-37	26.6	13.4	21.3	30.4
38-50	19.6	39.1	39.3	42.9
51-63	39.3	43.3	29.5	7.1
>64	8.0	1.5	9.8	10.7
Sex				
Male	83.6	87.0	86.5	69.6
Female	16.4	13.0	13.2	30.4
Religion				
Christianity	69.0	27.5	44.3	57.1
Islam	29.5	60.9	33.7	12.7
Traditional	1.6	11.6	2.3	25.0
Educational Status				
None	27.9	27.5	24.6	28.6
Adult Literacy	9.8	5.8	32.8	23.2
Primary	32,2	21.7	29.5	23.2
Secondary	16.4	17.4	29.5	23.2
Tertiary	13.1	27.5	6.6	8.9
Sources of Land				
Inheritance	29.5	50.7	50.8	58.9
Lease	31.1	11.6	16.3	7.1
Purchase	24.6	14.5	26.2	16.0
Gift	14.8	23.2	6.6	15.5
Agricultural Holding				
1-5	57.4	26.1	27.8	75.0
6-10	18.0	20.3	9.8	16.1
11-15	11.5	10.1	13.1	3.6
16-20	3.3	20.3	9.8	0.0
>20	9.8	23.3	39.3	5.4
Type of Farming				
Arable Cropping	10,0	11.0	12.0	13.0
Permanent Cropping	11.0	15.0	1.0	1.0
Arable +Permanent	60.0	55.0	8.0	7.0
Livestock only	6	4.0	16.0	20.0
Livestock +Arable	10.0	13.0	63.0	58.0
Livestock + Arable +Permanent Crops	6.0	7.0	0.0	0.0

4.2 Farmers' Accessibility to Their Farm when Violent Conflict De-Escalated in Rainforest and Savannah Zones

Table 3 indicates that a very low percentage 24.0% of farmers in core conflict area of rainforest zone had access to their farms when conflict de-escalated compared to 96.5% of the farmers in outside conflict areas that had access to their farms. Similarly, in savannah zone, majority (78.0%) of farmers in core conflict area, 100.0% in the outside conflict areas had access to their farms land. The implication of this finding is that in core conflict area of rainforest areas 76.0% of farmers were displaced from their farm while 22.8% of farmers were displaced from their farm in savannah zone when conflict de-escalated.

Table 3. Farmers having access to their farms after the conflict in the two agro-ecological zone

Access to Land	Rainforest				Savannah			
	Core		Outside		Core		Outside	
	Freq	%	Freq	%	Freq	%	Freq	%
Yes	15	24.0	68	98.5	47	77.0	56	100
No	46	75.5	2	1.5	14	23.0	-	-

4.3 Farmers' Farm Land Lost to the Violent Conflict in Rainforest and Savannah Zone

Conflict situation in any community often reduces productive activities of the conflicting parties. This often results into diversion of time, energy, material and human resources to fighting in conflict situations (Ugwuegbu, 1999). This assertion is confirmed in rainforest core conflict area as shown in Table 4 where only few farmers (27.9%) did not lose any of their farm land to the conflict compared to majority (98.6%) of farmers that did not lose greater proportion of their productive land to conflict in outside conflict areas. Also, in savannah zone majority (78.4%) of farmers in the core conflict area lost a greater proportion of their farm land to the conflict compared to fewer (15.8%) of farmers that did lose their productive farm land to conflict in outside conflict area. Consequently, the conflict had resulted in adverse effects on the farmers' productive activities since land meant for performance of productive activities was lost to conflict.

Table 4. Farmers productive activities (farm land) lost to conflict in the two agro-ecological zone

Farm land (ha)	Rainforest Core Conflict	Savannah Core Conflict
1-5	31.4	20.3
6-10	52.2	42.4
7-15	-	15.7
> 16	-	-

4.4 Farmers' Crops Production in the Two Conflict Strata in Rainforest and Savannah Zones

The crops mean production index in rainforest as shown in Table 2 reveals that farmers in core conflict area have lower crops mean yield weight for cocoa 295.0, kolanut, 266.5, maize 250.2 yam 227.0, cassava 238.0 compared to higher crops mean yield weight for cocoa 697, kolanut, 466.0 maize 683/0, sorghum 273.0, yam 374.2, cassava 715.5 in outside conflict area The violent conflict accounted for the low crop production in core conflict area, since 76.0% of the farmers were displaced (Table 3). Furthermore, data on Table 4 shows that 83.6% of them lost their productive farm land to conflict. Paradoxically, when crops mean production in core and outside conflict areas was compared in savannah zone; the result indicated that conflict impact was not severe on crops production. Farmers in the core conflict locations have higher mean yield because 77.0% of the farmers were not displaced by the conflict (Table 3). Also, harvesting period for arable crops require short duration and permanent tree crops require longer time may have accounted for the variation in conflict impact on crops production index in core conflict area of rainforest and savannah.

Table 5. Farmers crops production across conflict location after the conflict

Variables	Rainforest		Derived Savannah	
	Core	Outside	Core	Outside
	Mean No	Mean No	Mean No	Mean No
Cocoa	295.0	697.3	29.7	12.9
Kola-nut	266.5	466.6	26.0	0.0
Maize	250.0	683.5	697.0	792.0
Sorghum	-	273.2	575.0	742.0
Yam	227.0	745.6	423.0	629.0
Cassava	238.0	715.5	423.0	321.6

4.5 Livestock Production after the Conflict Across the Two Conflict Strata in Rainforest and Savannah Zones

The result presented in table 6 indicates that farmers in core conflict area of savannah recorded lower mean number of x = 29 for cattle, x = 180 for sheep and goat x = 547 for birds. However, outside the conflict area, farmers recorded higher mean number of 41 for cattle, higher mean number of x = 2007 for sheep and goat and x = 2179 mean number for birds. The observed decline in livestock production in core conflict area of savannah is attributed to the violent conflict since, 78.4% of the farmers had earlier expressed that they have lost their productive land to the conflict as shown in (Table 4). However, it is observed that farmers did not recover quickly from the impact of the conflict on their livestock enterprises because livestock maturity requires longer time unlike the arable crops in the same zone. The rainforest zone felt impact of the conflict on livestock production but it was not as severe as that of savannah zone as shown in Table 6.

Table 6. Farmers livestock production across conflict locations after the violent conflict

Variables	Rainforest		Derived Savannah	
	Core	Outside	Core	Outside
	Mean No	Mean No	Mean No	Mean No
Cattle	6.5	5.2	29.0	40.7
Sheep and goat	14.2	62.2	180.2	2007.0
Birds	29.5	90.1	547.0	2179.0

4.6 Hypothesis 1

There is no significant difference between farmers' production level in core conflict areas and outside conflict areas after the conflict in Rainforest and savannah zone.

Six crops standardized mean yield weight for conflict strata were computed in order to test hypothesis which established that there is a significant difference between the farm output yield of farmers in core conflict area and outside conflict areas of rainforest and savannah zones. The difference is statistically significant in rainforest zone (t cal = 8.87; > 1.96). The higher mean index of 3,208 kg recorded in the outside conflict zone confirms the impact of the violent conflict on crop production in core conflict zone where lower crops mean yield index of 1,276 kg was recorded while it is assumed that other factors remain constant. Data from savannah zone indicates that there was no significant difference between farmers' standardized six crops mean yield index in core conflict area and outside conflict areas after the conflict (t cal = 0.27, < 1.96). This is further corroborated by mean yield of farmers, where core conflict area recorded higher (2,706.3 kg) yield index than mean yield index in outside conflict area (2,613.4 kg).

Table 7. Test of difference between crops production means in core and outside conflict areas of the two ecological zones

Zones	Location	Mean	Calculated T-Value	Tabulated T- Value	Decision
Savannah zone	Core	92.9	0.27	1.96	*NS
	Outside				
	Core	1932.2	8.97	1.96	* S
Rainforest	Outside				

*NS= Not Significant.

*S= Significant.

4.7 Hypothesis 2

There is no significant difference in livestock production mean number between core and outside conflict areas in rainforest and savannah zones

Pertaining to livestock production level in the two ecological zones as shown in Table 8, there is a significant difference between average number of sheep and goat as well as bird, kept by farmers in core and outside conflict areas. The test result indicates that the difference is statistically significant for sheep and goat in rainforest (t = 2.32; > 1.96) and savannah (t cal = 10.98, > 1.96). Also, average number of birds is significantly different in savannah (t = 10.91; t > 1.96) and rainforest (t = 2.62; t > 1.96) as shown in Table 8. These findings reveal that in core conflict area the violent conflict wiped out livestock holdings of farmers, which is a major source of animal protein to rural farm families. Consequently, many farmers are likely to suffer from malnutrition and other ailments. Meanwhile, one of the major effects of the violent conflict is the decline in the income farmers realize from the sale of extra livestock. Hence, food security in the village as a result of keeping small livestock has been distorted as well as reduction in the source of protein.

Table 8. Test of difference between sheep and goat production means in core and outside conflict areas of the two ecological zones

Zones	Location	Livestock	Mean	Calculated T-Value	Tabulated T- Value	Decision
Savannah zone	Core	Cattle	29.0	1.88	1.96	*NS
	Outside					
	Core	Sheep		10.98	1.96	* S
&goat 1826.8						
	Outside					
	Core	Birds	1692.0	8.91	1.96	*S
	Outside					
Rainforest	Core	Cattle	1.3	1.83	1.96	NS
	Outside					
	Core	Sheep &	47.7	2.32	1.96	S
	Outside	goat				
	Core	Birds	60.6	2.62	1.96	S
	Outside					

*NS= Not Significant.

*S= Significant.

5. Conclusion and Recommendation

Based on the findings of this study it can be concluded that farmers in the two agro-ecological zones were in the adventurous age. The age when young people explore new horizon for green pastures of which attempt to block their ambition may result into personal and inter-group conflict in the society. It was also found that majority of the farmers in rainforest zone acquired farm land through purchase, gift and least whereas majority of the farmers acquired farm land through inheritance in savannah zone. It was found that majority of farmers in core conflict areas did not have access to their farms when conflict de-escalated unlike in savannah area. In the 2 agro-ecological zone farmers lost their productive land to the conflict. Consequently crops production was lower in rainforest area while there is reduction in number of livestock produced in savannah areas. In order to prevent and minimised occurrences of violent conflict in the 2 agro-ecological zones, the following are recommended: Agricultural Inputs Supply Company (A.I.S.C) in the two states should endeavor to focus on supply of farm inputs to farmers at subsidized rates and prevent farmers from purchasing adulterated and very expensive farm inputs from the private input supply agencies in their areas. Capacity building for farmers should be encouraged through formation of rural cooperative societies or related associations to facilitate farmers' access to loan facilities with fewer burdens for collateral security requirements.

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Developing of Local Peanut Based on Multiculture That Tolerant to Abiotic and Biotic Conditions With Multigamma Radiation Method (Nuclear)

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Abstract

The main problems investigated in these research are 1) the developing of local peanut variety from west Sumba through breeding with multigamma radiation method (nuclear) and carefully selection on two varieties of peanut, i.e erect peanut and creep peanut by multiculture principle, in order to obtain the primer seed of local peanut variety that can increase production, 2) developing of primer seed of local yellow corn, in order to tolerant to abiotic and biotic conditions. The main method of research is application of multigamma radiation that supported by other methods comprised of observation/survey, sampling, multiculture, analysis, comparison, and interpretation.

The results of research are two primer varieties of local peanut seed from East Sumba with principle multiculture by multigamma radiation and carefully selection, and the primer seed of sweet local corn that tolerant to abiotic and biotic conditions, in order that production results of peanut's farmer and local corn in East Sumba specially and NNT generally, can significantly optimally increase, to support the stamina and safety of National food. The average production is obtained 5.7 ton/ha and 4.5 ton/ha for erect peanut, or the average production increase 43.86% for creep peanut and 42.22% for erect peanut.

On the second planting (first cleansing), the research on the second year, obtained the increase of average production about 45.84% (percent) of dried pod for erect peanut and 46.67% (percent) of dry pod for creep peanut. The increase of production potential on the second planting, the research on the second year in succession is 52.29% (percent) dried pod dried pod for erect peanut, and 52.22% (percent) dried pod for creep peanut. The increase average of production on the second year of research is 40.25% (percent) dried pod for erect peanut, and 40.42% (percent) dried pod for creep peanut. The increase average of production potential 48.84% (percent) dry pod for erect peanut, and 49.95% (percent) dried pod for creep peanut.

Keyword: abiotic-biotic, breeding, developing, multiculture, multigamma

1. Introduction

Peanut (Binomial Arachis Hypogaea L) is the second legumes in Indonesia after soybean has an important benefit because it's seed contain high protein and fat (Dean et al., 2009). In 2003, FAO report that oil product of peanut reach to 10% market of world oil (non crude oil). Peanut suits to be cultivated with multiculture system, because it's illumination limited and can be produced two kinds of commodities in the same time. Multiculture is combination of two or more plants at one area in the same time (Warsana, 2009), like as corn and peanut, corn and soybean, etc.

Peanut generally cultivated by the farmer there are two kinds: 1) creep peanut with characteristics: grow to side direction, the main stem is long, pod on the joints of stem, and genrally has long age. It's grows to creep to all directions in form of circle with diameter up to 150 cm. 2) erect penut. Characteristics of erect peanut: grow bolt upright, pod on the the joint of stem near clump, the age of plant is short and compact flowered, the average of plant high is 50 cm. On generally, the primer or superior variety of peanut has several physical characteristics (KDMBP2IPT, 2009; PT. Nasa, 2009): 1) high production, 2) the age of plant is shorter (between 85 up to 90 days),

3) product is stability, 4) tolerant to germ specially viruses (Damicone et al., 2010), 5) tolerant to plant disease like as agromyza, phaedonia inclusa, lamprosema litura, riptortus linearis, etiella zinkkenella, and nezara viridula (Branch et al., 2008; Chapin et al., 2010; Tubbs et al., 2010), 6) tolerant to abioic conditions, 7) the quality of seed increase (content of protein and fat) 8) power of grow is haigh (95%), 9) shiny skin, 10) water concentration 9%-12% (Irwan, 2006). Multigamma radiation technique cause fenotive and genetic effect like as the changing of structure dan composition of chromosome and also molecular of deoxiribo nuclei acid (DNA) on several kinds of plants. Mutation on the plant is spontaneously changed of genetic matters in cells caused by: 1) rearrangement occurred on chromosome structure, 2) changing in genetics, 3) segments duplication of chromosome loss (Darussalam, 1989). The breeding of plant to obtain superior seed variety is necessary chosen efective mutagen i.e mutagen that cause small changing or damage of physiology but large genetic effect on the plant (Isleib et al., 2008; Holbrook et al., 2009; Nigam et al., 2009), like as multigamma or gamma radiation (physical mutagen).

Dosage standard of gamma or multigamma radiation used on breeding of plants according to International Atomic Energy Agency and Darussalam are: 1. Mutation on the plants: 100 rads-3000 rads, 2. Mutation on seeds of plants: 1000 rads-4000 rads, 3. Grow stimulation (cereals plants): 250 rads-1000 rads (IAEA, 2003, 2004, 2006., Darussalam, 1989).

1.1 Radiation Effect on DNA

DNA structure formed of double helicks which composed from bundle between phosphate group and dioxiribo sugar that form of strand DNA, and bundle between nitrogen bases which connect to two strands DNA. A large part broken of DNA are broken on bases, bases lost, the bundle between bases has broken, and the bundle of sugar and phosphate has broken, in order that, occurred broken on one strand is called single strand break (***ssb***).

This damage can be quickly reconstructed without mistake by enzymatic repairs process with using strand DNA that is not break as mold (Bennet, 1996; Hall, 2000). Cell can do the contruction process to the broken of DNA in a few hours, but can be not perfect, mainly to the broken of DNA is called double strends breaks (***dsb***) or break of two strand DNA. The reconstruction process with mistaking causes mutaion of abnormality genetics and chromosome (Bennet, 1996; Hall, 2000). Figure 1, illustrates the broken on DNA as consequnce of radiation.

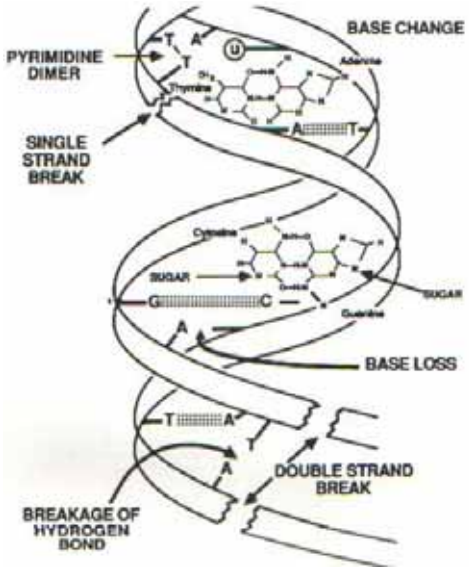

Figure 1. The broken of DNA as consequ-ence of radiation.

1.2 Radiation Effect on Chromosome.

Multigamma radaition causes changing of chromosome structure. Normally, chromo-some comprises of upper-arm and fore-arm connected by a centromer. Multigamma radiation causes the forming of: 1) assentric fragment (formed of chromo-some fragment without centromer), 2) disentric chromosome (chromosome has two centromers), 3) ring chromosome, 4) translocation (removal of genetic matter betwen chromosome arm) (Hall, 2000). Fregquency of disentric chromosome and ring increase with creasing of commulative doses on area which

is high radiation (Crose, 1987). The both effect is important case in breeding of Peanut to oabtain superior seed with specific characteristics.

Production of peanut per hectare is relatively low in every year. It is influenced by soil factor that harder and extreme weather, dry condition, germ, availability of superior seeds, etc. Peanut as a part of food self-supporting of Indonesia is a commodity which still low production in farmer level (Liptan IP2TP, 2004). For increasing these production is needed system completing of peanut cultivation with application of cultivation technology and modern technology for supplying of superior seeds (Domer, 2009). This method is application of multigamma radiation for breeding of local peanut to obtain several superior seeds on multiculture system, that tolerant to abiotic and biotic conditions, specially dry condition, in order that attained optimal production. The supplying of superior seeds of peanut and corn is one problem to the farmer specially in East Sumba, and generally Indonesia, because environment condition factors, like as dry conditions, hard soil, high calcium, high salt, micro and macro-elements are poor, germ, etc. The developing of peanut from East Sumba and corn have an opportunity to obtain superior seeds of peanut and corn that tolerant to those conditions above, in order that can increase production of the farmer in regional or national level.

The superiority of this method is obtained superior seeds variety of plants in relatively short time and there are several superior verieties as a breeding product with characteristics are differenced to initial variety. The aims of these research: 1) to develop local peanut from East Sumba based on multiculture through breeding with multigamma radiation method (nuclear) and carefully selection to obtain two kinds superior seed: a) creep peanut, and b) erect peanut, that tolerant to abaiotic and biotic conditions, 2) to develop primer seed of local yellow corn as a product of multigamma radiation on research in 2009 for incresing of production.

2. Methods

The main instruments used in this research comprose of: 1) Multigamma/gamma radiation source, 2) Counter of radiation dose, 3) Analysis equipments of water concentration, fat, and protein, 4) Tractor, 5) Equipments proponent like as: crowbar, mattock, pail, pourer, water tube, germ sprayer, shovel, hammer, saw, etc. Research location in Kupang East Nusa Tenggara at four locations : Bolok, Baumata, Oesao, and Fukdale for the first year, and ten locations for second year: two location at Bolok, two location at Baumata, two locations at Oesao, two locations at Fukdale, and two locations at Bakunase.

The methods of research consist of Observation/ Surveying, sampling, radiation, selection, comparison, and interpretation. The steps of research comprise of: 1) Observation/ surveying to establish location research and selection of two kind samples of local peanut form East Sumba. Observation and measurement several kinds of initial plant of peanut (plant high, mass of 100 pods, plant age, mass of 100 seeds), analysis of protein, fat, and water amount, 2) Design and preparation of sample garden, 3) Peanut sample irradiated by multigamma radiation source on doses 1000 rads-4000 rads and gamma source on dose 0,2 kG, 4) Plant seeds after irradiated with multiculture system, 5) Pour plant necessary while growth, 6) Weed garden and fertilize of plant, 7) Observation and measuring physiscs and chemical characteristics of plant, 8) Harvesting and drying of seeds, 9) To compare physical and chemical characteristics bettwen initial plant and generation plant, interpretate, and conclude of analysis results.

Collecting and data analysis is done through observation that focused on several cases (tolerant to germ, growth at land with haigh calcium, high salt, poor of micro-micro elements, tolerant to dry condition, etc). On harvest period, is done last selection, measuring of plant high and size of pod. After hervest, mass of each group 100 seeds and 100 pods measured. Bihind that, analize protein, fat, and water amount of initial plant and generation plant. The all physical and chemical characteristics and analysis results compared between initial plant and generation plant.

3. Result and Discussion

Figure 2, shows two examples growth of local peanut from East Sumba as a result of multigamma radiation on multiculture system. The corn seed used is local sweet yellow corn as the result of multigamma radaition, crossing, and carefully selection on research in 2009 (Pasangka & Jaelani, 2009). Those examples on Figure 2 show that peanut and corn which grew with multiculture system can be growing rapidly on garden with several conditions laike as dry conditions, high salt, haigh calcium, and poor of macro-micro elements. Figure 3, shows the growth example of generation local peanut on second year research (cleansing).

Figure 2. The growth example of local creep peanut as a result of multigamma radiation and superior sweet yellow corn (multiculture) on flowered period until age resemble to young harvest, which fertile grow at garden with high calcium and high salt

Figure 3. The growth example of generation local peanut on second year research (cleansing)

4. Observation and Measuring

Physical and chemical characteristics inspected and measured on local peanut as a result of multigamma radation during research go on included in Table 1, while superior sweet yellow corn included in Table 2. Production of local peanut from East Sumba as a result of multigamma radaition on research in first year included in Table 3, production of local peanut at ten planting locations on first step and the second step of research in second year include in Table 4 and Table 5. Based on data in Table 1, can be proposed that developing of local peanut from East Sumba with multigamma radiation based on multiculture can increase mean production as large as 43.86% for creep peanut and 42.22% for erect peanut. Increasing potential of production reach 32.20% for creep peanut and 27.66% for erect peanut. Production of corn on multiculture system is 14.85 tons/ha young harvest (research on first year).

Table 1. Observation and measuring result of physical and chemical characteristics of local peanut as a result of multigaam radiation

No	Description	Initial variety	Generation variety
1	Growth	10 days after planting (dap)	(4-6) days after planting
2	Mean sprouting capacity	88 %	95 %
3	Adaptation at garden with high calcium and high salt	non fertile grow	fertile grow
4	Adaptation to dry condition and poor micro-macro elements.	non adapt	adapt
5	Stem colour	green	green
6	Leaf colour	green yellowish	green
7	Flower colour	yellow	yellow
8	Seeds colour	red- brownish	red-brownish, red, brown
9	seeds measure	creep peanut: middle erect peanut: small	creep peanut: large erect peanut: middle
10	Form of plant	1) creep 2) erect	1) creep, 2) erect
11	Form of seeds	creep peanut: oval erect peanut: circle	creep peanut : oval, tapering, pointed erect peanut: circle, oval
12	Growth	fertile less	fertile
13	Taste	sweet and deliciously oily	sweet and deliciously oily
14	Grow type in time flowered	1) creep, 2) erect	1) creep, 2) erect
15	The age of flowered	26-33 days after planting	20-33 days after planting
16	The age of ald pods	creep peanut: 180-225 days after planting, erect peanut: 92-108days after planting	creep peanut: 105-120 days after planting, erect penut: 90-95 days after planting.
17	Plant high	creep peanut: 28 cm - 51 cm, erect peanut : 35 cm-57 cm	creep peanut: 42 cm - 63 cm. erect peanut:74 cm - 105 cm
17	Mean of plant high	creep peanut: 39 cm creep long: 25 cm- 51 cm mean crep long: 40.64 cm erect peanut: 46 cm	creep paenut: 53.88 cm erect peanut: 78.60 cm creep long: 59 cm-82 cm. mean creep long: 67.14 cm
18	Form of pods	creep peanut: rather waist erect peanut: waist.	creep peanut: rather flat. erect penaut: rather waist
19	The number of pods per plant	crep peanut: 18-36 pods erect peanut: 10-31 pods.	creep peanut: 32-123 pods. erect peanut: 25-76 pods.
20	The number of seed per pods	creep peanut: 1-2 seeds. erect peanut: 1-3 seeds.	Creep peanut: 1-3 seeds. erect peanut: 2- 8 seeds.
21	The mean mass per 100 seeds	creep peanut : 40.8 grams. erect peanut: 38.5 grams.	creep peanut: 78.7 grams. erect peanut: 68.4 grams.
22	The mean mass per 100 pods	creep peanut: 142.0 grams. erect peanut: 120.2 grams.	creep peanut: 257.6 grams. erect penaut: 249.5 grams.
23	Carbohydrate amount	creep peanut: 15.23 %. erect peanut: 13.84 %.	creep peanut: 15.86 %. erect peanut: 14.25 %.
24	Fat amount	creep peanut: 46.12 %. erect peanut: 33.86 %.	creep peanut: 46.98 %. erect peanut: 34.18 %.
25	Water amount	(9-12) %	(8.7-11.9) %
26	Mean Production	creep peanut: 3.2 tons/ha dry pods. erect peanut: 2.6 tons/ha (dp).	creep peanut: 5.7 tons/ ha dry pods (dp). erect peanut: 4.5 tons/ha (pk)
27	Potential production	creep peanut: 4.0 tons/ha (dp) erect peanut: 3.4 tons/ha (dp)	creep peanut: 5.9 tons/ ha (dp) erect peanut: 4.7 tons/ha(dp)
28	Adaptation to germ	non adapt.	adapt
29	Growth with multiculture system	non fertile and small pods	fertile and large pods

Table 2. Observation and mesuring result of physical and chemical characteristics of superior local sweet yellow corn

No	Observed and measured variables of plant of superior sweet yellow corn
1	Seeds fast grow.
2	Mean sprouting capacity : 92%
3	Fertile grow at garden with high calcium, high salt, poor micro-macro elements, and dry conditions (abiotic conditions).
4	Adapt to dry condition.
5	Adapt to germ.
6	The number of sheet leaf: 8-12.
7	Form of leaf is short and strong.
8	Form of plant: erect and strong.
9	Pods hair: white (young), brown (old).
10	The mean of high plant: 235 cm.
11	The age of young harvest : 60 days - 68 days after planting.
12	Measure stem of an aer of corn: long: 19.10 cm - 33.12 cm, diameter: 4.65 cm- 8.92 cm, mean diameter 6.45 cm
13	Mass of stem an ear of corn: 310.90 grams - 415.70 grams.
14	Seeds colouri: yellow.
15	Mean mass per 1,000 seeds: 385.00 grams.
16	Mean of stem an ear corn: 369.04 grams.
17	Taste: sweet.
18	Production potential 15 tons per ha multiculture system with young harvest.
19	A part of plant have two stem an ear of corn (60%).
20	Large seeds.
21	Form of dry seed: shrivel.
22	Protein amount: 10.75%

Table 3. Production of local peanut from East Sumba as a result of multigamma radaition on research in first year

Num	Locations	Kind of peanut	Production (tons/ha)	Mean production (tons/ha)	Production potential (tons/ha)
1	Fukdale	Creep	5.8		
		Erect	4.6		
2	Baumata	Creep	5.5	5.7	5.9
		Erect	4.3		
3	Bolok	Creep	5.6		
		Erect	4.4	4.5	4.7
4	Oesao	Creep	5.9		
		Erect	4.7		

Table 4. Production of local peanut at ten planting locations on first step of research in second year

Num.	Locations	Kinds of peanut	Production (tons/ha)	Mean production (tons/ha)	Production potential (tons/ha)
1	Bolok (2)	Creep	4.40		
		Erect	3.46		
2	Baumata (2)	Creep	4.30	4.86	6.00
		Erect	3.50		
3	Bakunase (2)	Creep	4.50		
		Erect	3.53		
4	Oesao (2)	Creep	5.10		
		Erect	4.62	3.98	4.80
5	Fukdale (2)	Creep	6.00		
		Erect	4.80		

Table 5. Production of local peanut at ten planting locations on second step of research in second year (first cleansing)

Num	Locations	Kinds of peanut	Production (tons/ha)	Mean production (tons/ha)	Production potential (tons/ha)
1	Bolok (2)	Creep	4.75		
		Erect	4.12		
2	Baumata (2)	Creep	4.80	5.46	6.84
		Erect	3.95		
3	Bakunase (2)	Creep	5.20		
		Erect	4.25		
4	Oesao (2)	Creep	5.70		
		Erect	5.12	4.58	5.45
5	Fukdale (2)	Creep	6.84		
		Erect	5.45		

Figure 4a, shows pods still hang on it's stem which dominated 3-4 seeds per pod with the number of pods per tree revolve between 25 up to 76. The main production of peanut from East Sumba as a result of multigmma radiation reached at four planting location with multiculture system is 5.7 tons/ha for creep peanut and 4.5 tons/ha for erect peanut. Production interval reached at four planting location: 5.5 tons/ha up to 5.9 tons/ha for creep peanut and 4.3 tons/ha up to 4.7 tons/ha for erect peanut. Maximum product potential is 5.9 tons/ha for creep peanut and 4.7 tons/ha for erect peanut (Research on first step, the second year).

Figure 4a. Generation erect peanut

Figure 4b. Generation erect peanut

Figure 4a. The example of erect local peanut from East Sumba as a result of multigamma radiation has harversted, It shows pods to be dominated by four seeds per pod about 55% and three seed per pod about 35%, and 10% two seeds per pod. The number of pods per tree revolve between 25 up to 76 pods , the age of harvest about 90-95 dys after planted. Figure 4b shows the example of erect local peanut as a result of multigamma radiation has harversted with four seeds per pod and compact contents.

Figure 5a shows example of creep local peanut from East Sumba as a result of multigamma radiation has harvested with two seeds per pod, compact contents, and large seed. Figure 5b is example of seeds of creep local peanut as a result of multigamma radiation which carefully selected for continuously developing. Figure 6 shows example of superior local corn (sweet yellow corn) selected for continuously developing with multiculture system.

Figure 5a. Pods of generation creep peanut

Figure 5b. Seeds of generation creep peanut

Figure 6. Superior sweet yellow corn as a result of multigamma radiation (local corn)

The results of observation and measuring of physical and chemical characteristics show that local creep peanut and local erect peanut from East Sumba that developed with multigamma radiation on multiculture system, tolerant to abiotik conditions like as dry conditions, high salt, high calcium, and poor of micro-macro elements, and tolerant to biotic conditions like as germ. Based on data in Table 1 and Table 2, can be proposed that seeds of local peanut from East Sumba as a result of multigamma radiation have grow power is higher than initial local peanut and growth is more quickly than initial local peanut, flowered time is qucikly and harvest time is more quickly than initial peanut. Economizing time obtained as large as 41.67% up to 46.70% for superior creep peanut and 2.17% up to 12.04% for superior erect peanut.

4.1 Tolerant to Germ

Based on observation results from firts growth until to harvest time clearly shown that growth of superior local peanut tolerant to germ. This case is shown by soft leaf sisnce growth on both kinds of peanut.

4.2 Adaptation Characteristics

On first step and the second step planting ofresearch in second year is shown that the both kinds of superior peanut as a result of multigamma radiation for all varieties chosen, growing rapidly at several conditions laike as dry condition, high salt, high calcium, etc.

4.3 Grow Ability

Superior seeds of peanut have mean grow ability about 96% and superior seeds of sweet yellow corn about 92%. The grow ability of initial peanut: 88%. It is shows the increase of grow ability as big as 9.05% on the all varieties of superior local peanut as a result of multigamma radaition.

4.4 The Increase of Production

Production of superior varieties of peanut significantly increase, i.e the increase of mean production is 42.22% dry pods for erect peanut and 31.91% dry pods for creep peanut (research on the first year). On the first step planting of research in second year obtained potential increasing production 29.17% dry pods for erect peanut, and 33.33% dry pods for creep peanut. On the second step planting of researh in second year (first cleansing) obtained increase of mean production 43.23% dry pods for erect peanut and 41.39% dry pods for creep peanut. Production potential increasing on second step planting of research in second year is 37.61% dry pods for erect peanut and 41.52% dry pods for creep peanut. The increasing of mean production of research on second year about 38.95% dry pods for erect peanut and 37.78% dry pods for creep peanut. The mean increasing potential 33.39% dry pods for erect peanut and 37.43% dry pods for creep penut. The increasing of corn is also significantly, i.e 15 tons/ha young harvest (multiculture system).

4.5 Conclusion and Suggestion

Based on explain above, can be proposed conclussion: 1) Developing of local peanut based on multiculture with multigamma radaition and carefully selection can increase mean production of the peanut as big as 40.59% dry pods for erect penaut and 34.85% dry pods for creep peanut (mean production: 4.39 tons/ha dry pods for erect peanut and 4.93 tons/ha dry pods for creep penaut). 2) Developing of local peanut based on multiculture with multigamma radaition (nuclear) and carefully selection produces superior local peanut and has a certain quality with physical and chemical caharacteristic complete. There are several varieties of primer seed obtained in this research. The researcher suggest in order that the all varieties can be continuously developed to obtain superior seed of peanut to be cultivated as widespread as possible by the farmers.

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Effect of Dietary Garlic Source on Feed Utilization, Growth and Histopathology of the African Catfish (*Clarias gariepinus*)

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Abstract

The effects of garlic on the growth, survival and histology of *Clarias gariepinus* were examined during the period of eight weeks. One hundred and eighty (180) fingerlings with initial mean weight of 3.90 ± 0.02 were stocked at 15 fish per net happa (0.8 cm x 0.6 cm x 0.4 cm) suspended in an earthen pond of (12 m x 12 m x 1.5 m).

Triplicate groups of fish with garlic feed inclusion were fed at 3% body weight with four Iso-nitrogenous diet (40% crude protein) in Treatment Diet1 (TD1) (control), Treatment Diet2 (TD2) 10%, Treatment Diet3 (TD3) 20% and Treatment Diet4 (TD4) 30% respectively. At the end of the experiment, mean weight gain (MWG), Feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and total protein consumed (TPC) were statistically close among the Treatments.

The highest MWG (53.63 ± 0.63) was recorded in fish fed with T4 while the lowest was recorded in fish fed T1 (41.73 ± 0.63). Specific growth ratio (SGR) was high in fish fed T4 (3.04 ± 0.06) and low in T3 (2.32 ± 0.04). The Treatment with the highest Feed conversion ratio (FCR) was T3 (2.57 ± 0.03) and the lowest was in T1 (2.11 ± 0.05). The overall best Treatment was Treatment 4 with 30% inclusion level of garlic source.

The histological examinations show no visible lesion in the liver and gut of all the Treatments except Treatment 3 with diffuse vacuolar degeneration of the hepatocytes, the gills in Treatment 4 has the epithelial cells of the secondary lamellae fused and proliferated.

Result obtained in this study indicated that 30% garlic inclusion rate in a compounded feed helps in feed utilization and growth Performance with no negative effect to the tissues.

Keywords: garlic, histology, fingerlings, iso-nitrogenous feed, treaments

1. Introduction

The supply of qualitative animal protein in sufficient quantity and at affordable cost has continued to remain a dream yet to be realized. It is a perennial problem and a major challenge to the livestock industry in most developing countries. High costs of feed due to shortage and unavailability of conventional feedstuffs for compounding livestock rations has been the major cause of rising cost of animal products (Sarkiyay, 2010).

Efforts aimed at increasing animal protein supply must necessarily address the competition between man and livestock for feed sources which has often resulted into shortage of such conventional feedstuffs like maize, soya beans and groundnut cake for compounding livestock feeds (Omage et al., 2008). This limitation imposed by scarcity of the conventional feedstuffs has made it necessary to source for alternative and cheaper feed materials to supply nutrients in livestock rations. Such materials would totally or partially substitute the expensive and relatively unavailable conventional feedstuffs and this will directly reduce production cost and improve profitability. It has been studied and reported that in intensive culture of fish breeding in which the fish are fed artificial feeds, the major recurring cost is the cost of feed which is about 60-75% of the operating cost for every cycle. The cost of feeding fish amounts to over two thirds of the operating cost of a fish culture in an intensive system (Eyo, 1990). Garlic is originating in Asia Minor and spontaneously grows in southern Europe, but in cultures, it could be found all over the world. It's a rich source of calcium, phosphorus and vitamin B1; it has a high content of carbohydrates and as a consequence a high nutritive value. Garlic also contains iodine salts which have

a positive effect on the circulatory system and rheumatism, silicates which have a positive effect on the skeletal and circulatory system and sulfur salts with positive effects on the skeletal system, cholesterolemia, and liver diseases. Garlic also contains vitamin complex B, vitamins A, C and F (Drăgan, 2008).

Garlic has the following effects: lower the cholesterol and the triglycerides, ameliorates atherosclerosis, has a hypotensive, coronary dilator, antioxidant and anti cancer effect.

Garlic contains sulfur containing compounds. Alliin is converted to the anti-microbial active allicin, when the bulb is cut or bruised. Ajoene, which is a secondary degradation product of alliin, is presumably the most active compound responsible for the anti-thrombotic activity of garlic (Wichtl, 2004) the fresh bulb contains alliin, allicin and volatile oils. When the garlic clove is crushed, the odorless compound alliin is converted to allicin, via the enzyme allinase. Allicin gives garlic its characteristic pungent smell (Skidmore-Roth, 2003). Garlic has also been shown to have antioxidant properties, which could have a protective nature against gastrointestinal neoplasias, against blood clots (anti-platelet action) due in part to the compounds alliin and ajoene, which have fibrinolytic activity. Ajoene inhibits thromboxane synthesis through the inhibition of the cyclo-oxygenase and lipoxygenase enzymes (Schulz et al., 2004).

Therefore, this study aims to determine the utilization and additive nature of garlic in the feed with various inclusion levels and to examine the histopathological effect of garlic on the visceral organs of the fish species.

2. Materials and Methods

One hundred and eighty (*Clarias gariepinus*) fingerlings with average weight of 3.90 ± 0.02 g were allowed to acclimatize to the environment for one week, and were starved for 24 hrs prior to being placed on experimental system. Ten fingerlings randomly selected samples were sacrificed for carcass analysis before the commencement of the experiment.

The feeding trial was carried out in 12 net happas (0.8 m x 0.4 m x 0.6 m) suspended by bamboo poles with kuralon rope in an earthen pond of size (12 m x 12 m x 1.5 m). Each Treatment was replicated thrice with a control treatment. The swampy nature of the earthen pond had water recharging it from the underground water.

Fifteen (15) fish were selected randomly into each happa and weigh with the use of a sensitive weighing scale (METER TOLEDO FB602) and fed at 5% of their body weight twice daily for a period of 8 weeks between the hours of 07:00-08:00 and 16:00-17:00 GMT. Fish were batch weighed weekly with a sensitive electronic scale and the feeds were adjusted accordingly with their increasing biomass, mortality was monitored daily.

Table 1. Experimental Layout

T1R3	T2R2	T3R2	T2R1
T3R3	T3R1	T4R2	T1R1
T4R3	T2R3	T4R1	T1R2

Treatment 1- control diets (0% inclusion); Treatment 2- Garlic (10% inclusion); Treatment 3- Garlic (20% inclusion); Treatment 4- Garlic (30% inclusion).

Table 2. Ingredient composition of experimental feed diet

Ingredients	T1	T2	T3	T4
Maize	29.67	30.10	30.45	31.47
Fish meal	27.03	26.84	26.64	26.43
Groundnut cake	27.03	26.59	25.54	23.43
Soybean meal	13.52	13.42	13.32	13.22
Garlic	-	0.3	1.2	2.8
Vit. Premix	1	1	1	1
Dicalcium phosphate	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00

Table 3. Proximate composition of experimental feed diet

Parameters	T1	T2	T3	T4
Moisture	7.6 ± 0.01[c]	6.9 ± 0.01[d]	8.33 ± 0.01[b]	8.6 ± 0.01[a]
Dry	92.98 ± 0.02[b]	93.1 ± 0.06[a]	91.66 ± 0.01[c]	91.4 ± 0.01[d]
Fat	19.57 ± 0.01[a]	15.52 ± 0.05[c]	15.28 ± 0.01[d]	16.13 ± 0.01[b]
Ash	9.26 ± 0.01[a]	8.35 ± 0.01[b]	8.11 ± 0.00[c]	7.92 ± 0.01[d]
F.C	29.54 ± 10.34	38.04 ± 0.02	36.71 ± 0.12	36.62 ± 0.01
C.P	3.56 ± 0.01[a]	2.26 ± 0.03[b]	2.16 ± 0.01[c]	2.06 ± 0.01[b]
CHO	28.91 ± 0.01[b]	29.95 ± 0.05[b]	29.41 ± 0.02[c]	30.67 ± 0.01[a]

Means along the same row with different superscripts are significantly different ($p<0.05$).

2.1 Growth Performance

$$\text{Percentage weight gain PWG (\%)} = \frac{(\text{Final mean body weight})}{(\text{Initial mean body weight})} \text{x } 100$$

$$\text{Specific growth rate, SGR} = \frac{\text{Ln W2} - \text{LnW1 x } 100}{\text{Time (days)}}$$

W1= initial weight gained; W2= Final weight gained; Ln= Natural logarithm.

$$\text{Protein efficiency ratio} = \frac{\text{Mean weight gain}}{\text{Average protein fed}}$$

$$\text{Feed conversion ratio} = \frac{\text{weight of feed (g)}}{\text{Weight gained}}$$

$$\text{Mortality rate} = \frac{\text{No of fish dead at the end of the experiment}}{\text{No of fish at the beginning of the experiment}} \text{x } 100$$

$$\text{Survival rate} = \frac{\text{No of fish remaining at the end of the experiment}}{\text{No of fish at the beginning of the experiment}} \text{x } 100$$

Feed conversion ratio, FCR this is obtained by dividing the total weight of the food administered the total increase in weight gained by the fish over a period of time.

$$\text{SGR} = \frac{\text{Ln W2} - \text{LW1}}{\text{Time (days)}} \text{X100}$$

W_1= Initial weight gain; W_2 =Final weight gain; L_n = Natural logarithm; Time = Number of days of experiment

$$\text{PER} = \frac{\text{Fish weight gain}}{\text{Protein gain}}$$

3. Results and Discussion

The initial and final carcass analysis of the fish is represented in Table 3.

Table 4. Carcass Analysis of experimental fish

Parameters	Initial	T1	T2	T3	T4
Moisture	78.62 ± 0.30 [c]	71.96 ± 0.01 [d]	81.41 ± 0.01 [b]	81.56 ± 0.01 [b]	85.03 ± 1.67 [a]
Dry	21.38 ± 0.01 [a]	18.54 ± 0.01 [c]	18.59 ± 0.03 [b]	18.61 ± 0.01 [b]	16.64 ± 0.01 [d]
Fat	2.79 ± 0.05 [b]	6.45 ± 0.12 [a]	2.42 ± 0.01 [c]	1.76 ± 0.01 [d]	1.45 ± 0.01 [e]
Ash	1.44 ± 0.01 [c]	2.17 ± 0.01 [a]	1.55 ± 0.01 [b]	1.26 ± 0.01 [d]	1.12 ± 0.02 [e]
F.C	0.98 ± 0.21	1.08 ± 0.01	1.06 ± 0.00	1.02 ± 0.01	1.00 ± 0.01
C.P	37.84 ± 0.17[c]	41.62 ± 0.08 [a]	39.44 ± 0.01 [b]	39.28 ± 0.01 [b]	38.03 ± 0.01 [c]
CHO	1.22 ± 0.05 [a]	0.92 ± 0.01 [d]	1.08 ± 0.00 [bc]	1.12 ± 0.01 [b]	1.04 ± 0.01 [c]

Means along the same row with different superscripts are significantly different ($p < 0.05$).

The moisture content of the fish carcass obtained after the experiment was high in three Treatments compared to the initial value. Treatment 1 was the lowest (71.96%), followed by Treatment 2 (81.41%), and Treatment 4 having the highest moisture content (85.03%). However, Treatment1 recorded the highest crude protein (41.62%) and the lowest was recorded in Treatment 4 (38.03%). Final Carbohydrate of fish carcass ranged between 0.92% in Treatment 1 to 1.12% in Treatment 3. The final dry matters in all the Treatments are lower than the initial values.

Table 5 shows the growth response of fish to different Garlic inclusions in the feed and the mean weekly values of physico-chemical parameters during the experiment are represented in Table 6

Table 5. Growth response of fish to different Garlic inclusion in the feed

Parameters	0% Inclusion T1	10% Inclusion T2	20% Inclusion T3	30% Inclusion T4
Initial Weight (g/fish)	3.90 ± 0.02	3.90 ± 0.04	3.90 ± 0.03	3.90 ± 0.02
Final Weight (g/fish)	35.00 ± 2.32^{a}	79.36 ± 1.92^{c}	80.49 ± 0.29^{b}	86.10 ± 0.39^{d}
Weight Gain (g/fish)	41.73 ± 0.63^{a}	43.96 ± 2.38^{c}	43.96 ± 0.93^{a}	53.63 ± 0.63^{b}
AFC (g/fish/day)	105.71 ± 0.14^{b}	155.43 ± 0.57^{c}	103.9 ± 0.71^{a}	159.58 ± 0.4^{d}
FCR	2.51 ± 0.01^{c}	2.33 ± 0.02^{b}	2.38 ± 0.02^{a}	2.11 ± 0.05^{a}
SGR	2.32 ± 0.04^{a}	2.79 ± 0.02^{b}	2.38 ± 0.02^{a}	3.04 ± 0.06^{d}
TPC	33.19 ± 0.59^{a}	55.62 ± 1.86^{d}	37.77 ± 0.39^{b}	55.26 ± 0.02^{d}
PER	0.75 ± 0.03^{a}	0.85 ± 0.01^{b}	0.91 ± 0.01^{d}	0.72 ± 0.01^{a}
Survival	50.77 ± 0.33^{a}	65.94 ± 0.44^{b}	70.40 ± 0.35^{c}	72.71 ± 0.61^{d}

Means along the same row with different superscripts are significantly different ($p<0.05$);
AFC: Average Feed Consumed, FCR: Feed Conversion Ratio, SGR: Specific Growth Rate, TPC: Total Protein Consumed, PER: Protein Efficiency Ratio.

In Table 5, Treatment 4 with 30% garlic inclusion level had the highest final weight per fish (86.10 ± 0.39 g), followed by Treatment 3 with 20% (80.49 ± 0.29 g) and the least in (control) Treatment 1 with 0% inclusion (35.00 ± 2.35 g), and all Treatments were significant ($p < 0.05$).

The Average feed consumed/fish/day was significantly different ($p < 0.05$) with the highest value in Treatment 4 (159.59 ± 0.4), followed by Treatment 2 (155.43 ± 0.57), Treatment 1 (105.71 ± 0.41) and the least was Treatment 3 (103.9 ± 0.71).

Treatment 4 (2.11 ± 0.05) had the best feed conversion ratio followed by Treatment 2 (2.33 ± 0.02) and 3 (2.38 ± 0.02), the least in Treatment 1 (2.51 ± 0.41).

The specific growth rate was significantly different ($p < 0.05$) in Treatment 4 (3.04 ± 0.06) and Treatment 2 (2.79 ± 0.02) while there was no significant different ($p > 0.05$) in Treatment 3 (2.38 ± 0.02) and Treatment 1 (2.32 ± 0.04) respectively.

There was a significant difference ($p < 0.05$) in the protein efficiency ratio in Treatment 3 (0.91 ± 0.01) and Treatment 2 (0.85 ± 0.01). This agrees with the work of Gabor (2010) on the effects of Some Phytoadditives on Growth, Health and Meat Quality on Different Species of Fish.

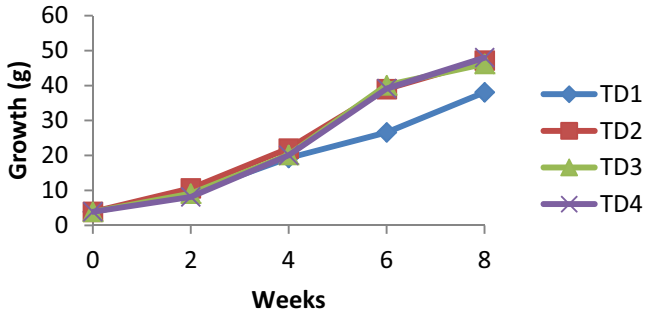

Figure 1. Growth response of *C.gariepinus* fingerlings fed with garlic base diets for eight weeks

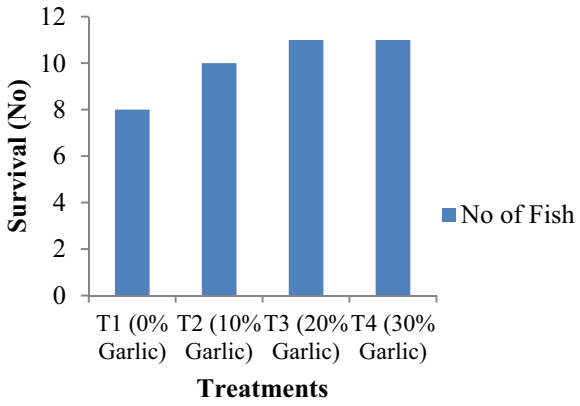

Figure 2. Mean survival rate of different treatments

There was significant difference ($p < 0.05$) in the survival rate of *Clarias gariepinus* fingerlings fed varying inclusion levels of garlic for the 8 weeks. The highest survival was obtain in Treatment 4 (72.71 ± 0.61), followed by Treatment 3 (70.40 ± 0.35), Treatment 2 (65.94 ± 0.44) and the least in Treatment 1 (50.77 ± 0.33). This was in support by Ashraf (2008) finding on survival of *Clarias gariepinus* in a net happa suspended in an earthen pond with varying stocking density. Increase in garlic inclusion of feed resulted in higher fish survival. This is similar to the findings of Ayotunde et al. (2005) in the work on toxicity of aqueous extract of drumstic, *Moringa oleifera*, to fingerling and adult catfish *Clarias gariepinus.* The mean survival rate of Treatments at the end of the experiment was highest in Treatment 4 (73%) while the lowest was recorded in Treatment 1 (51%).

Table 6. Mean weekly values of physico-chemical parameters during the experimental period

Weeks	pH	Dissolve oxygen (Mg/L)	Temp (^{o}C)
0	7.20 ± 0.06	6.17 ± 0.03	26.00 ± 1.15
1	7.31 ± 0.12	6.30 ± 0.20	26.53 ± 0.27
2	7.45 ± 0.32	6.23 ± 0.33	27.17 ± 0.33
3	7.41 ± 0.11	6.53 ± 0.13	27.37 ± 0.07
4	7.25 ± 0.43	7.40 ± 0.80	26.40 ± 0.35
5	7.15 ± 0.20	7.47 ± 0.73	28.01 ± 0.39
6	7.55 ± 0.55	7.25 ± 0.14	27.71 ± 0.19
7	7.26 ± 0.20	7.33 ± 0.33	27.44 ± 1.12
8	7.80 ± 0.30	7.40 ± 0.20	26.78 ± 0.77

The Mean weekly values of physico-chemical parameters during the experimental periods were within the acceptable range of rearing *Clarias gariepinus.* This conforms to the result of Adekoya *et al.* 2004 and FAO, 1992 recommended values for a successful catfish production system.

4. Statistical Analysis

All data obtained were subjected to one-way ANOVA test Where ANOVA revealed significant differences ($P < 0.05$), Duncan's multiple-range test (Zar, 1996) was applied to characterize and quantify the differences between treatments using SAS software for windows (SAS, 2009).

4.1 Histopathology (Control)

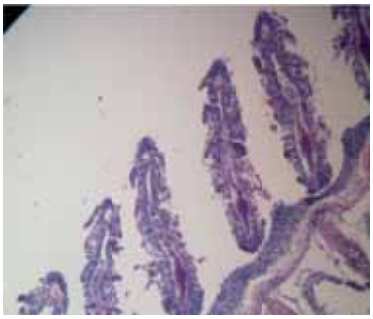

Plate 8
Gill (TD1)
No visible lesion (No physical damage)

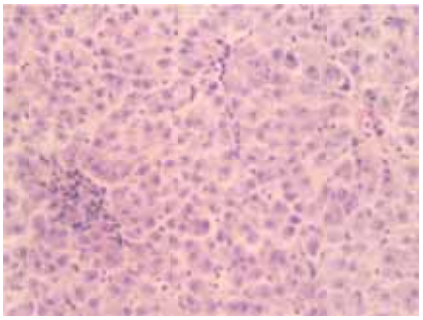

Plate 9
Liver (TD1)
No visible lesion (No physical damage)

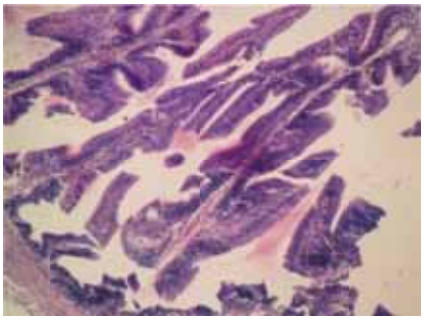

Plate 10
Gut (TD1)
No visible lesion (No physical damage)

4.2 Treatment 2

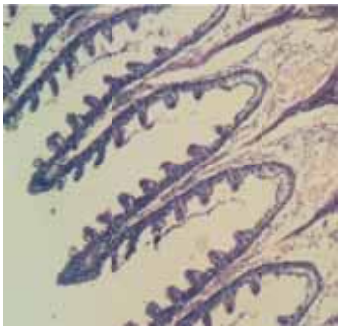

Plate 11
Gill
No visible lesion (No physical damage)

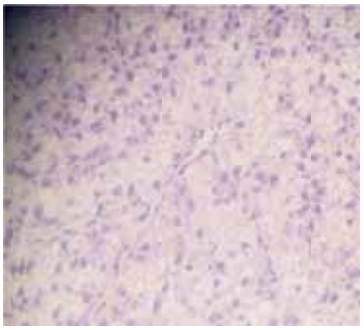

Plate 12
Liver
No visible lesion (No physical damage)

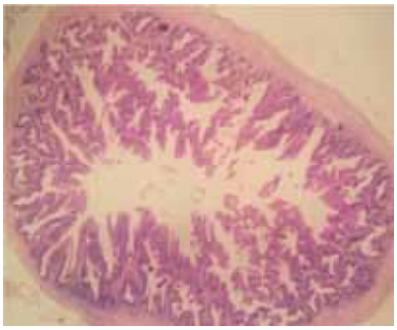

Plate 13
Gut
No visible lesion (No physical damage)

4.3 Treatment 3

Plate 14
Gill
Moderate diffuse proliferates or Hyperplasia of the second lamellae With vacuolation of the epithelial cells

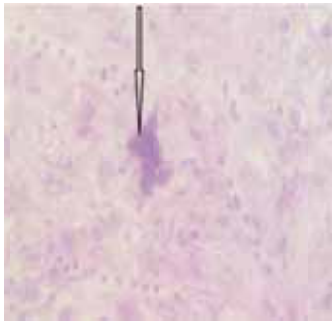

Plate 15
Liver
Moderate diffuse vascular degeneration

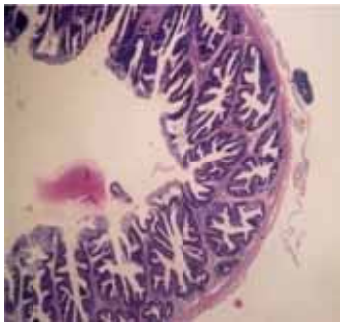

Plate 16
Gut
No visible lesion (No physical damage)

4.4 Treatment 4

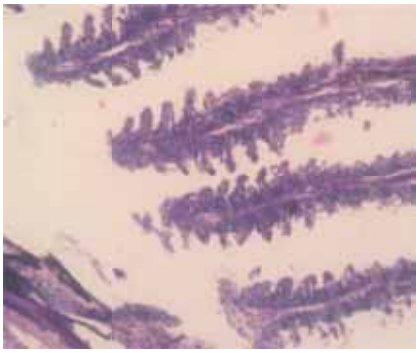

Plate 17

Gill

Fusion and proliferation of the gill

Epithelial cell of the ill lamillae

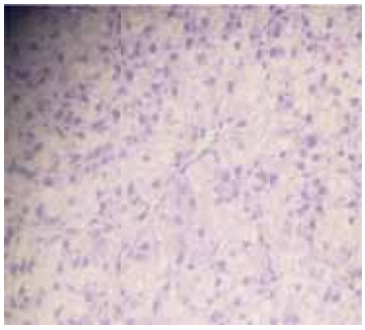

Plate 18

Liver

(No visible lesion i.e. no physical damage)

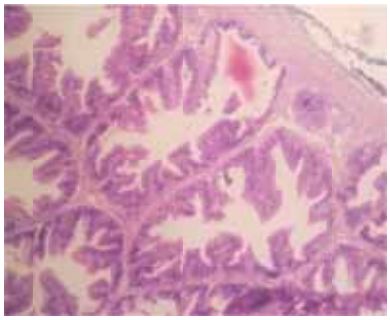

Plate 19

Gut

(No visible lesion i.e no physical damage)

4.5 Histopathology of the Gills

The gills participate in many important functions in the fish such as respiration, osmoregulation and excretion. The result in Treatment 1 (plate 8) and Treatment 2 (plate 11) shows no visible lesion i.e. physical damage in the gills which conform to the submission of Hassan et al. (2007), The finding was related to Anthonio et al. (2007) in the work on the histopathological changes in the normal gills epithelium of Nile Tilapia (*O. niloticus*) exposed to waterborne copper.

The lesion in the gills of Treatment 3 (plate 14) was manifested in the 20% inclusion level. The anomalies include diffuse proliferated hyperplasia of the secondary lamellae with vacuolation of the epithelial cells i.e. an unusual growth in a part of secondary lamellae caused by excessive multiplication of the cells. This is similar to the work of Sayed et al. (2007) on the histopathological alterations in the gills of adult catfish exposed to 4-Nonylphenol.

Treatment 4 (plate 17) shows gills with fusion and proliferation of the epithelial cells of the gills lamellae. This is similar to the findings of Dutta et al. (1996), where there were many alterations such as increase in mucous and chlorine cell number and size, necrosis, rupture of epithelium, desquamation, deformed secondary lamellae and Oedema.

Pleuranen et al. (1994), any discontinuity of epithelial lining of the gill lead to a negative ion balance and to changes in the haematocrite and mean cellular haemoglobin values of the blood.

Part et al. (1985) noticed similar result in the histology of the gills of rainbow trout where there was increased ion permeability and sodium efflux of gill epithelial cells due to ethoxylate nonyphenol.

4.6 Histopathology of the Liver

The liver is an organ most associated with the detoxification, biotransformation process and functions as blood supply due to its position (Carmago, 2011).

In the present study, Treatment 1 (plate 9), Treatment 2 (plate 12) and Treatment 4 (plate 18) shows no visible lesion in the liver. They possess normal histological structures of the liver.

Examination of the liver section of Treatment 3 (plate 15) shows moderate diffuse vascular degeneration of the hepatocytes resulting in the loss of ability of the fluid carrying vessel to deteriorate thereby reducing its function.

Similar results were recorded by Uguz et al. (2003) who reported a significant increase in the kupffer cells after one week of 4-Nonylphenol exposure. Hughes et al. (2000) and Uguz et al. (2003) reported that the disappearance of the cell membranes in the liver could be due to the lytic activity of alkylphenols.

4.7 Histopathology of the Gut

The result shows normal gut in all the treatments examined. Treatment 2 (plate 13), Treatment 3 (plate 16) and Treatment 4 (plate19) shows no visible lesion. No alterations in the gut of all the treatments administered with different level of garlic as observed in Treatment 1 (Control).

This is in agreement with the findings of (Fatma, 2009) in the work histopathogical studies on *Tilapia zillii* and *Solea vulgaris* from Lake Quarum.

Ayotunde et al. (2011), observed no visible lesion on control fish in the work histological changes in *O.niloticus* exposed to Aqueous extract of *Moringa oleifera* seeds powder.

5. Conclusion

Results show that garlic can be conveniently used as a complete phyto-additives in fish diet of African catfish (*Clarias gariepinus*). In general, the results obtained showed no negative effect on the growth of African catfish and histology of the viscera organs suggesting that it is essentially good for growth and utilization.

From the results, it is clear that there was no negative impact on the survival rate in the use of garlic and there was efficient utilization of feed on weight gain, total protein consumed, feed conversion ratio and moderate feed intake. The significant of the research to fish farmers is that natural growth promoters have fewer disadvantages compared to artificial growth promoters for artificial growth promoters could be bio-accumulated to the final consumers.

6. Recommendation

For suitable aquaculture practices, majorly in developing countries where the level of awareness on fish drug is low, the use of garlic at higher level 30% is recommended, for the highest inclusion gave the best result.

Further study should be carried out on the most suitable phyto-additives for efficient utilization and immune activity with corresponding analysis on their tissue and blood compositions.

There could be trial on higher inclusion level from 40% upward to further ascertain the maximum possible derivable garlic additive limit in fish compounded feeds.

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Genetic Divergence Among *Gerbera* spp. Genotypes Based on Morphological Traits

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Abstract

Gerbera is one of the most important ornamental flowers marketed worldwide. Information on the genetic variability of this species represents an important resource for improvement programs that rely on the selection of promising genotypes. The aim of the present study was to assess and characterize the genetic divergence among 32 gerbera accessions based on morphological traits by means of multivariate analysis. A total of 21 traits, nine quantitative and 12 qualitative, were analyzed using clustering analysis and the principal component method. Although the quantitative and qualitative traits were analyzed separately, joint analysis allowed for a more reliable interpretation of the genetic variability and also permitted the identification of wide dissimilarity among the investigated accessions. With regard to the quantitative traits, four main groups were observed, which was in partial agreement with the result of the analysis of canonical variables. The qualitative trait analysis, on the other hand, resulted in the formation of five groups. The traits that contributed most significantly to variability in gerbera were total width of the trans florets set and number of flowers. The data obtained in the present study may be applied directly to improve the design of controlled crosses between gerbera accessions and to better explore the hybrid effect in the process of genetic improvement in this species.

Keywords: dissimilarity, *Gerbera* spp., morphological descriptors

1. Introduction

Gerbera (*Gerbera* spp.), which belongs to the Asteraceae family and originates from Southern Africa and Asia, is an herbaceous, vivacious plant that grows to a height of approximately 45 cm. The elongated leaves are arranged in rosettes, reaching up to 40 cm in length. Although juvenile leaves are round, adult leaves are characterized by slight incisions or divisions in the leaf margins. Furthermore, leaf blades exhibit variations in size and color depending on the cultivar. The flower buds originate in the axils of some leaves, develop large scapes, and exhibit a terminal inflorescence known as a capitulum. The floral stem is slightly hairy, and its length and diameter vary depending on the cultivar, plant age, and growth conditions. Some long-stem cultivars grow to approximately 60 cm in height and are appropriate for use as cut flowers, whereas the most compact cultivars are used as pot flowers (Infoagro, 2005).

Gerbera propagation can be sexual (through seeds) or asexual (vegetative); the latter is accomplished using stolons originating from adult plants and micro-propagation. Vegetative propagation is critical for the maintenance of characteristics of plants of interest, especially in the case of hybrids (Souza et al., 2005).

The Brazilian and international markets for ornamental plants continue to expand. In the first five months of 2011, the value of Brazilian exports and imports of ornamental plants reached $7.60 million (USD) and $15 million (USD), respectively. The main products exported by Brazil were seedlings of ornamental plants

(69.01%), followed by bulbs, tubercles, rhizomes, and other dormant components (12.14%) (Junqueira & Peetz, 2011).

The ornamental plant market is extremely dynamic and demands constant novelties. To meet such needs, advances in genetic improvement programs aligned with the consumers' demands are crucial (Filliettaz & Andréa, 2007). The aim of the genetic improvement of ornamentals is to develop plants exhibiting novel and commercially valuable characteristics and plants that are more competitive with respect to cultivars already available in the market. In other words, the new cultivars must exhibit some advantage relative to those that are already available.

The success of improvement programs hinges on the genetic variability of the species and the process of selection applied to the species. For genetic diversity to be used and ultimately monetized, the accessions must be characterized, documented, and identified for their morpho-phenological, molecular, and agronomic traits. This information allows breeders to identify potentially promising genotypes for use in improvement programs (Borém & Miranda, 2005).

In this regard, multivariate analysis techniques represent an alternative tool for clustering and/or describing a group of individuals because they assess the full set of descriptors simultaneously. Furthermore, these strategies consider sets of genotypes in the context of a complex of variables of interest for breeders, allowing for the selection of genotypes based on several aspects, particularly agronomic ones (Castineiras, 1990). Techniques such as regression (Beale et al., 1967), discriminant analysis (Mardia et al., 1979), principal component, and canonical analysis (Cruz et al., 2004) have been used for the selection or removal of variables aimed at the genetic improvement of plant species (Albuquerque, 2011).

Because morphological characterization is one of the first steps in the identification of accessions, the present study sought to characterize the genetic variability of gerbera accessions by estimating their genetic divergence and by clustering the accessions through multivariate analysis of quantitative and qualitative morphological traits.

2. Materials and Methods

2.1 Plant Material and Morphological Evaluation

In the present study, 32 accessions of gerbera were used (Table 1) from the germplasm bank of the Pró-Clone company, which is based in São Paulo, Brazil.

The experimental design was entirely randomized, with 32 treatments (accessions) and two repetitions of each accession. Assessment began when the plants reached the commercial stage, which corresponds to the time of blossoming, i.e., when two rows of disk flowers are open. Analysis of the nine quantitative traits was performed first, with the findings expressed as means, maximum and minimum values, standard deviation, and coefficient of variation.

The accessions were assessed by means of morphological characterization, including nine quantitative and 12 qualitative traits. The measurement of the quantitative traits was performed according to the official gerbera descriptors provided by the Ministry of Agriculture, Livestock and Food Supply (Ministério de Agricultura, Pecuária e Abastecimento-MAPA) (2005): 1- length of leaves, 2- width of the trans florets set, 3- length of outer ray florets, 4- width of outer ray florets, 5- number of leaves, 6- stem length, 7- stem diameter, 8- capitulum diameter, and 9- number of capitula per assessed accession.

The qualitative traits were assigned sequential numerical codes according to the official gerbera descriptors (MAPA, 2005) and modified as follows: 1. depth of the incisions at the central third of leaves: shallow (3), medium (5), deep (7); 2. hairs on the upper surface of leaves: present (1) and absent (2); 3. intensity of the green hue on the upper surface of leaves: light (1) and dark (2); 4. level of the apex of the outer ray florets relative to the apex of the involucre: below (1), same level (2), and above (3); 5. shape of the outer ray florets: narrow-elliptic (1) and narrow-obovate (2); 6. shape of the apex of the outer ray florets: sharp (1) and round (2); 7. number of colors of the outer ray florets: one (1) and two (2); 8. dark disk: absent (1) and present (2); 9. main color of stigma: white (1), yellow (2), orange (3), pink (4), red (5), purple (6), and brown (7); 10. main color of anthers: yellow(1), orange (2), pink (3), red (4), purple (5), and brown (6); 11. color of flowers: yellow (1), white (2), red (3), orange (4), and pink (5); 12. type of capitulum: simple (1), semi-double (2), and double (3).

Table 1. Inflorescence type and capitulum color of 32 gerbera genotypes used in the morphological analysis

Genotype name	Plant code*	Inflorescence type	Color
Igor	C1	Semi-double	Pink
Terra Fame	C2	Simple	Yellow
Golden G.	C4	Semi-double	Yellow
Igor	C5	Semi-double	Pink
Kozak	C6	Semi-double	Dark orange
Selvagem	C7	Simple	Red
Pink Elegance	C8	Double	Pink
Golden G.	C9	Semi-double	Yellow
Golden G.	C10	Semi-double	Yellow
Deranagem	C12	Semi-double	Pink
Terra Fame	C13	Simple	Yellow
Golden G.	C14	Semi-double	Yellow
Deranagem	C15	Semi-double	Pink
Cariba	C16	Semi-double	Red
Igor	C17	Semi-double	Pink
Terra fame	C18	Simple	Yellow
Mystique	C19	Simple	Orange
Golden G.	C20	Semi-double	Yellow
Selvagem	C21	Simple	Orange
Cariba	C22	Semi-double	Red
Terra Fame	C23	Simple	Yellow
Golden G.	C24	Semi-double	Yellow
Terra Fame	C25	Simple	Yellow
Golden G.	C26	Semi-double	Yellow
Terra Fame	C27	Simple	Yellow
Terra Fame	C29	Simple	Yellow
Terra Fame	C31	Simple	Yellow
G32	C32	Simple	Yellow
Monique	C34	Semi-double	Red
Orça	C40	Simple	White
Orça	C42	Simple	White
Pacific	C45	Simple	White

*Identical genotypes are clones of a given cultivar and represent individuals from a single lineage that originated from the vegetative reproduction of the reference genotype.

2.2 Statistical Analysis

Analysis of the quantitative and qualitative traits was performed separately. The first requirement of a desired trait is the ability to differentiate cultivars for this trait. For this purpose, the quantitative traits were subjected to descriptive analysis. Multivariate analysis was subsequently performed using the Mahalanobis distance (D2) (Hair, 2005). Clustering was performed using the Unweighted Pair-Group Method Using an Arithmetic Average (UPGMA) (Sneath & Sokal, 1973) to assess the potential of traits for genotypic discrimination when considered

as a whole. Canonical analysis was also used for clustering, and the relative importance of the traits was estimated by Singh's (1981) method.

For clustering based on the qualitative traits, the recorded values were transformed into binary data, and the simple matching coefficient was applied to analyze the dataset. The dissimilarity matrix was calculated for subsequent classification using the hierarchical method, and a dendrogram was created using the UPGMA algorithm, as recommended by Rohlf (1970).

The cophenetic correlation coefficient (CCC) was calculated to test the efficiency of each clustering method, with a lower CCC value indicating a greater efficiency. All analyses were performed using Genes version 7.0 software (Cruz, 2006).

3. Results and Discussion

3.1 Morphological Variability for Quantitative Traits

To verify the morphological diversity based on quantitative descriptors, the mean, maximum and minimum values, standard deviation, and coefficient of variation of nine morphological traits in 32 gerbera accessions were analyzed. The data were collected over the same trial period from plants grown under the same environmental conditions. Given the observable morphological variability among the accessions, one can therefore infer that the accessions represent different genotypes.

The largest coefficient of variation was found for the number of capitula (49%) and number of leaves (47%); the lowest was found for capitulum diameter (12%) (Table 2).

Table 2. Descriptive analysis of nine quantitative gerbera descriptors

Traits	Mean	Max	Min	SD	cv (%)
Length of leaves (cm)	17.64	27.00	9.00	3.48	20.00
Width of the trans florets set (cm)	30.11	54.39	14.70	8.41	28.00
Length of outer ray florets (mm)	42.13	69.27	31.40	6.45	15.00
Width of outer ray florets (mm)	8.98	12.16	3.27	1.90	21.00
Number of leaves	19.48	61.00	6.00	9.09	47.00
Stem length (cm)	34.90	57.00	11.27	8.49	24.00
Stem diameter (mm)	5.61	10.90	2.27	1.19	21.00
Capitulum diameter (cm)	9.60	13.16	7.00	1.15	12.00
Number of capitula	1.77	5.00	1.00	0.86	49.00

Max, Maximum; Min, Minimum; SD, Standard deviation; cv, Coefficient of variation.

According to Rocha et al. (2006), the coefficient of genetic variation indicates the presence of variability among evaluated accessions, with higher values corresponding to greater heritability and, consequently, the odds of finding superior individuals that will afford genetic gains during the process of selection. Further evidence supporting this assertion was provided by Ram et al. (2005), who found a high coefficient of genotypic variation and high levels of heritability for plant height, stalk diameter, and number of capsules per plant in the species *Silybum marianum* G.

3.2 Clustering of Quantitative Traits

For the quantitative traits, the average Mahalanobis distance among accessions (D2 = 103) was used as a criterion for clustering (Figure 1).

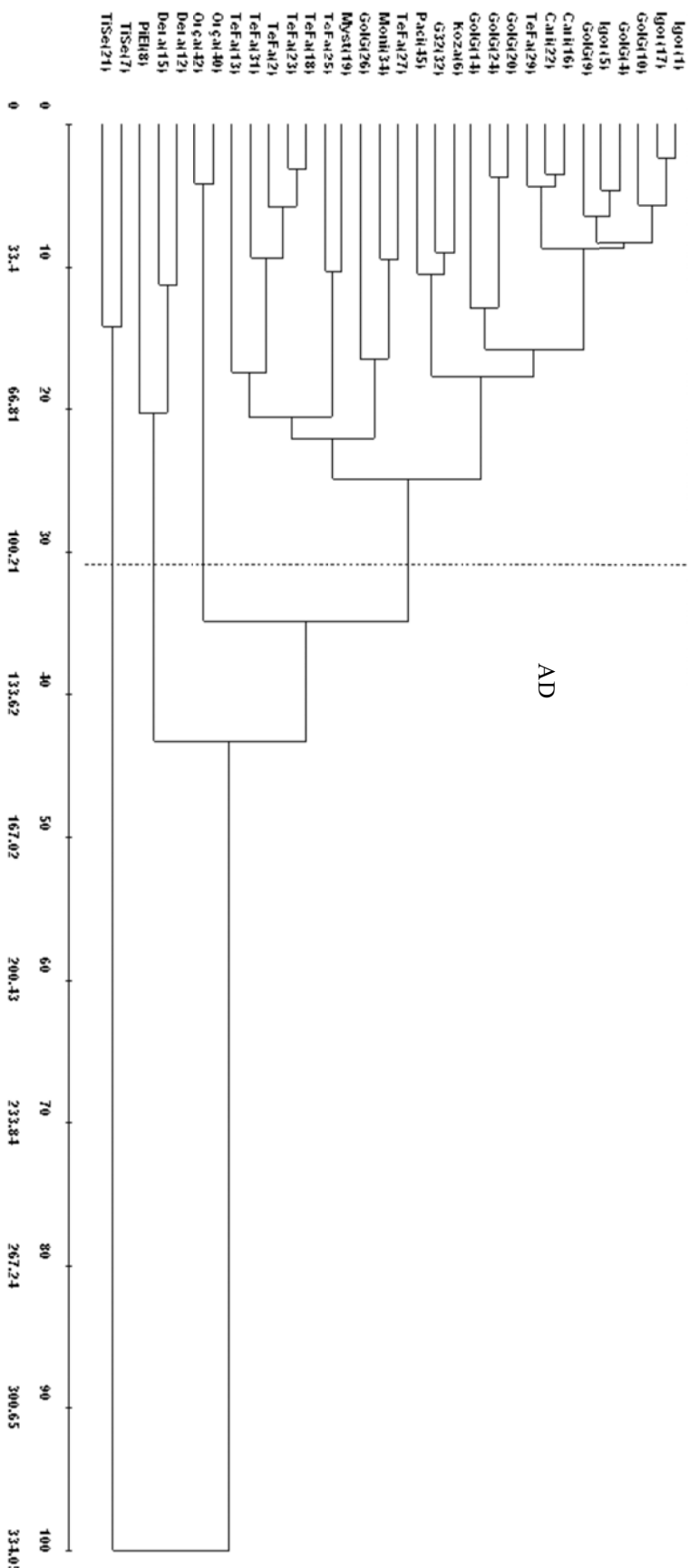

Figure 1. Clustering dendrogram of 32 gerbera accessions generated by UPGMA based on the analysis of quantitative traits using the Mahalanobis distance. AD, Average dissimilarity

Four main groups were created in the dendrogram, with the first including most of the accessions: Igor (1, 5, and 17); Golden G. (4, 9, 10, 14, 20, 24, and 26); Cariba (16 and 22); Terra Fame (2, 13, 18, 13, 25, 27, and 29); G32 (32); Pacific (45); Monique (34); Kozak (6); and Mystique (19). The second group included the Orça accessions (40 and 42); the third group included the Deranagem (12 and 15) and Pink Elegance (8) accessions; and the fourth group included the wild type accessions (7 and 21). In spite of some genetic distance, the similar accessions are clones of a given cultivar and represent individuals of a single lineage that originated by vegetative propagation. The fact that they are not identical may be indicative of mutation events over the course of several vegetative propagations.

The quantitative morphological traits that exhibited greater relative contribution were total width of the trans florets set, number of leaves, stem diameter, length of leaves, width of outer ray florets, stem length, number of capitula, and diameter of capitula (Figure 2). The first four traits explained 69.22% of the variability.

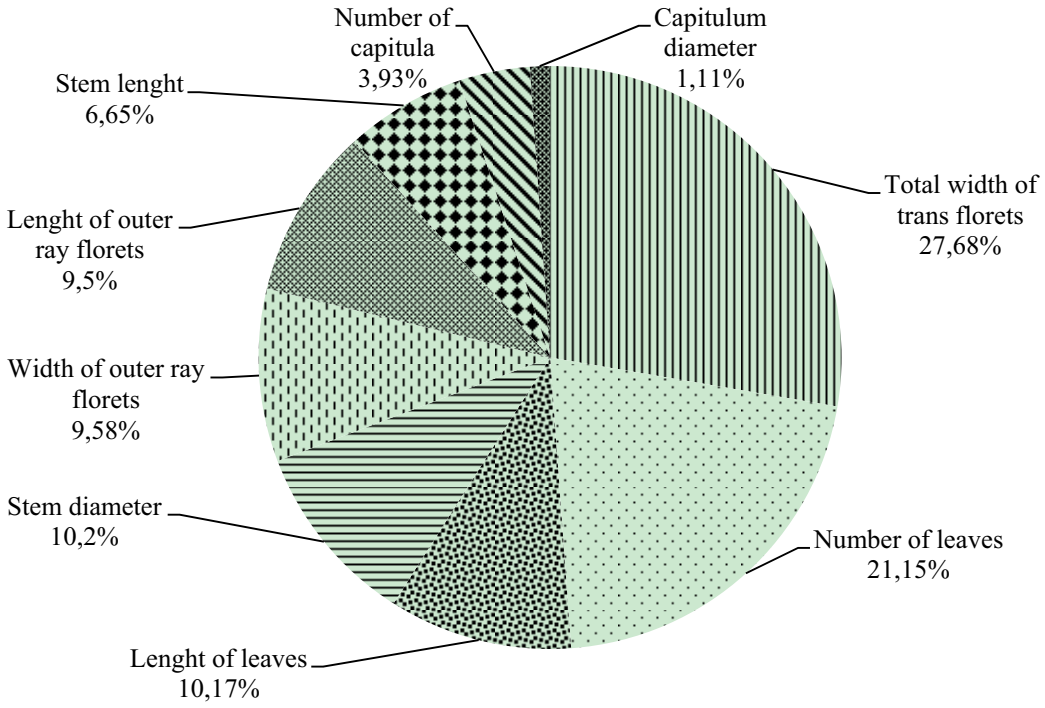

Figure 2. Relative contribution (%) of the quantitative traits assessed in gerbera

In a study on gerbera, Cardoso et al. (2007) also found that the total width of the trans florets set was one of the most relevant traits explaining variability.

The largest divergence was found between accessions wild type (clone 21) and Pink Elegance (clone 8), with an average distance of 6.58. The most similar were accessions 23 and 18, both corresponding to Terra Fame, with a distance of 1.04. Cardoso et al. (2007) also found wide genetic diversity between Pink Elegance and Tipo Selvagem in a study on the genetic divergence of 13 gerbera genotypes.

For the average width of the trans florets set, the largest divergence was found between accession 8 (53.17 mm) and accession 21 (15.07 mm). For the average number of leaves, Pink Elegance (8) exhibited eight leaves, and wild type exhibited 46 leaves. For leaf length, Pink Elegance and wild type exhibited averages of 15.75 cm and 11 cm, respectively.

The stem diameter trait varied from 3.04 mm to 5.55 mm for wild type and Pink Elegance, respectively. According to Hermans et al. (2006), stem diameter increases rigidity and plays an important role in preventing flowers from falling over under the following conditions: windy conditions in the cultivation field, transportation from the field to the treatment and selection site, packaging, and post-harvest processing. For cut flowers in general, carbon reserves in the stems also increase the potential longevity of flowers. Thus, stem length and diameter greater, the longer is the post-harvest durability.

Cophenetic correlation analysis (r = 0.84) was used to determine the consistency between the clustering analysis and the dissimilarity matrix. Although the stem length trait did not contribute significantly to variability, it nevertheless represents an important trait in the selection of genotypes to be used as cut flowers.

3.3 Canonical Variables of Quantitative Traits

In addition to the clustering data, complementary analysis based on canonical variables (CV) was performed. Traits exhibiting higher percentages of variance were used to verify the dispersion of the genotypes on Cartesian coordinates to simplify the visualization and interpretation of the results, as recommended by Cruz and Cameiro (2006).

Analysis of the canonical variables showed that approximately 74.46% explained the total variance. VC1, VC2, and VC3 explained 44.93%, 18.83%, and 10.70%, respectively, and thus fit a three-dimensional graphical representation (Figure 3). Observation of the distribution of genotypes in each group revealed partial agreement between the UPGMA clustering analysis and the canonical variables, as four groups were formed and some accessions clustered together regardless of the algorithm employed.

Accessions 7 and 21 (wild type) clustered closely to one another (Figure 3) and distantly from the remainder of the accessions, corroborating the dendrogram results (Figure 1). A similar result was found for accession 8 (Pink Elegance) and accessions 12 and 15 (both Deranagem), which also clustered closely to one another using both clustering methods. Again consistent with the dendrogram results, accessions 40 and 42 (both Orça) clustered closely to one another. However, accessions 13 (Terra fame) and 6 (Kozak), which were mutually distant in the dendrogram, were close to one another in the canonical analysis. The remainder of accessions belonging to group 1 in the dendrogram also clustered into one single group in the canonical variable analysis.

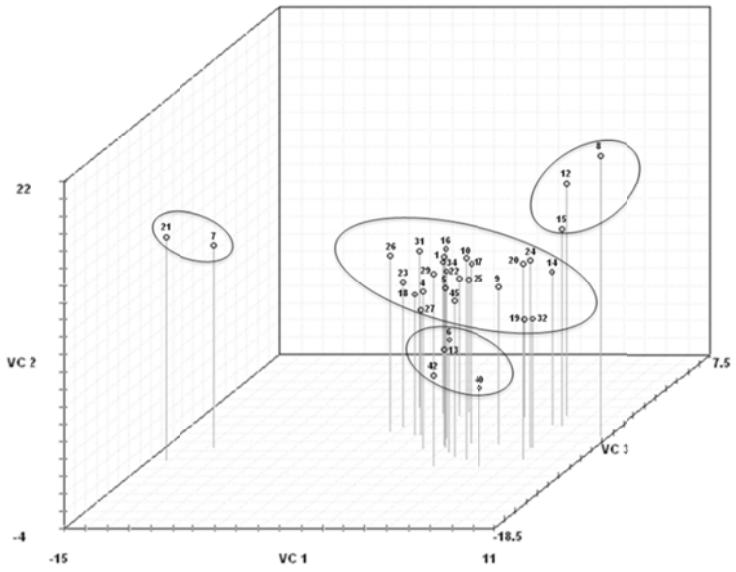

Figure 3. Dispersion graph of the 32 gerbera genotypes using the first three variables

The genetic variability of gerbera found in the present study was also observed by Cardoso et al. (2007) in a study investigating divergence in seven accessions using qualitative and quantitative descriptors; some of these were also incorporated in the present study.

3.4 Clustering of Qualitative Traits

To complement the quantitative trait variability analysis, dissimilarity analysis was performed using qualitative descriptors. Such descriptors are extremely important in the identification of accessions with the greatest economic potential and in the design of crosses to produce gerbera cultivars (Neitzke et al., 2010).

Analysis of the qualitative traits allowed for the creation of a dendrogram using the UPGMA algorithm (Figure 4), which revealed five groups based on the average dissimilarity among the investigated accessions (AD = 0.28).

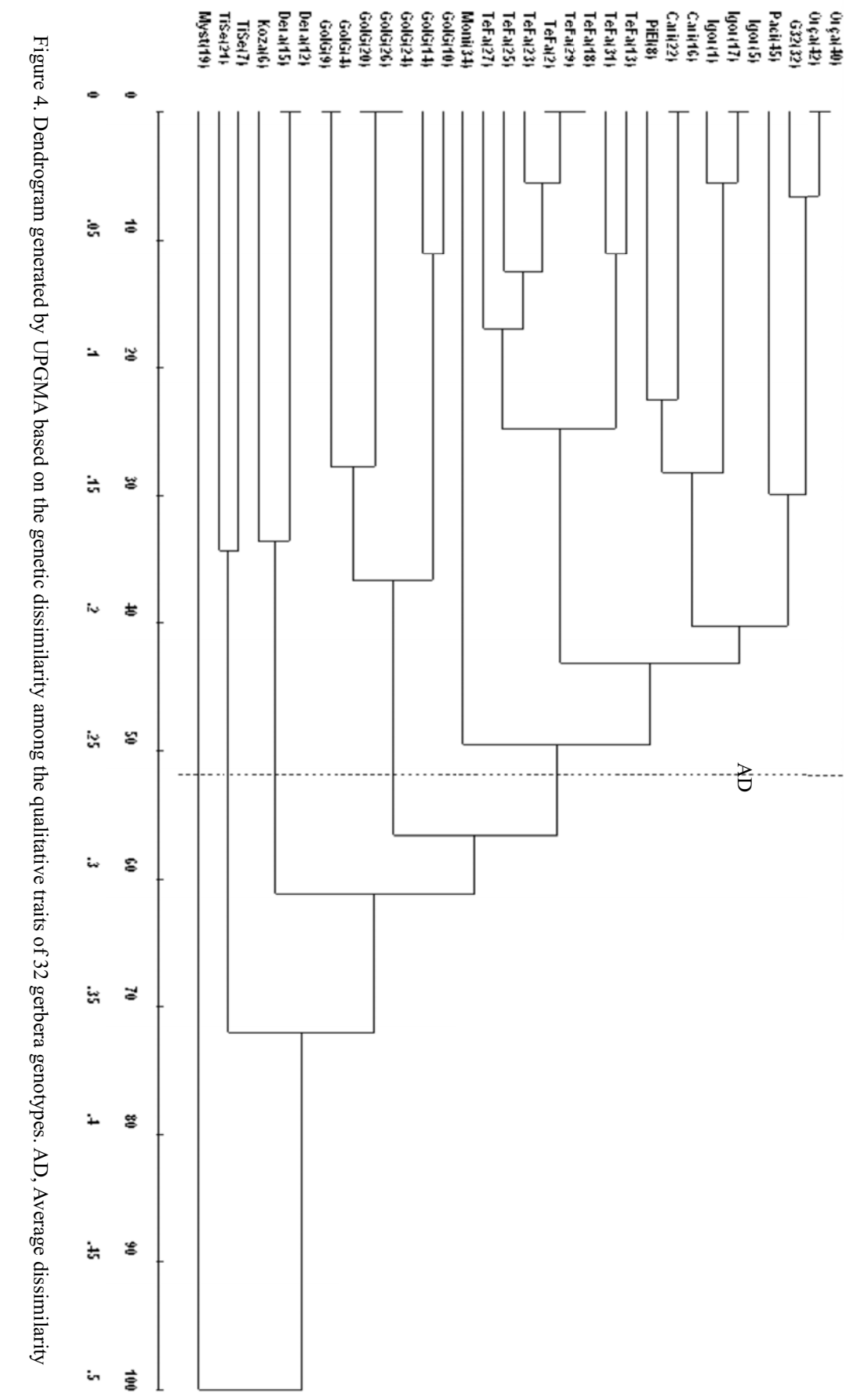

Figure 4. Dendrogram generated by UPGMA based on the genetic dissimilarity among the qualitative traits of 32 gerbera genotypes. AD, Average dissimilarity

The first group clustered the largest number of accessions, including Orça, G32, Pacific, Igor, Cariba, Pink Elegance, Terra Fame, and Monique. With the exception of Monique, these accessions were monomorphic for the following traits: hairs on the upper surface of leaves (present), depth of incisions at the central third of the leaf blade (shallow), and level of the apex of the outer ray florets relative to the involucre apex (same level). The second group included all of the Golden G. accessions, which were monomorphic for almost all the traits, except for depth of the incisions at the central third of the leaf blade and hairs on the upper surface of leaves. The third group consisted of the Deranagem and Kozak accessions, which were monomorphic for all traits except for dark disk color, main color of the stigma, main color of the anthers, and color of flowers. The fourth group was composed of the wild type accessions, which are not commercially available and were monomorphic for the following traits: green color of leaves, level of the apex of the outer ray florets relative to the involucre apex, and color of flowers. A Mystique accession was isolated in group five and was the only accession whose outer ray florets exhibited two colors.

The correlation coefficient obtained (r = 0.81) was very close to that obtained with the quantitative data (r = 0.84), indicating a good fit and agreement between the clustering analysis and dissimilarity matrix.

The mutually closest accessions with distances of 0.0 were as follows: 2 (Terra Fame) and 12 (Deranagem), 2 and 29 (both Terra Fame), 4 and 9 (both Golden G.), 5 and 17 (both Igor), 12 and 15 (both Deranagem), 16 and 22 (both Cariba), 18 and 29 (both Terra Fame), 20 and 24 (both Golden G.), 20 and 26 (both Golden G.), and 24 and 26 (both Golden G.). The most distant accession was Mystique, with a distance of 0.5. The latter differed from the other accessions insofar as it did not exhibit hairs on the upper surface of leaves.

The results obtained highlight the importance of analyzing both quantitative and qualitative traits insofar as it helps ensure that inferences about inter-accession variability are more reliable.

The importance of studies on genetic divergence for plant improvement reflects the fact that the superiority of hybrids is proportional to the genetic distance between the corresponding progenitors. Therefore, in species where this relationship is observed, breeders have access to a quick and simple criterion for selecting parents for use in hybridization programs, as suggested by Moreira et al. (1994). Unfortunately, few studies on genetic divergence have been conducted with gerbera, as most of the improved genotypes are traditionally developed by private companies (Sparjaaij, 1976). The type of analysis performed in the present study represents a practical and quick approach that may improve decision-making in the design of crosses between highly divergent genotypes, as greater heterotic effect is expected between the most genetically contrasting populations (Falconer & Mackay, 1996).

As with the present study, countless other studies in different species have shown that analysis of genetic divergence by means of multivariate procedures, such as the Mahalanobis generalized distance and graphical dispersion of mutually concordant canonical variables, is efficient for the discrimination of genotypes. In a study assessing genetic diversity among pepper accessions (*Capsicum* spp.), Sudré et al. (2006) confirmed the efficiency of multivariate analysis in the characterization of genetic variability among the accessions. Bertini et al. (2010) studied genetic divergence among coriander genotypes (*Coriandrum sativum* L.) and successfully used multi-categorical variables in the discrimination of genotypes. The applicability of that analysis model was corroborated by Ferrão et al. (2011) in the quantification of genetic divergence among pepper accessions and by Amorin et al. (2007) in their study on the genetic divergence of sunflower accessions. These examples provide evidence for the efficiency of multivariate analysis in the genetic discrimination of individuals and clustering; thus, homogeneity can be exhibited within groups, and heterogeneity can be exhibited between groups. However, the assessment of morphological and phenological traits is time-consuming and may be influenced by the environment. For this reason, an alternative strategy for the study of genetic variability is based on the use of molecular markers. Although studies utilizing molecular markers to assess variability in gerbera are scarce, two examples are those by Da Mata et al. (2009) and Rezende et al. (2009), which utilized random amplified polymorphic (RAPD) markers. In addition, Benemann et al. (2012) identified 17 polymorphic simple sequence repeat (SSR) markers from gerbera expressed sequence tag (EST) databases, allowing for correlations to be made with the morphological traits assessed in their study. Some studies conducted in several species, including plum tree (Shimada et al., 1999), almond tree (Kadkhodaei et al., 2011), chestnut (Serdar et al., 2011), and olive tree (Zaher et al., 2011), have been able to correlate molecular data with morphological and agronomic traits.

Based on the results obtained, it is possible to conclude the following: that there was wide genetic dissimilarity among the 32 gerbera accessions for the quantitative and qualitative traits assessed; that the multivariate techniques employed exhibited partial agreement for the demonstration of genetic variability; that the traits that contributed most to variability were total width of trans florets and number of leaves; and that the genetic

variability found among the 32 gerbera accessions may be used to perform controlled crosses in genetic improvement programs to develop new cultivars.

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Assessment of Actual Irrigation Management in Kalâat El Andalous District (Tunisia): Impact on Soil Salinity and Water Table Level

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Abstract

The objective of this work is to assess water and soil salinity evolution in the irrigated area of Kalâat El Andalous. Soil salinity, crops yield, water table level and drainage water flow were monitored during the period May 2008-June 2010. The results showed that during irrigation season (May-September 2008), the supplied water amounts for drip irrigated crops (tomato, melon and squash) were higher than crop water requirements. In fact, the soil water content was always equal or higher than the field capacity. Average root zone (0-60 cm) electrical conductivity of the saturated past extract (ECe) was 2.3 dS m^{-1}, 2.8 dS m^{-1} and 3.0 dS m^{-1} in May 2008, May 2009 and May 2010 respectively. But at the irrigation season end, higher electrical conductivity (8.4 dS m^{-1}) was recorded in the upper layer (0-30 cm). Along rainfall season, a soil salinity decrease was recorded in fact the average electrical conductivity reached 2.0 dS m^{-1}. In order to reduce soil salinization (due to accumulated salts during irrigation season), farmers use crop rotation including rain fed crops and bare soil. Allover irrigation season, the highest drainage discharge (192 l/h) was recorded on July 2008 when the maximum irrigation water amount was diverted. Water table level shows a sustained rise when irrigation is relatively frequent during summer.

Keywords: drip irrigation, soil salinity, soil water content, drainage, water table, crop yield

1. Introduction

In arid and semi-arid areas, irrigation is used to maximize crop yields by minimizing water stress in the root zone. However, this is often done an ad-hoc manner. Excess of water supplies may cause rising of ground water table which may carry salts from subsurface to surface layers through capillary rise and evaporation (Turhan & Baser, 2001). Soil salinization induced by capillary rise of shallow groundwater into the rooting zone plays a major role, nullifying pre-season salt leaching efforts, entailing yield decrease and seriously threatening economic growth and development (Grieve et al., 1986; Smets et al., 1997; Singh, 2004; Murtaza, 2006). Such evapo-concentration phenomenon associated with saline irrigation water is the main cause of soil salinization in irrigated districts (Stuyt, 2000). The salt accumulation in the soil profile is a widespread problem that seriously affects crop productivity throughout the world. More than 50% of the salinized areas in the Mediterranean basin are located in Algeria, Morocco, Spain, Tunisia and Turkey (Aragüés et al., 2011).

The use of drip irrigation may bring about a potential threat of the secondary soil salinization because no salt can be discharged from soil profile and salt build-up on the soil surface may be on the rise after long-term application of drip irrigation (Zhou & Ma, 2005). Hence, it is essential that farmers have a clear understanding about irrigation practices' impact on the soil moisture content, on soil salinity and on crop yields. In fact, optimal irrigation management is supposed to maintain favorable soil water content, prevent salinity stress, and save water resources as much as possible. In Tunisia, Kalâat El Andalous irrigated district is one of the most affected area by salinization due to shallow groundwater level. This study aims to assess water and soil salinity evolution under the main frequently irrigated crops (tomato, melon and squash), rain fed crop (wheat) and bare soil in Kalaât El Andalous district.

2. Materials and Methods

2.1 Experimental Site

The irrigated area of Kalâat El Andalous (latitude: 37°2′ to 37°6′ N; longitude: 10°5′ to 10° 10′ E) is located on the end part of the Medjerda watershed (Figure 1), with an average annual potential evapotranspiration (ETP) of 1400 mm and an average annual rainfall of 490 mm. Irrigation in Kalâat El Andalous district was launched since 1992 on a flood area that covers 2905 ha but the effectively irrigated surface varied over time, the maximum (about 1000 ha) was observed in summer. The district was divided into plots of 5 ha supplied by a flow rate of 3 l/s. All the irrigated area was equipped by a pressurized irrigation network and a subsurface drainage system with a length of 180 m and a depth of 1.5 m and spaced at intervals of 40 m. The drainage outlet is below sea level, and the drainage waters are rejected in the Mediterranean Sea through a pumping station (SP4).

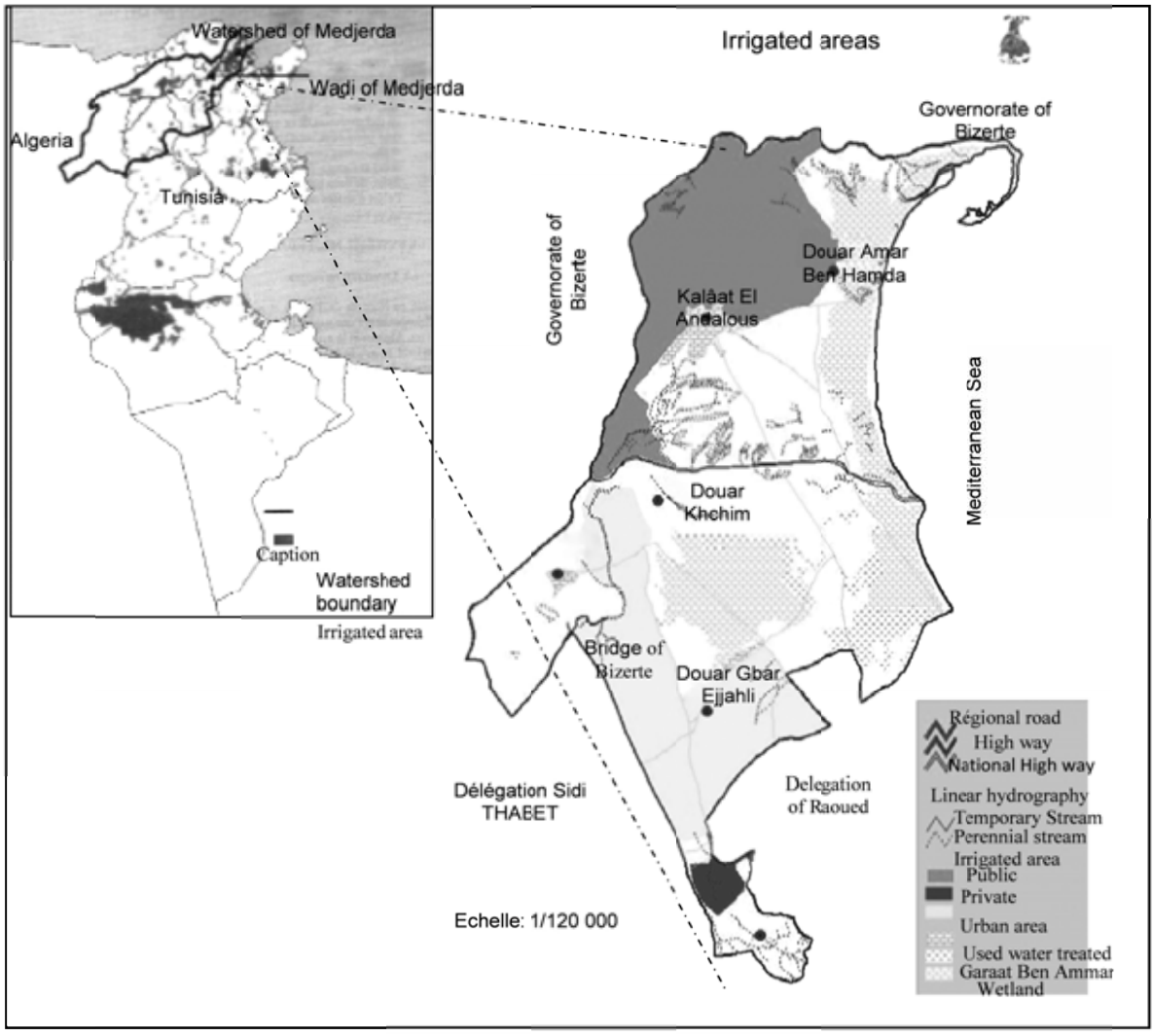

Figure 1. Irrigated area of Kalâat El Andalous

The soils have a fine texture, ranging from silty-clay to clayey-silt. The soil pH ranged from 7.3 to 8.9, and the average bulk density is about 1.5 g/cm^3. The hydraulic conductivity varied between 0.2 cm/h and 3.6 cm/h. Some physical and chemical characteristics of the soil in the experimental site were determined (Table 1). The irrigation water is pumped from Medjerda River which average Electrical conductivity reached 3.6 dS m^{-1}. The general characteristics of the irrigation district (Aragüés et al., 2011) were presented in Table 2.

Table1. Soils characteristics of Kalâat El Andalous area

Soil profile depth (cm)	SP (%)	Volumetric water content (%)		% OM	% $CaCO_3$	pH
		WP	FC			
0-30	50	20	35	4.6	42.2	8.9
30-60	52	15	32	1.8	43.5	8.8
60-90	57	26	42	1.3	44.0	8.6
90-120	67	26	44	1.9	46.2	7.3
120-150	55	27	44	1.3	36.8	8.5
150-180	60	27	45	2.9	48.0	8.5

SP: Saturation percentage, WP: Wilting Point, FC: Field Capacity, OM: Organic Matter.

Table 2. Water budget of Kalâat EL Andalous irrigation district during the hydrological year (2007/2008)

Parameter	Value
Irrigation (I, mm)	1187
Precipitation (P, mm)	676
Reference ET(ET_0, mm)	1412
Crop ET (ET_c, mm)	975
Surface drainage (Q, mm)	411

A shallow (1.4 m deep) and salinized (EC = 5.8 dS m^{-1}) water table covers all the nether part of the district. Such water resource is unsuitable for irrigation.

This study was carried out during May 2008-June 2010 in a farm plot of 2.38 ha (170 m x 140 m) equipped with drip irrigation system and drained by three subsurface pipes D_1, D_2 and D_3. Tables 3 and 4 showed the cropping pattern and the irrigation system characteristics respectively. Irrigations were practiced daily from 05/05 to 07/08 for tomato and from 15/05 to 25/07 for melon. Subsequently, irrigations were made once every two days. For squash, irrigation frequency was maintained on alternate days from 31/05 until 26/06, it was daily from 27/06 to 11/07 then once every two days during the rest of the irrigation period. Irrigation duration ranges between 1.5 h/day and 4 h/day for tomato, between 3 h/day and 3.5 h/day for melon and squash.

Table 3. Cropping patterns during the period: May 2008-June 2010

Period	Soil occupation
May 2008-September 2008	Irrigated crops: tomato (1.0 ha), melon (1.0 ha) and squash (0.38 ha)
October 2008-November 2008	Bare soil
December 2008-June 2009	Rain fed wheat
July 2009-November 2009	Bare soil
December 2009-June 2010	Rain fed wheat

Table 4. Field cropping and irrigation system's characteristics during the period (May 2008-September 2008)

Crops	Scientific Name	Field size (ha)	Date of plantation	Row spacing (m)	Emitter spacing (m)	Average emitter discharge (l/h)
Tomato	*Lycopersicum esculentum*	1.0	3 May 08	1.5	0.4	2.1
Melon	*Cucumis mela L. CV. Sancha*	1.0	17 April 08	1.5	0.8	1.5
Squash	*Cucurbuta maxima*	0.38	25 May 08	1.5	0.8	1.9

2.2 Measurements

Field measurements included supplied water volume and salinity, drained water discharge and salinity, water table level and salinity, soil water content and salinity.

The supplied volumes V (m^3) are determined as:

$$V = NqT10^3 \quad (1)$$

Where N is the number of emitters per hectare, q is the average emitter discharge (l/h), and T refers to the irrigation duration. The fixed emitters' discharges were measured weekly. The irrigation time was estimated according to farmer declaration and our survey. Daily climatic data were collected on a meteorological station located near the experimental plots. Reference evapotranspiration (ETo) was calculated using Penman-Monteith method (Allen, 1998). Crop evapotranspiration (ETc) was calculated as:

$$ET_c = ET_0 K_c \quad (2)$$

where K_c is crop's coefficient (Allen, 1998).

As given in Figure 2, rainfalls are negligible during the irrigation season (May-September).

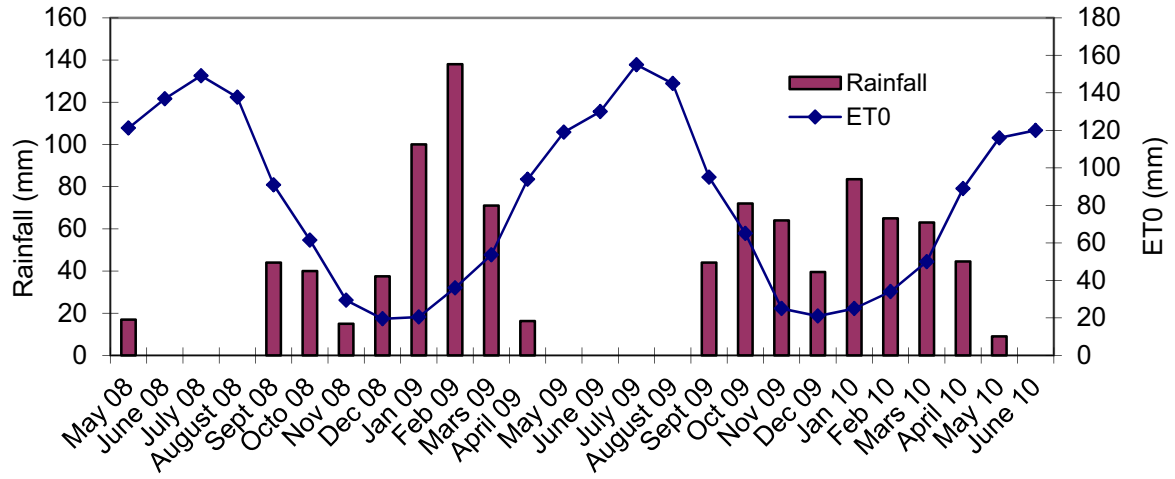

Figure 2. The repartition of precipitation and reference evapotranspiration (ET_0) during the study period

The water table levels were measured monthly using two piezometers. The first is localized in the plot, and the second is 50 m near the plot. Groundwater was sampled monthly for Electrical Conductivity (EC) measurements. In order to compute the removed (from the study area) salts amounts, monthly drained water discharge measurements were made on the subsurface pipes (D_1, D_2 and D_3) ends. Furthermore, soil sampling was carried out in order to assess soil salinity (ECe) evolution in irrigated (tomato, melon and squash), rain fed (wheat) and in bare soil plots. Within each plot, three spots were randomly chosen where samples were done on the 0-30 cm, 30-60 cm, 60-90 cm, 90-120 cm, 120-150 cm and 150-180 cm layers. These measurements were fortnightly during the irrigation period and monthly for the rest of the season.

For stored water computing, soil water contents (SWC), before (on 06/14) and after (on 07/12) irrigation, were determined gravimetrically in tomato and melon plots. Within each plot, three spots were randomly chosen where sampling was made on the 0-10 cm, 10-30 cm, 30-50 cm, 50-70 cm and 70-90 cm. For roots distribution and crop yields' estimates, three representative plants from each plot (tomato, melon and squash) were randomly chosen where some agronomic parameters were determined namely: the root length, average fruit number per plant and average fruit weight.

Soil, groundwater and drained water samples were analysed to determine electrical conductivity and pH. The calcium (C_{ca}), magnesium (C_{Mg}) and sodium (C_{Na}) concentrations were determined on saturated soil extracts (Black, 1965) and then sodium adsorption ratio (SAR) was calculated using the relationship:

$$SAR = \frac{C_{Na}}{(C_{Ca} + C_{Mg})^{0.5}} \quad (4)$$

Gathered data were analysed using descriptive statistics (average value, minimum value, maximum value, coefficient of variation and standard deviation).

3. Results and Discussion

3.1 Water Irrigation Volume, Salt Amount and Soil Water Content

The daily supplied water volume varied during the irrigation season, from 4 to 16 mm for tomato, from 3 to 9 mm for melon and from 4.0 to 5.5 mm for squash crops. In tomato and squash plots, the maximum supplied water volumes were recorded in July: 4143 m^3/ha and 1130 m^3/ha respectively. The applied water amount decreased in August, mainly because the crop water requirement decreased. But these diverted amounts remained higher than the crop water requirements (Table 5).

Allover the irrigation season, the cumulated rainfalls were only 17 mm. Thus, the applied salt amounts (by irrigation water) reached 24, 12 and 7 tons/ha in tomato, melon and squash plots respectively (Table 5). These results agree with those previously obtained in this region. In fact, Slama et al. (2004) recorded a total water amount equal to 10000 m^3/ha for drip irrigated tomato.

Table 5. Diverted water volumes and accumulated salt mass

Crops	Beginning of irrigation	End of irrigation	ET_c (mm)	Diverted water amounts (mm)	Accumulated salt mass due to irrigation (tons/ha)
Tomato	5 May 2008	15 September 2008	449	1030	24
Melon	15 May 2008	16 August 2008	332	503	12
Squash	1st June 2008	15 September 2008	285	299	7

Allover irrigation season, the water content was always higher or equal to field capacity (34%) (Figure 3). In fact before irrigations, the average water content profile was higher than 30% and 33% in tomato and in melon plots respectively. The most important changes were observed on 12/07 within 0 - 10 cm where the soil water content increased from 34 % to 39% underneath the emitter, from 32% to 36% at 10 cm and from 32% to 35% at 20 cm away of the emitter. In the deep layers (60-90 cm), water content distribution was approximately constant (≈ 37%) throughout the soil profile. Rawlins and Rotas (1975) and Guohua et al. (2009) reported that compared with the border-irrigation, frequently drip and sprinkler-irrigated field help to maintain higher soil water content.

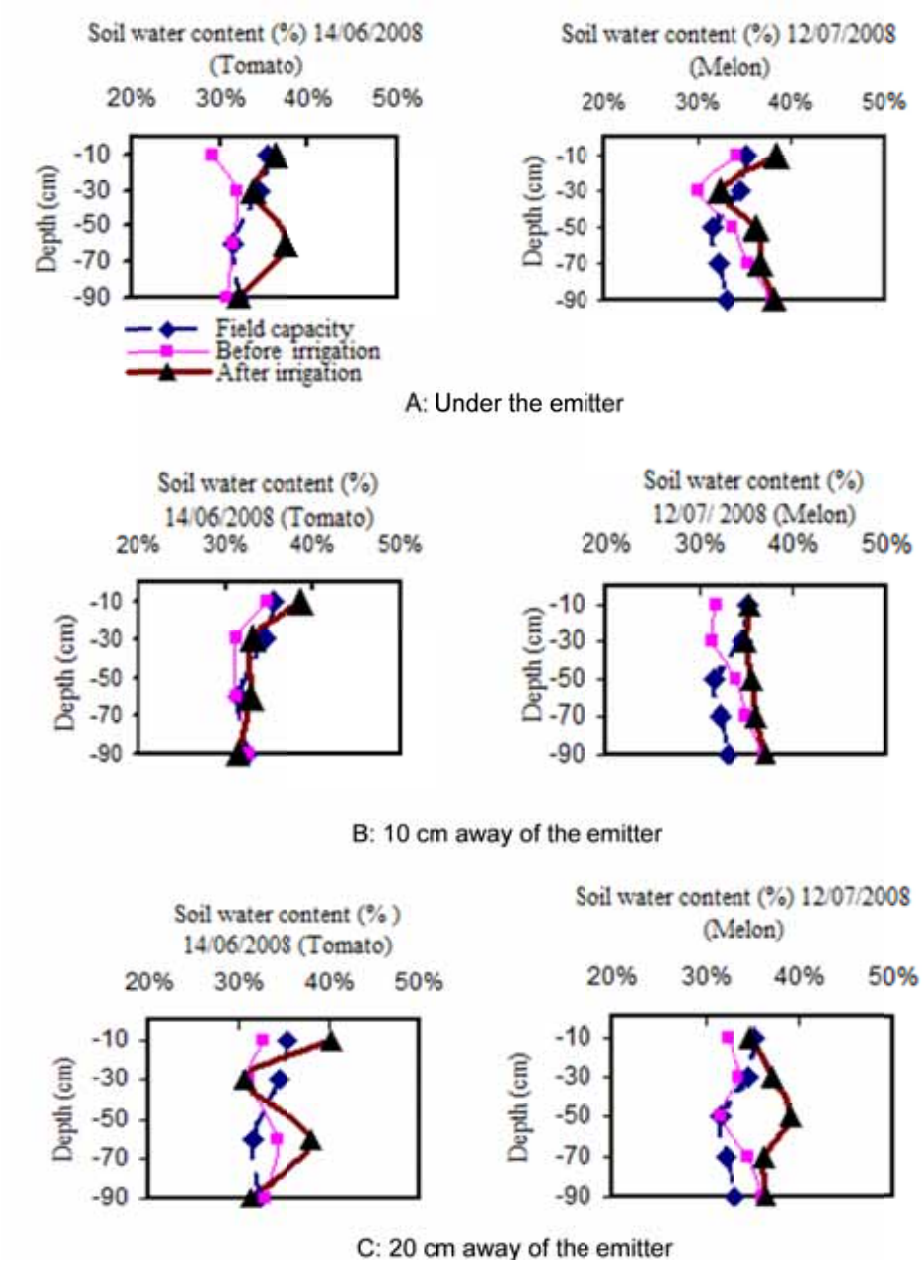

Figure 3. Volumetric water content profiles before and after irrigation: A: under the emitter; B and C: 10 cm 20 cm away from the emitter respectively. All data are averaged values of three soil samples

3.2 Soil Salinity Profiles

Table 6 lists the descriptive statistics of the electrical conductivity (EC) at 31 measurement points, including minimum, maximum, mean, standard deviation and coefficient of variation (CV) during the period May 2008-June 2010. The EC variations are more pronounced in the upper layer (0-30 cm, CV = 45%) than in the dipper layer (150-180 cm, CV = 8%). Whereas, along two years, the soil profile salinity showed little variation.. In fact, within the root zone (0-60 cm), EC average values were 2.3, 2.8 and 3.0 dS m^{-1} in May 2008, May 2009 and May 2010 respectively (Figure 4). But these EC variations were more pronounced if only the irrigation season was considered. In fact, EC in the first layer (0-30 cm) increased from 1.9 up to 7.0 dS m^{-1}, from1.6 up to 8.4 dS m^{-1} and from 2.9 up to 7.7 dS m^{-1}(within tomato, melon and squash plots respectively (Table 7).

Table 6. Some statistics of the soil electrical conductivity (dS m^{-1}) during the period: May 2008-June 2010

	Depth (cm)					
	-30	-60	-90	-120	-150	-180
Samples number	31.0	31.0	31.0	31.0	31.0	31.0
Average	3.3	3.3	3.3	4.0	5.1	5.6
Min.	1.6	2.1	1.9	2.0	3.6	5.0
Max.	8.4	4.9	5.1	6.3	6.5	6.9
Standard deviation	1.5	0.8	0.7	0.8	0.7	0.4
Coefficient of variation	45%	23%	20%	19%	15%	8%

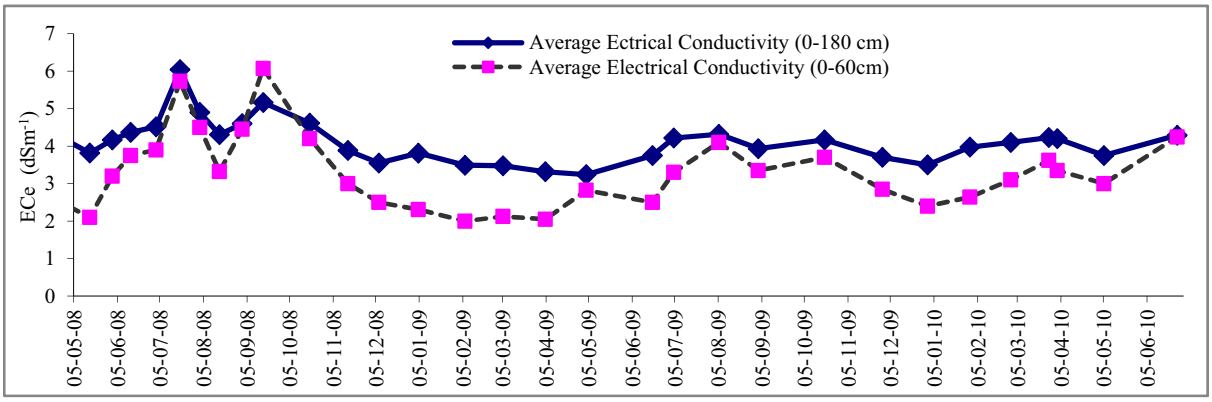

Figure 4. Average soil profile electrical conductivity during: May 2008-June 2010

In irrigated plots, the soil electrical conductivity (EC) changed allover crop growth stages. In fact, the root zone (0-60 cm) EC averaged value increased (between the beginning and the end of irrigation season) from 2.0 dS m^{-1} up to 5.3 dS m^{-1}, to 3.3 dS m^{-1} and to 6.8 dS m^{-1} in tomato, melon and squash plots respectively. But, the highest EC values: 8.4 dS m^{-1}, 7.0 dS m^{-1} and 7.7 dS m^{-1} were recorded on 19/07 and on 16/09 in melon, tomato and squash plots respectively. So irrigation water supply caused a progressive salt accumulation over irrigation season. In the three (melon, tomato and squash) plots, such salinization was especially recorded in the top layer (0-30 cm).

The roots' distribution for tomato along soil depth was as fallow: 20% within the (0-30 cm), 60% within the (30-40 cm) and 20% within the (40-60 cm) layers.

Along rainfall season, a decline in soil electrical conductivity was observed. In fact, EC values were: 3.8, 3.4, 3.5 and 3.9 dS m^{-1}, measured on 4/01/2009, 6/02/2009, 29/11/2009 and on 30/01/2010 respectively. The lowest value (1.6 dS m^{-1}) was recorded under rainfall conditions.

In the three irrigated plots, EC within the rooted layer (0-60 cm), ranged between 2.7 and 6.0 dS m^{-1}. While in the rain fed wheat and bare soil plots, EC (within the 0-60 cm layer) ranged between 1.6 and 4.1 dS m^{-1} and between 2.9 and 4.0 dS m^{-1} respectively. Therefore, this salt accumulation was essentially due to an inadequate irrigation management. The inherent alkalinisation risks are obvious because the recorded maximum SAR values reached: 16.1, 15.1, and 10.7 in the irrigated squash, tomato, and melon plots respectively. Whereas in the rain fed wheat and in bare soil plots, SAR recorded values were: 4.5 and 6.2 respectively.

Table 7. Soil electrical conductivity variations during irrigation season

Crops	Date	Electrical conductivity (dS m^{-1})					
		Depth (cm)					
		-30	-60	-90	-120	-150	-180
Tomato plot	02/05/2008	1.9	2.8	3.9	3.6	6.2	6.2
	16/05/2008	2.0	2.2	2.8	3.8	6.1	6.0
	01/06/2008	3.1	3.3	2.9	4.0	5.9	5.8
	14/06/2008	4.0	3.5	3.2	4.5	5.5	5.5
	02/07/2008	4.1	3.7	4.0	4.2	5.2	5.9
	19/07/2008	6.5	4.9	5.1	6.3	6.5	6.9
	02/08/2008	5.1	3.9	4.1	4.2	6.1	6.0
	16/08/2008	4.1	2.6	2.6	5.6	6.0	5.0
	01/09/2008	5.0	3.9	3.0	4.0	5.9	5.8
	16/09/2008	7.0	3.7	4.3	3.4	5.5	5.7
Melon plot	02/05/2008	1.6	2.3	2.0	3.8	5.2	5.9
	16/05/2008	1.8	2.5	2.3	3.9	5.7	6.0
	01/06/2008	1.9	2.1	2.3	3.9	5.6	5.7
	14/06/2008	3.0	2.1	2.4	4.0	5.9	6.2
	02/07/2008	5.1	3.3	3.9	3.9	5.9	6.1
	19/07/2008	8.4	3.8	3.5	4.9	6.5	6.8
	02/08/2008	5.3	3.8	3.2	4.5	6.1	6.3
	16/08/2008	4.1	2.6	2.6	5.6	7.7	6.9
Squash plot	02/05/2008	2.9	2.7	4.1	4.5	5.1	5.2
	16/05/2008	2.7	2.6	4.1	4.9	5.4	5.3
	01/06/2008	2.8	2.5	4.0	5.0	5.3	5.5
	14/06/2008	3.7	3.5	3.3	4.5	5.3	6.1
	02/07/2008	3.8	3.6	3.4	5.2	5.4	6.0
	19/07/2008	3.9	3.7	3.5	5.9	6.3	6.5
	02/08/2008	3.7	3.8	3.5	5.1	6.1	6.2
	16/08/2008	3.8	4.1	3.9	5.5	5.4	5.7
	01/09/2008	4.0	4.3	4.2	5.2	5.3	5.8
	16/09/2008	7.7	6.1	4.4	4.4	4.7	5.6

3.3 Crop Yield

Measured crops' yields are shown in Table 8. In 2008, Harvests began on August the first and the 15th for melon and tomato, and on September the first for squash. Tomato recorded yield was only 50 tons/ha: this result is significantly lower than Tunisian national average yield (80 tons/ha). According to Reina-Sanchez et al., (2005), tomato fruit is the most sensitive organ to the salinity, indeed significant yield reduction was recorded with irrigation water electrical conductivity higher than 2.5 dS m^{-1}. Ayers (1977) reported that using irrigation water with EC equal to 2.3, 3.4 or 5.0 dS m^{-1} reduces tomato yield by 10, 25 and 50 % respectively. Cuartero and Fernandez-Munoz (1999) recorded a decrease of tomato fruit weight and number when irrigated with water which EC is equal to 2.5 dS m^{-1}. Campos et al., (2006) compared the effects of five water salinity levels (1, 2, 3,

4, and 5 dS m^{-1}) on industrial tomato yield. They concluded that total yield was reduced by 11% upon each unit increase of water salinity while fruit quality improved with increasing water salinity.

Table 8. Recorded and average national yields of some crops

Crops	Average recorded yield (tons/ha)	Average national yield (tons/ha)
Tomato	50	80
Melon	43	60
Squash	60	70
Wheat	1.6	2

3.4 Drainage Discharge

The average flow rates were measured at the end of subsurface drainage pipes (D_1, D_2 and D_3) in tomato, melon and squash plots. Results obtained at several times (on 05/05, 31/05, 14/06, 12/07, 30/07, 16/08, 04/09 and on 16/09) during irrigation season (2008) are shown in Table 9. Measured drainage flow rate varied (between 1.5 and 3.2 l/mn) according irrigations amounts and frequencies. Indeed, maximum drainage discharge was observed on July: when the maximum water volume was supplied. After the irrigation season, the drainage flow rate decreased sharply and annulled on the beginning of October. Drainage water electrical conductivity ranged between 4 and 8 dS m^{-1}. Consequently, the salt masses leached by D_1, D_2 and D_3 were 2.6, 1.9 and 1.7 tons/ha respectively. Tedeschi et al. (2001) found that average flow rate of the drainage water reached 26.7 l s^{-1} and 31.3 l s^{-1} respectively during non-irrigated and irrigation season. They concluded that exported salts mass was linearly correlated ($p < 0.001$) with drained water volume that depended ($P < 0.001$) on irrigation supplies.

During rainy season, the subsurface pipes outlets were flooded.

Table 9. Irrigation water amounts and average drainage flow rates during irrigation season

Irrigation period	Irrigation water amounts (m^3/ha)			Average drainage flow rate (l/mn)		
	Tomato	Melon	Squash	Tomato (drain D_1)	Melon (drain D_2)	Squash (drain D3)
May	1567	400	-	0.6	0.2	0.3
June	3030	2120	820	0.4	0.4	0.7
July	4143	2170	1130	3.0	3.2	1.5
August	1260	340	680	0.6	0.6	0.8
September	300	-	360	0.1	0.0	0.2

3.5 Water Table Level and Salinity

The water table levels were measured monthly using two piezometers localized in the irrigated plot (piezometer 1) and at 50 m away (piezometer 2). Water table levels measurements on piezometer 1 showed that groundwater was 1.3 m deep on 2 May 2008. But because of over irrigation supplies (between May and July 2008), the water table level rose up to 1.2 m on 12 July 2008. At the end of irrigation season (September 2008), the water table was 1.3 m deep. The maximum depths: 1.5, 1.8 and 1.7 m were recorded on 01/11/2008, on 05/07/2009 and on 26/06/2010 respectively. While during the winter season groundwater level increased up to 1.2 m and reached 1.3 m on May 2009 (Table 10), the same value observed on May 2008. Hence, groundwater shallow depths likely contributed to salt build-up in the soil through evapoconcentration process. Feng et al., (2005) reported that after irrigation season, the groundwater level remarkably rose from 2.9 to 1.3 m below soil surface.

Table 10. Water table level and salinity variation

Date	Piezometer 1		Piezometer 2	
	Level (m)	Salinity (dS m^{-1})	Level (m)	Salinity (dS m^{-1})
02/05/2008	1.3	3.8	1.7	3.5
14/06/2008	1.3	3.5	1.8	3.4
12/07/2008	1.2	2.8	1.7	3.0
16/08/2008	1.3	3.1	1.8	3.4
16/09/2008	1.3	3.2	1.7	3.6
01/11/2008	1.5	3.5	1.8	3.8
06/12/2008	1.4	3.6	1.6	3.7
06/02/2009	1.2	5.8	1.5	5.6
02/05/2009	1.3	4.8	1.6	4.6
05/07/2009	1.8	5.5	1.9	5.4
16/08/2009	1.8	5.7	2.0	5.3
01/11/2009	1.6	5.3	1.8	5.2
30/01/2010	1.4	4.9	1.6	5.0
27/03/2010	1.5	5.4	1.7	5.2
07/05/2010	1.4	5.5	1.6	5.4
26/06/2010	1.7	5.3	1.9	5.2

On the beginning of irrigation season (05/05), the water table EC was equal to 5.5 dS m^{-1}. On 12/07 (current irrigation season), measured water table EC was 2.8 dS m^{-1} (equal to irrigation water EC). Allover irrigation season, water table EC remained lower than 3.6 dS m^{-1}. This decrease is due to the important irrigation water amounts that reached the groundwater. Since November the first, water table EC reached 5.8 dS m^{-1} on 06/02. It should be noted that the rainfall recorded along the year was 462 mm while the diverted water amount (was 1030 mm for tomato, 503 mm for melon and 299 mm for squash crops. As previously discussed, the broad irrigation water supplies were responsible for the significant water table rise which contributed to the soil salinity leaching. Therefore, Kalaat Landalous water table exhibits seasonal level and salinity variations, especially due to inadequate irrigation management.

4. Conclusions

This study was carried out during May 2008-June 2010 in a farm plot of 2.38 ha divided into: irrigated crops (tomato, melon and squash), rain fed crop (wheat) and bare soil. The supplied water amounts were higher than the total crop water requirements. Hence, during the irrigation season, the soil water content was always more than or near the field capacity. Therefore, water table level showed a sustained rise when irrigations were relatively frequent, and drainage flow rates increased accordingly. Whil along two years, the soil salinity varied slightly, but it was not the case over irrigation season. In fact, results showed an increase of the soil salinity essentially due to capillary rise of salt water from the water table. Soil salinity follow-up, on the three irrigated plots, showed that the most important concern was the top layer (0-30 cm) salinity increase. Fortunately, these accumulated salts (during irrigation season) were leached during the following rainfalls season.

In order to reduce an eventual soil salinization, farmers applied crops' rotation including rain fed crops and bare soil. Even though, using brackish water, wise irrigation management and regularly soil salinity monitoring are a sine qua non condition for soil and water resources sustainability.

Acknowledgments

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Mapping New Genetic Markers Associated with CMD Resistance in Cassava (*Manihot esculenta* Crantz) Using Simple Sequence Repeat Markers

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Abstract

Cassava mosaic disease (CMD) is the most serious disease in cassava–in India where it is grown for food, starch and sago purpose. The disease is best kept under control by exploiting the available host plant resistance, which was introgressed from *M. glaziovii* to cassava and it is known to be polygenic control. In the present study, an attempt was made to construct the genetic linkage map of cassava using SSR markers with the objective of mapping genes associated with CMD. Using single marker analysis (SMA), four CMD resistance markers were detected *viz*. SSRY28, SSRY235, SSRY44 and NS136. SSRY28 and SSRY235 were located on linkage group G and SSRY44 and NS136 on linkage group P of cassava genetic map developed by Fregene et al. (1997). Among the four markers, three (SSRY235, SSRY44 and NS136) are new markers associated with CMD resistance. The detection of markers SSRY44 and NS136 having association with CMD resistance is a new report indicating the possibility of having another genetic loci for CMD resistance in cassava in addition to the already established on linkage group G. This finding supports the polygenic control of CMD resistance.

Keywords: cassava, SSR marker, CMD, linkage map, F_1 mapping population, ICMV virus

1. Introduction

Cassava, *Manihot esculenta* Crantz., belonging to the family Euphorbiaceae, is one of the most important staple food crops in tropics and grown widely under diverse environmental conditions. This tuberous root crop has its origin in South America. It remains as the most reliable source of food for more than 700 million subsistence farmers in Africa, Asia and Latin America. The sub-Saharan Africa- currently accounts for 54% of the total world production of cassava (FAO, 2005). Cassava, in its calorie contribution in tropics, remains only fourth after rice, sugarcane and corn with a production of 202 million tonnes. India ranks first and seventh in the productivity (28 t/ha) and production (7 million tonnes) respectively in the world. Cassava is monoecious with 36 chromosomes, and is highly heterozygous due to its out-crossing nature. The crop is affected by various diseases and pests. Among them, cassava mosaic disease (CMD) is the most serious disease in Africa and India, causing yield loss ranging from 20 to 90 per cent. CMD is a viral disease caused by at least seven different geminiviruses. Transmitted of the virus by the vector whitefly, *Bemisia tabaci* is an extent of 5 per cent only. But the major spread of this disease is due to propagation of virus infected planting material.

The disease is best kept under control by exploiting the available host plant resistance, which was introgressed from *M. glaziovii* to cassava. The studies conducted by Hahn, Howland, and Terry (1980a); Hahn, Terry, and Leuschner (1980b) to establish the genetics of resistance to CMD indicated the possibility of several genes responsible for resistance. Akano, Dixon, Mba, Barrera, and Fregene (2002) reported that the resistance could be due to a major dominant gene (*CMD2*). Given the importance of *CMD2* to cassava production, and the limited knowledge on host plant resistance to geminiviruses, an effort was initiated to clone the gene. A bacterial artificial chromosome (BAC) library was constructed from the Nigerian variety, TME3, from which *CMD2* was first identified, and a fine map constructed around the region of the gene. Serial analysis of gene expression (SAGE) in

cassava revealed many differentially expressed genes of which beta-tubulin, elongation factor, importin, a transcription factor and rubridoxin were the most important (Anderson et al., 2004; Lopez et al., 2005).

The studies in the recent past on the genetics of resistance to CMD involving some of the African landraces and improved cassava clones indicated the possibility of several recessive genes responsible for CMD resistance (Lokko, Gedil, & Dixon, 2004; Lokko, Danquah, Offei, Dixon, & Gedil, 2005). Okogbenin et al. (2007) reported through marker assisted selection (MAS), the Latin American lines introgressed with dominant *CMD2* gene for cassava mosaic disease resistance. The resultant lines from introgression of the *CMD2* gene resulted in 14 genotypes combining CMD resistance and high yield are identified under African field conditions. The CMD resistance in two Nigerian cultivars *viz*., TMS 97/2205 and TMS 98/0505 was analyzed with SSR markers and in the field. Molecular data indicated, the CMD resistance in these lines was mediated by the *CMD2* gene. Okogbenin et al. (2012) reported a segregating F_1 population derived from a TMS 97/2205 x NR 8083 cross was screened using 530 SSR markers and identified (CMD3) a NS198 marker associated with CMD resistance, explaining 11% of the phenotypic variance. The combined QTL effect of CMD2 and CMD3 may account for the high level of resistance in TMS 97/2205.

Genetics of CMD resistance indicates the possibility of presence of several components responsible for CMD resistance. A better understanding on the components of resistance to CMD will be useful in breeding and in particular identifying genetic markers at DNA level to these individual components. The genetic approaches to mapping polyploid genomes with molecular markers have been reviewed by Ritter, Debener, Barone, Salamini, and Gebhardt (1991) and Wu et al. (1992). Single-dose restriction fragments (SDRF) is a class of marker useful for simplify the determination of allelism in highly heterozygous and polyploid crops (Wu et al., 1992). SDRFs are DNA markers that are present in one parent and absent in the other and segregate in a 1 : 1 ratio (absence: presence) in the progeny.

The overwhelming developments in molecular marker technology have generated various DNA marker systems. Among those systems, the SSR markers are being considered as the markers of choice. A total of 522 SSR markers were made available in cassava by Mba et al. (2001) and recently more SSR markers from new sources were available in CIAT (Fregene, Personal communication). The availability of these many markers in cassava will help to have genetic tags for various phenotypes in cassava. The objectives of the present study were to confirm the genetic locus already mapped and to identify new genomic region(s) for CMD resistance using SSR markers in a segregating population of CO2 x MNga-1.

2. Materials and Methods

2.1 Plant Materials

Two cassava varieties *viz*. MNga-1, a resistant variety and CO2, a susceptible variety, were selected based on field trials conducted at Central Tuber Crop Research Institute (CTCRI), Thiruvanthapuram and Tamil Nadu Agricultural University (TNAU), Coimbatore to develop the mapping population. CO2, a variety released from TNAU, Coimbatore, is highly susceptible to CMD, but possesses short plant type, middle branching, profuse flowering and good quality tuber with high starch content. MNga-1 (TMS30001) was developed at the IITA, Nigeria. It is a backcross derivative of cultivated cassava and wild *M. glaziovii*. It is a high yielding variety (29 t/ha) with tall plant type, top branching and good flowering.

2.2 Whitefly Vectors to Spread CMD

Whiteflies collected from the field were reared on tobacco under greenhouse conditions. The greenhouse reared whiteflies were released onto the susceptible parent CO2, grown under greenhouse conditions, to make the insects acquire the viral inoculum. The insects were allowed to feed on susceptible CO2, collected after 24 hrs and released on seedlings of CO2/MNga-1 in the greenhouse. The seedlings of susceptible CO2 infected with whiteflies were also planted along with F_1 seedlings to have the natural transmittance of virus across all the F_1 seedlings.

2.3 Screening for Resistance to CMD

The level of resistance to CMD resistance across F_1 seedlings of CO2 x MNga-1 was done six months after planting based on the scoring system adopted by Hahn et al. (1980b) and Akano et al. (2002). The level of resistance was determined based on the 1-5 scores established as per the severity of the symptoms developed, Score 1-Unaffected shoots, no symptoms in leaves; Score 2-Mild chlorosis, mild distortions at bases of most leaves, while the remaining parts of the leaves and leaflets appear green and healthy; Score 3-Pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets; Score 4-Severe mosaic distortion of two thirds of most leaves and general reduction of leaf size and stunting of shoots and Score 5-Very

severe mosaic symptoms on all leaves, distortion, twisting, mis-shapen and severe leaf reductions of most leaves accompanied by severe stunting of plants. At six months stage all the seedlings were pruned and allowed to grow for three more months to record the severity of the symptom expression.

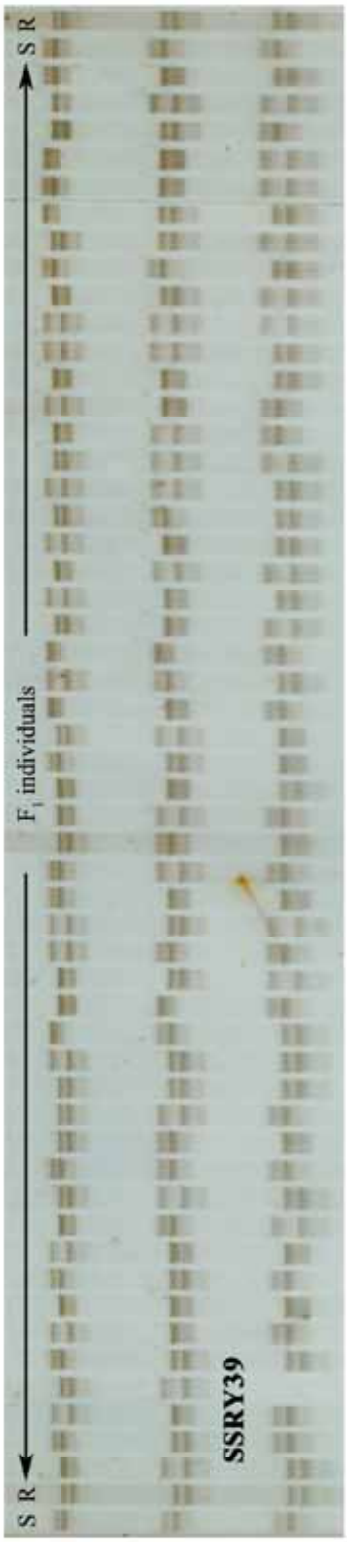
2.4 DNA Extraction

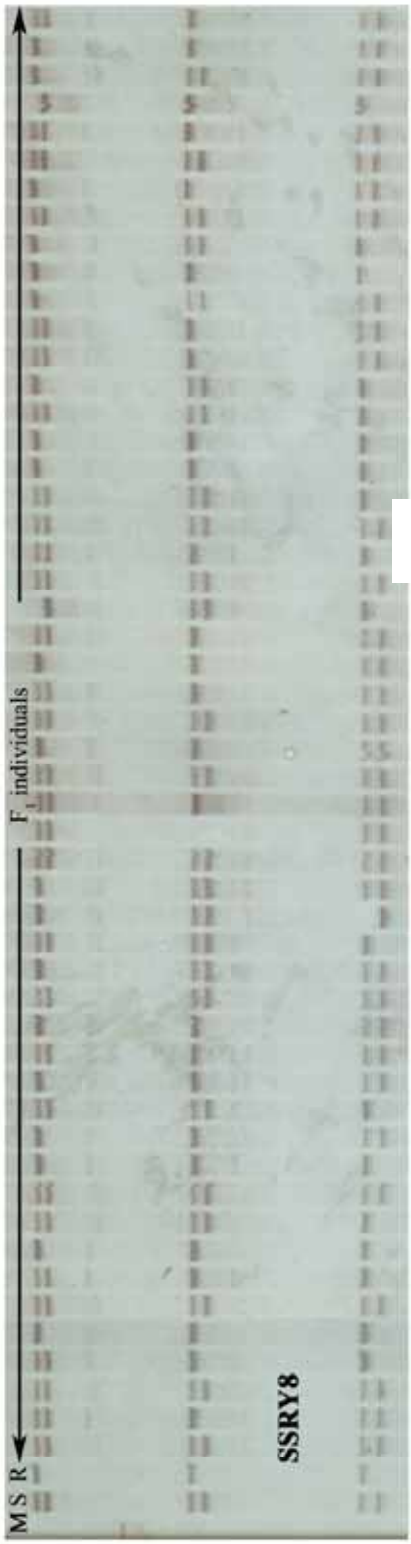
Genomic DNA of the parents and 141 F_1s were isolated from young fresh leaves adopting the procedure developed by Dellaporta, Wood, and Hicks (1983). The DNA of individual sample was quantified by using a fluorometer (DyNA Quant TM200, M/s Hoefer Pharmacia, Biotech Inc., USA) and its quality was checked on 0.8 per cent agarose gel. The final DNA concentration of all the samples was adjusted to 25 ng/µl.

2.5 Simple Sequence Repeat (SSR) Analysis

A total of 75 SSR primer pairs representing loci covering all 18 linkage groups as established by Mba et al. (2001) were synthesised from M/s Sigma Aldrich Inc. Out of 75 SSR primers, 19 primers were selected from linkage group G of Fregene et al. (1997) linkage map, where CMD resistant gene was already mapped. The primer pairs were used to identify polymorphic markers between CO2 and MNga-1. The SSR primer pairs producing polymorphic markers were surveyed on the seedlings of the 141 F_1 progenies to establish their segregation patterns because of the heterozygous nature of parents. PCR conditions were maintained as described by Mba et al. (2001). The PCR reaction was conducted in volumes of 20 µl containing 25 ng genomic DNA, 0.2 µM each of forward and reverse primers, 50 µM dNTPs, 1 X buffer (10 mM Tris-Hcl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$) and 0.3 Unit of Taq DNA polymerase (M/s Bangalore Genei Pvt. Ltd, Bangalore).

PCR amplifications were performed on a PTC100 (M/s MJ Research Inc.) Thermal Cycler with a PCR profile of 94ºC for 5 min followed by 30 cycles of 1 min at 94ºC, 2 min at 58ºC, and 2 min at 72ºC with a final extension for 5 min at 72ºC. A volume of 8 µl of loading buffer (98 per cent formamide, 10 mM EDTA, 0.005 per cent each of xylene cyanol and bromophenol blue as tracking dyes) was added to each of the amplified product and the denatured at 94ºC for 5 min, snap cooled using ice and separated on 5 per cent denaturing polyacrylamide gels (PAGE) containing 7 M urea at a constant current of 100 W. Multiplex loading of amplified products was followed based on the amplified product size range. Three sets of amplified products from the parents and F_1s were loaded at an interval of 15-30 mins when the amplified products were distinctly different for their size ranges. The patterns of amplified products across the samples were resolved by silver staining following procedure described by Panaud, Chen, and McCouch (1996) (Plate 1).





M : Marker S : CO2 R : MNga-1

Plate 1. Segregation pattern of SSRY39 and SSRY8 primers.

2.6 Data Scoring and QTL Analysis

The scoring of markers for each of the primer pairs was done as described by Wu et al. (1992). χ^2 test was carried out to establish the expected 1:1 ratio for all the SSR loci. Single marker analysis was performed by one way ANOVA to identify SSR markers associated with resistance to CMD. A significant F-test ($P < 0.01$) indicated association of marker locus with phenotype.

3. Results and Discussion

Out of 70 primer pairs, 51 primer pairs produced 89 possible patterns of segregating markers. These markers were established based on the method described by Wu et al. (1992) for SDRF markers. These 89 segregating markers were used for linkage map construction. Of the 89 markers, 9 markers (SSRY23c, SSRY23d, SSRY1, SSRY4d, SSRY20b, SSRY24a, SSRY24c, SSRY22 and SSRY303c) deviated from the regular 1:1 segregation pattern and these nine markers were not considered for linkage map construction. Forty seven markers were found to be linked into14 different groups spanning 412.9 cM and the rest of 42 markers remained unlinked. Markers were randomly distributed on the 14 linkage groups. The distance between the markers on the map also varied greatly across the different linkage groups.

Screening of 141 progenies and the parents *viz.* CO2 and MNga-1 to assess their levels of resistance to CMD indicated wide variation for the level of resistance among the progenies. CO2 and MNga-1 were identified as highly susceptible and resistant to CMD respectively. The number of plants under each category of damage score ranged from 9 (score5-highly susceptible) to 73 (score1-resistant). The number of plants falling under each category of damage score are as follows: 73 for score 1, 25 for score 2, 22 for score 3, 12 for score 4 and 9 for score 5. Based on CMD resistance screening, 73 resistant and 68 progenies susceptible to CMD and it is segregating for CMD resistance 1:1 ratio. Frequency distribution of the F_1 progenies coming under various categories of damage scores is shown in Figure 1.

Single marker analysis (SMA) was carried out using the damage scores and the marker segregation patterns 141 progenies to identify putative SSR markers linked to CMD resistance. SMA was carried out in the present study since complete genetic map could not be constructed with the available number of polymorphic markers to carry out interval analysis. The SMA between marker and phenotype resulted in the identification of four markers having association with resistance to CMD. Those four markers were SSRY28, SSRY235, SSRY44 and NS136. SSRY28 and SSRY235 were on the linkage group #5 and #11 respectively of this study (Figure 2). The other two markers showing significant association with CMD resistance were SSRY44 and NS136 on linkage group #13 and #14 respectively (Figure 3). The outcome of one-way ANOVA between markers and phenotype is given in Table 2.

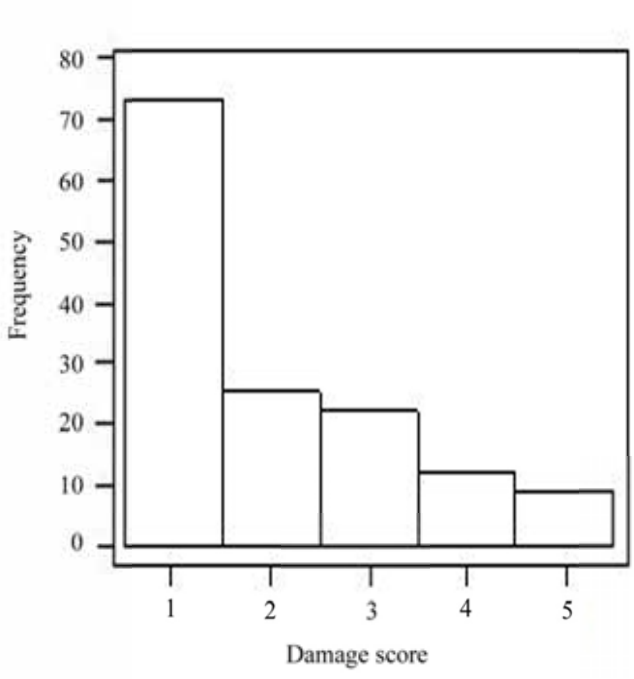


Figure 1. Frequency distribution of phenotypic values of CMD resistant F_1 mapping population

Table 2. One way ANOVA for SSR markers associated with resistance to CMD

Linked markers	R square	F (calculated)[f]	P-value	F (critical)
SSR28[a]	0.031	4.378	0.038	3.910
SSR44[b]	0.040	5.812	0.017	3.910
SSR235[d]	0.037	5.267	0.023	3.910
NS136[d]	0.032	4.669	0.023	3.910

[f] - calculated using single factor ANOVA.

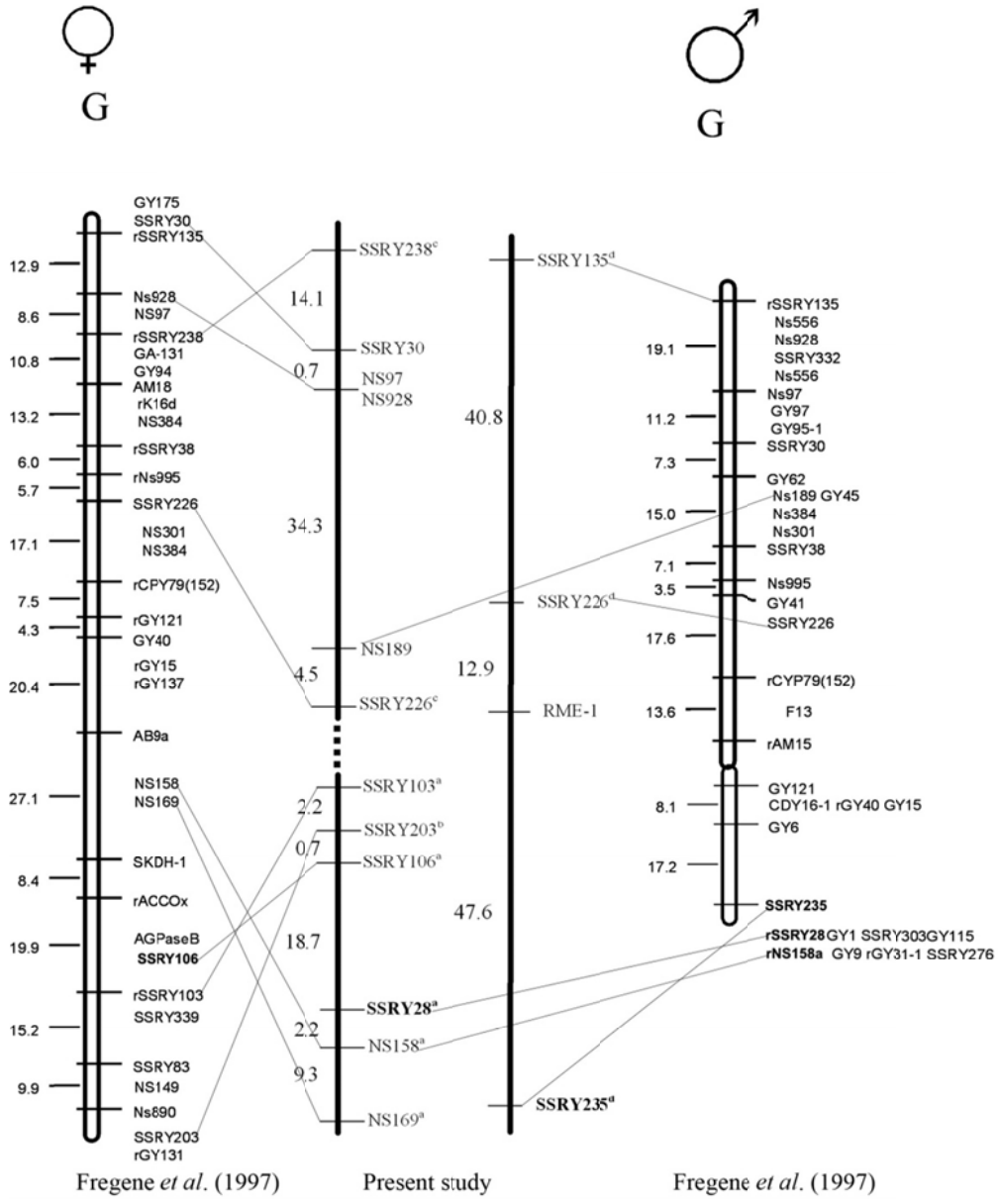


Figure 2. Combined linkage map of linkage group #5, #11 and #12 along with linkage group G of Fregene et al. (1997)

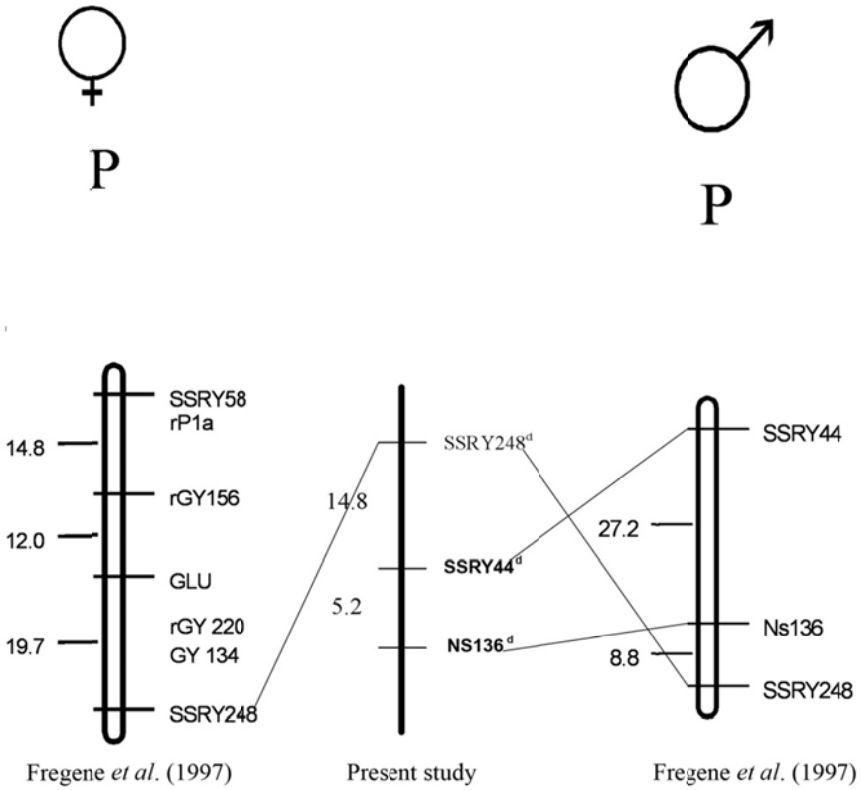


Figure 3. Linkage map of linkage group #14 along with linkage group P of Fregene et al. (1997)

In the present study, an attempt made to construct genetic maps CO2/MNga^{-1} using available 89 markers. All the 89 segregating markers were used to construct the linkage map which resulted in the grouping of 47 markers into 14 linkage groups. The other 42 markers remained unlinked. This could be because of less number markers screened and the target was to construct the linkage maps for the groups harbouring genetic loci associated with CMD resistance based on the earlier reports. The marker loci belonging to the linkage groups *viz*. #5, #11 and #12 in the present study were already mapped to a single linkage group G and similarly linkage group #14 mapped to group P (Fregene et al., 1997). The constructed genetic map containing only 47 linked SSR loci and the linkage map thus generated needs further saturation. The expected number of eighteen linkage groups for a comprehensive linkage map of cassava (2n = 36) was less than the 18 linkage groups, out of which four linkage groups had only two markers, and five linkage groups had only three markers.

The major objective of the present study was to detect the genomic regions associated with resistance to CMD caused by ICMV begomoviruses. The screening of parents and progenies for their levels of resistance to CMD revealed a continuous variation for the resistance to CMD. However, the frequency distribution of 141 progenies was skewed towards high level resistance indicating the influence of major genes. Based on CMD screening, resistance is segregating for CMD resistance 1:1 ratio. It indicates, in the resistant parent (MNga-1), CMD gene is in heterozygous condition and due to that it segregates in the F_1 progenies. This is confirmed by self pollinating the MNga-1 resistant parent and it segregate for resistance in 3:1 ratio in the S_1 progenies. The polygenic control of resistance to CMD was reported earlier by Hahn et al. (1980b); Lokko et al. (1998); Fregene, Bernal, Duque, Dixon, and Tohme (2000) and Lokko et al. (2004). However, Akano et al. (2002) reported that the resistance to CMD was under the control of single dominant gene.

The association of resistance to CMD with specific SSR markers adopting SMA by one-way ANOVA, resulted in the detection of four markers *viz*. SSRY28, SSRY235, SSRY44 and NS136. SSRY28 and SSRY235 are located on linkage group G and SSRY44 and NS136 on linkage group P of cassava linkage map (Fregene et al., 1997). Among the four markers, three (SSRY235, SSRY44 and NS136) are new markers associated with CMD resistance. Fregene et al. (1997) identified markers *viz.* SSRY235, SSRY28 and NS158 on linkage group G closer to each other. The strong association of SSRY28 and SSRY235 located on the same linkage group G with resistance to CMD established the possibility of having a major QTL for resistance to CMD in that region. In the same manner, SSRY44 and NS136 loci on linkage group P showed association with resistance to CMD indicating the control of resistance to

CMD by more than one locus. In the present study, apart from confirming the association of SSRY28 and SSRY235 with resistance to CMD, additional markers having association with CMD resistance was also established. These markers include, SSRY44 and NS136, which mapped to linkage group P of Fregene et al. (1997). The detection of markers SSRY44 and NS136 having association with CMD resistance is a new report indicating the possibility of having another genetic loci for CMD resistance in cassava in addition to the already established on linkage group G (Fregene et al., 1997). This finding supports the polygenic control of resistance to CMD as established by Hahn et al. (1980a, 1980b).

The first report of identifying a DNA marker for resistance to CMD was the detection of SSRY40 with *CMD1* gene by Fregene (2000). Similar attempts to detect the DNA markers associated with resistance to CMD led to identification of SSRY28 by two independent groups involving the Nigerian landraces *viz*. TME3 (Akano et al., 2002) and TME7 (Lokko et al., 2005). Further saturation of TME3 resistance source with more number of SSR markers and RAPD markers, two more tightly linked markers *viz*., NS158 and RME-1 were identified (Moreno Tomkins, & Fregene, 2004). However, Lokko et al. (2005) identified two more DNA markers SSRY106 and E-ACC/M-CTC (an AFLP marker) having strong association with resistance to CMD. By considering the results of the present study and the studies of Akano et al. (2002) and Lokko et al. (2005), it is very clear that the genomic regions around the marker *viz*. SSRY28 harbours one of the major gene conferring resistance to CMD irrespective of resistance sources. The SSR markers found to be associated with the CMD resistance *viz*. SSRY28 from previous studies (Akano et al., 2002; Moreno et al., 2004; Lokko et al., 2005) and NS158 and SSRY106 from the present study are on the same linkage group covering a distance of 20.9 cM. Lokko et al. (2004) identified seven different SSR markers associated with resistance to CMD in population derived from TMS30572 and TME117 and established the contribution of both the parents towards the resistance to CMD. Okogbenin et al. (2012) identified (CMD3) a NS198 marker associated with CMD resistance, explaining 11% of the phenotypic variance. The detection of several markers from different parts of the cassava genome having association with resistance to CMD unequivocally establishes the polygenic control of resistance to CMD with minor effect.

The availability of various genetic resources from TME and TMS could be used to impart CMD resistance to various elite cassava genotypes by backcross breeding. Identifying markers for the genes responsible for resistance to CMD in these genetic resources is expected to speed up the process of backcross breeding in cassava. The markers could help in future cloning of these genes.

In conclusion, in the present study, apart from the genomic region harbouring *CMD2* on the linkage group G, new genomic region having association with CMD resistance was established on the linkage group P. Established clearcut diagnostics both at phenotypic and molecular level to determine the varying levels resistance to CMD in mapping population will facilitate detection of new genetic loci for CMD resistance. Identification of more genetic loci, otherwise known as QTLs, is expected to change the strategies in breeding for CMD resistance in cassava, to have durable resistance in the progenies. Considering the availability of above genetic and genomic resources, the results from the present study involving a set of 141 progenies and limited SSR markers could be fine tuned to explore the possibilities of mapping genes associated with resistance to CMD. This could be achieved by screening the population in different environments of cassava growing areas. Moreover, the genetic map with more markers available at present and to be made available in future and the mapping population generated from CO2/MNga-1 in the present study could remain as the source for mapping genes associated with other agronomically important traits *viz.* early maturity, high dry matter, low cyanogens and high yield.

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An Immunological Investigation of Leaf Development in *Capsicum frutescens*

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Abstract

The objective of this project was to investigate the developmental regulation of cell wall components and development of leaves of *Capsicum frutescens*. In order to this, a growth curve was constructed of lengths and widths of fifty *Capsicum frutescens*leaves measured until growth had stopped (20 days). From the curve, four developmental stages were selected and harvested for further study. Cell wall proteins from each developmental stage were then extracted. The protein concentration present in each selected stage was then calculated from a Bovine Serum Albumin (BSA) standard curve. Then an immunological (Immunodot Blots) study was carried out to detect various cell wall associated epitopes present in the extracts.

Keywords: *Capsicum frutescens*, immunological, leaf development

1. Introduction

The Capsicum genus belongs to the family Solanaceae. Species of Capsicum are used as condiments, spices, ornamentals and in pharmaceutical therapies. Capsicums are native to South America and are cultivated in warm, dry climatic conditions, but cannot tolerate frost (Barceloux, 2008; Lozzio et al., 2008). The term Capsicum is believed to be derived from Greek word 'Kapsimo' which means bite (in terms of taste). Globally Capsicum is called by a variety of names e.g. pepper, chilli, chile, cayenne, paprika, aji, jalapeno, pimento, sweet pepper or bell pepper, red and green peppers (Kothari et al., 2010; Sanatombi et al., 2010). The genus Capsicum includes around 25 wild and 5 domesticated species. Worldwide, Capsicum annuum was the first pepper to be introduced (Siviero, 2002). Capsicum species are basic ingredients in many cuisines as a flavourant, colourant and to add taste (Ravishankar et al., 2003; Kothari et al., 2010). According to Kelly and Kelly (2001), chillies have been used since 3400 BC for domestic purposes. Hence, it is one of the oldest cultivated crops (Wallin, 2004). Globally, chillies were spread via colonisation mainly by the Portuguese. The Portuguese started establishing their colonies in India in the 16th century and by 1512 had established a chilli trade in all major cities of India. Chillies are thought to have introduced to China by Buddhist monks and from India to Srilanka (Wallin, 2004). The leaf plays an important role during plant development and is the main centre of photosynthesis and respiration. The leaf consists of the blade or lamina, stalk (petiole), nodes, axils (Becky, 2004). Leaf development consists of five phases. In phase I, leaf position is determined. Phase II determines leaf length, phase III, determines leaf width, phase IV determines leaf enlargement and phase-V determines leaf thickness. Meristematic tissue therefore regulates both leaf shape and size. The leaf in the early stages of morphogenesis is termed the 'primordium'. The shoot apical meristem (SAM) gives rise to leaf primordia in a regular pattern, with a definite position and arrangement according to the phyllotoxy (Byrne, 2005). Morphologically, scientists have classified two types of leaves- microphyllls and megaphylls. Microphylls are present in lycophytes and horsetails and are lacking complex venation patterns and parenchymatous tissues. Megaphylls have larger larger leaves, complex venation as in ferns and higher plants (euphyllotypes) (Piazza et al., 2005).

2. Material and Methods:

2.1 Plant Materials

The chilli pepper plants (*C. frutescens*) used in this investigation were grown under greenhouse conditions consisting of 16 hours of light and 8 hours of dark at temperatures of between 15 to 25°C from April to August 2011.

2.2 Growth Curve and Chilli Leaf Harvestion

The lengths and widths of fifty Capsicum frutescens (chilli) young leaves were measured and recorded on a daily basis. The chilli leaves were measured starting from lengths of 30, 31, and 32 and widths of 11, 12 and 13 until they reached their full length (growth had stopped or slowed significantly). Two growth curves were then constructed using average lengths and widths of the chilli leaves and used to select four different developmental stages of growth for further study. The chilli leaves from the selected stages were then harvested and 30 mg of chilli leaves from each stage was placed into Eppendorf tubes, labelled and stored at -20°C.

2.3 Extraction of Cell Wall Proteins from Chilli Leaves

200 µl of extraction buffer (Appendix 1) was added to the Eppendorf tubes containing 30 mg of chilli leaves and homogenised (by using Eppendorf grinder). The samples were then centrifuged at 13,000 rpm for 5 minutes and the supernatant transferred into a fresh sterile Eppendorf tube and the pellet discarded. This step was repeated. 800 µl of absolute ethanol (80% v/v) was added to each of the 200 µl samples to precipitate out carbohydrates (cellulose, hemicelluloses, pectin) and heavily glycosylated glycoproteins extensions (hydroxyproline-rich glycoproteins, HRGP) and arabinogalactin proteins (AGP's)]. The sample was then incubated overnight at 4°C. The precipitated sample was centrifuged again at 13, 000 rpm for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 100 µl of sterile distilled water (SDW). The protein extract was stored at -20°C, prior to future usage.

2.4 Protein Assay

2 µl of each of the protein samples (stage 1 - 4) were placed into a 1.5 ml Eppendorf tube with 798 µl of SDW to make a 400X dilution. 200 µl of BIORAD dye reagent was added to each tube and mixed by gentle inversion of the Eppendorf tubes. 800 µl of SDW and 200 µl of BIORAD dye reagent was put into another Eppendorf tube and used as a control. After 10 minutes, the samples were transferred to cuvettes and the optical density of each of the samples was measured using a spectrophotometer. The absorbance for the samples was read at 595nm, using the control as a blank. The absorbance of each protein samples was then compared to a standard absorbance curve for Bovine Serum Albumin (BSA) (Appendix 3, Table.7) to determine the protein concentration for each sample (Bio-Rad laboratories, 2005).

2.5 Immuno-Dot Blots

The protein samples from the four stages were diluted to the lowest protein concentration (1 µg/µl) by the addition of sterile distilled water. A 1 cm grid was then drawn onto nitrocellulose membrane and cut into 1 cm x 6 cm rectangles. One square was cut diagonally to indicate the position of the four different stages. 1ug of each protein sample was dotted onto the nitrocellulose membrane and 1µl of SDW was used as a negative control. These strips of nitrocellulose membrane were then left to air dry for 30 minutes. The strips of nitrocellulose membrane were then soaked in 1 x TBS pH 7.4 (Appendix 2) for 5 minutes and then a blocking solution consisting of a 5% solution of skimmed milk powder in 10ml of 1 x TBS was added to the membranes and left for one hour on an orbital shaker at room temperature. 200 µl of the primary antibody (1:4 dilutions) was added to the membranes in the blocking solution and then left for further 2 hours on the orbital shaker at room temperature. The membranes were subsequently washed four times in 1 x TBS for five minutes each time. After the membranes were washed, another 10ml of blocking solution was added and then 10 µl of secondary antibody (goat anti-rat IgG alkaline phosphatase conjugate) was added to the membranes (1: 1000 dilution) and returned to the orbital shaker for 1 more hour. The membranes were then given 5 - 6 minutes washes in 1x TBS and this step was repeated three times. 10ml of alkaline phosphate buffer (Appendix 2) containing 66 µl of nitro blue tetrazolium (NBT) and 33 µl of 5-bromo-4-chloro-3-indolyl-phosphatase (BCIP) was added to the membranes to detect bound antibodies. The membranes were then observed, while they developed. Once the dots had fully developed, the membranes were rinsed in SDW and left to air dry on a filter paper. Then the membranes were photographed.

3. Results

3.1 Growth Curve for Chillileaves

The standard deviations for leaf length are calculated. From the growth curve, four developmental stages were selected for the further experiments (Table 1)

1) Days 3 (40 ± 2.73mm)-stage 1,
2) Days 6 (60 ± 3.9mm)-stage 2,
3) Days 9 (80 ± 7.58mm)-stage 3 and
4) Days 18 (114.1 ± 8.74mm)-stage 4.

Table 1. Stages of *Capsicum frutescens* leaf development selected for further study

Stage	No. of days of growth (from, 30, 31, 32)	Mean (mm)	Standard Deviation (±)	95% Confidence Intervals (mm)
1	3	40	2.73	± 38-42
2	6	60	3.9	± 58-62
3	9	80	7.58	± 78-82
4	18	114.1	8.74	± 110-120

Table 2. Protein concentration of samples from the absorbance readings plotted against BSA standard curve

Tube No.	Sample stage	Absorbance (595nm)	Mean	Protein concentration from graph (µg / µl)	Protein concentration of sample (µg / µl)
1	1	0.274 0.290	0.282	4	2
2	2	0.454 0.448	0.451	10	4
3	3	0.672 0.662	0.667	17.5	7
4	4	0.457 0.451	0.454	10	4

3.2 Protein Assay

A Bovine Serum Albumin standard curve was plotted to determine the protein concentrations of various samples pertaining to stages 1 to 4. From the four stages, absorbance values were read at the wavelength 595 nm. The samples were then diluted to a concentration of 1 µg/µl for the further studies. The absorbance values were read for twice and the average plotted against the standard Bovine Serum Albumin curve and the concentration of protein samples the determined.

3.2.1 Dilution Factor Correction

1) From the stage1, mean absorbance value obtained was 0.282 read at 595 nm (Table 2). This absorbance value within the protein concentration 4 µg/µl. As this sample was diluted to 400 times, it had to be converted to dilution factor to arrive at protein concentration of sample stage 1 as follows.

$$4/1000 \text{ x } 400 = 1.6 \text{ µg / µl} \approx 2 \text{ µg/µl}$$

2) From the stage 2, mean absorbance value obtained was 0.451 read at 595 nm (Table 2). This absorbance value within the protein concentration 10 µg/µl. As this sample was diluted to 400 times, it had to be converted to dilution factor to arrive at protein concentration of sample stage 2 as follows.

$$10/1000 \text{ x } 400 = 4 \text{ µg/µl}$$

3) From the stage 3, mean absorbance value obtained was 0.667 read at 595 nm (Table 2). This absorbance value within the protein concentration 17.5 µg/µl. As this sample was diluted to 400 times, it had to be converted to dilution factor to arrive at protein concentration of sample stage 3 as follows.

$$17.5/1000 \times 400 = 7\ \mu g/\mu l$$

4) From the stage 4, mean absorbance value obtained was 0.454 read at 595 nm (Table 2). This absorbance value within the protein concentration 10 µg/µl. As this sample was diluted to 400 times, it had to be converted to dilution factor to arrive at protein concentration of sample stage 4 as follows.

$$10/1000 \times 400 = 4\ \mu g/\mu l$$

3.3 Immuno-Dot Blots

Immuno-dot blots were carried out to detect the presence of epitopes present in the leaf cell wall extracts from developmental stages 1 to 4. Primary monoclonal antibodies named JIM-5, 7, 8; LM-5 and 6 were used for epitopes detection (Table 4). These blots were performed in duplicate and the results analysed on the basis of an intensity of epitopes. In the tables 4and 5, a high intensity is represented in terms of '+' signs. As the '+' signs increase, the intensity kindly increase. High intensity represented high binding levels of the specific primary antibody to the epitopes. The variations in the intensities of different samples infer that epitopes were developmentally regulated.

Table 3. Samples dotting on nitrocellulose membrane with control upon specific antibody

Antibody Used					
JIM 5	Control	Sample 1	Sample 2	Sample 3	Sample 4
	1 µl	1 µl	1 µl	1 µl	1 µl
JIM7	Control	Sample 1	Sample 2	Sample 3	Sample 4
	1 µl	1 µl	1 µl	1 µl	1 µl
JIM8	Control	Sample 1	Sample 2	Sample 3	Sample 4
	1 µl	1 µl	1 µl	1 µl	1 µl
LM5	Control	Sample 1	Sample 2	Sample 3	Sample 4
	1 µl	1 µl	1 µl	1 µl	1 µl
LM6	Control	Sample 1	Sample 2	Sample 3	Sample 4
	1 µl	1 µl	1 µl	1 µl	1 µl

Table 4. Summary of Immuno dot blots intensity for the four developmental stages

No.	Antibody Used	Replicates	Leaf Stages					Intensity observation
			1	2	3	4	Control	
1	JIM 5	1	++	++	+++	++++	-	Increasing
		2	++	++	+++	++++	-	
2	JIM7	1	++	+	+	+	-	Decreasing
		2	++	+	+	+	-	
3	JIM8	1	+++	+++	+++	+++	-	No Change
		2	+++	+++	+++	+++	-	
4	LM5	1	+++	++	++	++	-	Decreasing
		2	++	++	++	++	-	
5	LM6	1	+++	+++	+++	+++	-	No Change
		2	+++	+++	+++	+++	-	

(+ Low intensity, ++ Medium intensity, +++ High intensity, ++++ Very high intensity, - No binding).

According to obtained results, average intensities to each sample were calculated to observe the intensity levels of the antibodies expression (Table 4).

Table 5. Average intensity to each blot sets

No.	Antibody Used	Average Intensity (Leaf Stages)					Intensity observation
		1	2	3	4	Control	
1	JIM 5	++	++	+++	++++	-	Increasing
2	JIM7	++	+	+	+	-	Decreasing
3	JIM8	+++	+++	+++	+++	-	No Change
4	LM5	+++	++	++	++	-	Decreasing
5	LM6	+++	+++	+++	+++	-	No Change

(+ Low intensity, ++ Medium intensity, +++ High intensity, ++++ Very high intensity, - No binding).

Samples 1 to 4 developmental stages expressed epitope conveyed the presence of cell wall epitopes in all the samples. JIM 7 and LM 5 antibodies expressed in first blot and inferred that these epitopes were developmentally regulated. In general, JIM 5 (John Innes monoclonal antibody-JIM) binds to pectin and recognizes partially methyl esterified epitopes of homogalacturone and unesterified homogalacturone. JIM 7 recognises methyl esterified pectin. Hence it detects methyl esterified pectic in sample 1. LM 5 recognises beta-D galactone epitopes in sample one and the intensity decreasing from second to fourth sample. This inferred that the samples were developmentally regulated. JIM 8 recognises arabinogalactans (AG) and AG detects in sample 1 to 4 without any change in intensity. Immuno-dot blot results are shown in figure 1 to 5 according to each epitopes respectively (JIM 5, 7, 8 and LM 5 & 6 with control).

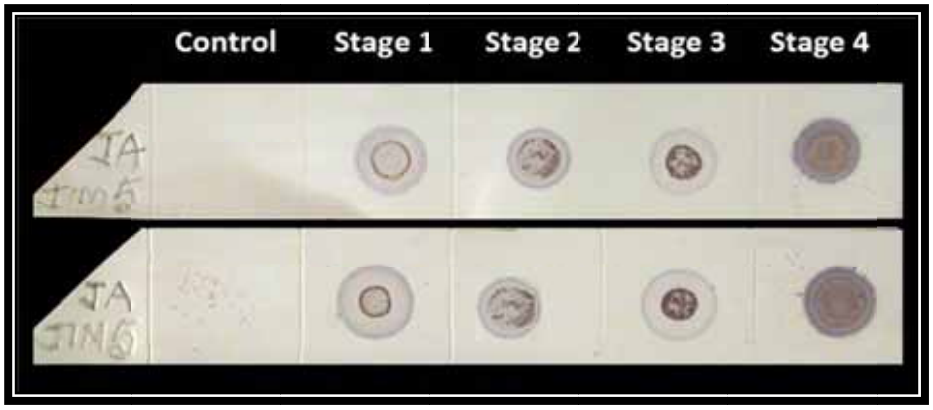

Figure 1. Photograph of Immunodot-blot for JIM5

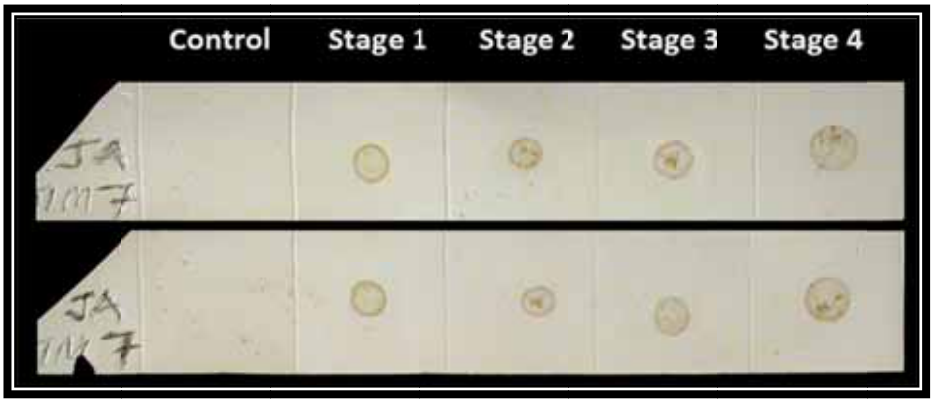

Figure 2. Photograph of Immunodot-blot for JM7

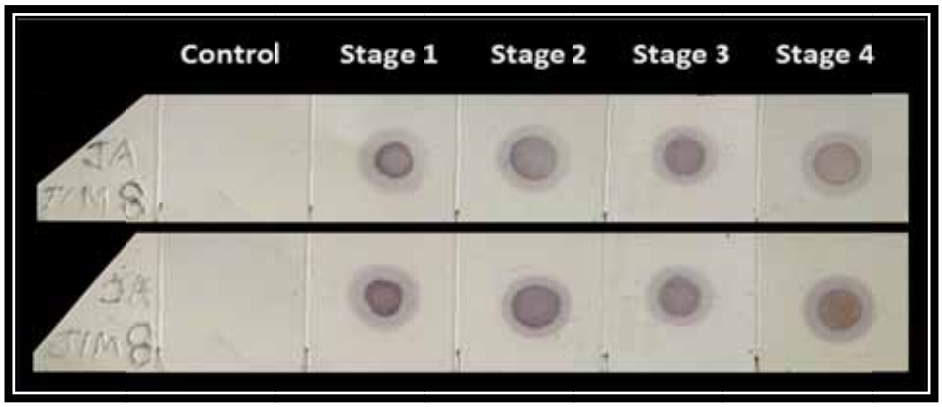

Figure 3. Photograph of Immunodot-blot for JM8

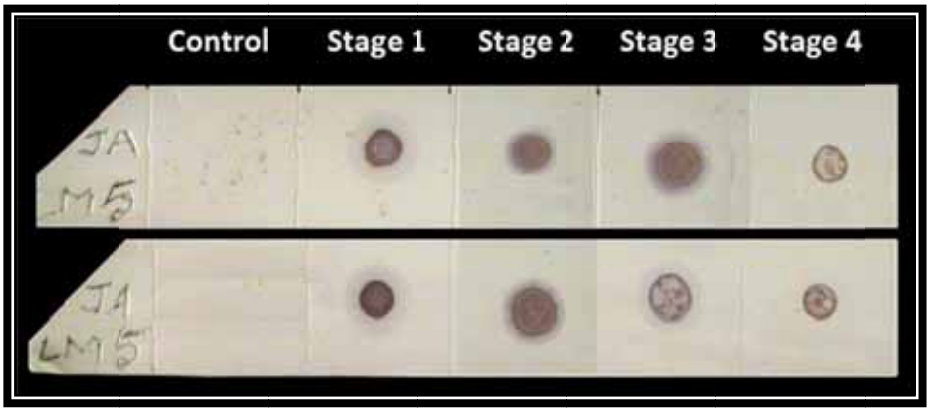

Figure 4. Photograph of Immunodot-blot for LM5

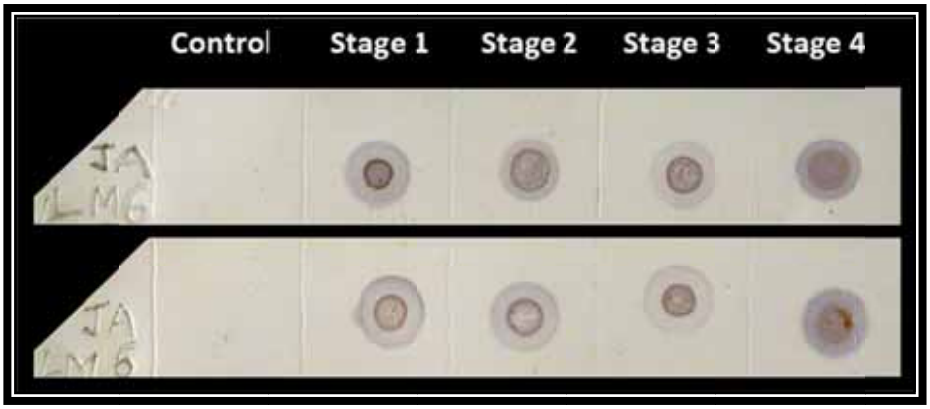

Figure 5. Photograph of Immunodot-blot for LM6

As per the results obtained, the four samples expressed various antibodies finely and conveyed that the epitopes were developmentally regulated according to their respective stages.

4. Discussion

Leaf is a principle organ of photosynthesis, respiration and is divided into different portions like blade and petiole-stalk. Leaf is responsible for photosynthesis (food manufacture) and respiration (usable energy production). Leaves are the repositories of food and water (Tsukaya, 2002). In response to environmental conditions, leaves themselves modified to various kind of forms to sustain. Leaves are used in multidiscipline fields like food, medicine, fossil fuel and many (Lozzio et al., 2008*;* Ravishankar et al., 2003). *Capsicum* is a crop having economic importance around the globe especially in the countries like India, Mexico and China.

immunological techniques were performed to investigate developmental changes occur in cell wall composition in leaves of *Capsicum frutescens* according to their developmental selective stages. Studies pertaining to crystallography, spectroscopy, microscopic analyses and many are the basis for cellulose structure elucidation in the cell walls of the plants. Analytical tools like IR (Infra Red), NMR (Nuclear Magnetic Resonance), XRD (X-ray Diffraction), TEM (Transmission Electron Microscopy) and Atomic Force Microscopy (AFM) are providing atomic level resolutions of cellulosic matrix present in the cell wall of plant tissues (Harris et al., 2010). Pectic components of cell wall are identified by monoclonal antibodies which are highly efficient and reliable. In the cell walls of higher plants, pectins are major components that play a key role in cell adhesion (Jones et al., 1997). From the results it can be concluded that JIM5, JIM7 and LM5 epitopes were developmentally regulated and the functions of each epitope were depicted in tabular form (Table 6).

Table 6. Panel of Monoclonal antibodies used in Immuno dot blots

Monoclonal Antibodies	Epitopes Recognised	Reference
JIM 5	Anti- Homogalacturonan (unsterified pectin)	Knox et al., 1990
JIM 7	Anti- Homogalacturonan (methyl esterifed pectin)	Willats et al., 2000
JIM 8	Arabinogalactans	Yi-qin et al., 2004
LM 5	Anti- β- 1, 4-D- Galactan	Knox, 2008
LM 6	Anti- α- 1, 5- D- Arabinan	Seifert & Roberts, 2007

JIM5 detected unesterifiedpectins present in the samples. As JIM7 expressed in decreasing order with a higher intensity in the stage 1, inferring that the four developmental stages detected methyl un-esterified pectins and were developmentally regulated. LM5 recognised β-D-Galactan with higher intensity in stage 1 and the intensities were decreased from stage1 to 2 and from the 2nd stage, intensities were stable as no change was observed. The epitopes of JIM8 and LM6 were recognised but the intensity observation remained the same without any detectable change. These results conveyed that the stages from 1 to 4 were developmentally regulated.

In primary cell walls, celluloses and hemicelluloses are the prime polysaccharides. Structurally celluloses comprising of 1, 4-β-D-glucose residues and hemicelluloses are branched polysaccharides and are homologous to celluloses as their backbone is composed of 1, 4 linked β-D-Hexosy-1-residue. In primary cell walls, the abundant hemicelluloses present are xyloglucans. Gluronoxylan, arabinoxylan, glucomannan and galactomannan are the other hemicelluloses present in the secondary cell wall (Brett & Waldron, 1996).

5. Conclusion

*Capsicum frutescens*is an important agro-economic crop which could benefit from the research to improve quality, yields and production levels by using the various plant biotechnological methods. Solanaceae members such as *L. esculentum* (tomato), *N. tobaccum* (tobacco) and *S. tuberosum* (potato) are the model systems have been subjected to biochemical and immunological investigations (Ochoa-Alejo & Ramirez-Malagon, 2001). Advanced techniques such as genetic transformation, genetic modification can improve the crop qualities and these crop cells are capable of regenerating organs by cell, tissue and organ cultures and eventually to a whole plant in vitro.

According to a study done by Ochao-Alejo and Ramirez-Malagon (2001) conferring resistance to pests and diseases to the plants is highly difficult by recombinant DNA techniques. Till now, much progress has been done in terms of genetic improvement of *Capsicum*. Still a long way is ahead to improve the quality traits. Many efforts are being put to combat various kinds of diseases especially viral induced diseases. But these efforts are still not satisfactory. Various strategies have been employed to protect plants against viruses such as protein mediated resistance and satellite RNA mediated resistance. To block the progress of virus infection, protein based approaches are reliable.

In RNA based resistance, viral DNA is degraded by using plants post-transcriptional gene silencing mechanism. Advanced technique like designing artificial microRNA (miRNA) is used against pathogenic viruses. Artificial miRNA down regulates the gene expression in plants. So this technique is exploited to confer resistance in plants to combat against pathogenic virus (Kothari et al., 2010).

A variety of investigative techniques such as immuno dot blots have depicted the presence and importance of developmentally regulated cell wall associated proteins. Further research work is mandatory to identify, diagnose and their role in the developmental regulation of proteins and synthesis of new cell wall components.

It could be quite inevitable that all the new cell wall proteins and their genes must be identified and characterised. But the difficulty here is their functions and molecular interactions. Prior to their function, knowing the protein position is a million dollars question. Inculcating mutants by antisense RNA technology is exciting but is a difficult experiment and is raising many concerns regarding ethics. So these concerns must be answered in the upcoming days (Showalter, 1993).

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Appendix 1: Cell wall extraction buffers

Stock solutions

A - 0.5 M EDTA (500 ml)

93g of dissodium EDTA-2H20

Dissolved slowly in 400ml of SDW

Adjust pH to 8.0 with approximately 10g of NaOH pellets

B - 2M NaCI (100 ml)

11.2g NaCI

Dissolved in 100 ml SDW

Autoclave

C – 1 M Tris-HCl pH 8.0 (100 ml)

12.11 g Tris base

Dissolved in 80 ml SDW

Adjust pH with Concentrated HCI

Autoclave

Protein extraction buffer

A-10 mM EDTA

B-10 mMNaCl

C- 0.2 M Tris-HCl (pH 8.0)

For preparing 1 ml protein extraction buffer

200 µl 1M Tris-HCl (pH 8.0)

5 µl 2M NaCl

20 µl 0.5 M EDTA

775 µl SDW

1 ml Extraction buffer

900 µl Proteinextraction buffer

100 µl 10% Nonidet

1 µl M DTT

Appendix 2: Immuno-dot blots Solutions

10 x TBS (Tris buffered saline) (500 ml)

40 g NaCl

1 g KCI

15 g Tris base

Dissolve in 300ml of SDW

Adjust pH to 7.4 with concentrated HCI

Make up volume to 500 ml with SDW

Autoclave

Alkaline phosphate buffer (500 ml)

2.5ml 1M Tris-HCl pH 9.5

2.92 g NaCl

0.508 g MgCl2

Make up volume to 500 ml with SDW

Appendix 3: BovineSerumAlbumin (BSA) proteinassay

Table 7. BovineSerum Albumin (BSA) protein assay

Tube	Final BSA protein concentration (µg/µl)	Volume of Stock (µg)	BIORAD dye Reagent (µl)	Volume of SDW (µl)	Absorbance (595nm)	Mean
1	1	2	200	1798	0.116 0.122	0.119
2	5	10	200	1790	0.318 0.33	0.324
3	10	20	200	1780	0. 459 0.451	0.455
4	20	40	200	1760	0.760 0.752	0.756
5	25	50	200	1750	0.841 0.853	0.847
Control	0	0	200	1800	0 0	0

Stock Solution (µg/µl).

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The Revitalisation of Water Resources for Sustainable Agricultural Development in South Africa: A review

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Abstract

The promotion of efficiency and sustainability of water use have been enshrined in the National Water Act (Act 36 of 1998) of South Africa. Currently there is no immediate intractable water crisis facing South Africa, although this is undoubtedly based on the assumption that the existing water resources will be managed effectively. In enhancing revitalisation, the design and supervision of water resources must be linked with the country's development approach for sustained agrarian reform. It cannot, however, be applauded that the broad social and economic objectives, or even the more specific objective of water security, in South Africa are being fully realized at moment. However, to understand the contemporary issues in revitalisation of water resources for sustainable agricultural development, the paper first describes the various phases of irrigation development in South Africa and highlights the past and present measures taken by the government to ensure equity of access to water. Secondly, it also highlights water security; water demand and supply management; water productivity and water governance in South Africa.

Keywords: revitalisation, water resources, sustainable agriculture, governance and water security

1. Introduction

South Africa is the 29th driest country out of 139 countries with 1 110 m^3 of water per capita in 2005 (Muller et al., 2009). The rainfall pattern is erratic and unequally distributed across the country. Water resource base supports intensive and subsistence agriculture, mining activities and other social and economic activities in both rural and urban areas of the country. The sphere of water flow is divided into green water (vapour) flows and blue water (run-off) flows. Currently, emphasis has been placed on the management of blue water, which is used for irrigation, mining and industries, and domestic as against green water which is erroneously assumed that it is of less value. Green water also sustains the ecosystem services like rain-fed agricultural production, forestry, fibres and grasses for livestock. Today, rain-fed agriculture which mainly depends on green water is embraced and practiced on 97% of the available agricultural land in Sub-Saharan Africa (SSA) notwithstanding, the positive postures of irrigated farming (FAO, 2002). It is unjustifiable to rely entirely on irrigation as a universal remedy for intensification of agricultural production and sustainability (FAO, 2002). The expansion of irrigation schemes has been in indeterminate state over the past 20 years, coupled with the degradation of irrigated crop land, mismanagement, difficulties in revitalisation and maintenance of infrastructures (FAO, 2002). Currently, South Africa is dependent on blue water (run-off or surface water) for its agricultural production and water security improvement. However, studies from "Water Reconciliation" asserted that blue water handiness is inadequate to support a developing economy (NWRS-2, 2012). In South Africa, the growth of blue water occurs in few water management areas whilst majority of the water management areas have no water. However, in some basins or watersheds were enough water is available, for instance the UThukela, Mzimvubu and Pongola, the proximity to areas of demand is not ideal coupled with the exorbitant cost of transmissions per cubic metre to areas of need (DWA, 2010). In addition, development of new dams and purchase of water resource equipment are costly coupled with arduous procedure that may take a decade from commencement to commissioning (DWA, 2010). In most municipalities, water that is classified as "non-revenue water" (free water) accounts for more than 37%, and water losses accounts for approximately 50%; while losses from domestic and irrigation schemes accounts for 60%. In relations to loss in revenue, these losses amounts to about R11 billion per annum at the municipal level alone (NWRS-2, 2012). Water use through irrigation has decreased substantially from 80 to 50% in the last 25years (De Villers et al., 2004,

Muller et al., 2009). Therefore, it is imperative to improve and revitalise water resources for sustained agricultural development and water security.

South Africa is water-scare, therefore, revitalization and increasing water availability for agricultural production becomes very necessary to assist in the generation of sustainable living. According to (Hamdy et al., 2003), the more we conserve water for agricultural production, the less the need for investment in water infrastructural development and the greater the local food security, and more water for agriculture, more for domestic and industrial uses. Revitalising water resources is therefore just one part of a wider unified sustainable rural development strategy. Soil depletion and insecurity of water resources has affected the livelihood of majority of people and the prospect of food security, with adverse consequences on sustainable agriculture. The ultimate problem for human race is to safeguard and manage the water resource base on which income, food, and raw materials production depends. The international community has acknowledged the need for a unified method to the security and sustainable management of land and water resources, as highlighted in "decision III/11 of the Conference of the Parties to the Convention on Biological Diversity" which encompasses all interested parties at local, national level, farmers, peasants and non-governmental organizations (UN, 1997). The term 'water resources and water use' are used to succinctly illustrate in this review, that water use in agriculture is not only limited to irrigation but also encompasses the water that is harvested, soil water conservation and water for livestock production. The review paper therefore, dwells on a range of strategies and policy aspects adopted to revitalise the water resources for sustainable agricultural development in the following perspectives: Smallholder irrigation development, water resources in South Africa, water security and management, water demand and supply management, water productivity and water governance.

1.1 Smallholder Irrigation Development

The growth and development of smallholder irrigation schemes in South Africa have been characterised by definite government policies and different forms of technology which was associated with economic development (Backeberg & Groenewald, 1995). The smallholder irrigation in South Africa existed in phases

1.1.1 Peasant and Mission Diversion Phase

The peasant and mission phase which started in the 19th century was the first smallholder irrigation scheme to be developed and it was linked to missionary activities in South Africa (Bruwer & Heerden, 1995). This phase also coincided with the existence of the individual diversion scheme era which was private and the technology used involves river diversion to farms (Backeberg & Groenewald, 1995).

1.1.2 Smallholder Canal Phase

The canal phase of smallholder irrigation was developed together with the schemes using storage dams. The primary motive for establishing both schemes was to provide black and white families the opportunity to use agrarian activities as a means of livelihoods (Backeberg & Groenewald, 1995). However, many of the smallholder irrigation schemes developed at this period were built after the Second World War and it was mainly aimed at providing African families residing in the "Bantu Areas" with a full livelihood (The Commission, 1955). The "Bantu Areas" which were formed as a result of the Land Act of 1913 and the Land and Trust Act of 1936, limited land ownership by the black South Africans to these zones. The irrigation water was obtained from the river and diverted to the field by a concrete canal conveyance system. Averagely, the plot size on the schemes ranges from 1.28 to 1.71 hectares for the black farmers while the white counterparts had an average plot size of 8 hectares to 20 hectares (Van Averbeke et al., 2006). The variance in plot size between black and white farmers indicated that irrigation design was carried out under the notion that black farmers and their families needed less land than the white families to reach a complete livelihood (Backeberg & Groenewald, 1995). This second phase of irrigation development lasted from 1930 to 1960 and it was noted as the public storage irrigation scheme. In 1952, about 122 smallholder irrigation schemes have been built or under construction, with total land area of 11 406 hectares comprising a sum of 7 538 plots holders (The Commission, 1955). According to The commission (1955), a total of 54 051 hectares of land in these areas had the prospect for irrigation development and could settle almost 36 000 farming families, representing about 216 000 farmers. A total of 18 200 hectares of irrigated land was developed during this phase (Arcus Gibb, 2004).

1.1.3 Independent Homeland Phase

The independent homeland phase of smallholder irrigation development which formed an essential part of economic development of the homelands existed from 1970 to 1990. The homeland which had its origin from the "Bantu Areas" made provision for both traditional and language cluster. The homelands were granted autonomy but dearth of development and poverty was very pronounced in the inlands and therefore it became pertinent to

improve their economy by the creation of new irrigation schemes as a development strategy ((Beinart, 2001). However, funding of these irrigation schemes was provided by South Africa government (Lahiff, 2000). According to Arcus Gibb (2004), not less than 64 of the existing irrigation schemes, with a total land coverage of 13 000 hectares, were built at this phase. Nevertheless, the number of smallholder irrigation schemes that existed at this period may be more, as records were not properly documented. This phase was noted for purposeful and centralised scheme management, large irrigation scheme with technical development (Backeberg & Groenewald 1995). The large scheme which was more than 500 hectares were established mainly in the Eastern Cape and comprised of the scheme situated at Keiskammahoek, Tyefu, Xonxa and Ncora (Van Averbeke et al., 1998). The large schemes established during the sovereign homeland period were costly to maintain. However, following the democratisation of South Africa in 1994, the provincial governments decided to dissolve the agricultural homeland parastatals that were inherited. This decision led to the total collapse of production in the large schemes (Laker, 2004).

1.1.4 Irrigation Management Transfer (IMT)

The primary focus of irrigation development in the early 1990s was for food security and poverty alleviation. The study carried out by Arcus Gibb (2004) showed that they were approximately 62 new irrigation schemes established at that period. The Independent Development Trust played a major role in the funding of these irrigation projects but later the Provincial department of Agriculture, public works and health department took over the funding (Van Averbeke & Mei 1996). At this period, the Reconstruction and Development Programme (RDP) as an economic development programme of government mandated for community based projects collaborated with Irrigation Management Transfer (IMT) to enhance the living condition of the people and to empower them to take over the control and maintenance of the existing irrigation projects in their areas. The primary aim of the IMT is to transfer entirely the duty of supervision, operation and maintenance of irrigation schemes to the farmers. However, the transfer of these responsibilities requires revitalisation of the irrigation schemes. In many parts of the world, IMT were also implemented as an approach to irrigation scheme management with the aim of increasing the lucrativeness of irrigated agriculture and to reduce the continuing government spending on the operation and maintenance of the irrigation schemes (Shah et al., 2000). The Revitalisation of these irrigation schemes stems from the WaterCare programme that was launched in 1998. The aim of the WaterCare programme was to revitalize selected smallholder irrigation schemes not only in the area of infrastructure but also in governance and productivity (Arcus, 2005).

2. Water Resources in South Africa

Rainfall in South Africa is usually irregular and occurs during the summer with almost all agricultural activities been dependent on irrigation. The total annual surface water run-off resulting from rainfall is approximately 50 150 million m3 per year, however, with the construction of dams average water storage capacity of 27 000 million m3 has been attained (DWAF, 2009). The annual water usage in South Africa stood at 22, 400 million m3 while the percentage used for irrigation is approximately 50%. For instance, the average yearly rainfall is about 500mm with the Central and Eastern regions of South Africa experiencing summer rainfall, while the Western region of the country experience winter rainfall (DAF 2010). Most crops has varying degrees of water requirement, for example while about 15kg of maize grain is produced with one millimetre of water consumed (Du Plessis, J., 2003), cabbage and soya bean production cycle water requirement vary from 380 to 500 and 500mm to 900mm respectively (DAF, Cabbage/Soya bean Production guide lines 2010). The National water Act (Act 36 of 1998), which became effective in October the same year, produced a framework for ensuring that South Africa's water resources are managed in such a way to benefit its citizenry. The fundamentals to be considered in ensuring that this happens include (DWAF, 1998): "Meeting the basic human needs of present and future generations; promoting equitable access to water; redressing the results of past racial and gender discrimination; promoting the efficient, sustainable and beneficial use of water in the public interest; facilitating social and economic development; and providing for the growing demand for water use". These fundamental recipes recognise that equilibrium has to be struck in the allocation between different groups of users. Social and economic growth will be enhanced when water is allocated and used efficiently. The Water Users Associations (WUAs) was formed primarily to allow communities to pool their financial and human effort together to be able to carry out water related activities. It is stipulated that the WUA would assist in water distribution role, maintenance, and collection of water supply charges (DWAF, 2000).

3. Water Security and Management

Water security has been defined as "the reliable availability of an acceptable quantity and quality of water for health, livelihoods and production, coupled with an acceptable level of water-related risks" (Grey & Sadoff, 2007).

In order to secure water availability and use, South Africa adopted a new water policy which climaxed the promulgation of National Water Act-NWA (Act 36 of 1998). The new NWA permits the government to remain as the trustee of the nation's water resources and also functions in the public trust to guarantee that water is "protected, used, developed, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons" (DWAF, 2000). The freedom to use water is however, granted to users, who must be registered and licensed after paying the prescribed fee. Nevertheless, the main idea of water management under the new Act is decentralization with security measures in water distribution for basic human requirement and development purposes. In ensuring the effective devolution of water, Nineteen Water Management Areas have been delineated countrywide under the auspices of Catchment Management Agencies (CMAs) with the mandate to ensure the sustainable use of water resources in their areas of operation, in line with the aims of the Act. At the community and smallholder farmers' levels, every user are allowed to take water for "reasonable domestic use, gardens and livestock watering" (but not for commercial purposes) without registration, licensing or paying fee, as specified in the Act.

4. Water Demand and Supply Management

Water scenery is a resource in itself, supporting many activities, particularly irrigation agriculture, nature conservation, and construction, as well as other livelihoods of the community. Water is obviously essential to economic growth and sustainable development of a country. In water demand management, each user sector has a variety of alternatives for control. This control is necessary because of limited groundwater supply resulting from surface runoff (Schreiner, 1998).

4.1 Household Category

The demand management system at the household category encompasses tariff adjustment upwards (to discourage consumption); changes in living arrangements (use of smaller plot size); and control of water losses through leakages at the Municipal distribution level; other demand management activities are: maintenance of household water fittings; and the regulation of the water use efficiency of household by creating awareness and encouraging the use of devices such as machines used for washing clothes (DWAF 2002).

4.2 Industrial Category

The water demand control and accounting in industries are made by proper metering. The "Government Notice R 22355 dated 8/06/2001" published in terms of Water Acts, is an essential document that oversees both industrial and local consumption of water (DWAF (2002). The guidelines specify that water to any consumer must be measured by means of a water-volume measuring apparatus (meter). The accessibility of water and consistency of supply, rather than costs, are usually the main concerns of industries. For big industries however, the policy stipulates that such industry must cover the full costs of making water available and this has assisted government to focus on water use efficiency. In addition, the policy allowed many industries to treat municipal waste water for reuse in their operations.

4.3 Agriculture Category

Agriculture is the largest water user in South Africa and measures have been put in place to regulate and manage its demand in the sector. As exemplified in most smallholder irrigation projects, there has been considerable progress in the efficacy of water use. For instance water scheduling practice which entails the timing of irrigation event and the control of irrigation water released has been adopted in most smallholder irrigation schemes. In addition, the use of advanced equipment and the cost placed on irrigation water indirectly act as an encouragement for improving the efficiency of water use. Improved efficiency in water use would make more water accessible for agricultural production thus enhancing better returns on investment. The adoption of stiff water pricing may lead to disastrous effect for poor farmers and most urban household. However, water marketing and pricing arrangement are considered beneficial in the search for higher water productivity (Jury & Vaux, 2005).

4.4 Water Supply Management

The utilisation of water resources in South Africa is increasing and has necessitated concerted effort towards sustained supply. However, increases in water supply have been achieved through the following: (i) Storage: South Africa's storage size is quite well established, with a dam capacity of over 32 billion m3. This is approximately two-thirds of the average yearly flow in all its rivers. (ii) Water transfer: Water transmission, both within one river basin and from other river basins to another has been developed broadly in South Africa. The notable example is the Vaal River, which is augmented with water conveyed from the Orange River through the Lesotho Highlands Water Scheme. Related transfers happened in other areas such as Gauteng, the Nelson Mandela Metro in the Eastern Cape and other areas in North West and Limpopo provinces. (iii) Recycle of wastewater: The recycling of

waste water is another supply management options which is extensively practice in South Africa. (iv) Naturally endowed water is also a source of additional water supply. In South Africa, legislation has been put in place to conserve enough water in rivers for environmental sustenance. The maintenance and sustainability of "environmental flows" has been come imperative with a view of protecting aesthetic attractions, such as the country's national parks.

5. Water Productivity

Water productivity is the totality of water used within a system, measured in terms of physical, economic or social values. "It is the measure of the economic, livelihood or biophysical output derived from the use of a unit of water" (Machibya et al., 2004). Water could be put into various uses such as irrigation, brick preparation, mining and domestic. The physical dimension denotes the output such as yield of crop for every amount of water exhausted in producing this yield. Economic measure is feasible when the physical output is converted to monetary terms or value under market situation. The social productivity is realistic when the value of water is likened to social benefits that are obtained as exemplified by the number of jobs created as a result of the existence of water or the worth of decent health realised through good sanitation by using water (DWAF 2000). The improvement of water productivity is hinged on the following conditions (i) "increasing the saleable yield of the crop for each unit of water transpired by it; (ii) reducing all outflows (e.g. drainage, seepage and percolation), including evaporative outflows other than the crop stomata transpiration; and (iii) increasing the use of rainfall, stored water, and water of marginal quality" (National Water Resource Strategy) (NWRS-2, 2012). The first condition has to do with the need to raise crop yield. The second condition is the intention to reduce losses of water while the third condition implies adoption of substitute for water resources. "The Comprehensive Assessment of Water Management in Agriculture (2007)" measured water productivity at the following levels: "crop/plant or animal; field/pond; farm/ agricultural enterprise; irrigation system; basin and landscape". Water resource management should have a way of distributing, reassuring efficient practice and overseeing the use of water in obliging the collective necessities of the society (Lenton & Muller, 2009). There have been recorded feats in encouraging rainwater harvesting technologies in a variety of measures from "on-farm micro-catchment" to communal "macro-catchment" method in Sub-Saharan Africa (Malesu et al., 2007). There is also possibility for additional investment to encourage "up-scaling and out-scaling" of water harvesting initiatives while inducement programmes such as "working for water" might be a platform to develop water harvesting in South Africa (Poras et al., 2008). According to Lenton and Muller (ibid) results of enhanced water management will typically be discovered outside the water sector and there must be a linkage between planning of water resources and sustainable progress in the society.

6. Water Governance

Water Governance encompasses an array of political, social, economic and managerial processes over which varied interests are expressed, inputs are engrossed, decisions are made and applied, and decision makers (government) are answerable in the management and delivery of water resources at diverse levels of the society (Nowlan & Bakker, 2007). Water governance is all about drawing and implementation of the right laws and policies from health, food security to land use and conservation of the natural environment on which water resources absolutely depend (UNDP, 2012) Ideal water governance entails obviousness, involvement, transparency, fairness, liability, consistency, awareness, unification and virtuous decision making (NWRS-2, 2012). In South Africa, there have been pragmatic changes in the governing structure and institutional arrangements since 1994. Despite these seeming changes, the water sector is still wallowing in numerous challenges (NWRS-2, 2012). These analysis, nurtures the government confidence that these crises can be ward off by refining water resource use and management. However, the undertaking to surmount these challenges will involve substantial changes in the ways water resources are developed, allotted, and administered. The proposal enunciated by the National Water Resource Strategies (NWRS-2, 2012) was to design and sustain enviable changes in the water sector to address the financial, environmental and capacity limitations inherent in the sector. Even though much has been accomplished in the water sector since 1994, the performance and results of the sector in relation to the sector objective have not been encouraging. However, considerable improvement in increasing access to water and cleanliness (sanitation) among some communities has been made, but the impact of this progress has not been felt as majority of South Africans are without water. Water stress and distribution of water for agriculture and industrial use are still highly lopsided and unequal (NWRS-2, 2012), with vagueness and dearth of transparency with respect to institutional roles and responsibilities contributing to meagre performance. Feebleness of governance is another underlying factor affecting agricultural water productivity (Booth, 2005). In ameliorating these challenges, it is pertinent to engage both official and implementation concerns in the water resource sector. The National Water Act of 1998 and the Water services Act are presently under review with an understanding of overcoming a number of challenges confronting the water sector (NWRS-2, 2012). An ideal

water governance rest on robust and responsible sector management, a strong supervisory structure, and operative water management bodies with distinct roles and responsibilities.

7. Conclusion

Integrated water resource management and revitalisation is important keystone to sustainable agricultural development. It is necessary to address the policy framework and supportive actions at both farm and watershed levels. The termination of the traditional gap in improving water productivity does not necessarily mean the transfer of better know-hows to farmers, but entails putting proper institutional arrangements in place to allow farmers access to water use efficiency. Management of water resources involves contribution of all stakeholders, investing in infrastructure and the unrelenting effort and commitment of men and women in all segment of the society (Lenton & Muller, 2009). The Millennium Development Goals on alleviation of poverty, food safety and ecological security are fundamental pointers to sustainable livelihood. The paper therefore, suggest that there is no immediate intractable water crisis facing South Africa; although this is clearly based on the assumption that the existing water resources will be managed efficiently. It is imperative that water losses should be reduced by embracing water loss reduction process such as: water supply and demand management, enhanced water governance and optimum use of available water resources (blue water, green water, rainwater harvesting, re-use of water, and desalination process). In the interim, it cannot however, be said that the country's broad social and economic objectives, or even the more specific objective of water security, are being fully realized. Nevertheless, the DWAF has therefore, introduced a programme for the promotion of Water for Growth and Development (WfGD) Framework. The necessity for such a framework is exemplified across the dimensions of social parity, livelihoods and agricultural productivity. With poverty as a social malaise that is taking its turn among majority of the citizenry, it is desirable that many opportunities inherent in the water sector should be utilised effectively for development and enhanced livelihood. However, if water security is to be achieved, revitalisation and the development of water resources must be guaranteed via action plan for the benefit of all. It is only then, will South Africa be seen as "water secured". The good news is that the DWAF has further initiated a strategy referred to as Water Conservation and Demand Management (WC&DM), geared towards revitalisation of water use and productivity (DWAF, 2000).

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Resistance of Cowpea Genotypes to *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) when stored is mainly attacked by bruchid Coleopterans. The control of these pests is done primarily through chemicals, however, with problems related to the selection of resistant insects, alternative forms of control are searched. Seeking an alternative control, the objective this work was to evaluate the resistance of cowpea genotypes to the attack of *Z. subfasciatus*. In the first selection of genotypes were evaluated 35 cowpea genotypes, being observed the variables: number of eggs, number of insects emerged and weight loss (%). In this first selection of cowpea were selected 10 genotypes. In the second selection of genotypes, the 10 most resistant from the first selection were used, but with eight repetitions per genotype, besides evaluating the egg viability (%) and instantaneous rate of population growth. The cowpea genotypes showed significant differences for resistance to attack by *Z. subfasciatus*. The most resistant to *Z. subfasciatus* were BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5.

Keywords: weevil, *Vigna unguiculata*, stored grain insects, postharvest

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume cultivated in semi-arid of Africa, Brazil and United States. In Brazil, the crop is very important in the North and Northeast, which have a tradition in its cultivation, trade and consumption. The crop presents increasing advancement in the Brazilian Midwest, where cowpea cultivation has been conducted in a mechanized form, and there is a great demand for upright cultivars (Rocha et al., 2009). Among the largest producers in Brazil stand out states: Amazonas, Pará, Maranhão, Piauí, Ceará and Rio Grande do Norte (Medeiros et al., 2007).

In Brazilian Northeast, the cowpea crop has great importance to agricultural development in both the economic and nutritional aspects. It is the staple food in the diet of the poor, exerting social function in supplying the nutritional needs in this section of the population (Teófilo et al., 2008), besides fixing hand labor in the field. (Távora et al., 2003).

The cowpea grains have good energy levels, with excellent protein content, 23-25% on average (Amaral et al., 2005). It is rich in lysine and other essential amino acids, however, poor in sulfured amino acids, methionine and cysteine. It constitutes an excellent source of niacin and also contains reasonable quantities of hydrosoluble vitamins such as riboflavin, pyridoxine, folacin, iron, zinc and phosphorus mineral elements (Silva et al., 2002). Thus, cowpea is a food that meets basic nutritional needs of the low-income population (Amaral et al., 2005).

The cowpea stored is mainly attacked by bruchid beetles. In the subfamily Bruchinae, the insects are associated with grains of many plants, consisting in a family of agricultural importance. The Mexican bean weevil *Zabrotes subfasciatus* (Boheman, 1833) is originating of the New World, being distributed in tropical and subtropical regions of Central and South Americas (Dendy & Credland, 1991; Haines, 1991), Africa, Mediterranean and India (Oliveira & Vendramim, 1999). The eggs of this insect are deposited on the surface of the grains and the larvae develop inside, causing considerable weight loss, reduction in germination, decreased nutritional quality and commercial devaluation (Oliveira & Vendramim, 1999). Furthermore, the metabolism of the larvae causes an increase of the temperature in the storage containers, which favors the development of fungi capable of producing toxins (such as aflatoxin) dangerous to the health of the consumer (Amevoin et al., 2007).

The use of chemical pesticides is a common preventive measure to protect stored grain from insect damage. Many pesticides are effective at relatively low doses and may offer long-term protection, ranging from 6 to 12 months (Athanassiou et al., 2004). However, some of these pesticides, especially organophosphates have high toxicity to mammals, and the residues left can cause health problems because they are conventional neurotoxins that affect the human nervous system (Athanassiou et al., 2009). Thus, the use of varietal resistance against the attack of *Z. subfasciatus* has been the subject of scientific research by offering an alternative measure of control.

Some studies show that is viable for control *Z. subfasciatus* in cowpea (*Vigna unguiculata*) and common bean (*Phaseolus vulgaris*), the use of genetically resistant cultivars (Mazzoneto & Boiça Junior, 1999; Boiça Júnior et al., 2002; Barbosa et al., 2011). These researches represent a direct benefit to the producer by reducing postharvest losses.

Thus, the objective of this study was to select cowpea genotypes resistant to attack of *Z. subfasciatus*.

2. Materials and Methods

2.1 Rearing of Insects

The insects were reared for several generations in cowpea (Sempre Verde cv) grains, these were packed in glass containers (1.5 L) closed with perforated plastic lids, lined on the inside with a thin cloth, allowing gas exchange. The rearing of insects and experiments were conducted at 27 ± 2ºC, 70 ± 10% relative humidity and 12 h photophase.

2.2 Cowpea Genotypes

Were used 35 cowpea genotypes from Active Germplasm Bank of Embrapa Meio-Norte: BRS Tracuateua, BR17-Gurgueia, TE67-304G-12, Monteiro, BRS-Nova Era, BRS-Urubuquara, BRS-Paraguçu, BRS-Guariba, BRS-Milenio, BRS-Marataoá, BRS-Rouxinol, Canapuzinho nº10, Inhuma nº12, Pingo de ouro-1-2 nº13, Paulistinha nº15, 35 TVX 5058-09C, 38 Vaina Blanca, 39 Californiablackeye, 40 BRS-Guariba, 21 MNC99-537F-1, 22 MNC99-537F-4, 23 MNC99-541F-5, 24 MNC99-541F-8, 25 MNC99-542F-5, 26 MNC00-553D-8-1-2-2, 27 MNC00-553D-8-1-2-3, 28 MNC99-557F-2, 29 MNC01-627F-14-2, 30 MNC01-627F-14-5, 31 MNC03-720C-20, 32 MNC03-720C-31, 33 MNC03-731C-21, 34 MNC03-732C-5, 36 MNC05-784B-38-2 e 37 MNC05-832B-234-5.

2.3 Experimental Protocol

The grains were packed in plastic bags at -5°C (in a freezer) to eliminate possible latent infestations. Before installing the experiments, the grains were removed from the freezer, placed in plastic recipients covered with thin tissue and kept in the laboratory for six days to come into equilibrium moisture content (Lima et al., 2002).

The treatments consisted of grains of each genotype (10 g) infested with five couples *Z. subfasciatus* (0-48h old), left for seven days to oviposition. The grains were placed in plastic recipients (300 ml) with transparent lid, lined on the inside with a thin cloth. The experiments were evaluated for 40 days.

2.3.1 First Selection of Genotypes

The experimental design was completely randomized with four replications for each genotype under no-choice test. To evaluate the resistance were observed following parameters: number of eggs, number of insects emerged and weight loss (%).

2.3.2 Second Selection of Genotypes

The experimental design was completely randomized with eight replications for each genotype under no-choice test. However, were used the most resistant genotypes from the first selection. To evaluate the resistance were observed following parameters: numbers of eggs, number of insects emerged, weight loss (%), egg viability (%) and instantaneous rate of population growth. For calculating this was used the equation: ri= [Ln (Nt/N0)/ΔT],

where Nt is the final number of adults; N0 is the initial number of adults transferred and Δt is the change in time (Walthall & Stark, 1997). The positive value of ri indicates a population growth increase; ri = 0 means that the population is stable; and a negative value of ri indicates a population decline to extinction (Stark & Banks, 2003).

2.4 Statistical Analysis

The data were analyzed by analysis of variance (ANOVA), while the significant means were compared by Scott-Knott test (Scott & Knott, 1974) at the 5% level of significance through the statistical program Sisvar 5.0 (Ferreira, 2011).

3. Results and Discussion

3.1 First Selection of Genotypes

The values obtained for the number of eggs present significant difference between them, showing that genotypes BRS Tracuateua and 31 MNC03-720C-20 obtained the lowest oviposition in relation to the others (Table 1). The most resistant genotypes obtained on average 61.93% less eggs than the most susceptible.

Table 1. Number of eggs, number of insects emerged and weight Loss on 35 cowpea genotypes attacked by *Z. subfasciatus*

Genotypes	Number of Eggs	Number of Insects Emerged	Weight Loss (%)
BRS Tracuateua	58.00 c	41.25 d	2.7 d
31 MNC03-720C-20	60.00 c	40.00 d	2.8 d
BRS-Milenio	95.00 b	69.75 d	5.0 d
26 MNC00-553D-8-1-2-2	102.50 b	83.50 c	6.3 d
Monteiro	102.75 b	67.00 d	5.3 d
36 MNC05-784B-38-2	103.50 b	80.75 c	5.7d
37 MNC05-832B-234-5	105.50 b	59.75 d	4.3 d
32 MNC03-720C-31	107.00 b	90.00 c	6.6 d
34 MNC03-732C-5	107.25 b	90.75 c	7.7 b
29 MNC01-627F-14-2	108.25 b	90.50 c	6.2 d
39 Californiablackeye-27	112.25 b	88.50 c	10.3 b
BRS-Marataoá	120.50 b	95.50 c	7.0 c
33 MNC03-731C-21	122.75 b	99.75 c	8.8 c
BRS-Nova Era	123.75 b	98.50 c	7.7 c
30 MNC01-627F-14-5	126.50 b	105.75 b	7.1 c
27 MNC00-553D-8-1-2-3	127.25 b	99.75 c	9.3 b
BRS-Urubuquara	131.25 b	84.75 c	5.8 d
28 MNC99-557F-2	138.75 a	85.25 c	6.3 d
23 MNC99-541F-5	142.50 a	113.50 b	8.8 c
38 Vaina Blanca	144.50 a	111.25 b	9.7 b
BR17-Gurgueia	145.25 a	118.00 b	8.3 c
24 MNC99-541F-8	147.75 a	108.75 b	8.2 c
BRS-Rouxinol	148.00 a	123.25 b	10.0 b
22 MNC99-537F-4	148.00 a	110.00 b	8.1 c
25 MNC99-542F-5	154.50 a	110.00 b	10.0 b
BRS-Guariba	157.25 a	124.25 b	8.3 c
TE97-304G-12	159.25 a	141.75 a	11.5 b
BRS-Paraguaçu	161.00 a	138.75 a	9.1 c
35 TVX 5058-09C	164.50 a	141.25 a	13.7 a
40 BRS-Guariba	167.50 a	145.00 a	11.3 b
Canapuzinho nº10	170.75 a	135.50 a	15.0 a
21 MNC99-537F-1	175.50 a	145.25 a	11.2 b
Pingo de ouro-1-2 nº13	175.50 a	151.50 a	14.5 a
Paulistinha nº15	177.75 a	145.50 a	12.1 b
Inhuma nº12	185.00 a	142.25 a	14.7 a

*Means followed by the same letter in the column do not differ significantly by Scott-Knott test at 5% probability.

Mazzoneto and Boiça Junior (1999) seeking alternative methods for the control of *Z. subfasciatus* in beans, conducted tests with and without choice using genotypes: Goiano Precoce, Onix, Diamante Negro, Iapar MD 808, Preto 143, 2357, 2306, 2174, 2044, 2041, 2037, 133, 115, 2037, 2374E 2395 and concluded that genotype Preto 143 showed resistance to non-preference for oviposition on free choice test, and the genotypes 2374, 2395, 2174, 133 e 155, showed resistance of the non-preference for feeding and/or antibiosis. In this study, genotypes BRS Tracuateua and 31 MNC03-720C-20 presented a lower number of eggs, probably because they have antibiosis resistance, since the test was performed without choice.

There was significant difference in the number of insects emerged, being that BRS Tracuateua, 31 MNC03-720C-20, BRS-Milenio, Monteiro, 37 MNC05-832B-234-5 and 37MNC05-832B-234-5 presented the lowest number of insects emerged. These genotypes had on average 40-67 insects emerged, having an emergence 61.88% lower than genotypes with higher number of insects emerged. The genotypes TE97-304G-12, BRS-Paraguaçu, 35 TVX 5058-09C, 40 BRS-Guariba, Canapuzinho nº10, 21 MNC99-537F-1, Pingo de ouro-1-2 nº13, Paulistinha nº15 and Inhuma nº12 showed the highest insect emergence (Table 1).

Barbosa et al. (2000) studied the stability of resistance *Z. subfasciatus* in four genotypes of common bean and found that the highest average number of adults emerged was observed in cultivars Goiano Precoce e Porrillo 70, differing from ARC4 e ARC1, genotypes with protein arcelin. Miranda et al. (2002) observed that genotypes ARC1 e ARC4 presented a number of adults emerged significantly lower than those presented by genotypes ARC2 and ARC3 and genotypes without arcelin. In this research, was observed a lower number of insects emerged in genotype 31 MNC03-720C-20. This may have occurred due to some adverse effect for insect development inside the grain, probably related to antibiosis caused by this type of protein.

In this study, 11 genotypes showed the lowest weight loss, being BRS Tracuateua and 31 MNC03-720C-20 less attacked, suggesting the possibility to have antibiosis resistance. The consumption of grain is positively related to the number of insects emerged. Thus, the largest insect emergence reflects in increased consumption. The opposite can also occur due to the presence of substances capable of inhibiting the feeding content inside the grains by the larvae of *Z. subfasciatus*. The presence of inhibitory substances feeding in weevil is reported in the literature, for example the arcelin which confers resistance to *Z. subfasciatus* (Oriani & Lara, 2000) and trypsin inhibitors responsible for antibiosis in some cowpea genotypes (Gatehouse et al., 1979).

The genotypes Canapunzinho nº10, Pingo de ouro-1-2 nº 13 and 35 TVX 5058-09C were the most consumed, demonstrating do not possess or have low levels deterrents substances to insects.

The resistance of BRS Tracuateua, 31 MNC03-720C-20, BRS-Milenio, 26 MNC00-553D-8-1-2-2, Monteiro, 36 MNC05-784B-38-2, 37 MNC05-832B-234-5, 32 MNC03-720C-31, 34 MNC03-732C-5 and 29 MNC01-627F-14-2 can also be explained by the fact they presented rough texture, which may lead to a lower preference for oviposition by the females of *Z. subfasciatus*. Lima et al. (2001) identifying cowpea genotypes resistant to *Callosobruchus maculatus* (F.), observed that morphological causes and tegument texture affect the preference for oviposition. Nwanze et al. (1975) observed higher oviposition of *C. maculatus* on smooth grain than rough grain.

3.2 Second Selection of Genotypes

Were observed similarities in relation to the first selection, since the genotypes showed significant difference in the number of eggs, number of insects emerged and weight loss (%).

The genotypes BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5 had the lowest oviposition with 17.87, 31.12, 30.62 and 27.87 eggs, respectively (Figure 1A). In these genotypes the average of eggs was 32.77 which represents 46.9% less eggs than susceptible genotypes. Botelho et al. (2002) studying strains of *Phaseolus vulgaris*, observed that strain ARC 3 was the least oviposited, with 308 eggs. In this research, the cowpea genotype with less eggs was BRS Tracuateua, being the most resistant to *Z. subfasciatus* in relation to oviposition.

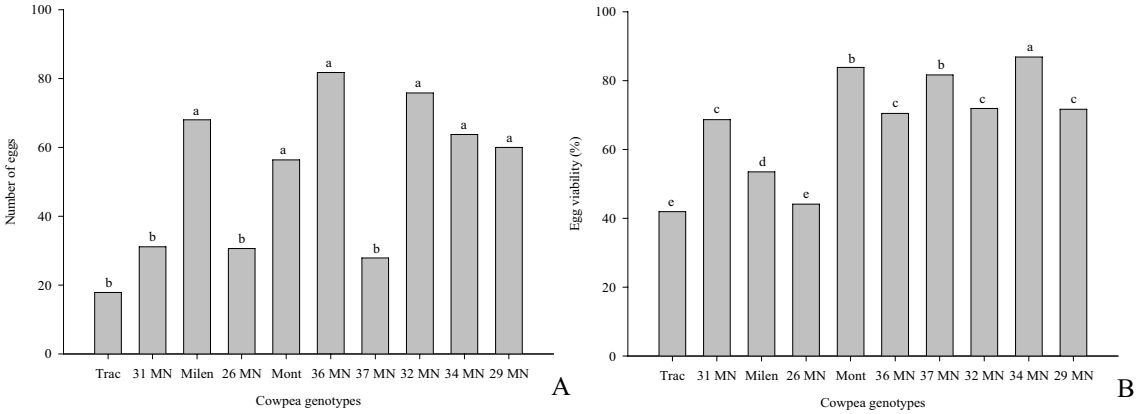

Figure 1. Effect of cowpea genotypes in embryonic development and oviposition of *Z. subfasciatus*. A) Number of eggs. B) Egg viability (%). Means followed by the same letter do not differ significantly by Scott-Knott test at 5% probability

The genotypes BRS Tracuateua and 26 MNC00-553D-8-1-2-2 allowed the lowest egg viability of *Z. subfasciatus* (Figure 1B). Ribeiro-Costa et al. (2007), seeking to verify the development of *Z. subfasciatus* in genotypes with and without arcelin, demonstrated that genotypes as ARC1 and ARC2 containing this protein showed higher resistance, since obtained a lower preference for oviposition, and a low percentage of viable eggs. In this research, low egg viability observed in the least susceptible genotypes can also be explained by presence of proteins that can cause negative effects on embryonic development of insects.

Sales et al. (2005) observed 95% emergence of adults of *Z. subfasciatus* in seeds of cowpea susceptible, thus presenting egg viability above 90%. In this research, the egg viability was 76.41% in the genotypes 36 MNC05-784B-38-2, 32 MNC03-720C-31 and 34 MNC03-732C-5, the most susceptible to oviposition. The mean egg viability was 43.03% in the genotypes BRS Tracuateua and 26 MNC00-553D-8-1-2-2.

The genotypes BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5 had the lowest number of insects emerged, with the insect emergence ranging from 7.50 to 22.75 adults (Figure 2A). This low number of insects emerged may be due to larval mortality caused by proteins with potential insecticide such as vicilins present in some leguminous plants. These proteins bind to chitinous structures of the midgut (peritrophic membrane) interfering in the assimilation of nutrients, which may cause the insect death (Amorim et al., 2008).

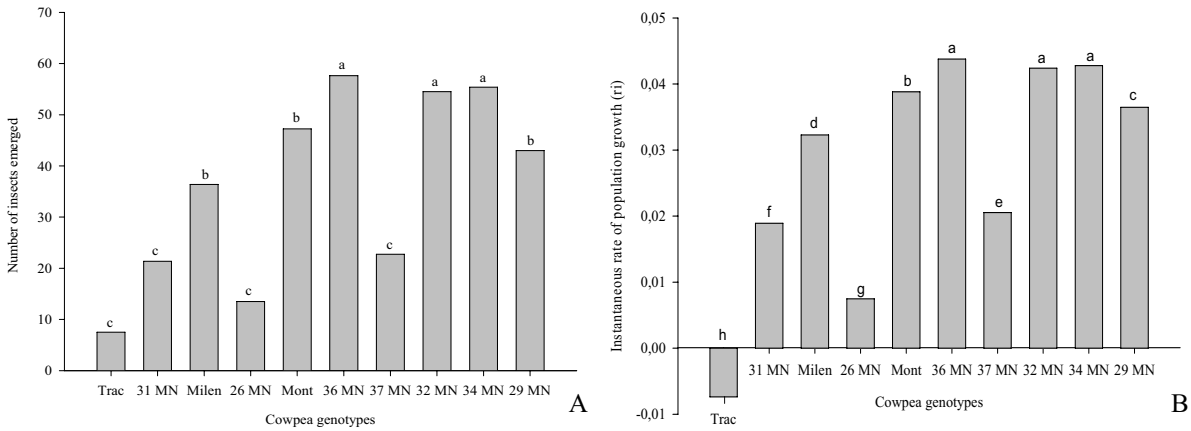

Figure 2. Population growth of *Z. subfasciatus* in cowpea genotypes. A) Number of insects emerged. B) Instantaneous rate of population growth (ri). Means followed by the same letter do not differ significantly by Scott-Knott test at 5% probability

Almost all cowpea genotypes allowed the population of *Z. subfasciatus* continue growing, except BRS Tracuateua which showed negative value of the instantaneous rate of population growth (Figure 2B). According to Stark and Banks (2003) the population is declining and will reach to extinction. This result showed that genotype BRS Tracuateua was effective to control *Z. subfasciatus*.

The genotypes 34 MNC03-732C-5 and 29 MNC01-627F-14-2 had the highest weight loss (%), and therefore, were the most consumed (Figure 3). These genotypes were 67.13% more consumed than BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5, which had an average 18% weight loss. Shafique and Chaudry (2007) suggested that the low insect population and low weight loss of grain can be used as one attribute of the resistance to insects.

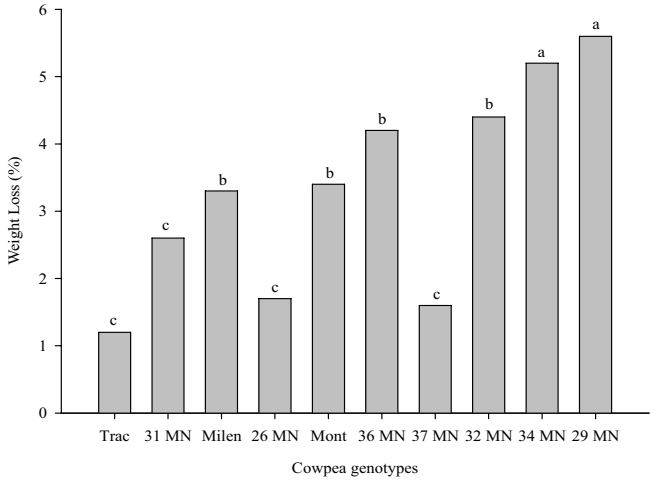

Figure 3. Weight Loss of cowpea grain when subjected to attack *Z. subfasciatus*. Means followed by same letter do not differ significantly by Scott-Knott test at 5% probability

Our results showed that the release of cowpea genotypes resistant to *Z. subfasciatus* can be an important tool to help control this pest. Furthermore, selection of cowpea genotypes resistant to insects can also be used as a parameter in plant breeding programs.

4. Conclusions

The cowpea genotypes most resistant to *Zabrotes subfasciatus* were BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5.

The genotype BRS Tracuateua has great potential to control *Zabrotes subfasciatus* because it was the most resistant in all parameters evaluated.

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Profitability and Adoption of Watermelon Technologies by Farmers in Moro Local Government of Kwara State, Nigeria

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Abstract

The study was carried out to determine the adoption rate of watermelon as an alternative crop to the melon the farmers in Moro Local Government Area, Kwara State Nigeria are used to grow traditionally. Unfortunately, melon had remained a poor marketing commodity over a long period of time due to price fluctuation. Data collected were analyzed using descriptive statistics and gross margin. The results indicate that the majority (80.87%; n = 93) trained in year 2007 are still active in watermelon cultivation. The adoption of watermelon technologies was influenced by extension packaging styles, compatibility with known methods of melon cultivation, relative advantages, good market price and ready market. Budgetary analysis revealed a profitability of watermelon with gross margin of ₦253,850.00 per hectare. The benefit/cost ratio (BCR) was 3:1. The study identified poor extension visit to the farmers after the training. It therefore recommended that the local government should acquire more tractors for hiring to the farmers while subsidy is required in other to reduce the cost of land clearing. Finally, government should as a matter of urgency repair, grade and open up more road network that will facilitating easy transportation of farm produce and reduce cost of transportation.

Keywords: watermelon, training, adoption, profitability

1. Introduction

Melon (*Citrullus colcynthis*) also known as honeydew (Mohammed, 2011) is a major crop in Moro Local Government Area (MLGA) of Kwara State, Nigeria. It is an annual crop planted twice in a year, as an early and late season crop due to the bimodal nature of the raining season in the area. Melon belongs to the family of *curcurbitaceae* and it is planted for its'seed oil (Mohammed, 2011). It is also a major source of different delicacies in food preparations among tribal groups in Nigeria. Melon flesh is bitter, not edible and cannot be cooked (Lagoke et al., 1983). Melon cultivation is common among the small scale farmers and is inter-planted with crops like cassava, maize, yam, pepper (Rice et al., 1986) in order to maximize utilization of the land resources and increase returns from the production systems (Mohammed, 2011). Melon cultivation has not enjoyed a high level of technological improvements such as the use of new hybrid seeds, fertilizer and processing. According to Mohammed (2011) an average yield of 57.70 kg was obtained on a sole cropping field in Ifelodun Local Government Area Kwara State. Melon cultivation and processing are labor intensive. The matured and harvested fruits are collected on several spots on the farm depending on the farm size. The processing involves breaking the pod, fermentation, scooping the seeds from the pods, washing and sun-drying. Problems associated with marketing of melon include low price and unstable market prices. According to Yusuf et al. (2008), the gross return per hectare (ha) on melon was ₦12, 638.61 with the total cost put at ₦8, 838.74 on average giving a net farm income of ₦3, 799.87 per ha. Ayodele et al. (2007) also reported profit of ₦3, 619.01 on a hectare of melon farm in Ibadan. Mohammed (2011) in his findings on the socioeconomic analysis of melon cultivation in Ifelodun Local Government Kwara State, observed the difference between the gross return and the total cost of production that gave a gross margin of N1, 263.81 per ha. As a result of these factors, most farmers could not sell their produce. Many complained of having produce of between three to four years in storage without any hope of immediate sales. Based on these multiple challenges, Fayolam Farms situated at Bielesin village, via Bode Saadu, Moro LGA of

Kwara State conducted a simple survey in 2007 in order to introduce an alternate crop to the farmers that could bring in the needed income for improved livelihood.

Watermelon (*Citrullus lanatus L*) was chosen after considering several factors within the framework of the diffusion process of new technology that must include relative advantage, compatibility, complexity, triability and observability (Rogers, 2003). The common shared problem, poor marketing and price fluctuation under the social system of diffusion (Rogers, 2003) was addressed as joint problems to be solved towards providing better marketing opportunities for an alternate crop in watermelon. In Nigeria, watermelon grows well both in the humid and drier savanna agro ecologies. The largest production of the crop comes from the northern part of Nigeria where a suitable agro ecology is found (Adekunle et al., 2007). Moro Local Government Area (MLGA) of Kwara State is located in the humid area suited for watermelon cultivation. Watermelon is relished by many people across the world as a fresh fruit (Adekunle et al., 2007). The fruit is 93% water, with small amounts of protein, fat, minerals, and vitamins (Namdari, Mohammedi, & Mobtaker, 2011). Watermelon is known to be low in calories, it contains Vitamins C and Awhich helps address night blindness, eye problems, dry skin, eczema and psoriasis (Bendich & Olson, 1989). It contains potassium which is believed to help in the control of blood pressure and possibly prevent stroke (Adekunle et al., 2007).

The diffusion of the watermelon technologies to the farmers was a tripartite arrangement involving Fayolam Farms, the initiator that provided the logistics, fertilizers and demonstration plot, a seed company that provided different varieties of watermelon seeds adapted to the ecological zone and agro-allied company that provided the herbicides, pesticides and knapsack sprayers. The training workshop was conducted on the 18-19 March, 2007.

Farmers were trained with the following modules (a) Land preparation for watermelon cultivation using minimum tillage; (b) Pre and post emergence weed control methods using different types of herbicides; (c) planting (distance and number of seeds per hole); (d) disease and pest control; (e) fertilizer application; (f) How to use a knapsack sprayer, handling and maintenance; (g) harvesting; (h) storage and; (i) marketing tips. The training was practically oriented and conducted in Yoruba language, being the local language. Three years after the introduction of the watermelon technologies, the training is now evaluated to:

1) Examine the socioeconomic characteristics of the respondents;

2) To compare the farm size holding of farmers in 2007 and 2010;

3) Determine the profitability of watermelon production by the respondents in the study area.

2. Methodology

2.1 Study Area

The Kwara state lies between latitudes 7°45′ N and 9°30′ N and longitudes 2°30′ E and 6°25′ E and covers a total land area of about 332,500 square kilometers (Paul & Oluwasina, 2011). The state has a population of about 2.37 million people (National Population Commission (NPC), 2008). It is exclusively in the hinterlands. The weather is humid tropical (Jimoh & Adeoye, 2011). The state shares boundary with Ondo, Oyo, Osun, Niger and Kogi States in Nigeria and an international border with the Republic of Benin along its northwestern part (Kwara State Government (KWSG), 2003). The mainstay of the state's economy is agriculture. The study area is within Lanwa District in Moro Local Government Area (LGA). Moro LGA was created out of the Ilorin Native Authority in 1976 (KWSG, 2012). The headquarters is at Bode Saadu which is about eighty five kilometers from Ilorin, the state capital. Moro LGA is rural, mostly comprised of local populations with low literacy level and low income (Ajibade et al., 2005). It has an area of 3272 km square and a population of 108,792 at the 2006 census. It is populated by rural farmers. The local government area is endowed with good climatic conditions, sizable expanse of arable and rich fertile soils. The vegetation which is mainly wooded guinea savannah, well suited for the production of a wide variety of staples like yam, melon, groundnut, cassava, maize, cowpea, fruits and vegetables. Rice, sugarcane, locust beans, shea butter trees, cashew, and mango are also significant cash crops. Common to the area are local poultry, guinea-fowls, goats rearing mostly by the women and cattle by immigrant Fulani and Bororo who are settled amongst the people.

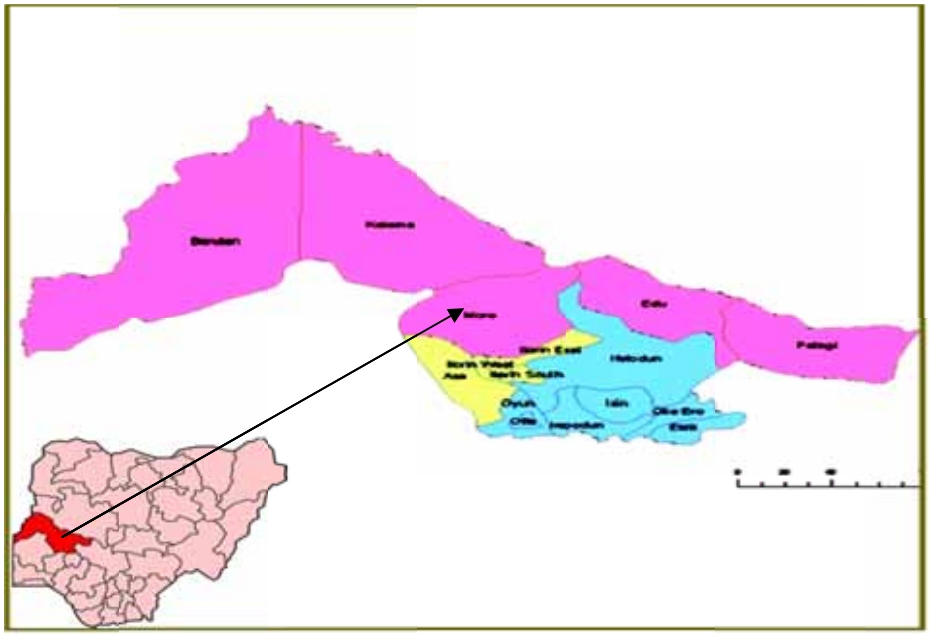

Figure 1. Showing the maps of Kwara State and Nigeria beside. Arrowed is the study area

2.2 Sample Size

The population consisted of all 115 farmers trained in watermelon technologies in the year 2007, who were drawn from eight villages that included: Fallah, Bielesin, Olokiti, Gata, Bode-Saadu, Alagbon, Lasaki, and Olokiti-Nla. Data (through structured interview schedule) was received and analyzed from a sample of 93 farmers representing 80.87% response rate. Purposive sampling procedure was used since they were known target respondents. The instrument was structured interview schedule that solicited information from the respondents on the socioeconomic profile of the watermelon farmers, age, gender, marital status, educational status, occupation, farming experience, farm size and household size.

The second part of the questionnaire solicited information on the cultivation practices by the farmers on watermelon after the training, the size of farmland in 2007 and 2010, the challenges in cultivation practices, quantity of inputs used (seed, fertilizer, herbicides, pesticides), labor cost, yield per hectare (number of fruits/ balls), and revenue realized per hectare. The instrument was field tested for content and face validity by extension staff of the Kwara State Agricultural Development Programme (KWSADP). In order to obtain a high measure of internal consistency to determine the validity and reliability of the questionnaire, the instrument was pilot tested with watermelon farmers located at Kainji. The items that were found to be highly reliable and valid were used. The level of reliability of the instrument was calculated using Cronbach's alpha coefficient ($\alpha = 0.91$).

Data collected were analyzed using descriptive statistics which include frequency counts, percentages and mean scores. Farm budget analysis was used to estimate the cost of production, total revenue and gross margin for the farmers. The Gross margin, total cost and Net farm income were calculated using the following formula

$$GM = TR - TVC \quad (1)$$

$$NFI = GM - FC \quad (2)$$

Where

GM = Gross margin, TR = Total revenue, TVC = Total variable cost;

NFI = Net farm income, FC = Fixed cost;

Depreciation on farm tools was calculated using the straight line method as follows:

Depreciation = (cost of purchase - salvage value) /usable life.

3. Results and Discussion

3.1 Socioeconomic Characteristics of Respondents

Table 1 shows the socioeconomic characteristics of the respondents. The result indicates that the majority of the respondents are male (75.26%; n = 70) while female (24.74%; n = 23) are less. This follows the traditional pattern that the male is dominant in the farming system in that community. The result also shows that the majority of the respondents (76.4%; n = 71) are in the age bracket of less than thirty to fifty years which is active with few (23.07%; n = 22) belonging to fifty-one years and above. The mean age was 37.5 years indicating that the majority are able bodied men and women who are still in their active year. Age plays significant role in farming as it determines the strength of the farmers' ability to carry out tedious and rigorous work as defined by some activities in watermelon cultivation, for example, mounting of knapsack sprayer at the back. The majority (77.4%; n = 72) were married with a very low proportion (11.83%; n = 11) as either single or (10.78%; n = 10) widowed. The number of households of the respondents indicates that majority (66.68%; n = 62) are having household population of less than five to eight with the mean value of 6.65. The majority (78.49%; n = 73) of the respondents had gone through one form of formal education at various levels of primary to tertiary while very few (21.51%; n = 20) did not have any form of formal education. It has been confirmed that education plays significant role in the adoption rate of technologies by creating positive mental attitudes (Benor et al., 1997). Adetiba (2005) and Kehinde (2005) also confirm that education was key to enhanced productivity among farming households in the humid forest, dry savannah and moist savannah agro-ecological zones of Nigeria.

3.2 Farm Size Holding of Farmers in 2007 and 2010

Figure 1 showed that between 2007 and 2010, the majority (99.14%; n = 114) of the respondents who were clustered on farmland sizes ranging from 0.1 to 1 hectares (ha) (male, 25.2%; n = 29, female 20%; n = 23), 1.01-2ha (male, 40.87%; n = 47, female 4.3%; n = 5) and 2.01-3 ha (male 8.7%; n = 10) were able to increase their farmland sizes by various hectarage. In 2010, the farm land sizes indicates 0.1 to 1ha (male, 9.68%; n = 9, female, 11.83%; n = 11), 1.01-2 ha (male, 30.10%; n = 28, female 9.68%; n = 9), 2.01 – 3ha (male, 20.43%; n = 19, female, 3.03%; n = 3), 3.01-4 ha (male 12.9%; n = 12), 4.01-5 ha and 5.01 ha and above (male 1.07%; n = 1 each). The gradual expansion in farmland sizes could be attributed to the successes recorded by the farmers in terms of market availability and profits realized from the watermelon cultivation. Meanwhile, of the total 115 farmers trained in year 2007, the majority (80.87%; n = 93) are still active in growing watermelons indicating the acceptance of the watermelon cultivation as an alternative to melon cultivation.

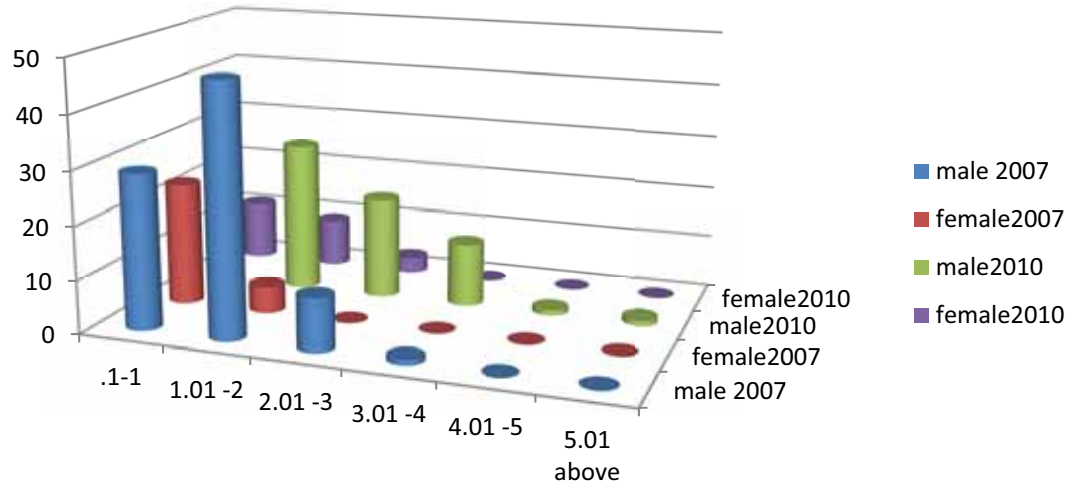

Figure 2. Farmland size holding of farmers in 2007 compared with 2010 on watermelon cultivation

Table 1. socioeconomic characteristics of respondents in the study area N = 93

Items	Frequency	Percentage	
Male	70	75.26%	
Female	23	24.74%	
Total	93	100%	
Age according to gender			
Age	Male	Female	Mean
≤ 30	17 (18.3%)	8 (8.6%)	
31-40	20 (21.5%)	6 (6.5%)	
41-50	16 (17.2%)	4 (4.3%)	
51-60	12 (12.9%)	4 (4.3%)	
> 60	5 (5.4%)	1 (1.07%)	37.5 years
Total	70 (75.26%)	23 (24.74%)	
Farming experience according to gender			
Farming experience	Male	Female	Mean
≤ 5 years	32 (34.4%)	4 (4.3%)	
6-10 years	13 (13.98%)	6 (6.5%)	
11-15 years	6 (6.45%)	4 (4.3%)	
16-20 years	15(16.12%)	2 (2.15%)	
> 20 years	4 (4.3%)	7 (7.52%)	9.89 years
Educational status			
No formal education	9 (9.68%)	11 (11.83%)	
Primary education	19 (20.43%)	8 (8.6%)	
Junior secondary school	20 (21.50%)	2 (2.14%)	
Senior secondary school	17 (18.3%)	2 (2.14%)	
Tertiary education	4 (4.3%)	-	
Total	70 (75.26%)	23 (24.70%)	
Marital status			
Married	52 (55.91%)	20 (21.50%)	
Single	11 (11.83%)	-	
Widowed	7 (7.52%)	3 (3.22%)	
Total	70 (75.26%)	23 (24.70%)	
Household size			Mean
≤ 5	29 (31.18%)		
6-8	33 (35.5%)		
9-10	17 (18.3%)		
> 10	14 (15.03%)		6.65
Total	93		
Occupation	Male	Female	
Farming	16 (17.2%)	08 (8.60%)	
Non-farming	10 (10.8%)	04 (4.30%)	
Trading	07 (7.52%)	11 (11.83%)	
Okada rider	26 (27.95%)	-	
Civil service	05 (5.40%)	-	
Others	06 (6.50%)		
Total	70 (75.26%)	23 (24.70%)	
Extension visit			
Yes	23%		
No	77%		

Table 2. Pattern of cropping practices used by the respondents (N = 93)

Characteristics	Categories	Percentage
Cropping system adopted	Sole cropping	62.6
	Inter- cropping	37.4
Inter-cropping system	Watermelon and cassava	50
	Watermelon and maize	41.18
	Watermelon and other crops	8.82
The reasons adduced for inter-cropping	Reduced risk of plant failure	73.6
	Increase in income	81.9
	Weed control	48.5
	Improve soil fertility	53.9
	Effective land use	42.4
Reasons for adopting sole-cropping	Good management	73.6
	More profit	51.3
	Convenient in farm management	69.8
Watermelon variety planted	Sugar baby	94.9
	Charleston Gray	5.1
Cultural practices	Direct seeding	100
	Seedling	-
	Weed control	
	Manual	39.5
	Use of herbicides	60.5
	Pest and diseases control	
	Chemical control	87.3
	Non use of chemical control	12.7
Labor	Family labor	41.93
	Hired labor	58.06

Source: Field survey 2011.

Table 2 shows that the majority (62.6%; n = 58) planted watermelon as sole crop while the remaining (37.4%; n = 35) practiced intercropping of watermelon with maize (41.18%; n = 14), cassava (50%; n = 18), and other minor crops (8.82%; n = 3). The popularity of sole cropping may be as a result of the training which emphasized sole cropping practices. The respondents also gave the following reasons for engaging in sole cropping to include better management (73.6%; n = 43), increased profit (51.3%; n = 30) and ease of farm management (69.8%; n = 40). Reasons for inter-cropping include: reduced risk of crop failure (73.6%; n = 26), lead to increase in income (81.9%; n = 29), weed control (48.5%; n = 17), improves soil fertility (53.9%; n = 19), and effective land resource use (42.4%; n = 15). Sugar baby variety was the most popular (94.9%; n = 87) to a Charleston Gray variety (5.1%; n = 6). The reasons for this may be attributed to its characteristics of big size, sweetness, ability to store very well and command a high price in the market.

The cultural methods employed by the respondents reflected the training pattern that they were taken through with few yet to adopt the entire technologies. All respondents made use of direct seeding (100%; n = 93). Weed control was mostly done by the use of herbicides (60.5%; n = 56) and manual (39.5%; n = 37). Manual weeding demanded an average of 2-3 weeding before harvesting. Manual weeding is mostly affected by accidental cutting of vines, destruction of flowers and stepping on young fruits. Pest and disease control was mostly done by the use of chemical (87.3%; n = 81) and non -utilization of chemical control method (12.7%; n = 12). The use of chemical control of pest and diseases is needed because of the high level of susceptibility of watermelon to diseases and

pests and this has to be done on a weekly basis at the onset of fruiting. The majority (73%; n = 68) of the respondents owned knapsack sprayers while others rent from colleagues. The majority (58.06%; n = 54) of the respondents relied on hiring labor for their farm operations while others (41.93%; n = 39) depend on family labor. This result is closely in agreement with the findings of Ala and Bala (2011) in their study on the profitability of watermelons production and marketing in Kirfi local government areas of Bauchi State, Nigeria, which revealed that 60% of the labor employed was hired while 40% was family labor. Meanwhile, respondents reported low (23%) visit by extension agents, thus contributing to the inadequacy of solving their technical challenges.

Table 3. Average costs and returns per hectare for sole watermelon

Items of costs/returns	Total (₦)	% of TVC
Gross return (₦)	377, 500.00	100
Variable cost (₦)		
Land clearing and preparation	28, 500.00	23.05
Stumping and packing	3, 500.00	2.8
Plow	4, 250.00	3.44
Seeds	5, 500.00	4.45
Planting	5, 000.00	4.04
Herbicide and application	12, 300.00	9.95
Fertilizer and application	13, 300.00	10.77
Insecticides and application	6, 300.00	5.1
Harvesting	8, 500.00	6.9
Transportation	12, 500.00	10.11
Total variable cost (TVC) (₦)	123, 650.00	100
Gross margin (TR-TVC)	253, 850.00	
Fixed cost		
Land rent	4, 500.00	
Depreciation	3, 724.00	
Net margin /ha or Net Farm Income (GM-FC)	245, 626.00	
Benefit / Cost Ratio (BCR)	3 : 1	

Source: Field survey 2011.

3.3 Costs and Return Structure

Table 3 represents the average cost and returns per hectare for sole cropping of watermelon. The total variable cost was ₦123, 650.00/ha with a gross margin generated at ₦253, 850.00. The benefit cost ratio was 3:1 indicating high profitability of watermelon cultivation in the area. This is a strong indication why many of the farmers are now opting for watermelon cultivation after the training. There was no report of poor marketing as compared to melon where most farmers are having melon in storage for up to three to four years due to price fluctuation and non-availability of buyers.

The total cost structure indicates that land preparation (23.05%), herbicides and application (9.95%), fertilizers and application (10.77%), and harvesting (6.9%) accounted for high variable cost. These variables included cost of hiring labor. It implies that hired labor is still highly required for efficiency in any small-holding farming system in Nigeria. Transportation cost (10.11%) was also significant.

4. Conclusions and Recommendations

From the results obtained from this study, it can be concluded that watermelon production is profitable in this situation and the adoption rate of the watermelon technologies was high and encouraging. The contributory factors to the change by the melon farmers in watermelon production could be attributed to the availability of inputs, markets and the profit margin that accrued to the farmers. These indicate compelling factors / drive for farmland expansion of the farmers. Based on the results from the study, the following recommendations are made:

1) That the local government should aquire more tractors for hiring to the farmers while further subsidy is required in other to reduce the cost of land clearing.

2) Government should as a matter of urgency repair, grade and open more access roads to reduce the cost of transporting agricultural goods.

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Effect of Postharvest Treatments and Storage Conditions on Quality Parameters of Carrots

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Abstract

The purpose of this study was to examine differences between postharvest treatments, either washed (hot water, H_2O_2 and Na_2OCl) or non-washed (control) carrot roots and the effect of different storage conditions, S_1 (0°C and > 95% RH) or S_2 (0-2°C and < 90% RH) on the compositional changes. Losses of mass, β-carotene and vitamin C in carrot taproot (*Daucus carota* L. cv.'Maestro F_1') were monitored during 160 days of cold storage (in both cold room) plus 20 days at 20°C (market simulation). At the end of 180 days of storage the percentage mass loss ranged from 3.1 to 33.2% depending on the storage condition and disinfection treatment. Loss of β-carotene during storage was higher in the S_2 (28.2-46.9%) than in the S_1 cold storage (7.8-20.7%). The vitamin C loss in carrot root inside the S_1 cold room ranged from 2.0% to 18.2%, while the vitamin C loss was significantly higher (20.7%-52.3%) under simple refrigerated cold storage (S_2). Our experimental results indicate that prestorage root washing (Na_2OCl) significantly reduced weight loss, while hot water treatment maintaining a quality (β-carotene and vitamin C). Storage at cold room (S_1) after these treatments, is a practical strategy for reducing weight loss, β-carotene and vitamin C contents in the carrot during prolonged storage.

Keywords: *Daucus carota*, cold room, mass loss, β-carotene, vitamin C

1. Introduction

Carrots (*Daucus carota* L.) are, in general, one of the best sources of β-carotene in our diet, and they provide 17% of the total β-carotene intake in human nutrition (Alasalvar et al., 2001). β-carotene is the principal precursor of vitamin A, which is involved in vision, cell differentiation, synthesis of glycoproteins, mucus secretion from the epithelial cells, and overall growth and development of bones. The harvested carrot root is an underground organ that has been dug out of the soil while it was in full metabolic activity. The carrot has low metabolic activity at low temperatures, as shown by the low respiration rate (Stoll & Weichmann, 1987) and can be stored for 6-8 months without loss of quality under optimal storage conditions (Ilić et al., 2009).

The two basic conditions, as recommended by previous researchers are a temperature of 0°C and a relative humidity of 98% (Afek et al., 1999; Eshel et al., 2009). The most significant changes in postharvest quality are weight loss, bitterness, bacterial deterioration, rooting and sprouting. All of these changes can be prevented by different methods including cold storage, postharvest treatment and chemical applications. Chlorination of process water is one of the primary elements of a proper postharvest sanitation program. Washing carrots with cold chlorinated water (4°C) and warm tap water (50°C), respectively, provided good microbiological safety paired with improved sensorial properties (Klaiber et al., 2005). In the last few years, carrot growers in Israel usually applied combined application with stabilized hydrogen peroxide (Tsunami® 100) or a yeast commercial product (Shemer™) and have begun to brush carrots before storage to remove the outer peel of the root (Eshel et al., 2009). Since carrots are important source of vitamin C and carotenoids in the human diet, it is appropriate to determine to what extent these compounds are lost during storage. Loss of β-carotene during carrot storage was observed to be higher in the cellar than in the cold storage (Fikselova et al., 2010). Singh et al. (2001) have reported vitamin C and β-carotene losses after storage, with higher losses for vitamin C. A thirty-day carrot storage resulted in a significant reduction in vitamin C content (47%) and β-carotene content of 11% (Matejkova & Petrikova, 2010). The aim of

this study was to determine the effectiveness of prestorage treatment in maintaining the quality of carrot during prolonged cold storage.

2. Material and Methods

2.1 Plant Material

The carrot (*Daucus carota* L.) cv. 'Maestro F_1' is a commercial hybrid for open field production during fall, autumn and winter. Uniformly sized taproots at their full maturity stage, about 150 g, were picked directly from a field in the south part of Banat (village Debeljača). The soil conditions were well drained and sandy, and drip irrigation was used. Cultural practices, such as land preparation, planting and plant protection of the crop, were as is the standard in this area. Mature carrots were harvested in the fall and sent into storage immediately after harvest. Taproots without defects or diseases, of same size, shape and injury free were selected for the experiment. The carrots went into storage on November 10, 2011 and the study was terminated on April 20, 2012.

2.2 Postharvest Treatment

The following postharvest washing treatments have been conducted: 1-hot water washing and brushing (50°C for 1 minute); 2-1% H_2O_2 (tap water for 1 minute); 3-175 ppm Na_2OCl (tap water for 1 minute); and 4-control, non-washed roots (with soil).

2.3 Storage Condition

Following treatment, the taproots were stored for 160 days at different storage conditions. The taproots were stored at 0°C, in a cold room (S_1) with high relative humidity (RH 95-98%) in the dark, or (S_2) in a cooling room with a temperature of 0-2°C and uncontrolled conditions of relative humidity (RH 79-94%). For each postharvest treatment and storage regimen, 25 roots per replicate were sampled for analysis, with 4 replicates analysed in total. After 160 days storage at either temperature, the taproots were transferred to 20°C during 20 days (simulates marketing practices). The analysis of β-carotene and vitamin C has been carried out after 90, 160 and 180 days of storage.

2.4 Samples and Analysis

Ten grams of scraped carrot were extracted with 100 ml of 95% hot ethanol in 400 ml erlenmeyer flask, for 30 minutes. The obtained yellow extract was diluted with water to 85% of ethanol and cooled down to room temperature. This solution was then transferred to a separatory funnel and mixed well with 50 ml of petroleum ether. The lower, alcoholic phase, was washed with petroleum ether in portions of 2 ml, until the upper layer became colorless, which indicates that all carotenes are extracted. Petroleum ether layers have been collected and washed with 85% ethanol to remove possibly present xanthophylls. The collected petroleum ether layers were concentrated under vacuum at temperature below 40°C. The remaning oil residue was kept in dark at 4°C.

HPLC analysis of extracts was carried out with the Agilent 1100 Series system, Waldborn, Germany (pump, detector, software). The LC column Zorbax-Eclipse XDBC18; 4.6 mm × 250 mm, 5 μm was used. Mobile phase was a mixture of acetonitrile : methanol : ethyl acetate, 6:2:2 v/v, flow rate 1 cm^3/min. The injection volume was 20 μl using DAD Agilent 1200 Series detector, monitored wavelength was 474 nm (Cvetkovic and Markovic, 2008).

2.4.1 Preparation of Extracts for Analysis

The extracts (8 cm^3) of different concentrations were evaporated to dryness with rotary vacuum evaporators at room temperature, and the residues were dissolved in *n*-hexane (2 cm^3). Extracts were filtered through a 0.45 μm Millipore filter immediately before HPLC analysis. Chromatogram of β-carotene standard in concentration of 0,00625mg/cm^3 is shown on (Figure 1).

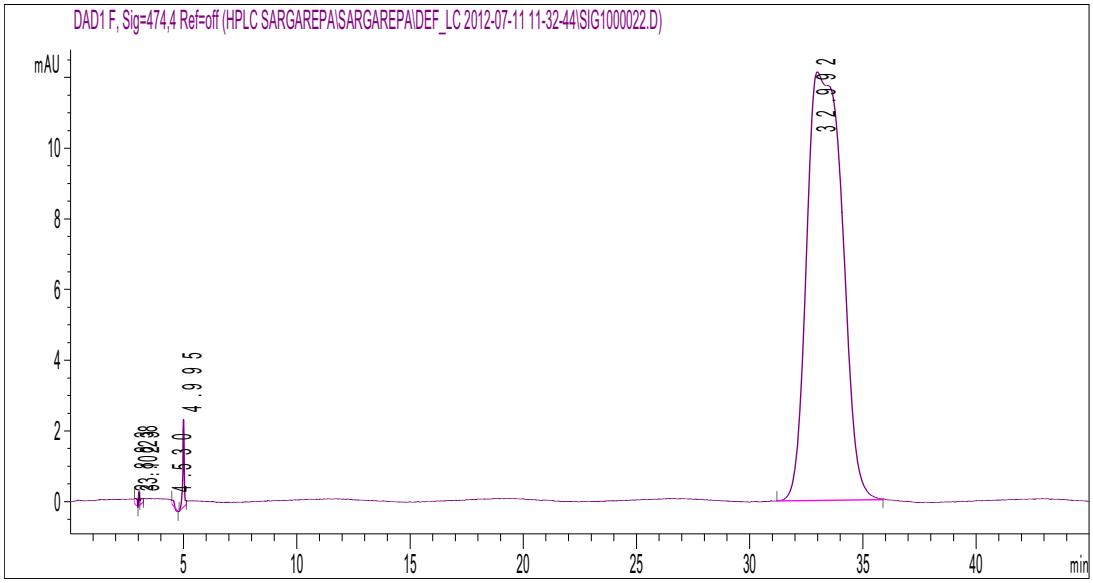

Figure 1. HPLC chromatogram of β-carotene (c = 0,00625mg/cm^3)

2.4.2 Calibration Curve for β-carotene Standard

β-Carotene was dissolved in *n*-hexane just before HPLC analysis, and diluted to appropriate concentrations (1.56-100 mg/cm^3) for calibration curve preparation of (Cvetkovic & Markovic, 2008). The external standard method was used to determine the β-carotene concentration in the extracts. Calibration curve obtained from HPLC analysis of β-carotene in different concentrations is shown on (Figure 2).

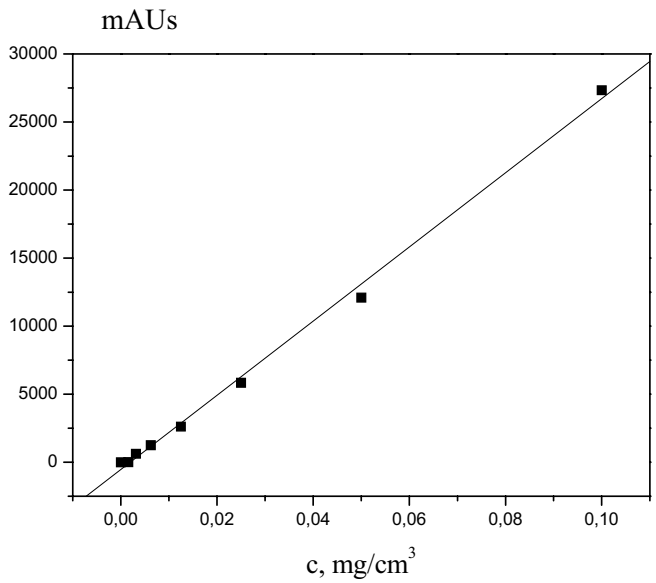

Figure 2. The calibration curve for determination of β-carotene content in carrot, based on HPLC analysis

2.5 Vitamin C

Vitamin C content was determined by Tillman's method'. The method is based on the extraction of L-ascorbic acid from the analysing sample by means of the oxalic acid and conversion of 2.6-dichlorphenolindophenol into dehydroascorbic acid.

2.6 Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical (St. Louis, MQ, USA), Aldrich Chemical Co. and Alfa Aesar (Karlsruhe, Germany).

2.7 Data Analysis

All data were subjected to one- or two-way statistical analysis at P = 0.05 using JMP6 Statistical Analysis Software Program (SAS Institute Inc. Cary, NC, USA).

3. Results and Discussion

Carrots have low metabolic activity at low temperatures, as shown by the low respiration rate. However, carrots are sensitive to wilting if not protected from water loss. The moisture content of the refrigerated carrots decreased with time of storage. The relative humidity measured inside the S_1 sophisticated cooling room conditions ranged from 95% to 98%, whereas the relative humidity under simple refrigerated storage varied from 79% to 94%. The low relative humidity under the refrigerated storage may have contributed to the decrease in moisture content of the carrots with time.

Mass losses (shriveling) and fungal diseases were the most important causes of postharvest losses of carrot in Serbia. At the end of 180 days of storage the percentage mass losses ranged from 3.1% to 33.2% depending on the storage condition and disinfection treatment. Thus, the weight loss of carrot roots inside the S_1 sophisticated cooling room conditions ranged from 3.1% with chlorinated (175 ppm Na_2OCl) prestorage treatment to a maximum weight loss of 6.8% in control (unwashed-control roots). A different trend was observed under simple refrigerated storage, where the lowest mass losses (20.7 %) were recorded in treatment with hydrogen-peroxide, but more significantly the highest mass losses (33.2%) were observed with hot water treatment (Table 1).

Table 1. Effect of postharvest treatments and storage conditions on mass losses (%) during storage

Storage condition	S_1-(0°C; > 95% RH) days after harvest			S_2-(0-2°C; < 90% RH)		
Treatment	90	160	180	90	160	180
Control	0.86b	3.00b	6.77 c	12.48b	18.96b	24.00 b
Hot water	3.01a	4.01a	4.85 b	21.80a	29.76a	33.24 c
H_2O_2	1.42b	1.73c	3.12 a	11.71b	18.08b	20.69 a
Na_2OCl	2.30a	3.02b	3.42 a	11.00b	17.52b	21.25 a

Similar results were found by Sudimac et al. (2012) who showed that the percentage mass losses ranged from 15% to 35% depending on the cultivars and disinfection treatment. Commercial storage of carrots resulted in mass losses of 15% fresh weight over a 3-month period (Ng et at., 1998). The effects of sanitation treatments were highly effective at reducing disease decay (*A. alternata*) in comparison with control (12%). In view of microbial reduction and maintenance of sensory properties, the use of cold chlorinated water proved to be effective for washing carrots (Sudimac et al., 2012). The rate of water loss of a carrot is affected by the surface area of the root, the water vapour pressure deficit and air velocity (Correa et al., 2012). Water loss due to transpiration results in shrivelling, loss of bright colour and increased risk of postharvest decay. An 8% weight loss is reported to make carrots unsaleable (Robinson et al., 1975).

β-caroten and vitamin C levels in the fresh carrot decrease during storage. Since carrots are an important source of carotenoids and vitamin C in the human diet, it is appropriate to determine to what extent these compounds are lost during storage. The carrot root is associated with the presence of certain carotenoids, xanthophylls or anthocyanins. In orange carrots, α-carotene accounts for 15% to 40% of the total carotenoid content, while β-carotene accounts for 45% to 80% and γ-carotene for 2% to 10% of the total carotenoid content (Tang, 2010). At the end of 180 days of storage the percentage of β-carotene loss resulted in a significant reduction, particularly under simple refrigerated storage. Depending on the disinfection treatment the loss of β-carotene varied. Thus, loss of β-carotene in carrot roots inside the S_1 sophisticated cooling room conditions ranged from 4.1% with hot water prestorage washing treatment to a maximum of 20.7% in treatment with Na_2OCl (Table 2).

Table 2. Effect of postharvest treatments and storage conditions on β-carotene (μg/g f.w) changes

Storage condition		S_1-(0°C; >95% RH)			S_2-(0-2°C ; <90% RH)		
		days after harvest					
Treatment	Time of harvest	90	160	180	90	160	180
Control	**39.74**	38.80a	37.93a	36.65b	37.90a	34.05a	28.53a
Hot water		39.21a	38.92a	38.12a	37.15a	32.10b	23.02b
H_2O_2		37.54b	35.10b	33.52c	35.02b	31.55b	24.79b
Na_2OCl		36.86b	34.45b	31.51c	34.10b	28.17c	21.09c

A similar trend was observed under simple refrigerated storage, where the lowest β-carotene loss (28.2%) was recorded in control roots and the highest β-carotene loss was observed in Na_2OCl treatment (46.9%).

Our findings that β-carotene is lost during storage and that this depends on the storage conditions is in agreement with findings by Fikselova et al. (2010), who similarly found that loss of β-carotene during carrot storage is higher in the cellar than in cold storage. The mean loss of β-carotene in dry matter for cold storage was 13.57-14.28%, compared to 20-27.3% in the cellar (Fikselova et al., 2010). In Nantes carrots, stored at 2°C and 90 percent relative humidity, α-carotene and β-carotene levels increased slowly through 100 to 125 days and then decreased (Lee, 1986). Losses in total carotenoid content were reported in some vegetables, especially leaves. Both sweet pepper and parsley lost over 20 % of their total carotenoid content at cold room storage (7°C) for 9 days (Takama & Saito 1974). Carotenoid losses amounted to 60 and 80 % at 15°C and 17°C, respectively. Leek lost about 53% of its total carotenoid content within 3 days at both temperatures (Takama & Saito, 1974).

The content of vitamin C in carrot can be influenced by various factors such as genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling procedures. Temperature management after harvest is the most important factor to maintain vitamin C levels in vegetables; losses are accelerated at higher temperatures and with longer storage durations (Lee & Kader, 2000).

The losses of vitamin C that occur immediately after harvest. 90-day storage resulted in a significantly larger reduction in vitamin C content under simple refrigerated storage (2-32.5%) compared with the loss in a sophisticated cooling room (1-18.2%), Tab 3. A more pronounced vitamin C loss was observed by Matejkova and Petrikova (2010) after 30 days of storage, a 47% reduction on average.

At the end of 180 days of storage the percentage of vitamin C loss resulted in a significant reduction particularly under simple refrigerated storage. Depending on the disinfection treatment the loss of vitamin C also varied. Thus, the vitamin C losses of carrot root inside the S_1 sophisticated cooling room conditions ranged from 2.0% with hot water prestorage washing to maximum vitamin C losses of 18.2% in treatment with Na_2OCl. A similar trend was observed under simple refrigerated storage, where the lowest vitamin C loss (20.7 %) was recorded in treatment with hot water treatment, but the highest vitamin C loss was observed with hydrogen-peroxide treatment (52.3%), Table 3.

Table 3. Effect of postharvest treatments and storage conditions on vitamin C ($mg \cdot 100g^{-1}$) changes

Storage condition		S_1-(0°C; > 95% RH)			S_2-(0-2°C; < 90% RH)		
		days after harvest					
Treatment	Time of harvest	90	160	180	90	160	180
Control	**5.05**	4.95 a	4.72 a	3.28b	4.80 a	4.07 a	2.97b
Hot water		5.00 a	4.80 a	4.07a	4.95 a	4.20 a	3.52a
H_2O_2		4.48 b	4.35 b	3.08b	4.10 b	3.41b	2.56c
Na_2OCl		4.40 b	4.18 b	2.86b	4.30 b	3.99a	2.41c

Favell (1998) noted a 15% decrease of vitamin C in carrot after 14 days storage at 4°C. Fresh storage at ambient temperatures resulted in greater loss. For example, fresh peas stored at ambient temperatures lost 50% of their

ascorbic acid in 1 week, while fresh spinach stored at ambient temperatures lost 100% of its ascorbic acid in less than 4 days (Hunter & Fletcher, 2002).

4. Conclusions

This work confirms the existence of important differences between storage condition and postharvest washing treatments during carrot storage, regarding mass, vitamin C and β-carotene losses. Losses of mass, β-carotene and vitamin C during carrot storage were greater in the simple cooling room compared to the sophisticated cold storage. After 180 days of storage the levels of both β-carotene and vitamin C were significantly lower with the largest losses noted for vitamin C.

This research revealed that prestorage root washing (175 ppm sodium hypochlorite) significantly reduced weight loss, while hot water treatment maintained a good quality of β-carotene and vitamin C contents in the carrot.

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Agricultural Information Needs of Rural Women Farmers in Nkonkobe Municipality: The Extension Challenge

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Abstract

Access to agricultural information is vital for improving food security at the village level. This study accessed the agricultural information needs of women farmers in Nkonkobe Municipality of the Amathole District, Eastern Cape Province, South Africa. Data was obtained from 118 households. The women farmers were identified from four villages using the snowball sampling technique. Findings revealed that backyard gardening (87.2%; n = 103) was common in addition to the rearing of indigenous chicken (65.2%; n = 77) to complement food security. Most (80.5%; n = 95) were confronted with weed problems after applying cow dung as manure. There was a high report (70.3%; n = 83) of insect attack on leaves of cabbage, spinach and carrot, while seed dormancy was low (24.58; n = 29). Problems of fowls' theft (66.95%; n = 49) and fowl predators (40.68%; n = 48) were common. More than average (54.2%; n = 64) depends on friends, neighbors and farmers' colleagues for agricultural information but the majority (99.1%; n = 117) preferred extension workers coupled with farm demonstration for agricultural information. The study identified the importance of farmer-to-farmer model of technology transfer among farmers. It is recommended that farmer-to-farmer model could further be investigated to complement efforts of the extension services towards providing agricultural information to the smallholder farmers.

Keywords: agricultural information, rural women farmers, extension services, needs

1. Introduction

Women farmers play a significant role in the food security of households. In sub-Saharan Africa, women do about 80% of the farm labor (Bill and Melinda Gates Foundation, 2012). Banji and Okuade (2005) attributed 60% of the farm labor force to women who produce 80% of food and earn 10% of the monetary income but own just 1% of the farm assets. The report of FAO (2011) indicated that if women had the same access to productive resources as men, they could increase yields on their farms by 20-30%, lifting 100-150 million out of hunger. Furthermore, FAO (2011) reported that equal access to the resources by both male and female farmers will increase the total agricultural output in the developing countries by 2.5-4%, thereby contributing to both food security and economic growth. Thus, any effort designed to improve South Africa's agriculture generally and the lives of its smallholder farmers in particular must take cognizance of women's roles in livelihoods and food security

Information, according to Belkin and Pao (1989), is the product that emanates from processing, manipulating and organizing data in a way that creates value to the knowledge of the person receiving it. Though Stanley (1990) likened information as one of the basic necessities of life after air, water, food, and shelter, Rezvanfar et al. (2007) indicated that information is needed because of its significant effects on the living activities of man. Mudukuti and Miller (2002) suggested that in the information age, dissemination of information and applying this information in the process of agricultural production will play a significant role in the development of farm settlements. In farming entrepreneurs, Doss (1999) showed that access to appropriate information has had significant impact on agricultural productivity. In the same vein, the United Nations (UN) (2002), FAO (2004),

IFPRI (2004) and Des Castello and Braun (2006) concluded that achieving sustainable agricultural development is less based on material inputs but rather on the available knowledge and information appropriate for sharing with the farmers.

South African Agricultural Research Development (SARD, 2007) reported that there has been a steady decline in the number of rural inhabitants from 44.9% in 1996 to 42.5% in 2001 because of economic and declining agricultural opportunities in urban and rural areas respectively. The situation implies that the rural communities require information on various facets of rural livelihoods that will enhance opportunities and reduce vulnerabilities. In this regard, appropriate and scientifically researched information is needed on some of the challenges militating against good farming techniques, pest and disease control in crops and livestock, impact of climate change, storage and market hints. Appropriate agricultural information is, therefore, necessary towards helping the farmers, who are mostly the women, to satisfy their needs. However, the inequality in the distribution of resources between men and women has been linked with production inefficiency, yet interventions targeting smallholder farmers often fail to redress a women's lack of access to, and control of, important agricultural resources (Quisumbing & Pandolfelli, 2008). Whereas, access to reliable and adequate agricultural information by women farmers could address many of their needs and aspirations and enhance production efficiency and market accessibility.

Nkonkobe Municipality has been identified as having the challenges of poverty, high unemployment rate, and poor agricultural production (Vengayi, 2009). Breaking the poverty cycle requires identifying factors militating against achieving food security and improved livelihoods among the smallholder women farmers who constitute larger percentages of rural dwellers. Most of the rural women from disadvantaged areas are using their backyards to grow crops and farm livestock in order to feed their families and the community. As little as the land size of backyard gardening may be, farmers still require agricultural information that will enhance efficient and effective utilization of the land, manage soil and water, control pests and diseases and help solve other problems emanating from the farm. Unfortunately, there is scanty information pertaining to the agricultural information needs of women farmers that could be used to design appropriate extension intervention in the municipality. This study attempts to provide answers to the following questions**:**

a. What are the agricultural information needs of the women farmers in Nkonkobe Municipality?

b. What are the information seeking behavioral patterns of these women farmers that add value to their farming activities?

c. What role do extension workers play in the dissemination of agricultural information to these women farmers?

1.1 Purpose of the Study

This study was designed to investigate the agricultural information needs of the women farmers in Nkonkobe Municipality, Eastern Cape Province, South Africa.

1.2 The Specific Objectives

a. Describe the demographic characteristics and agricultural information needs of the women farmers in the Nkonkobe Municipality (NM).

b. Determine the agricultural information seeking behavioral patterns of women farmers.

d. Identify channels most preferred for seeking agricultural information by the rural women farmers.

e. Determine the relevance of the obtained agricultural information towards improving agricultural productivity.

2. Methodology

Nkonkobe Municipality was established in 2000 with an estimated total population of 131 071 and 28 259 households (Global Insight, 2008). The local municipality is made up of 21 wards. According to Global Insight (2008), approximately 74% of people living within the municipal area are indigent with the majority of the population residing in both villages and in urban settlements. Urbanization is mainly concentrated in Alice and Fort Beaufort. The ratio of urbanization (Urban/rural) has improved from 4:1 in 2001 to 2:6 in 2008 (Global Insight, 2008).

The target population consisted of women farmers from ward twelve of Nkonkobe Municipality. Ward twelve is made up of nine villages comprising Ngcothoyi, Magaleni, Bergplaas, Msobomvu, Woburn, Taylor, Melani, Skhutswana, and Lower Gqumashe. Four villages were randomly selected that included Lower Gqumashe,

Taylor, Melani and Woburn. A total of 144 copies of questionnaires was administered, but data were received and analyzed from a sample of 118 women farmers which represent 81.9 % of usable questionnaires. Data was collected using the snowball technique.

2.1 Validity and Reliability

The instrument was field-tested for content and face validity by an expert in Information and Communication in the Department of Communication, University of Fort Hare. The instrument was pre-tested at Hala village for reliability and validation. The internal consistency reliability result was 0.79 using the Cronbach's coefficient.

The instrument for analysis was made up of two parts. Part A addressed the demographic characteristics of the women farmers that elicited information on age, marital status, level of education, source of income, size of households and main farming occupation. Part B of the questionnaire elicited information on the types of agricultural information needs that will enhance agricultural productivity, most desired channels for seeking information, information-seeking behavioral pattern, ways that the agricultural information could benefit the farmers, sourcing information, the solution obtained from the sourced channels and steps taken on the problems. The structured interview schedule was used for collecting relevant quantitative data from the sampled respondents.

The data collected were analyzed using the SPSS version 20. The descriptive statistics tools used include frequency counts, percentages and means.

3. Results and Discussion

Table 1. Socioeconomic characteristics of respondents in the study area N = 118

Age	Frequency	%
25-35	9	7.6
36-45	36	30.5
46-55	46	39.0
56-65	23	19.5
66-75	4	3.4
Total	118	100.0
Marital status		
Single	43	36.4
Married	35	29.7
Divorced	12	10.2
Widow/widower	28	23.7
Total	118	100.0
Number in household		
2-3	48	40.7
4-5	57	48.3
6-7	13	11.0
Total	118	100.0
Education		
Primary	3	2.5
Some secondary	103	87.3
Completed grade 10	9	7.6
Completed grade 12	3	2.5
Total	118	100.0
Source of income		
Salary	46	39.0
Pension, social grant	55	46.6
Self employed	17	14.4
Total	118	100.0

Source: Field Survey 2012.

The descriptions of the demographic profile of the women farmers described in Table 1 indicate that many of the women farmers fell between age brackets of 46-55 years old (39%), followed by 36-45 years (30.5%), and 56-65 years (19.5%) SD (0.954). The marital status revealed those who are single as (36.4%), married (29.7%), widowed/ widowers (23.7%) and the divorced (10.2%). The number of households was highest for respondents with 4-5 children (48.3%), followed by 2-3 children (40.7%) and 6-7 children (11.0%), SD (0.658). The educational status revealed that the majority (87.3%) had some secondary school education but could not complete grade 10. This is followed by those who completed grades 10 and 12 which are 7.6% and 2.5% respectively. Very few (2.5%) had only primary school education, and SD (0.441). Many of the respondents (46.6%) depend on pension and one form of social grants such as sources of primary income; 39% were earning salaries for jobs ranging from nanny to junior staff and complementing it with farming. This is followed by 14.4% that are self-employed.

Table 2. Farming occupation

	Frequency	%
Livestock	3	2.5
Backyard gardening	22	18.6
Indigenous chickens	12	10.2
Livestock and backyard garden	16	13.6
Backyard garden and indigenous chickens	62	52.5
Livestock, backyard garden and indigenous chickens	3	2.5
Total	118	100.0

Source: Field Survey 2012.

Table 2 indicates that backyard gardening (87.2%; n = 103) was a common farming practice. A substantial number (65.2%; n = 77) kept indigenous chicken while livestock (18.6%; n = 22) mainly cattle, pigs, sheep and goat are, also, reared. The rearing of indigenous chicken by women contributes significantly to food security of the rural livelihood, and this finding was, also, reported by Gondwe (2004).

Table 3. Types of agricultural information needed

	Frequency	%
Insects attack on vegetables e.g. Cabbage	83	70.30
Rust of Spinach	37	31.36
Weed control and management, especially when organic manure (Cow dung) was applied	95	80.5
Seed dormancy problem during winter	29	24.58
Sudden death of chicks after hatching	18	15.25
Mice and Giant rat menace	23	19.49
Fowls predator problem	48	40.68
Expensive feeds for the scavenging fowls	53	44.91
Lice and mite problem in fowls	18	15.25
Soil fertility management	32	27.12
Fowl theft	49	66.95
Kid mortality in goats	16	13.56
Diarrhea problem in goat kids	28	23.73

Source: Field survey 2012.

The information needs of the women farmers varied as it was determined by the types of farming enterprises. Table 3 indicates that weed, (80.5%; n = 95) constitute a major challenge especially when cow dung was used as fertilizing materials. Some farmers (27.12%; n = 32) whose soils require replenishing actually made use of cow

dungs that are relatively available and cheap. The major challenge of tackling the growth of undigested weed seeds constitutes menace. Most of the farmers (24.58%; n = 29) that depend on planting seeds of vegetables directly during winter experienced poor and late germination. This could be attributed to the dormant nature of the seeds and the climatic condition prevailing. Sudden death of chicks (15.25%; n = 18), mice and giant rats attack (19.49%; n = 23), lice and mites (15.25%; n = 18), fowl theft (66.95%; n = 49) and fowl predators (40.68%; n = 48) were common problems for the scavenging birds. The report indicates a threat to food security of the resource-poor farmers considering the small number of flocks kept for household consumption. Most (44.91%; n = 53) who gave supplementary feeds complaint about the high cost of feeds for newly hatched chicks with the mother hens in brooding. Artificial brooding is practiced amongst some farmers to reduce predators' attack. Insect pests of vegetables (70.3%; n = 83) constituted another major problem. The farmers reported insects eating the leaves of cabbage, spinach and carrot thereby reducing yield and affecting the quality.

Table 4. Channels used mostly for obtaining Agricultural information

Channels	Frequency	%
Radio	1	0.8
Extension workers	21	17.8
Radio and extension workers	20	16.9
Radio, extension workers and demonstration	29	24.6
Ext workers, practical farm demonstrations and group meetings	9	7.6
Extension and practical farm demonstrations	38	32.2
Total	118	100.0

Source: Field survey 2012.

The information-seeking pattern of the women farmers was chiefly influenced by the technical knowledge of the source coupled with practical farm demonstrations. Table 4 indicates that the importance of extension workers serving as a channel of information was noted with overwhelming responses in all variables when calculated (99.1%; n = 117). The majority of the respondents believed that access to agricultural information through the extension workers supported with farm demonstrations will facilitate their learning thereby improving agricultural productivity.

The yearning for extension workers by the women farmers indicated the need for the government to employ more extension workers while the services of the nongovernmental organizations (NGOs) and private organizations that provide extension services are essential most especially at the village levels.

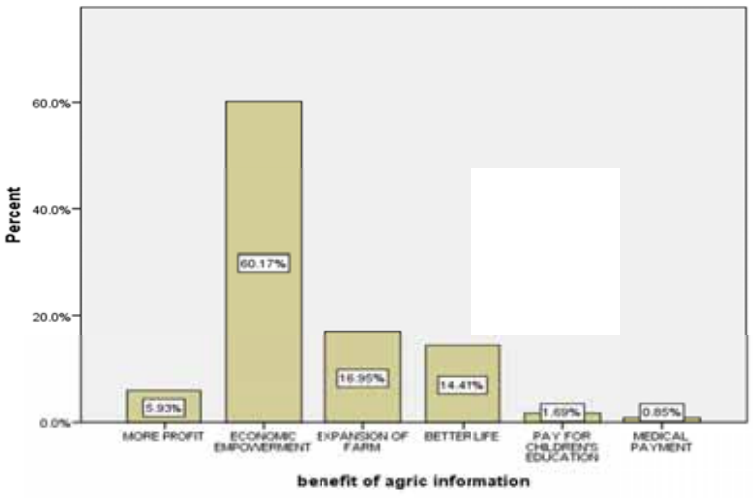

Figure 1. Frequency distribution of benefits of Agricultural information

Figure 1 showed that the majority of the respondents (60.2%) was of the opinion that accessing vital agricultural information will lead to their being economically empowered. This is followed by farm expansion (16.9%) and better life (14.4%). Other variables were believed to have been encompassed within economic empowerment framework. The women farmers believed that economic empowerment is an all embracing economic, social, cultural and political activities that make them to be relevant and recognized in the community. To them, being economically empowered implied that they are able to support their households with nutritious foods, good education, health care, and comfortably perform their social functions.

Table 5. Sources of information used by the respondents

Information sources	Frequency	%
Husband	1	0.8
Friends	30	25.4
Neighbor	27	22.9
Extension agents	8	6.8
Farmers organizations	3	2.5
Cooperative Society	2	1.7
Farmer's colleagues	7	5.9
Nobody	14	11.9
Don't know who to contact	21	17.8
Where I bought inputs	1	0.8
University community engagement	4	3.4
Total	118	100.0

Source: Field survey 2012.

Table 4 showed that the majority of the farmers (54.2% n = 64) depended on friends, neighbors and farmers' colleagues for agricultural information when faced with challenges on the farm. While 17.8%; (n = 21) of the women farmers did not know who to contact, (11.9%; n = 14) indicated they have contacted nobody for information, only 6.8%; (n = 8) had contact with extension services, and 3.4%; n = 4 sought information from the University community engagement officials. The trend where farmers relied on friends, neighbors and farmers' colleagues were also observed by Yahaya (2002), Tologbonse et al. (2006), Okwu and Dauda (2011), Achugbe and Anie (2011) and Rezvanfar et al. (2007) indicating the strong social dynamics of 'across the fence' contact when in need or facing challenges. The ease or proximity of the source could also be an enabling factor here. The low number of contacts with extension workers may be attributed to the inadequate number of extension workers operating in the municipality. Meanwhile, the findings indicate the importance of farmer-to-farmer extension model in technology dissemination. This model has proven for its potency in filling the extension gap in technology transfer to farmers in Nigeria (Kormawa, Ezedinma, & Singh, 2004). The authors identified that the model narrowed the gap with the technology transfer process among farmers. This was attributed to the participatory role of farmers in testing, watching and circulating information among themselves that ensured adoption. However, the little contact enjoyed by the women farmers from the extension agents and university community engagement yielded positive results.

4. Conclusion and Recommendations

Access to adequate, relevant and reliable agricultural information is an essential factor towards building a strong and virile agricultural foundation. The significant role played by women in food security requires that they receive support towards access to scientific and unbiased agricultural information. Women farmers had been classified to be highly vulnerable due to multiple challenges they faced in the rural setting. The challenges are surmountable if they could access the right types of agricultural information as this will reduce shocks that are inherent in vulnerability context.

This study indicates that most women farmers engaged in backyard farming and indigenous chicken production. They require agricultural information that is proven towards addressing multiple challenges on their farms and

livestock rearing. The study revealed that most farmers relied on their friends and neighbors for agricultural information. It is recommended that further study is needed towards understanding the farmer-to-farmer model of technology transfer that could be used to reach the small scale women farmers in South Africa.

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Effects of Enhanced Ultraviolet-B (UV-B) Radiation and Antioxidative-type Plant Growth Regulators on Rice (*Oryza sativa* L.) Leaf Photosynthetic Rate, Photochemistry and Physiology

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Abstract

Elevated UV-B radiation deleteriously affects rice yields. The impacts of plant growth regulator (PGRs; α-tocopherol, glycine betaine [GB] and salicylic acid [SA]) applications on higher plants have been the subject of many studies. However, little or no work has been carried out on rice responses to α-tocopherol, GB or SA under UV-B stress conditions. This study determined the effects of α-tocopherol (2.3 kg ha^{-1}), GB (2.0 kg ha^{-1}) or SA (12.9 g ha^{-1}) application on rice leaf photosynthetic rate (P_N), photochemistry and physiology under ambient and elevated UV-B conditions. Elevated UV-B decreased P_N (17%), quantum yield (8%), electron transport rate (9%), total chlorophyll concentration (8%), plant height (12%), number of leaves (17%), pollen viability (6%), phenolic concentration (46%) and yield (21%). The applications of α-tocopherol, GB or SA increased yield by 23%, 18% and 29%, respectively, under elevated UV-B. Application of PGRs increased leaf phenolic content thus rendering protection against elevated UV-B.

Keywords: α-Tocopherol, glycine betaine, phenolic content, rice, salicylic acid, UV-B

1. Introduction

Global stratospheric ozone depletion is elevating surface ultraviolet-B (UV-B) levels (Russell et al., 1996). Increase in UV-B levels can alter crop productivity as it affects photosystem II, the electron transport systems, enzymes, pigments, nucleic acids and growth regulators (Sullivan & Teramura, 1989; Caldwell & Flint, 1994). UV-B radiation can affect plants by inhibiting photosynthesis, damaging DNA, pollen and pollen tube development, and changing accumulation of biomass and partitioning (Caldwell et al., 1998; Feng et al., 2000). Elevated UV-B decreased leaf photosynthetic rate (P_N), thereby decreasing rice yields (Dai et al., 1994; Kumagai et al., 2001; Mohammed & Tarpley, 2009a, 2010, 2011a). Elevated UV-B decreases leaf stomatal conductance (Dai et al., 1992), chlorophyll content (He et al., 1993; Huang et al., 1993), rubisco content (Ziska & Teramura, 1992), nitrogen concentration, protein content (Hidema et al., 1996), chlorophyll fluorescence, and/or altered photosynthesis-related gene expression (Strid et al., 1996a,b), thereby decreasing P_N.

Apart from P_N, enhanced UV-B radiation can negatively affect plant morphology and phenology (Mohammed & Tarpley, 2011a), pollen viability, pollen germination, pollen tube growth, fertilization and fruit set, thereby decreasing yield (Feng et al., 2000, Koti et al., 2005). Carotenoids can protect the photosynthetic apparatus against enhanced UV-B by quenching highly reactive singlet oxygen and dissipating excess excitation energy (Nonnengiesser et al., 1996; Rakhimberdieva et al., 2004). Phenolics in the epidermal layer also play an important role in protecting the photosynthetic apparatus against UV-B (Meijkamp et al., 1999). Hence, an increase in carotenoid and/or phenolic concentrations protects photosynthetic tissues from enhanced UV-B radiation.

Alpha-tocopherol, GB or SA application enhances plant tolerance to abiotic stresses (DeLong & Steffen, 1998; Mohammed & Tarpley, 2011b, 2011c). The UV-B radiation has been shown to increase the peroxidation of lipids in plants (Predieri et al., 1995). The α-tocopherol present in the thylakoid membrane protects the structure and function of photosynthetic membranes by scavenging active O_2 species and peroxyl radicals produced as a result of stress (Fryer, 1992; Hess, 1993). In addition, exogenous application of α-tocopherol increases membrane stability under elevated UV-B (Pelle et al., 1990). Glycine betaine enhances stress tolerance by protecting enzymes (Paleg et al., 1981), photosystem II (Allakhverdiev et al., 1996), membrane integrity and increasing the antioxidant status

of the plant (Mohammed & Tarpley, 2009b). Salicylic acid enhances resistance to biotic and abiotic stresses (Lopez-Delgado et al., 1998) by increasing antioxidant capacity and phenolic content in plants (Rao et al., 1997; Mohammed & Tarpley, 2009, 2011a; Ghasemzadeh & Jaafar, 2012).

Genetic improvement and breeding for UV-B tolerant rice cultivars can be beneficial for rice adaptation to future climate conditions. However, genetic improvement and breeding for UV-B tolerance are long-term approaches. A short and easy way to negate the negative effects of enhanced UV-B is through the use of PGRs. The use of PGRs for the prevention and/or amelioration of various environmental stresses are a viable approach to make rice production more resilient to UV-B stress. Glycine betaine, SA, vitamin E, proline and choline are some of the PGRs which can induce stress-tolerance (thermotolerance, drought tolerance, cold tolerance and/or salinity tolerance) in various crop plants (Mohammed & Tarpley, 2011b). The research presented herein addresses the effects of α-tocopherol, GB or SA on rice leaf photosynthetic rate, photochemistry and physiology under UV-B conditions.

2. Material and Methods

2.1 Plant Material and Growing Conditions

Three independent experiments were laid out in complete randomized design. In each experiment there were three replications per UV-B and PGR combination. Rice inbred cultivar 'Cocodrie', was used in all three experiments. Plants were grown in pots (15 cm diameter x 17.5 cm height) filled with a clay-rich soil and were placed in square boxes lined with 6.0 mm thickness black plastic (FILM-GARD, Minneapolis, Minnesota, USA). Four seeds per pot were sown at a 2.5-cm depth. After emergence, plants were thinned to one plant per pot, which were maintained until maturity. The boxes were filled with water to approximately 2 cm above the top of the soil in each pot, 20 days after emergence (DAE). Nitrogen was applied at planting, 20 DAE and at the panicle-differentiation stage as described by Mohammed et al., (2007). At planting, urea-N was applied at the rate of 113.5 kg ha^{-1} along with 45.4 kg ha^{-1} of phosphorus (P_2O_5). The remaining nitrogen fertilizations were applied at the rate of 79.5 kg ha^{-1} of nitrogen in the form of ammonium sulfate at 20 DAE and at the panicle-differentiation stage. Mean day temperature and humidity in the greenhouse were monitored using standalone sensor/loggers (HOBOs, Onset Computer Corporation, Bourne, Massachusetts, USA). The greenhouse temperature and absolute humidity ranged between 27-35 ^{o}C and 14-16 g/m^3, respectively. The light intensity and CO_2 in the greenhouse during the day were measured using a light quantum meter (Quantum Meter, Apogee Instruments, Logan, Utah, USA) and LI-6400 (LI-6400, LI-COR Inc., Lincoln, Nebraska, USA), respectively. The light intensity in the greenhouse ranged between 600-800 μmol m^{-2} s^{-1}.

2.2 UV-B Radiation Treatments

In all three experiments, UV-B radiation from fluorescent sun-lamps were delivered to plants for nine hours from 0800 to 1700 h by UV-313 lamps (Q-Panel Company, Cleveland, Ohio, USA) driven by 40 W dimming ballasts in a square wave fashion. The lamps were wrapped with cellulose diacetate film (solarized 0.07 mm, JCS Industries Inc., La Mirada, California, USA) to filter out radiations below 280 nm. The cellulose diacetate on the lamps was changed at regular intervals to account for the degradation of the cellulose diacetate properties. The lamps were arranged on the aluminum frame to provide a uniform UV-B radiation over the canopy. Four UV-B lamps were used to supply the required dosage. The UV-B energy delivered at the top of the canopy was monitored daily with a UV meter (UVM, Apogee Instruments Inc. Utah, USA). Plants were exposed to UV-B dose of 5 (ambient) or 10 (enhanced) kJ m^{-2} d^{-1}, 20 DAE.

2.3 Plant Growth Regulator (PGR) Treatments

The PGRs, α-tocopherol (2.3 kg a.i. ha^{-1}), GB (2.0 kg ha^{-1}), and SA (12.9 g ha^{-1}) were applied at the rate of 300 μL per plant at boot stage of rice plant using a pre-calibrated perfume-bottle sprayer. The PGRs were dissolved in de-ionized water with 0.5% (v/v) surfactant (Latron AG-98 spreader activator, Rohm and Haas Company, Philadelphia, Pennsylvania, USA). The α-tocopherol and SA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and GB was supplied by Capstone Food Ingredients (Marion, Massachusetts, USA).

2.4 Leaf Photosynthesis

The net photosynthetic rate (P_N) of the penultimate leaves was measured using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA), 10 days after PGR treatments (DAT). The P_N was measured between 1000 h and 1200 h. When measuring P_N, the light intensity, temperature and CO_2 concentration in the leaf cuvette were set to 1500 μmol m^{-2} s^{-1}, 25°C and 390 ppm (ambient CO_2 concentration in the greenhouse), respectively.

2.5 Chlorophyll Fluorescence

Chlorophyll fluorescence is often used to evaluate the functionality of the photosynthetic system in chloroplast membranes under various stresses (Chen et al., 2010). Chlorophyll a fluorescence parameters, maximum quantum efficiency of photosystem-II (F_v/F_m), thylakoid membrane stability (F_o/F_m), quantum yield of PSII (Y), electron transport rate (ETR) and non-photochemical quenching (NPQ) were assessed by measuring fluorescence with a pulse-modulated fluorometer (OS5p, Opti-Sciences, Hudson, NH, USA). The minimal fluorescence (Fo), maximum fluorescence (Fm) and Fv/Fm were measured in 30 min dark-adapted leaves. For Y and ETR, plants were under a steady state of photosynthesis (plants were exposed to ambient sunlight for more than 5 hours), a prerequisite for measuring Y and ETR. A photosynthetically active radiation (PAR) clip (OS5p PAR Clip, Opti-Sciences, Hudson, NH, USA) provides the PAR measurements while measuring Y and ETR. While measuring the Y and ETR the range of PAR was 600-700 μmol m^{-2} s^{-1}. The coefficient of non-photochemical quenching of excitation energy (NPQ) was calculated using Klughammer and Schreiber's equations, where NPQ is [(Fm-Fms)/Fms] (Klughammer & Schreiber, 2008). The leaf chlorophyll fluorescence was measured 10 DAT.

2.6. Leaf Pigments

Avoiding the mid-vein, three leaf discs (0.65 cm diameter) were obtained from mid-blade of the penultimate leaves for chlorophyll and carotenoid determination, 10 DAT. The three leaf discs were placed in a 10 mL vial with 5 mL of dimethyl sulphoxide (DMSO) and incubated for 24 h in darkness. From the 10 mL vials, 200 μL of the extract was transferred to microtiter plates of polypropylene material. The absorbance of the extract was measured using a $PowerWave_X$ microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) at 480, 648 and 664 nm (Chappelle et al., 1992) to calculate the carotenoid, chlorophyll a and chlorophyll b concentrations using equations described by Lichtenthaler (1987). Values for total chlorophyll were obtained by summing up the values of chlorophyll a and chlorophyll b. The pigment concentrations were expressed on a leaf area basis, μg cm^{-2}.

2.7 Morphology and Pollen Viability

At harvest, plant height was measured, the numbers of productive tillers and viable leaves were recorded and dry weights were determined.

Pollen viability was measured using the staining procedure from Virmani et al. (1997) with minor modifications. The 1% iodine potassium iodide (IKI) stain was prepared by dissolving 1 g iodine and 2 g potassium iodide in 100 mL de-ionized water. Pollen was dusted from the plants on the Petri dish, 4-5 mL of 1% IKI stain was applied per Petri dish, followed by incubation for 12 hours. After incubation the pollen grains were observed under a microscope. The pollen grains were classified based on their shape and the extent of staining. The viable pollen grain is round and deep red stained (Virmani et al., 1997). The total numbers of pollen grains and sterile pollen grains were counted and pollen viability was expressed as percentage.

2.8 Grain Characteristics

Grain length, width, volume, surface area and chalkiness of brown (dehulled) rice were determined using a Winseedle (Regent Instruments, Inc. Quebec, Canada), which uses image analysis of scanned color images of the grain to calculate these parameters.

2.9 Leaf Phenolic Concentration

Avoiding the mid-vein, three leaf discs (0.65 cm diameter) were obtained from mid-blade of the penultimate leaves for chlorophyll and carotenoid determination, 10 DAT. The three leaf discs were placed in a 10 mL vial with 5 mL phenolic extractant, which is a mixture of methanol, water and hydrochloric acid in 7:2:1 ratio by volume (Mirecki & Teramura, 1984) and incubated for 24 h in darkness. From the 10 mL vials, 200 μL of the extract was transferred to microtiter plates of polypropylene material. The absorbance of the extract was measured using a $PowerWave_X$ microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) at 300 nm (Kakani et al., 2004), and the phenolic concentration was calculated using the equation, $C = 16.05 \times A$, where A is absorbance at 300nm and C is the phenolic concentration (g/mL of extract). The phenolic concentrations were expressed on a leaf area basis, μg cm^{-2}.

2.10 Data Analysis

Observations were analyzed using the Proc GLM procedure of SAS (SAS statistical analysis package version 9.2, SAS Institute, Cary, NC, USA) to test significant differences among the experiments (three repeats of an experiment), UV-B (two UV-B levels) and PGR treatments (4; untreated + 3 PGRs) for the parameters measured. Duncan's Multiple-Range Test (alpha level of 0.05) was used to separate the means. For the parameters measured, there were no significant differences among the experiments. Hence, for a parameter measured, values from

three experiments were used to obtain the mean and standard error (n = 9). The standard errors of the means are presented in the graphs as error bars.

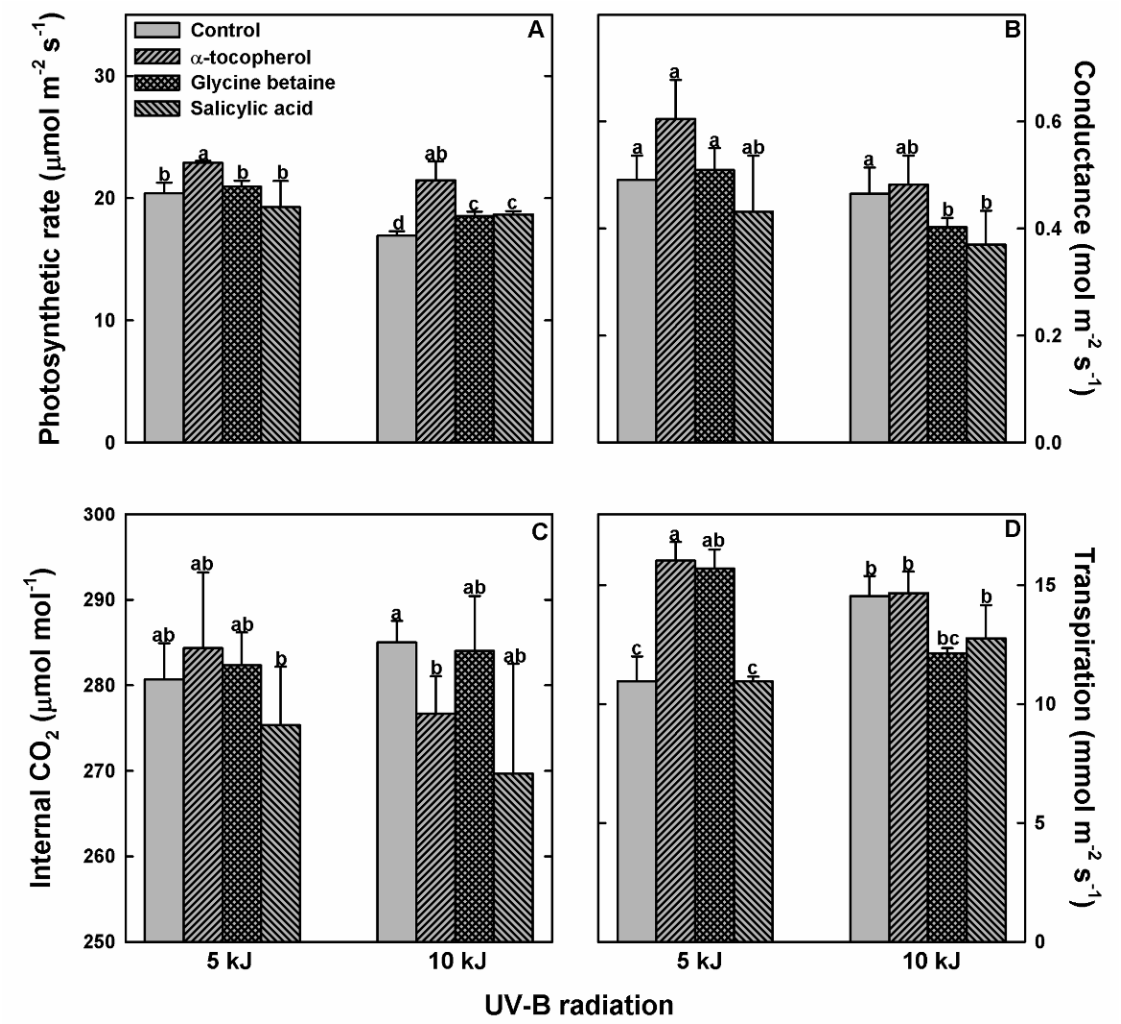

Figure 1. Effects of UV-B and plant growth regulators on rice leaf photosynthetic parameters. Bars with different letters for a particular parameter differed at $P \leq 0.05$

3. Results

3.1 Leaf Photosynthetic Parameters

There was no difference among the experiments for leaf photosynthetic parameters. The untreated plants grown under 10 kJ UV-B showed decreased P_N (17%) and increased leaf transpiration (33%), compared to untreated plants grown under 5 kJ UV-B (Figure 1a, d). The α-tocopherol-treated plants grown under 5 kJ UV-B showed 12% increased P_N, compared to untreated plants grown under ambient UV-B (Figure 1a). In addition, α-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 27%, 10% and 10% increases in P_N, compared to untreated plants grown under 10 kJ UV-B (Figure 1a). The GB- and SA-treated plants grown under 10 kJ UV-B showed 13% and 20% decreases in stomatal conductance, compared to untreated plants grown under 10 kJ UV-B (Figure 1b). The α-tocopherol-treated plants grown under 10 kJ UV-B showed 3% decrease in internal CO_2 concentration, compared to untreated plants grown under 10 kJ UV-B (Figure 1c).

3.2 Chlorophyll Fluorescence

There was no difference among the experiments for chlorophyll fluorescence. In addition, there was no difference between the UV-B treatments or among the PGR treatments for Fv/Fm and Fo/Fm (Figure 2a, b). The untreated plants grown under 10 kJ UV-B showed decreased Y (8%) and ETR (9%) and increased NPQ (15%),

compared to untreated plants grown under 5 kJ UV-B (Figure 2c, d, e). The SA-treated plants showed 12% and 10% increases in Y at 5 kJ and 10 kJ UV-B, compared to untreated plants (Figure 1c). In addition, SA-treated plants grown under 10 kJ UV-B showed 14% increase in ETR, compared to untreated plants grown under 10 kJ UV-B (Figure 2d).

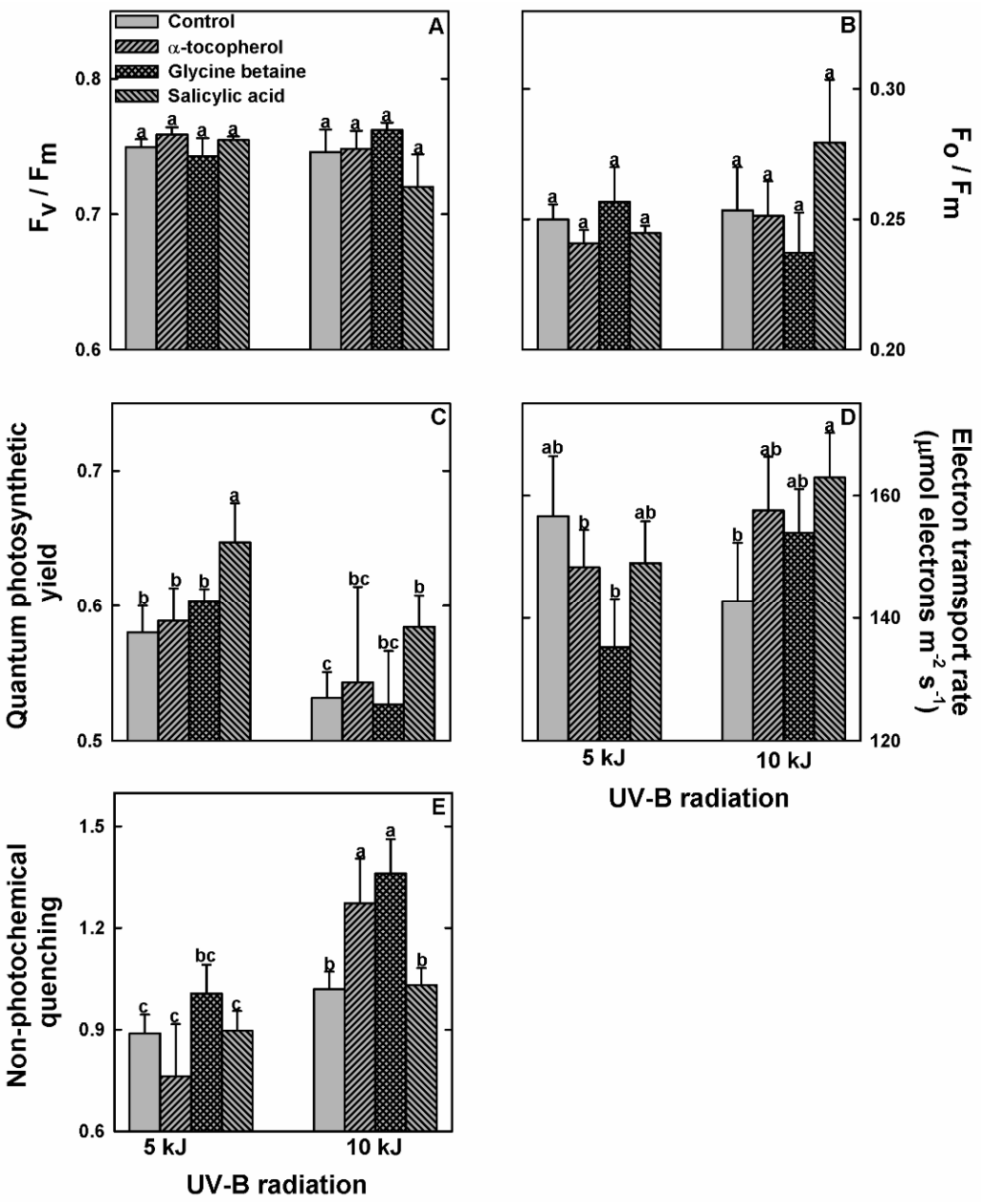

Figure 2. Effects of UV-B and plant growth regulators on rice leaf photochemistry. Bars with different letters for a particular parameter differed at $P \leq 0.05$

3.3 Leaf Pigments

There was no difference among the experiments for leaf chlorophyll or carotenoid concentration. In addition, the untreated plants showed no difference between the UV-B treatments for chlorophyll a, chlorophyll b, carotenoids concentrations or chlorophyll a/b ratio (Figure 3a, b, c, d). However, there was 8% decrease in total chlorophyll concentration at 10 kJ UV-B (Figure 3e). The α-tocopherol-treated plants showed 55%, 67% and 58% and 26%, 17% and 24% increases in chlorophyll a, chlorophyll b and total chlorophyll concentrations under 5 kJ and 10 kJ

UV-B, respectively (Figure 3a, b, e). In contrast, GB-treated plants showed 28%, 13% and 25% and 30%, 22% and 29% decreases in chlorophyll a, chlorophyll b and total chlorophyll concentrations under 5 kJ and 10 kJ UV-B (Figure 3 a, b, e). The GB-treated plants grown under 5 kJ UV-B and SA-treated plants grown under 10 kJ UV-B showed 26% and 28% decreases in carotenoids, compared to untreated plants (Figure 3c).

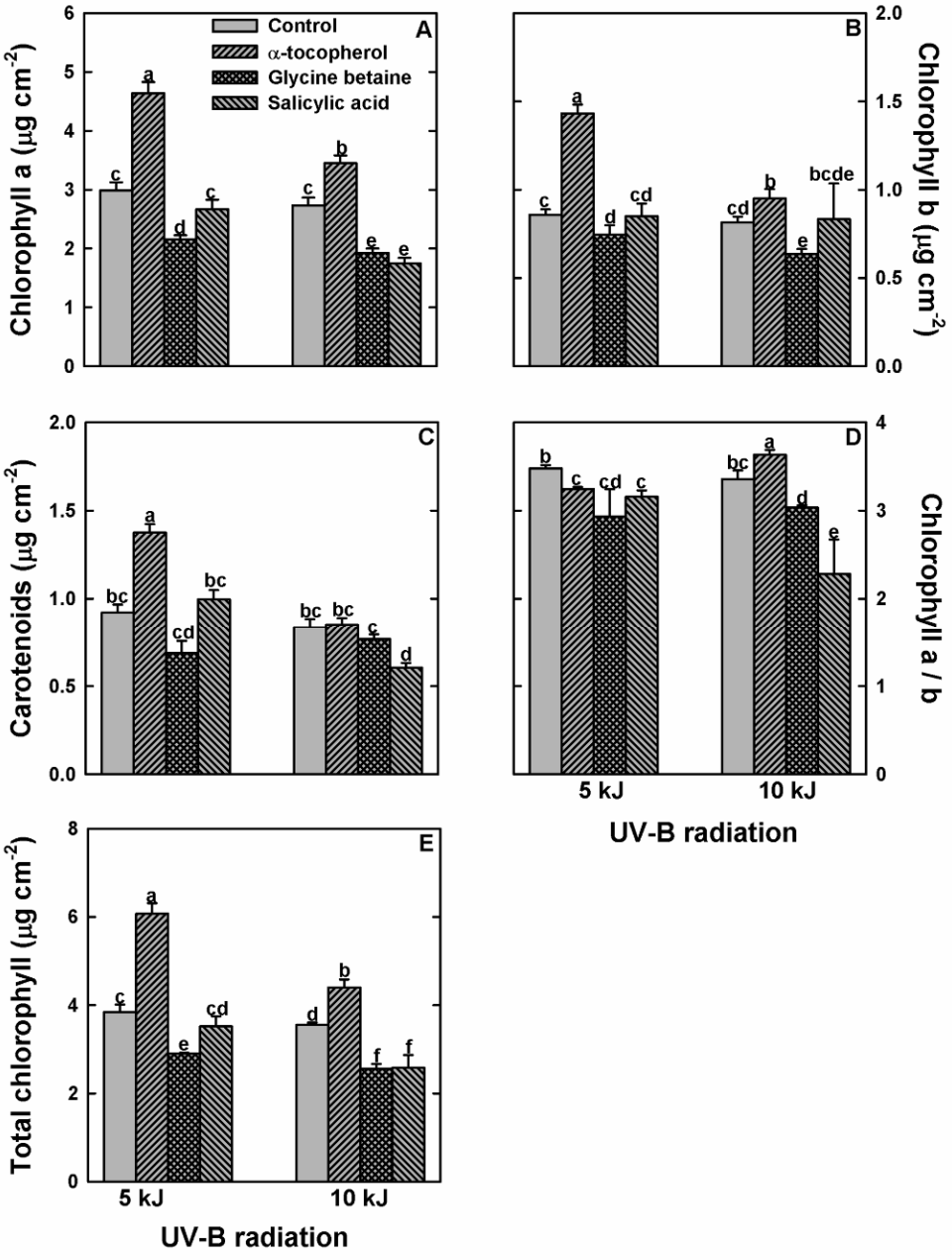

Figure 3. Effects of UV-B and plant growth regulators on rice leaf pigments. Bars with different letters for a particular parameter differed at $P \leq 0.05$

3.4 Rice Morphology, Pollen Viability and Dry Weight

There was no difference among the experiments for rice morphology or pollen viability. In addition there was no difference among the PGR treatments with respect to rice morphology or pollen viability (Figure 4a, b, c, d). However, untreated plants grown under 10 kJ UV-B showed 12%, 17% and 6% decreases in plant height, number of viable leaves per plant and pollen viability, compared to untreated plants grown under 5 kJ UV-B (Figure 4a, c, d). The untreated plants grown under 10 kJ UV-B showed 23% increase in shoot dry weight and

21% decrease in yield, compared to untreated plants grown under 5 kJ UV-B (Figure 5a, b). The SA-treated plants grown under 5 kJ UV-B showed 22% and 17% increases in shoot dry weight and yield, compared to untreated plants grown under 5 kJ UV-B (Figure 5a, b). The α-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 24%, 18% and 29% increases in yield, compared to untreated plants grown under 10 kJ UV-B (Figure 5b).

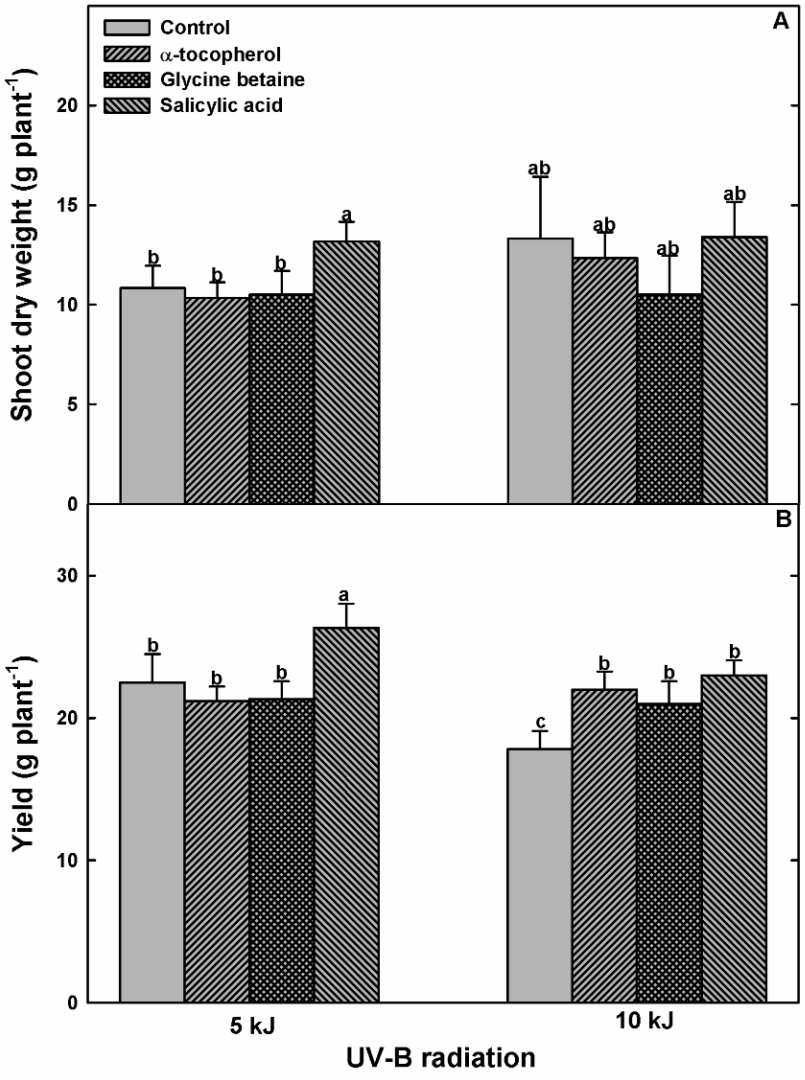

Figure 4. Effects of UV-B and plant growth regulators on rice morphology and pollen viability. Bars with different letters for a particular parameter differed at $P \leq 0.05$

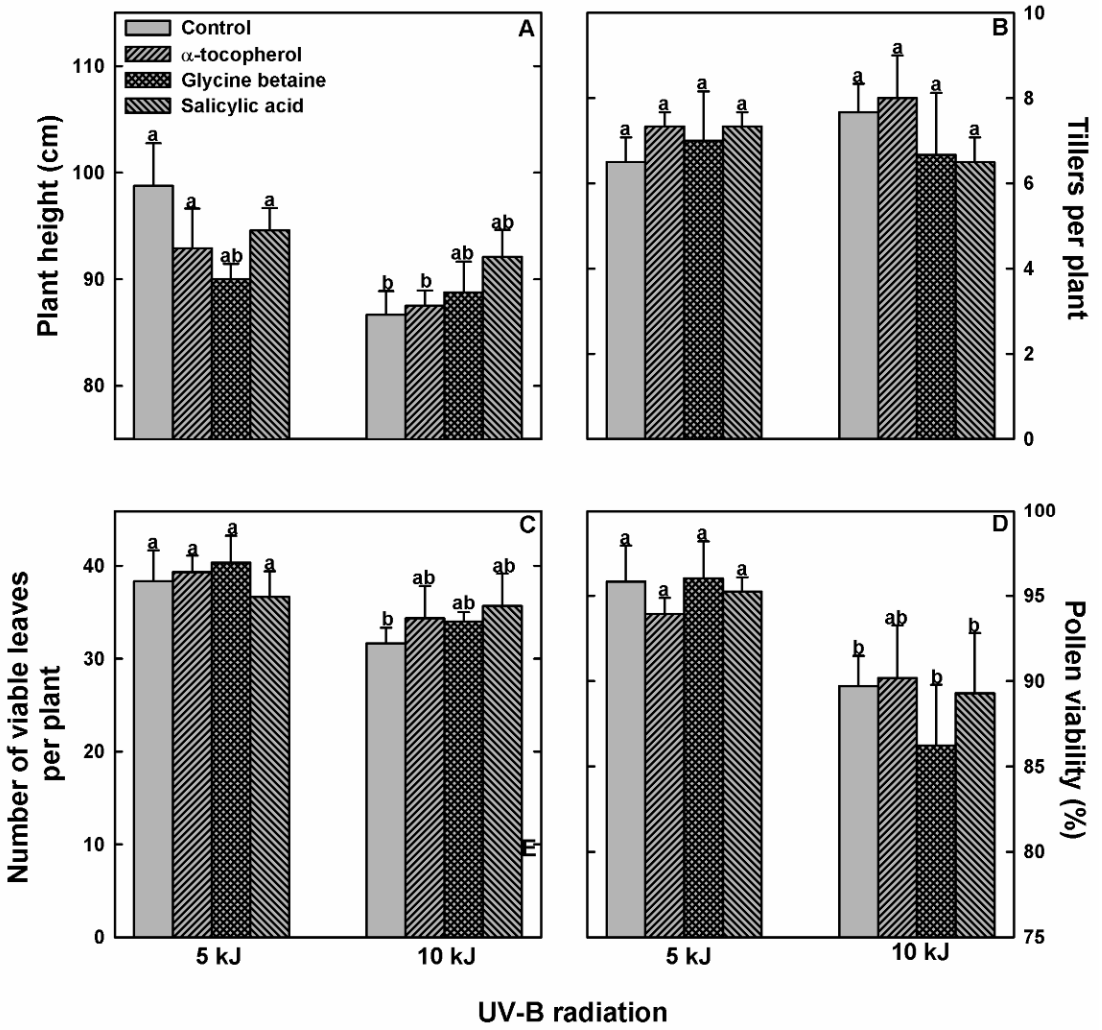

Figure 5. Effects of UV-B and plant growth regulators on rice shoot dry weight and yield. Bars with different letters for a particular parameter differed at $P \leq 0.05$

3.5 Grain Characteristics

There was no difference among the experiments for grain parameters. In addition there was no difference between the UV-B treatments or among the PGR treatments for grain volume or grain surface area (Figure 6c, d). However, untreated plants grown under 10 kJ UV-B showed 3% and 37% increases in grain width and grain chalkiness, compared to untreated plants grown under 5 kJ UV-B (Figure 6b, e). The GB-treated plants showed 2% and 3% decreases in grain length under 5 kJ and 10 kJ UV-B, compared to untreated plants (Figure 6a). The α-tocopherol- and GB-treated plants grown under 5 kJ UV-B showed 5% and 1% increases in grain width, compared to untreated plants grown under 5 kJ UV-B (Figure 6b). The α-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 2% increase and 3% and 1% decreases in grain width, respectively, compared to untreated plants grown under 10 kJ UV-B (Figure 6b). The SA-treated plants grown under 5 kJ UV-B showed 41% decrease in grain chalkiness, compared to untreated plants grown under 5 kJ UV-B (Figure 6e). The α-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 23%, 22% and 22% decreases in grain chalkiness, compared to untreated plants grown under 10 kJ UV-B (Figure 6e).

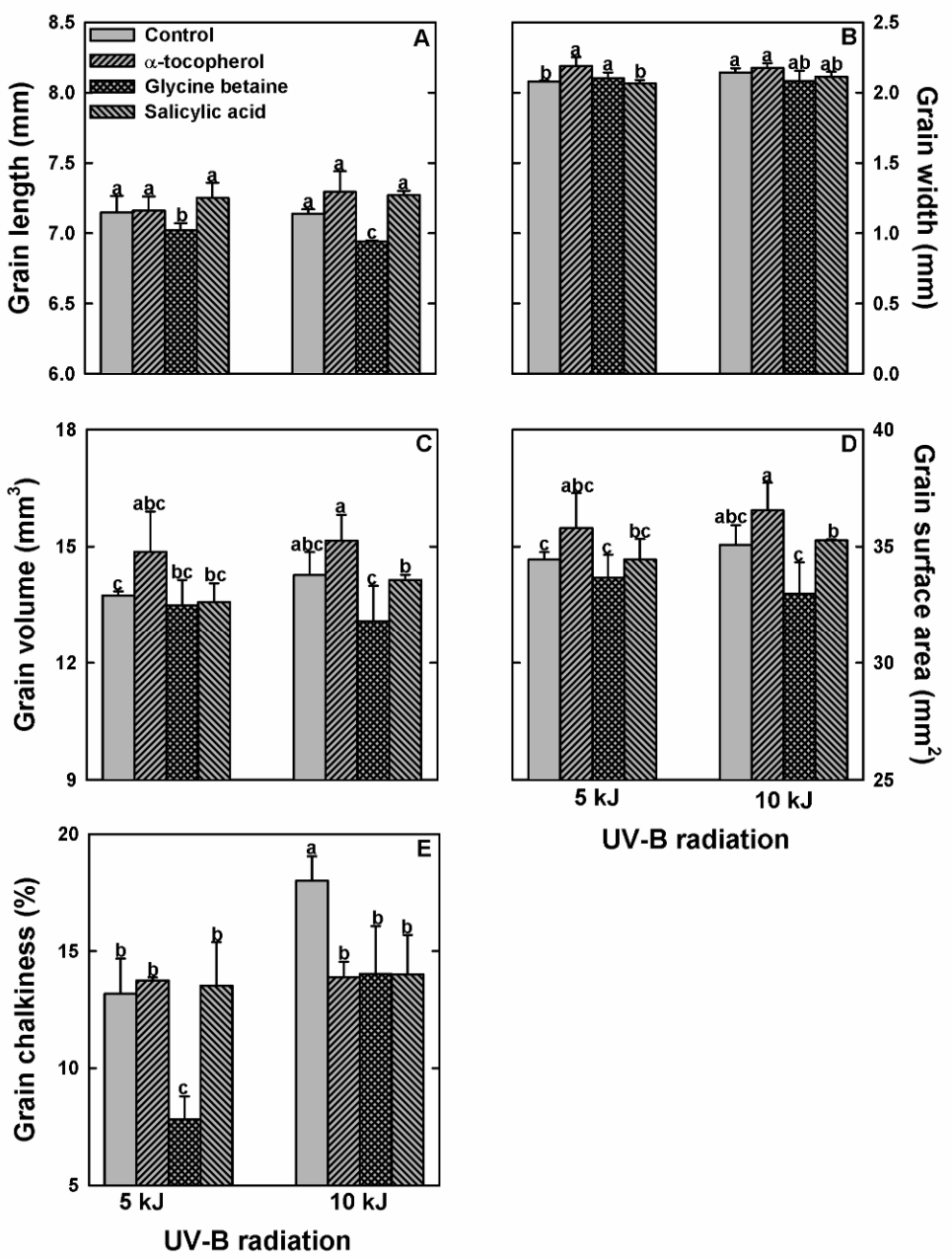

Figure 6. Effects of UV-B and plant growth regulators on rice grain characteristics. Bars with different letters for a particular parameter differed at $P \leq 0.05$

3.6 Leaf Phenolic Concentration

There was no difference among the experiments for leaf phenolic concentration. However, untreated plants grown under 10 kJ UV-B showed 46% decrease in leaf phenolic concentration, compared to untreated plants grown under 5 kJ UV-B (Figure 7). The α-tocopherol-, GB- and SA-treated plants showed 213%, 57% and 29% and 476%, 157% and 352% increases in leaf phenolic concentration under 5 kJ and 10 kJ UV-B, respectively, compared to untreated plants (Figure 7).

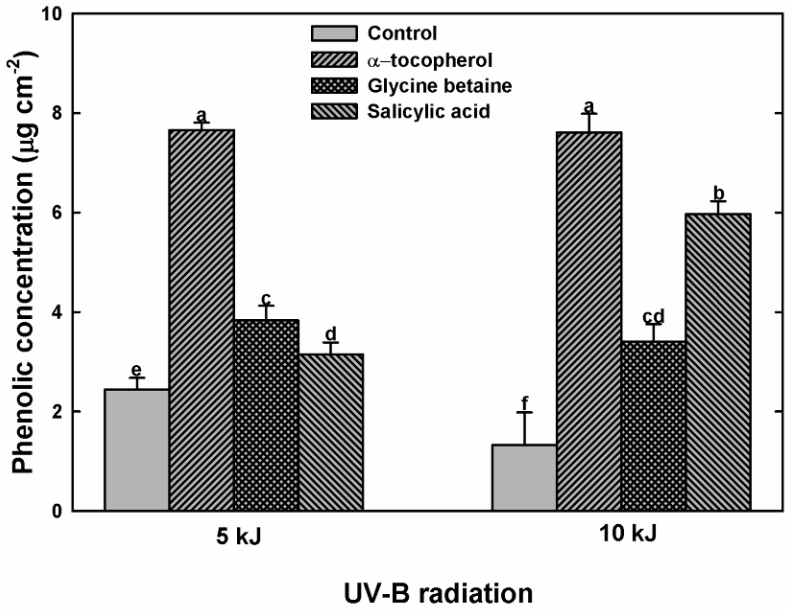

Figure 7. Effects of UV-B and plant growth regulators on rice leaf phenolic concentration. Bars with different letters for a particular parameter differed at $P \leq 0.05$

4. Discussion

The present study was conducted to improve our understanding of rice responses to α-tocopherol, GB and SA applications under UV-B stress conditions. Our results indicated beneficial effects of α-tocopherol, GB and SA application under UV-B stress conditions. In the present study, UV-B inhibited P_N. However, this decrease in P_N under enhanced UV-B was not associated with stomatal conductance or internal CO_2 concentration. In this study, P_N decreased due to decreased photosynthetic quantum yield and total chlorophyll concentration. The decrease in photosynthetic quantum yield under enhanced (10 kJ) UV-B might be due to damaged D1 protein in PSII (Gao & Ma, 2008) and decrease in total chlorophyll concentration might be due to chlorophyll degradation (Kakani et al., 2003). Previous studies have reported chlorophyll degradation as a result of enhanced UV-B (Huang et al., 1993; Kakani et al., 2003). The damage to chloroplasts and changes in photosynthetic pigments result in reduction of P_N (Teramura et al., 1990; Sullivan & Rozema, 1999).

In this study, enhanced UV-B decreased plant height and pollen viability. Decrease in plant height is one of the indicators of UV-B damage (Fiscus et al., 1999). Previous studies have shown decreased plant height as a result of enhanced UV-B in monocots and dicots (Tevini & Teramura, 1989; Dai et al., 1994). Decreased plant height under enhanced UV-B is due to decreased carbohydrate content (Zhao et al., 2003), damaged cell components, and interaction of growth regulators (Ensminger & Schafer, 1992). Enhanced UV-B also decreases pollen production, viability and germination (Feng et al., 2000, 2003; Koti et al., 2005), which are essential for seed/fruit set. Decreased seed/fruit production under enhance UV-B can occur due to decreased pollen production or viability (Koti et al., 2005). In this study, rice yield decreased under enhanced UV-B. Similar results were reported by previous studies with respect to yields under enhanced UV-B (Kumagai et al., 2001; Mohammed & Tarpley, 2009a, 2010, 2011a). In this study, enhanced UV-B increased grain chalkiness. Tsukaguchi and Iida (2008) stated that decreased carbon supply to the grain due to stress can lead to chalky kernels.

The epidermal layer is known to accumulate secondary metabolites, such as phenolics and flavonoids that absorb/screen UV-B and shield the underlying tissues against harmful UV-B radiation (Cen & Bornman, 1993; Olsson et al., 1998). In this study, leaf phenolic concentration decreased in plants grown under enhanced UV-B radiation. Previous studies have stated that leaf UV-B absorbing compounds, such as phenolic concentration, decreased when the plants are grown in relatively high PAR/UV-B (Wilson & Greenberg, 1993; Alexieva et al., 2001). The reduction in secondary metabolites (phenolics) might be due to reduction in photo-assimilation. Decreased photo-assimilation lowers the efficacy of the biosynthetic system to produce secondary metabolites (phenolics). Zhao et al. (2003) stated that UV-B-induced reduction of assimilate production leads to lower production of secondary metabolites.

In this study, application of α-tocopherol, GB or SA increased rice yield. The application of α-tocopherol, GB or SA increased leaf photosynthetic rate and pollen viability, thereby resulting in higher yield. Most of the abiotic stresses, including UV-B, produce reactive oxygen species (ROS). The ROS can increase lipid peroxidation, protein degradation, and DNA fragmentation leading to cell death (Farooq et al., 2008). Application of α-tocopherol, GB or SA can alter antioxidant levels in plants and detoxify superoxide radicals, thus preventing oxidative damage and protecting the membranes and enzymes (Pelle et al., 1990; Farooq et al., 2008b; Mohammed & Tarpley, 2009b). Previous studies have shown that GB or SA can increase photosynthetic rate by increasing photosynthetic pigments and carboxylase activity of Rubisco (Singh & Usha, 2003; Farooq et al., 2008). In this study, application of α-tocopherol, GB or SA increased leaf phenolic content, thus rendering protection to photosynthetic apparatus.

In conclusion, enhanced UV-B negatively affected leaf photosynthetic rate, photochemistry and physiology, thereby reducing rice yield; application of α-tocopherol, GB or SA increased rice yield under UV-B stress conditions. The application of α-tocopherol, GB or SA application increased leaf photosynthetic rate, pollen viability and leaf phenolic concentration, thus increasing rice yield under UV-B stress conditions.

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Marginal Lands: Concept, Assessment and Management

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Abstract

Marginal lands have received wide attention for their potential to improve food security and support bioenergy production. However, environmental issues, ecosystem services, and sustainability have been widely raised over the use of marginal land. Knowledge of the extent, location, and quality of marginal lands as well as their assessment and management are limited and diverse. There are many perceptions about what constitutes marginal lands and so clear definitions are needed. This paper provides a review of the historical development of marginal concept, its application and assessment. Challenges and priority research needs of marginal land assessment and management were also discussed.

Keywords: marginal land, land use, food security, bioenergy, land use, sustainability

1. Introduction

Marginal lands have received wide attention for their potential to increase food security and support bioenergy production (Brown, 1981; Tilman, Hill, & Lehman, 2006; Food and Agriculture Organization (FAO), 2008; Robertson et al., 2008). Marginal lands are typically characterized by low productivity and reduced economic return or by severe limitations for agricultural use. They are generally fragile and at high environmental risk (Barbier, 1989; Wiegmann, Hennenberg, & Fritsche, 2008). Currently, there is increasing interest in globally using marginal land for bioenergy biomass production in the face of limited arable land resources (Koonin, 2006; Milbrandt & Overend, 2009; Vuichard, Ciais, & Wolf, 2009). However, multiple concerns have been raised over environmental impacts, ecosystem services, and sustainability of marginal lands such as erosion, land degradation, biodiversity, and climate change mitigation (O'Connor et al., 2005; Intergovernmental Panel on Climate Change (IPCC), 2007; Searchinger et al., 2008; Fischer, Hizsnyik, Prieler, Shah, & Velthuizen, 2009). The debate on marginal land use is a serious topic associated with the trilemma of land use planning: food security, bioenergy, and environmental concerns (Lal, 2009; Tilman et al., 2009).

Although the concept of marginal land has been broadly applied, a generalized understanding and knowledge of marginal land concept, assessment and management are limited and diverse. This paper provides a multi-disciplinary review of marginal land concepts and their development across time and space. We aim to encourage a holistic rethinking of marginal land use issues in order to optimize their use, sustain productivity, lower environmental risks, and enhance ecosystem services.

2. Development of Marginal Land Concepts

The concept of marginal lands has evolved across time, space, and discipline. The concept is often interchangeably used with other terms such as unproductive lands, waste lands, under-utilized lands, idle lands, abandoned lands, or degraded lands (FAO, 1976; Lal, 1991; Sugrue, 2008; Wiegmann et al., 2008). The meanings of marginal land and its application domains vary across regions, countries and organizations by emphasizing their different management goals (FAO, 1993; Baldock, Beaufoy, Brouwer, & Godeschalk, 1996; Milbrandt & Overend, 2009; Dale, Kline, Wiens, & fargione, 2010; Tang, Xie, & Geng, 2010; United States Department of Agriculture – Natural Resources Conservation Services (USDA-NRCS, 2010).

Early concepts of marginal land emerged in the discipline of agricultural economics, and can be traced back to the 19th century. Ricardo (1817) mentioned the idea of marginal lands in his land rent theory. Different marginal cost

of lands would cause fluctuations in total production cost by shifting labor and capital, suggesting the farming trend between marginal lands and higher quality lands. The theory became the foundation of the marginal productivity theory. Hollander (1895) defined marginal lands as the poorest lands utilized above the margin of rent-paying land. Later, the concept of marginal land was systemically discussed by Peterson and Galbraith (1932) who examined the major variables associated with marginal lands, and proposed to dynamically determine locations of marginal lands. The three terms, physical marginal lands, production marginal lands, and economic marginal land were used under different backgrounds and concerns. In Europe, marginal lands have been defined as the land uses at the margin of economic viability (Strijker, 2005). Schroers (2006) defined more clearly an economically marginal land as "an area where a cost-effective production is not possible, under given site conditions, cultivation techniques, agricultural policies as well as macro-economic and legal conditions".

Physical marginality and production marginality of lands based on soil suitability and restrictions are often adopted by soil scientists and agronomists for the purpose of land use planning. Marginal lands generally refer to the areas not only with low production, but also with limitations that make them unsuitable for agricultural practices and ecosystem function (Heimlich, 1989; Hart, 2001). Some land limitations may not be directly associated with crop production, at least in the short term. Examples include highly erodible soil and ecologically sensitive areas. Prime farmland is defined as "the land that has the best combination of physical and chemical characteristics for producing food, feed, forage, fiber and oilseed crops". The opposite of prime farmland with restrictions of inherent soil characteristics are marginal lands (USDA-NRCS, 2010). Marginal agricultural land, marginal cropland, and marginal farming land are synonymously used in agricultural production terminology (G. Larson, Roloff, & W. Larson, 1988; Niu & Duiker, 2006). Marginal land concepts in agriculture are seldom applied to forestlands and grasslands.

High vulnerability of environment and ecological services of marginal lands have becomes issues of paramount concern (Baldock et al., 1996; Wood, Sebastian, & Scherr, 2000; Dale et al., 2010). Soil erosion has been a major issue of decreasing soil fertility and yield due to loss of fertile topsoil, but its impact on nonpoint source (NPS) pollution (e.g., sediment and nutrients) became another focus of marginal land management (Charbonneau and Kondolf, 1993; USDA-NRCS, 2010). Land capability classification was widely applied to agricultural management and land conservation by Natural Resources Conservation Services of the U.S. Department of Agricultural (USDA-NRCS). Eight classes together with four subclasses are used for grouping soils primarily based on their capability to produce common cultivated crops and pasture plants without deterioration (USDA-NRCS, 2010) (Table 1). The land capability classes from IV to VIII characterized by high soil erosion or with some restrictions were generally categorized as marginal lands (Hamdar, 1999). Because of multiple important ecosystem services that would be impaired by utilization (e.g. biodiversity, wildlife, habitats and soil carbon storage), wetlands were usually classified as marginal lands. Sustainability reflecting long-term preservation trends of land functions has become a key component of marginal land concept (Wiegmann et al., 2008; Lal, 2009). Hence, the marginal land concept has evolved to meet multiple management goals and to incorporate the trade-offs of environmental protection, preservation of ecosystem services and long-term sustainability (Baldock et al., 1996; Krcmar, van Kooten, & Vertinsky, 2005). P. Macdonald and A. Macdonald (2009) analyzed environmental limits and social-economic factors of marginal lands in the Highlands and Island of Scotland, and addressed the interactions between marginal landscapes and culture. Marginal land use planning would substantially alter communities and their development trends in the rural areas of developing countries (Sugrue, 2008). However, quantitative assessment of land marginality with respect to environmental suitability, ecological services, and sustainability is limited because of a lack of suitable metrics and criteria for multiple comparisons.

Table 1. Land capability classification (LCC) derived from USDA-NRCS (2010)

Class	Description
1	Slight limitations that restrict their use
2	Moderate limitations that restrict the choice of plants or that require moderate conservation practices
3	Severe limitations that restrict the choice of plants or that require special conservation practices, or both
4	Very severe limitations that restrict the choice of plants or that require very careful management, or both
5	Little or no erosion but have other limitations, impractical to remove, that restrict their use mainly to pasture, rangeland, forestland, or wildlife habitat
6	Severe limitations that make them generally unsuitable for cultivation and that restrict their use mainly to pasture, rangeland, forestland, or wildlife habitat
7	Very severe limitations that make them unsuitable for cultivation and that restrict their use mainly to grazing, forestland, or wildlife habitat
8	Miscellaneous areas have limitations that preclude commercial plant production and that restrict their use to recreational purposes, wildlife habitat, watershed, or esthetic purposes

3. Marginal Lands for Food and Bioenergy Production

Marginal lands provide necessary and candidate land resources for food production. Marginal lands account for about 36 percent of global agricultural land (1.3 billion ha), and support roughly one-third of the world's population (Wood et al., 2000). Cultivation of marginal lands is inevitable because of a shortage of prime agricultural lands in densely populated regions, as is essential in the developing countries due to the increasing demand on food (Laird, 1951; Nelson et al., 1997; FAO, 2008). To sustain crop production, marginal lands within the framework of land quality assessment program have become a major management target in countries with food shortages (FAO, 1993; Pieri, Dumanski, & Hamlbin, & Young, 1995). Several studies have suggested that enhancing food production will require the conversion of marginal lands to appropriate cropland management systems as well as restoration of degraded lands and ecosystems (Lal, 2004; Biggs, 2007). Twenty-five percent of recent increases in global wheat production were attributed to marginal lands in 1997 (Lantican, Pingali, & Rajaram, 2003). Therefore, the importance of marginal lands for food production should be considered together with the requirement of land conservation and sustainability (Hoag & Skold, 1996). Richardson, Bucks, & Sadler (2008) believed that marginal land in large farms under conservation practices could be profitable and sustainable for management through a national analysis of Conservation Effects Assessment Programs (CEAP) in the U.S.

Marginal lands are regarded as crucial for the second generation of bioenergy crop production (i.e., lignocellulosic biomass crops). Second-generation bioenergy crops provide an attractive option for avoiding land competition between first-generation bioenergy crops (e.g. corn and sugarcane) and food crops (Tilman et al., 2006; the Royal Society, 2008). Vogel (1996) reported that the bioenergy crop, switchgrass [*Panicum virgatum L.*] can reach high biomass, and simultaneously avoid erosion on marginal lands. Marginal lands become competitive for bioenergy production when environmental, economic, and benefits are considered (Hill, Nelson, Tilman, Polasky, & Tiffany, 2006). In the state of Tennessee, the U.S. alone, about 3.6 million ha of cropland eligible for CRP could be used for biomass production from cultivation of trees, native species, and improved grasses (Wells, Fribourg, Schlarbaum, Ammons, & Hodges, 2003). This offers a high potential of biomass production for bioenergy. Additionally, utilizing marginal land resources such as riparian and roadway buffer strips, brownfield sites, and degraded agricultural land could produce enough feedstocks to meet a maximum of 22% of all the energy requirements (Gopalakrishnan, Negri, Wang, Wu, Snyder, & Lafreniere, 2009). In China, about 45 million ha of marginal land could be used for biomass energy production (Tang et al., 2010). Using marginal land for bioenergy production in developing countries such as China and India would be the only solution to satisfy both food security and energy needs (Milbrandt & Overend, 2009). The Worldwatch Institute (2006) estimated that about 100 million to 1 billion ha of marginal lands are theoretically available for production worldwide. Establishment of biofuel plantations on these soils would also restore degraded soils, sequester SOC, improve soil quality, and benefit environment (Lal, 2009; Fisher, 2010). Fargione, Hill, Tilman, Polasky, and Hawthorne (2008) conclude that bioenergy production on degraded and abandoned agricultural lands would cause little or no carbon debt.

Pressure on biomass production for bioenergy is also driven by political goals in the European Union and the US. Globally, biofuels in the transportation sector are stated increase from a current rate of 2% up to 10% in 2020. In the US, 36 billion gallons of renewable fuels will be required with the Energy Independence and Security Act by 2022 (U.S. Congress, 2007). Undoubtedly, policies with subsidy programs or incentives will accelerate a transition of marginal lands into bioenergy production.

4. Marginal Lands Regarding Environmental Quality, Ecosystem Service and Sustainability

Concerns have been frequently raised regarding the impacts of marginal land use on environment, ecosystem services and sustainability. To avoid soil loss and nonpoint source pollution, the Conservation Reserve Program (CRP) of the U.S. has encouraged farmers to retire marginal farmlands from production (USDA, 2006). CRP lands based on the Environmental Benefits Index (EBI) rankings benefit ecosystem services such as wildlife habitat and biodiversity. Hence, if such marginal lands are improved for food or bioenergy production, environmental quality and ecosystem services would be potentially at risk. Long-term monitoring and management are required for soil sustainability and minimizing environmental risks resulting from these land use changes (Blanco-Canqui, 2010). Degradation of land and soils is a serious issue as suggested by an analysis of these North American prairie grass systems for lignocellulosic biofuel production (Gutierrez & Ponti, 2009). Some reports suggested that bioenergy production through land use change might not offset carbon emission (Righelato & Spracklen, 2007; Searchinger et al., 2008). A systems study of sustainability is needed to target the multifaceted challenges of marginal land use (Gopalakrishnan et al., 2009; Interagency Biomass Research and Development Board, 2008).

However, a number of studies indicated that bioenergy crop production on marginal lands can benefit environmental quality, ecosystem service and sustainability. Because of relatively low soil organic carbon (SOC) content and weak ecosystem services in marginal lands, growing bioenergy crops on such lands can minimize the potential of long-term carbon debt and biodiversity loss in comparison to land clearing (Fargione et al., 2008; Tilman et al., 2009). The use of marginal lands for bioenergy production with sound management practices could potentially increase soil carbon sequestration, enhance soil and water quality and support ecosystem services (Lal, 2004; Mensah et al., 2003; Johnson, Franzluebbers, Weyers, & Reicosky, 2007; Liebig, Schmer, Vogel, & Mitchell, 2008). In the process of becoming productive, these soils could be an effective repository of C through the enhancement of SOC (Lal, 2004). Mensah, Schoenau, and Malhi (2003) reported that conversion of marginal land to perennial grassland for bioenergy biomass production would increase the soil carbon sink, reduce erosion, and enhance ecological functions. Previous studies on bioenergy crops summarized by Anderson-Teixeira, Davis, Masters, and Delucia (2009) and Blanco-Canqui (2010) indicated that bioenergy crops such as switchgrass and miscanthus can build up soil organic carbon and improve soil and environment after land conversion from restored prairie or CRP lands. Therefore, to evaluate the success of land management strategies in harmonizing the goals of economic, environment and ecosystem service, we need a comprehensive approach to addressing the intertwined issues of economic, carbon-uptake and structural diversity indicators (Gorissen, Buytaert, Cuypers, Dauwe, & Pelkmans, 2010). Some methods of marginal land assessment derived from one or two land functions are inadequate for these multiple objectives. Accordingly, careful land use planning and management for marginal lands are required for sustaining production and environment (Barbier, 1989; Robertson et al., 2008).

5. Dynamic Characteristics of Marginal Lands

Many marginal lands are dynamic because of land use changes and social-economic impacts. Marginal lands are a transitional state of land resources, and very sensitive to natural processes and varied managements (Figure 1). Poor management of productive lands could cause land degradation. Marginal land can be enhanced or restored to productive lands by improving land functions. Another major driving force affecting the value of marginal lands is the market mechanism determined by socio-economic drivers. With the change in demand, the margin of land cultivation will advance or recede (Hollander, 1895). The decrease of land rent and increases of market demands change marginal profits and breakeven prices, and may finally lead to conversion of marginal lands into production. Places of economic paradox were employed to describe the dynamics and variability of marginal lands (Ritchie, 1950). Pollard (1997) summarized the trend of marginal land in Europe from the Middle Ages, and indicated that marginal lands have been significantly subject to economic development and food demands. Marginal land in Europe has been declining as a result of increasing labor costs and intensification of agriculture (Strijker, 2005). Certainly, policies, incentives and regulations are among driving variables causing land use change (Strijker, 2005; Perlack, 2007; James, Swinton, & Thelen, 2010). All these driving variables obviously push farmers to reclaim or abandon marginal lands because of changing breakeven prices. Hence, marginal lands may not remain marginal, depending specific circumstances. Transitional characteristics of marginal lands would be critical for describing marginal land dynamics, and should be fully considered when marginal lands are assessed and managed.

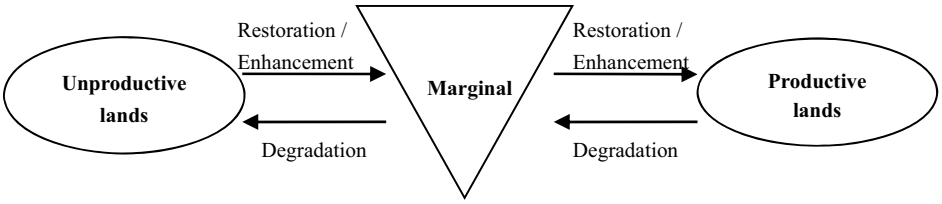

Figure 1. A transitional state of land uses – marginal lands

6. Marginal Land Assessment

Current methods for identifying marginal lands are qualitative and empirical, and some of them are very subjective. These methods reflect specific management goals on croplands across countries that vary with location and time. In the land use management framework of the Food and Agricultural Organization, United Nation (FAO, 1993), marginal land was simply classified as the lands with limitations such as erosion, wetlands, soil salinization, unfit for plant cultivation. Marginal farming land and prime farming land are used for farming suitability assessment by USDA-NRCS. Generally, the areas in land capability class IV-VIII that have severe limitations of production are classified as marginal lands (Hamdar, 1999). The farmlands registered in the Conservation Reserve Program (CRP) are generally treated as marginal lands to control soil erosion by the Food Security Act (Wells et al., 2003). Simply put, if one limiting factor of crop production such as soil, landscape and climate exists, the land is marginal (Biggs, 2007). Wastelands, paddy lands or lands fallow in winter are identified as marginal lands in China (Tang et al., 2010). Marginal land in the Marginal Lands Act 1940 of South Australia is simply classified as the land used to grow wheat, but unsuitable as the principle operations because of inadequate rainfall or other limitations (South Australia Land Act, 1940).

Some efforts for quantitative methods of marginal land assessment have been taken. On the basis of land capability classification, Larson et al. (1988) used a productivity index and an erosion resistivity index to identify marginal agricultural lands in Minnesota; however, other concerns such as wetland or soil limitations were not included. Smith, McDonald, and Thwaites (2000) developed a threat identification model for land sustainability assessment where marginal lands were identified with expert knowledge of local land management and their potential effects. Breuning-Masen, Reenberg, and Holst (1990) classified steep, wet and droughty soils as marginal agricultural land, and generated marginal land maps based on soil information in Denmark. Recent satellite data and historic information of land cover dynamics in Germany were used to detect the trend of abandonment of cultivation lands and further to identify marginal lands (Reger, Otte, & Waldhardt, 2007). In these assessment methodologies of marginal lands, qualitative physical functions or restrictions of soil and landscape in addition to production were considered, but remain very limited in terms of quantitative assessment for multiple land functions. Social and economic factors are seldom included because of their high variability and lack of available data.

Mapping studies associated with marginal land assessment were also conducted at different spatial scales. Based on the detailed information of soil databases, marginal agricultural land in the seven states of the U.S was mapped by Niu and Duiker (2006). High risk of organic carbon loss in marginal lands was delineated for land use decision making. Potential soil productivity map developed by Nizeyimana et al. (2001) provides a solid basis of land use management through the nation of the U.S. This map roughly provided the locations of potential marginal production area in the U.S. Milbrandt and Overend (2009) identified 12 categories of marginal lands using climate and soil restrictions, and generated a map of marginal land in the Asia-Pacific Economic Cooperation (APEC) countries with the global agro-ecological zones system of the United Nations Food and Agriculture Organization (FAO). Based on soil productivity, land slope and climate, global marginal agricultural land is estimated and delineated, and they indicated that high availability of this land for bioenergy production (Cai, Zhang, & Wang, 2011). Most of these mapping studies were mainly derived from soil and agricultural production analysis, but less on the aspects of environmental quality, ecosystem functions, and sustainability.

7. Challenges and Needs of Marginal Land Use Management

The concept of marginal land has evolved into a comprehensive term including multiple needs and concerns. With current knowledge, the concept of marginal lands can be further defined as the lands that are physically inaccessible or with soil and climate restrictions, or with high environmental risk and fragile ecosystem services, and with low production and therefore unprofitable. Following this concept, marginal lands are able to be assessed

quantitatively and the most suitable management practices applied. However, challenges of marginal land assessment and management remain and several research issues need to be addressed.

7.1 Criteria for Marginal Land Assessment and Management Recommendations

None of the current methods for identifying marginal land are widely accepted. Existing methods for marginal land assessment are mostly qualitative, and only address some concerns and management goals; some of them are very subjective. Without effective criteria for identifying marginal lands, land resources cannot be well assessed and managed. For example, at a global scale, the amount of marginal lands varied among different studies, 1.3 billion ha, 36 percent of agricultural lands (Wood et al., 2000), about 100 million to 1 billion ha (The Worldwatch Institute, 2006), and 385-472 million ha (Campbell, Lobell, Genova, & Field, 2008). Obviously, these numbers were estimated using different criteria to define marginal land. The constraints for marginal land assessment in developed countries are generally stricter than those in the developing countries (Sugrue, 2008; Wiegmann et al., 2008). While it may be challenging to establish global or unified marginal land criteria because of various management goals across region and countries, such criteria should be comparable and adjustable for a range of land use planning and policy making activities. As a major component of land resources assessment system, marginal land criteria should cover current requirements and concerns, and be derived from the existing criteria for water quality, air quality, soil and land quality or other tools for sustainable assessment. The criteria should also reflect the synergy of multiple land functions and management goals such as forested land, grasslands or rangelands, and other potential usable land resources besides crop land uses. Therefore, single index or criterion cannot fully meet these needs.

7.2 Land-Informatics Directing Marginal Land Assessment and Management System

Marginal land assessment and management involves multiple needs and concerns, and requires a systems approach to considering land functions and social-economic impacts. Major driving factors of marginal lands should include the demand of food supply and bioenergy, impacts of policies and incentives, and tradeoffs of environment, ecological services and sustainability. Current tools of soil quality and land quality assessment fail to meet the needs of marginal land management because of lack of comprehensive analysis of intertwined land functions and quantitative assessment. We outline here the essential ingredients of a land-informatics framework required to assist in the assessment, planning, monitoring of marginal lands (Figure 2). The framework incorporates land resources databases, land functions assessment, modeling and monitoring as well as decision-making tools. Best management practices and optimized production systems or scenarios of marginal lands can provide the synergistic analysis of land functions, needs and concerns on environment, ecosystem services and sustainability. Complimentarily, the land-informatics system is not only useful for marginal lands, but also can be extended to the other land resources management systems.

7.3 Marginal Land Use Policy and Regulations

Historically, land resources policies are associated with subsidy program and regulations in different regions and countries have played an important role in marginal land planning and management, for example, the Marginal Lands Acts 1940 in Australia (1940), the US-CRP implemented in 1985 (USDA, 2006), and the Energy Independence and Security Act of the US (U.S. Congress, 2007). These policies and regulations changed land use patterns and trends. However, most current policies on bioenergy biomass production have explicit bioenergy production goals, without any or with limited consideration of environmental effects, ecosystem functions and sustainability such as controlling nonpoint source pollutions (NPS) and mitigating climate change. Land use policy adjustment is critical (Strijker, 2005; Robertson et al., 2008; Acs et al., 2010). Meeting multiple goals with various tradeoffs in addition to production should be valued in the policies of marginal land use.

7.4 Priority Research Needs on Marginal Lands

Marginal land assessment and management are seriously challenged due to lack of clarified concepts, criteria, and effective quantification tools. The existing indices and indicators of soil quality and land quality that were developed for farming production are inadequate for assessing risks and tradeoffs of environment and ecosystem functions of marginal lands. The intertwined multiple land functions of marginal land further complicate assessment. Currently, the following research areas are critical for marginal land assessment and management:

- Developing and refining criteria of marginal lands. Criteria for marginal land assessment are urgent because of the current pressure of marginal land use transition for food and bioenergy crops. The existed indices of soil quality, land quality, environment and ecosystem indicated by Dumanski (1997) would provide a preliminary basis of criteria development.

- Establishing a land-informatics framework. The land-informatics framework will be employed to direct research, management and monitoring of marginal lands. A hierarchical assessment system based on diverse land functions and concerns is recommended for development under the framework of land-informatics. The Land-informatics framework will also contribute to the knowledge-base of scientific decision making and policy considerations
- Quantifying land functions based on land processes. Because of land use change and the optimizing needs for environmental quality, ecosystem services and sustainability in marginal land production systems, effective quantification of land functions will be crucial. Studies on the dynamics, resistance and resilience of marginal land functions with management practices in addition to static land quality are highly required. This requires long-term monitoring of land processes using careful experimental designs.
- Understanding land functions resulting from the emerging bioenergy crop managements. Quantifying and monitoring land function changes caused by varied bioenergy crop managements in different regions will aid in optimizing bioenergy crops and management scenarios for marginal lands and reduce environmental risks and loss of ecosystem functions.
- Integrating social-economic factors into dynamic marginal land assessment tools for land use planning. Impacts of market and subsidies or incentives need explicit consideration of quantitatively determining dynamics of marginal lands.

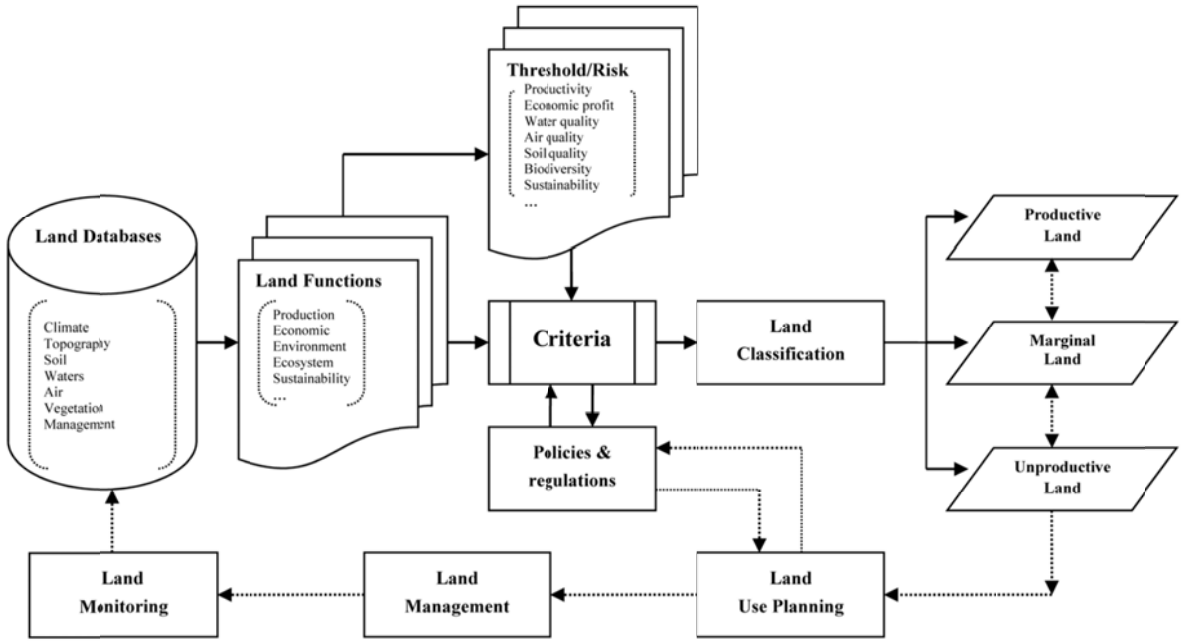

Figure 2. Land-informatics system directing assessment, management and monitoring of marginal land

8. Summary

The issue of marginal land use has triggered a wide discussion on future land use management for many countries and disciplines. Marginal lands play a critical role on the production of food and bioenergy under the growing pressure of limited land resources. However, a clarified concept and comprehensive understanding of marginal lands is needed that considers many intertwined land functions and social-economic impacts. Furthermore, effective comprehensive quantification assessment tools need continued development. Marginal land use planning can include multiple goals with best management practices and tradeoffs of concerns of environment, ecosystem services and sustainability under the framework of land-informatics. The debate on marginal lands will continue until our limited understanding of land functions and changes of management goals across time and space are improved.

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The Impact of Soil Erosion on Agricultural Potential and Performance of Sheshegu Community Farmers in the Eastern Cape of South Africa

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Abstract

Soil erosion is one of the unresolved problems of rural agriculture. This study investigates the impact of soil erosion on the agricultural potential and performance of Sheshegu community farmers in the Eastern Cape of South Africa. Structured interview scheduled was used to collect data from 50 respondents using simple random sampling. Findings revealed that most (62%) respondents are male, who are above 46 years old (68%). Most of whom (72%) had education above grade 7. Further, the majority of them (50.8%) depend on social grants as sources of income. Most respondents confirmed that erosion occurred naturally through heavy rainfall and persistent drought while human causes that facilitated erosion include farming activities, deforestation and indiscriminate bush burning that expose soil to impact of rain drop. Respondents affirmed that erosion contributed to poor health of livestocks due to lack of pasture grass to feed on, loss of grazing land and poor bush regrowth. It is recommended that awareness on the negative effect of human causes of erosion should be created while simple technologies on soil erosion control should be pushed to the farmers. Finally, edict on bush burning should be enforced to check indiscriminate bush burning.

Keywords: soil erosion, agricultural potential, farmers' perception

1. Introduction

Soil erosion is a critical global land degradation phenomenon affecting human beings since humanity's basic sources of livelihood is from the land. However, changes in land use worldwide have been recognized as capable of accelerating soil erosion (Chappell et al., 2010). Degraded soil is unproductive, which is also determined by the degree of severity to land damage. Soil worldwide is being degraded at a phenomenal rate. According to the Centre for Science and Environment (CSE) (1985), soil erosion affects between 25-30 per cent of the total land under cultivation in India. In South Africa over 70% of the nation's land surface has been impacted by varying levels and types of soil erosion (Pretorius, 1998; Garland et al., 2000; Le Roux, Newby, & Sumner, 2007). Similarly, FAO (1984) indicates that without any conservation measures, the total area of rain-fed cropland in developing countries in Africa, Asia and Latin America would in long-term get smaller by 544 million hectares because of soil erosion and degradation. On a global scale, the Food and Agriculture Organization (FAO) estimates that the loss of productive land through soil erosion globally is about 5-7 million ha/year (Kumar and Ramachandra, 2003). Many scholars projected that unless there is an adoption of better land management practices, about 140 million hectares of high quality soil, in Africa and Asia, would have been degraded as a result of soil erosion by 2010 (FAO, cited in Kumar & Ramachandra, 2003). This unveils the danger of soil erosion activities and the need for appropriate soil management practices, as well as a concerted effort in the fight against its effects.

According to Ojo and Johnson (2010) soil erosion is a dynamic geomorphic event operating on the landscape. Further, Jones (2007) defined soil erosion as "the wearing away of the land surface by physical forces such as rainfall, runoff water, wind, ice, temperature change, gravity or other natural or anthropogenic agents that abrade, detach and remove soil or geological material from one point on the earth's surface to be deposited elsewhere". However, Marsh and Grossa (2005) simply define soil erosion as the dislodgement of particles from the soil.

According to these definitions, there exist the gradual detachments of top-soil particles, which are transported by agents of denudation, such as water and wind, and subsequently deposited elsewhere. Toy, Foster and Renard (2002) explained that erosion can be "geologic" or "accelerated". Geologic erosion is naturally on-going within the earth crust. The erosion rate is so slow that it is of less importance to human beings. But accelerated erosion is caused by human-beings.The erosion rate is so high that it has resulted in different focuses by soil scientists and scholars in related disciplines.Therefore, this study is focussing on accelerated soil erosion activities in Sheshegu community.

Future world population requires increased food production (Pimentel, 2006). For example, the world population is said to have grown to 7.06 billion in middle of 2012, after having crossed the 7 billion mark in 2011 (Haub, 2012). Further, the 79.3 million people added to the overall global population each year has been consistent for nearly a decade (Engelman, 2010). This means that there is need to increase agricultural produce to feed this additional millions of people each year with food. Without the soil, this is not possible. Hence damage, through soil erosion or in any other forms, to the soil is an indirect damage to agricultural production and ultimately food security. According to Wall, Baldwin and Shelton (1987), the implication of soil erosion extends beyond the removal of valuable topsoil. In fact, crop emergence, growth and yield are directly affected through the loss of natural nutrients. Bathrellos, Skilodimou and Chousianitis (2010), commenting on how soil erosion could impact on farming, states that the main on-site impact of soil erosion is the reduction of soil quality which results from the loss of the nutrient-rich upper layers of the soil and the reduced water-holding capacity of many eroded soils.

Soil erosion has enormous negative impact on agriculture. Research shows that in most developing countries, especially Sub-Saharan Africa 60-70% of the population in the rural areas depend on agriculture to earn a livelihood (Loulseged & McCartney, 2000). The percentage of the population depending on agriculture is more in Ethiopia as about 85% of the population live in rural areas and they depend on agriculture to earn a livelihood (Düvel, Chiche & Steyn, 2003). It is therefore imperative that farmers' perception about the negative impact of soil erosion as it affects agricultural production be taken into consideration. This is because perception has a great influence in the type of behaviour displayed by farmers (Berelson & Steiner, 1964). According to Duvel (1991) among the reasons for most agricultural problems is the farmers' in-adoption or inappropriate adoption of agricultural technologies which are rooted in the perceptions displayed by the farmers. Perception, according to Lewin (1951), one of the foremost authors in the behavioural studies, as reported by Shaw and Constanzo (1970), is a behavioural product of individual life space or what he also calls the psychological environment. In this life space, perception plays a dominant role.

Therefore, this paper seeks, among other things, to unfold the impact of soil erosion on the agricultural potential and performance of farmers in Sheshegu community, in the Eastern Cape. The study also focuses on how farmers in the study area experience the impact of soil erosion on their agricultural productivity. The underlying questions are: do farmers view soil erosion in their area as a problem? If they do, how much of a problem do they see it to be? What are some of the underlying reasons for soil erosion problem in the area? And how does it affect their agricultural productivity?

2. Aim and Objectives of the Study

The aim of this paper is to have clear understanding of the challenges of soil erosion from the perception of the farmers and the necessity to implement conservation measures in the study area. The specific objectives are:

1) To identify farming practices that accelerates soil erosion in the study area.

2) To discuss the perception of the farmers in relations to the impact of soil erosion on the agricultural potentials and performance of the Sheshegu community.

3. Decription of the Study Area

Sheshegu is a small rural community in Nkonkobe Municipality, Eastern Cape Province of South Africa. It is located on the South West of Alice along R345 road to Peddie, and its position is on longitude 26° 44′47″.9 and 26° 50′ 55″.2E of Greenwich, and latitude 32° 53′ 06″.1S and 32° 56′ 36″.0S of the equator (Google earth, 2009). It is composed of six smaller villages, namely: Mpozisa, Skolwoni, Baluia, Lower Sheshegu, Sheshegu Fingo and Komkhalu respectively. Its lithology is of mudstone and sandstone, and it has a savannah type of vegetation. The inhabitants are mainly Xhosa speaking people relying on animal farming. Although the 2002 Census data indicates that the main land use type of Sheshegu is sheep farming (Statistics South Africa, 2002), there are other animals being reared in the area. Crop farming is hardly practised in the area due to the nature of the soil.

4. Methodology

The survey method of research was adopted for this study. Questionnaires were used during the data collection process. Data collection was through a one-on-one collection process, and the sampling technique adopted was the simple random sampling, in which the services of the researcher and three other survey assistants were employed. A Likert scale of 1-3 was adopted for the study, where 1, 2 and 3 represent insignificant, moderately significant and very significant respectively. A total of 50 farmers were interviewed and their responses form the basis of this study. Data analysis was executed using SPSS software package version 19 for both descriptive and inferential statistics.

4.1 Problem Conceptualization

The basis of problem conceptualization is based on the notion that "a problem well put, is a problem half solved" (Düvel, 1991). Problem conceptualization is a hypothetical construct providing a scientific basis for purposeful and systematic probing into the causes of a problem (Düvel, 1991). It is very relevant in agricultural studies, because it assists in the breaking down of agricultural problems into easily manageable units. The Düvel (1991) model was adopted for this study because of its particular relevance. It links human behaviour and its outcomes in an interrelated causal relationship with environmental factors through a decision making process (Ighodaro & Lategan, 2012).

4.2 Specific Variables Analysed (Dependent and Independent)

Table 1. Specific variables analysed in the study

Variables	Descriptions
Education level (independent)	Highest level of education as at the time of interview
Gender (independent)	Is the farmer a male or female?
Age of farmer (independent)	How old is the farmer as at time of interview?
Contribution of sources of income (independent)	Percentage contribution of various sources of income to total income of the farmer
Perceived severity of soil erosion (dependent)	Likert scale rating of farmers' perception on the severity of soil erosion
Perceived increase rate of soil erosion (dependent)	Likert scale rating of farmers' perception on increase of soil erosion in the past ten years
Perceived causes of soil erosion (independent)	Three main natural causes of soil erosion
	Three main human causes of soil erosion
Perceived impact of soil erosion on agricultural productivity and performance (independent)	Impact of soil erosion on farmers' profitability
	Impact of soil erosion on quality of product
	Impact of soil erosion on yield of crops
	Impact of soil erosion on farmers' sustainability

5. Results and Discussion

5.1 Demographic Profile of Farmers

Table 2 presents the demographic profile of the farmers in the study area. The results indicate that the majority (62%) are males while females (38%) are of the minority. The majority of the farmers are in the age group bracket of 46-55 years old (22%) and 66-75 years old (22%). The average age was 52.91 years old indicating the problem of ageing phenomenon (Ayinde, 2011) confronting the farming occupation nowadays. The youths are no longer interested in farming activities thereby migrating to the cities for white collar jobs. Similarly, majority (36%) of the farmers had educational attainment of between grades 7-10 while only 6% exceeded grade 12 showing that education level of farmers in the area is low. As reported, inadequate education and poverty are two key characteristics which impact on farmers' poor farming decisions and/ or perceptions which result to soil erosion (Pender & Hazell, 2000). Findings further indicated that most (50.80%) of the farmers depends on social grants as sources of income (Table 2).

From all indications in Table 2, apart from other income source which consists largely of the government welfare grants, farming (29.36%) seems to be the main source of income for farmers in the study area, which indicates the main human activity responsible for soil erosion in the area. Similarly Table 2 reveals that income from animal farming is largest, which seems to concur with the National Census data of 2002 that the main land use type of study area is sheep farming (Statistics South Africa, 2002) and thus the main farming type responsible for erosion in the area.

Table 2. Socioeconomic profile of Sheshegu farmers

Items	**Frequency**	**Percentage**	
Male	31	62%	
Female	19	38%	
Total	50	100%	
Age according to gender			
Age	**Frequency**	**Percentage**	**Mean**
<35	6	12	
36-45	10	20	
46-55	11	22	52.91 years
56-65	8	16	
66-75	11	22	
76-85	4	8	
Total	50	100	
Educational status	**Frequency**	**%**	
No formal education	6	12	
Grade 1-3	5	10	
Grade 4-6	10	20	
Grade 7-10	18	36	
Grade 11-12	8	16	
Diploma	3	6	
Total	50	100	
Sources of income			**Contributions (%)**
Government			13.94
Private			3.9
Farming			29.36
Self employed			2
Other sources (e.g grants)			50.8
Total			100
Types of farming practices by the farmers			**Contributions (%)**
Crop farming			5.85
Enclosed animal farming			30.05
Free-range animal farming			64.10
Total			100

Source: Survey research, 2010.

5.2 Basic Causes of Soil Erosion in the Study Area

Table 3 indicates the perceived main natural causes of soil erosion in Sheshegu community by the respondents. Findings reveal a wide variety of possible natural causes which are perceived to be responsible for the erosion in the research area. However, the basic ones in order of preference, are rainfall, drought, winds, and climate change. Speaking on the causes of soil erosion, Pimentel (2006) suggested that soil erosion results from actions that expose the soil to rainfall or wind. Similarly, citing Cook and Reeves (1976), Whitlow (1989) maintained that gully (which is one of the devastating forms of soil erosion) development is seen as the result of three sets of factors interacting together. These are (1) land use changes; (2) secular (long-term) climate changes; and (3) random frequency-magnitude variations such as inherent disadjustments in the long profiles of channels.

Table 3.Frequency distribution (%) of perceived natural causes of soil erosion in Sheshegu

Causes of erosion	**Priority 1**	**Priority 2**	**Priority 3**
Climate change/variation	4	10	6
Drought	38	14	10
Rainfall	36	39	25
Winds	16	18	20
Lack of adequate drainage	3	3	6
Nature of the soil	1	5	10
Slope/gradient of land	1	8	12
Bare land	1	3	11
Total	100	100	100

Source: Survey research, 2010.

Table 4 indicates that the types of agricultural activities on the field are the major factors responsible for the acceleration of erosion in the study area. Other human causes include building or construction, deforestation and indiscrimate veld/ bush burning. According to Zalidis et al. (2002), the detrimental effects of agricultural practices on soil quality include erosion, desertification, salinization, compaction and pollution. Ofomata (1975) confirmed that the intensive concentrations of domestic animals and the indiscriminate bush burning have increased the rate of soil erosion in Nigeria. Snyman (1999) maintained that although soil erosion is considered as a natural process, it is often accelerated by human activities such as clearing of vegetation (deforestation) or by overgrazing.

Table 4. Frequency distribution (%) of the perceived human causes of soil erosion in Sheshegu

Human causes	**Priority 1**	**Priority 2**	**Priority 3**
Agricultural activities	28	18	16
Animal/human footpath	8	12	8
Building purposes	20	24	16
Deforestation	20	20	8
Tradomedical uses	2	4	2
Veld fires/burning	14	12	10
Irrigation water	0	2	0
Improper soil management.	0	0	4
No response	8	8	36
Total	100	100	100

Source: Survey research 2010.

5.3 Perceived Impact of Soil Erosion on the Agricultural Potential and Performance in the Study Area

Table 5. The impact of soil erosion on Sheshegu farming operations

Impact of erosion on Sheshegu farming	F1	P1	F2	P2	F3	P3
Affects animal health (negatively)	9	18	18	36	6	12
Affects human health	-	-	3	6	-	-
Affect farmers' income	-	-	-	-	3	6
Drought	3	6	3	6	3	6
Development of gullies	-	-	2	4	2	4
Destruction of plants	-	-	-	-	1	2
Lack of shade for animals	-	-	1	2	-	-
Poor growth/production of crops	8	16	7	14	2	4
Shortage of grazing/farmland	23	46	2	4	5	10
Shortage of land for other uses	-	-	1	2	-	-
Siltation of dams	1	2	1	2	3	6
Unproductive soils	2	4	4	8	-	-
No response	4	8	8	16	25	50
Total	50	100	50	100	50	100

Source: Survey research 2010 (F= Frequency and P= Percent).

According to Table 5 above, the impact of soil erosion on the agricultural potential of study area was expressed in terms of three basic indicators: negative effect on animal health, shortage of grazingland and farmland and poor production of crops. The data collected proves that these three variables score the highest responses.

In the same table, the cumulative percentage responses of individual farmer in the study area are expressed as follows: perceived impact of soil erosion on Sheshegu farming operations indicates a 66% for negative effect on animal health, 60% for shortage of grazingland or farmland and 34% for poor crop growth. The implication of this is that agricultural production and overall rural development is under great danger. This is because, animals, which is the main farming type in Sheshegu is affected negatively, agricultural lands are progressively being reduced due to erosion activities, and growth of crops is being affected negatively as well.

From the study (Table 6), soil erosion was also perceived to impact significantly on different farming efficiency or performance aspects (profitability, product quality, yield and sustainability) in the study area. Farmers indicated that soil erosion impacted the most significantly on their profitability, product quality, yield and sustainability through the detrimental impact it has on grazingland, production of crops and on animal health, as depicted in the Table 6 below.

Table 6. Perceived impact of erosion on selected aspects of Sheshegu farming performance

Soil erosion impact on farming	Profitability			Product quality			Yield			Sustainability		
	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
Affects animal health	7	15	4	9	15	3	6	6	2	6	16	5
Affects human health	-	1	-	-	1	-	1	1	-	1	-	-
Affects farmers' income	1	1	1	1	1	1	1	1	1	1	1	1
Drought	2	3	2	3	2	3	3	3	3	3	2	3
Gully development	2	4	-	2	4	-	1	1	-	-	5	1
Destruction of plants	1	-	-	1	-	-	1	1	-	1	-	-
Lack of shade for animals	-	-	1	-	-	1	-	-	1	-	-	1
Poor growth of crops	10	6	-	8	6	1	8	8	2	10	5	-
Shortage of grazing/farmland	17	6	3	16	6	5	17	17	4	12	9	5
Shortage of land for other uses	-	1	1	1	1	-	1	1	-	1	1	-
Siltation of dams	1	2	-	1	2	-	1	1	-	1	2	-
Unproductive soils	2	3	1	4	2	1	3	3	-	5	2	-
No response	7	8	37	4	10	35	7	7	37	9	7	34

Source: Survey research 2010 (F= Frequency).

6. Conclusion

Amongst other things, soil erosion poses great danger to agricultural development anywhere in the world. The Eastern Cape, where this study was conducted, is regarded as one of the three most degraded provinces in South Africa. According to the findings in this study, the impact of soil erosion, in terms of its severity and rate on the agricultural potential and performance of farmers in Sheshegu community, is found to be negatively high. Similarly, the three most significant natural causes of erosion in the area are heavy rainfall, drought, and strong winds, while in terms of human causes, agricultural activities seem to enjoy the highest priority, followed by building purposes and deforestation activities. However, soil erosion impact is expressed in the form of negative effect on the health of animals, shortage of grazingland or farmland and poor growth of crops. It is therefore recommended that conserted efforts should be made towards educating the farmers on the danger inherent in inappropriate farming activities, indiscriminate bush burning and deforestaion. Edicts on bush burning should be enforced. Finally, simple methods of soil erosion control should be pushed to the farmers by extension services.

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Determination of Resistance to *Phytophthora infestans* on Potato Plants in Field, Laboratory and Greenhouse Conditions

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Abstract

An experiment was conducted to determine the host resistance of potato against *Phytophthora infestans* for twenty-five potato genotypes in 2010 and 2011 at Khumaltar, Lalitpur, Nepal using four assays: three for foliage resistance (field, whole-plant and detached leaf) and one for tuber resistance (tuber slice). An isolate of *P. infestans* collected from Lalitpur (LPR-1) was used for inoculation at a concentration of 3 x 10^3 sporangia ml^{-1} in all assays. Infected foliage area in the field and whole-plant assays, lesion size on detached leaves, and colony growth on tuber slice were all individually converted to 0-9 interval scale for susceptibility. Field assessment, considering the most robust measure of resistance, was used as benchmark for comparing the other assays. Sixteen of the genotypes had very little disease in the field (scale value <1) indicating they were probably expressing race-specific resistance, which has historically been short lived. Susceptibility levels measured in the whole-pant assay were highly correlated (r = 0.90) with converted field scale values, although the correlation was lower for the detached leaf assay (r = 0.63) and least for tuber-slice assay (r = 0.46). Low correlation in the detached leaf assay was assumed to represent lower resolution of the single-cycle assay. Low correlation in the tuber assay may have also reflected genetic differences as foliage and tuber blight resistance are not always correlated. Genotypes with extreme resistance in the field were frequently identified as having partial resistance in the other assays, which could mistakenly be interpreted as more durable field resistance. The consequences for selecting durable resistance are discussed.

Keywords: detached leaf, *Phytophthora infestans*, potato, resistance, susceptibility, tuber slice assay

1. Introduction

Potato late blight, caused by the Oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, is one of the primary problems faced by small-scale potato producers worldwide. In potato, the third largest global food crop after wheat and rice, per year yield losses and fungicide costs in developing countries alone were estimated at over 10 billion Euros (Haverkort, Struik, Visser, & Jacobsen, 2009). In addition to economic losses, the disease poses a threat for food security, human health and environment (Kromann, Taipe, Perez, & Forbes, 2009).

In Nepal, potatoes are grown across varying geographic areas ranging from 75 to 4700 m a s l with different planting seasons: November in low lands, September and January (two seasons) in the mid hills and February/March in the high hills. In Nepal, potato is grown on 182,600 ha for the total production of 2,508,044 Mt, with a productivity of 13.74 t ha^{-1} (MOAC, 2011) and potato contributes 9.4% of the national agriculture gross domestic product (AGDP) (MOF, 2010). Late blight is one of the major biotic constraints in potato production in Nepal. It has been estimated that monitory loss due to this disease was approximately 104 million US$ in FY 2009/2010, based on the total production of that year, an estimated 15% average loss and an average potato price of 176 US$ ton^{-1}. In addition, a large amount of money is routinely spent to manage the crop by frequently applying fungicides e.g., 10-15 times per crop in the autumn season in the Kathmandu valley (Sharma, Khatri-Chhetri, Dhital, Khatri-Chhetri, & Chand, 2007).

One of the most effective and efficient ways to control any plant disease is with host plant resistance.In the case of potato late blight, however, efforts to control the disease with host resistance have been limited by many factors,

including market preference of susceptible varieties, lack of seed distribution systems to diffuse new varieties and rapid evolution in the pathogen population toward new pathotypes which overcome race-specific resistance. Many cultivars released as resistant have lost their resistance against new pathotypes in the pathogen population. In contrast, resistance in some cultivars has held for longer time (Forbes, 2012). For example, Janakdev (CIP-720123), which is one of the most popular potato varieties in Nepal, has maintained its resistance for many years.

Resistance in potato to *P. infestans* has been previously classified as either vertical (generally absolute and race-specific) or horizontal (partial and effective against all pathogen races) (Van der Plank, 1963). Subsequent research demonstrated that race-specific resistance is governed by major resistance genes (R genes) and their interaction with pathogen effectors (Vleeshouwers et al., 2011). This categorization has been questioned because further research into the genetic and mechanistic basis of resistance have demonstrated that in some ways the two resistance types both involve a hypersensitive reaction (Kamoun, Huitema, & Vleeshouwers, 1999). Furthermore, the majority of research on resistance to *P. infestans* in recent years has focused on the simultaneous use (stacking) of several R genes newly discovered from wild potato species (Tan, Hutten, Visser, & Eck, 2010). Regardless of genetic and cellular mechanisms underlying resistance, for the purpose of this study, it is important to recognize that resistance phenotypes may be expressed in one of two ways: i) as apparent immunity, with no visible symptoms or with an indication of hypersensitivity, or ii) by varying levels of disease severity. Resistance of the second phenotype would appear to have less risk of being rapidly overcome by pathogen evolution (Forbes, 2012). The partial resistance phenotype is measured by a specific component of disease or a synoptic measure of overall disease severity through the season, such as the area under the disease progress curve (AUDPC). Recently, an interval scale for resistance in potato to *P. infestans* was proposed by Yuen and Forbes (2009) to quantify susceptibility of the partial phenotypic expression of resistance.

Resistance to tuber blight caused by *P. infestans* may (Platt, 1998) or may not (Black, 1970; Inglis et al., 1996; Dorrance & Inglis, 1997; Kirk et al., 2001) be correlated with foliar resistance. Therefore, it is essential to test breeding lines for susceptibility to *P. infestans*in both tubers and foliage (Douches et al., 2002).

The National Potato Research Program (NPRP) of Nepal regularly evaluates potato genotypes in multiple locations for resistance to *P. infestans* in replicated field trials, in which the effect of resistance on the polycyclic development of the disease can be assessed. Resistance can also be evaluated in more convenient and often less expensive monocyclic or oligocyclic green-house or laboratory assays in which one or more epidemic components, such as lesion expansion rate or sporulation, are measured (Dorrance & Inglis, 1997). This then leads to the question, to what degree do green-house and laboratory evaluations correlate with results obtained in the field?

The purpose of this investigation was two-fold: i) to identify new sources of resistance to *P. infestans*; and ii) to determine the efficiency of two non-field screening methods for foliage blight resistance and one method for tuber blight resistance and compare results from these assays with resistance levels established in the field.

2. Materials and Methods

2.1 Field Assay

Twenty-five potato genotypes, including one standard susceptible check, were evaluated for resistance to *P. infestans* at Khumaltar (1360 m a s l), a location representing the mid-hills agro-climatic conditions. Planting was done during the second week of September, which is the second or "fall" season in this region and the most conducive to disease development. Ten tubers of each potato clone were planted with a spacing of 60 cm between and 25 cm within rows, which were 2.5 m long, giving a plot size of 1.5 m^2. Healthy looking tubers of similar physiological age were used. Genotype LBr-40 and KufriJyoti were used in the experiment as resistant and susceptible checks, respectively. Desiree, a susceptible variety, was planted around the border of the experiment plots to increase inoculum pressure of *P. infestans*. Prior to planting, fertilizer (100:100:60 kg N, P_2O_5, K_2O ha^{-1} respectively along with 20 t ha^{-1} farmyard manure) was applied.Weeding, earthing-up and irrigation were applied two times at 30 and 50 days after planting. No fungicide was applied throughout the crop period.

At 25 DAP, a suspension of 3 x 10^3 sporangia ml^{-1} of *P. infestans* isolate LPR-1 was applied to plants using a 0.5 liter plastic atomizer. Disease severity scoring began with the first appearance of symptoms and continued until the susceptible check reached 100% of foliage area damaged. Scoring of the disease severity was based on a visual estimation of percent foliage damage and was done at 30, 41, 54, 60, 69 and 76 DAP. Percent severity values were converted to the area under the disease progress curve (AUDPC) using the midpoint formula (Campbell & Madden, 1990; Madden & Hughes, 1995).

2.2 Screen House Assay

The 25 potato genotypes, which were screened under field conditions, were also evaluated in a whole-plant inoculation assay in screen house, where temperatures ranged from 15 to 25^{o}C and relative humidity ranged from 75 to 90 percent. When plants were approximately at flowering stage, each plant was covered with a transparent plastic bag from 24 hr before until 48 hr after inoculation to maintain high relative humidity for better initiation of infection. Spray inoculum was prepared using isolate 'LPR-1'. A sporangial suspension was prepared from 5 days old cultures grown in Petri dishes. Five ml of inoculum at 3 x 10^3 sporangia ml^{-1} $plant^{-1}$ were applied to cover all expanded leaves of test plants using 100 ml glass atomizer. Sterile distilled water was applied on the check plants. After establishing infection, the plastic covers were removed and plants were misted three times daily at 10, 14 and 18 hr for 30 days after the plastic bags were removed. Late blight severity was recorded as percent foliage area damaged on each plant at 30 days after inoculation, using the same visual evaluation method as the field.

2.3 Detached-Leaf Assay

Plants grown in a screen house as described earlier were used for this assay. The apical leaflets of leaves growing in the middle of plants near flowering stage were taken for inoculation. Prior to inoculation, leaflets were washed with sterilized distilled water and placed abaxial surface up in moist chambers, made of transparent plastic boxes (20.3 x 25.4 x 12.7 cm) containing two layers of water-soaked blotting papers at 1.5 cm below and 3.0 cm above the slices.

One 50 µl drop of inoculum (3 x 10^3 sporangia ml^{-1}) was placed on each leaflet using a micropipette. Sterile distilled water was applied on the check leaflets. Three leaflets per genotype were used as sub-samples. Inoculated leaflets were incubated at 16 ± 0.5°C with 12 hr light cycle. On the 7th day after inoculation, the mean diameter of the lesion on each leaflet was measured as described by Nilsson (1981). The experiment was repeated twice.

2.4 Tuber Slice Assay

Three medium-sized, apparently healthy tubers of each of the 25 genotypes were washed with tap water, soaked in 2% sodium hypochlorite for 5 min, rinsed with distilled water three times and air-dried. Five-mm-thick sections from the middle of each tuber were cut with a sterilized knife. One 50 µl drop of inoculum with 3 x 10^3 sporangia ml^{-1}, prepared as for the whole-plant assay, was placed at the center of each tuber slice. Four tuber slices per genotype were replicated thrice. Slices were placed in plastic boxes, lined with moist paper towels and incubated at 16 ± 0.5°C with 12 hr light cycle for 6 days. Percent area of tuber slice colonized by *P. infestans* was estimated by visual observation on the 6th day. The experiment was done twice within 15 days under similar environmental conditions.

2.5 Standardization of Resistance Measures

An interval scale (0-9) as per Yuen and Forbes (2009) was used to standardize data from the field and the other assays as follows:

$$\text{Standard scale } (0-9) = \frac{\text{Severity of test plant}}{\text{Severity of control}} \text{X } 9.0$$

Where 'severity' was the AUDPC for the field, percent foliage damage in whole-plant, mean lesion size (cm^2) for the detached leaf and percent area covered by colony for the tuber slice assay. The formula above is for a control which has an assigned severity value of 9; if the assigned value were different, the numbers in the formula would change accordingly. In cases where experiments were repeated, the average of experiments was used.

3. Results

3.1 Field Assay

Late blight severity observations in the field started from 30 DAP and continued up to 76 DAP when the susceptible check KufriJyoti had 100% infection with an AUDPC of 2361 (Table 1). Many of the other genotypes were immune or had very little disease at the end of the evaluation period, with 16 of them having a susceptibility scale value of 1 or lower (Table 2). The susceptibility scale data from the field produced a clear bimodal frequency distribution indicating that many of the potato genotypes were probably incompatible with the local pathogen population (Figure 1). Several genotypes had values between 2 and 5 indicating moderate levels of resistance (Table 2).

Table 1. Reaction of potato genotypes to *Phytophthora infestans* isolate (LPR-1) under field, screen house, detached leaves and tuber slices inoculated conditions at Khumaltar, Lalitpur in 2010

Clones	AUDPC in field inoculation assays	Severity under screen house (%)	Lesion size in detached leaf assay (cm^2)	Area covered by colony (%)
PRP 35861.2	94	11.7	0.8	78.9
PRP-85861.12	93	18.3	1.2	4.4
PRP-25861.10	61	20.0	0.4	12.8
PRP-225861.2	120	43.3	0.7	66.1
LBr-40 (Resistant check)	0	0.0	0.0	4.0
PRP-85861.8	77	35.0	3.6	15.9
L 235.4	916	60.0	DM	91.1
PRP-276264.01	26	5.0	2.5	65.0
PRP-266264.01	0	0.0	3.2	41.7
BSUPO3	27	5.0	4.7	96.1
KufriJyoti (Susceptible Check)	2361	100.0	6.5	100.0
CIP-384321.15	28	5.0	0.7	23.3
PRP- 25861.1	39	18.3	1.9	12.6
CIP-388580.6	1054	50.0	4.8	22.8
CIP-394050.110	1287	58.3	4.7	20.8
CIP-393385.39	31	20.0	4.7	11.3
PRP-266264.15	0	11.2	4.8	25.0
Janakdev (CIP-720123) Check	1153	70.0	5.5	36.4
CIP-393280.57	452	5.7	0.8	65.0
PRP-25861.11	103	11.7	0.4	9.4
PRP-85861.11	49	16.7	4.8	7.2
CIP-393077.54	713	26.7	4.8	78.3
CIP-392657.8	29	15.0	0.5	16.1
CIP-385499.11	1335	90.0	5.8	77.2
CIP-389746.2	692	46.0	4.2	67.8
P value	<.001	<.001	<.001	<.001
LSD (0.05)	134.46	6.068	1.313	6.938
CV %	19.1	9.5	16.2	8.1

DM: Data missing.

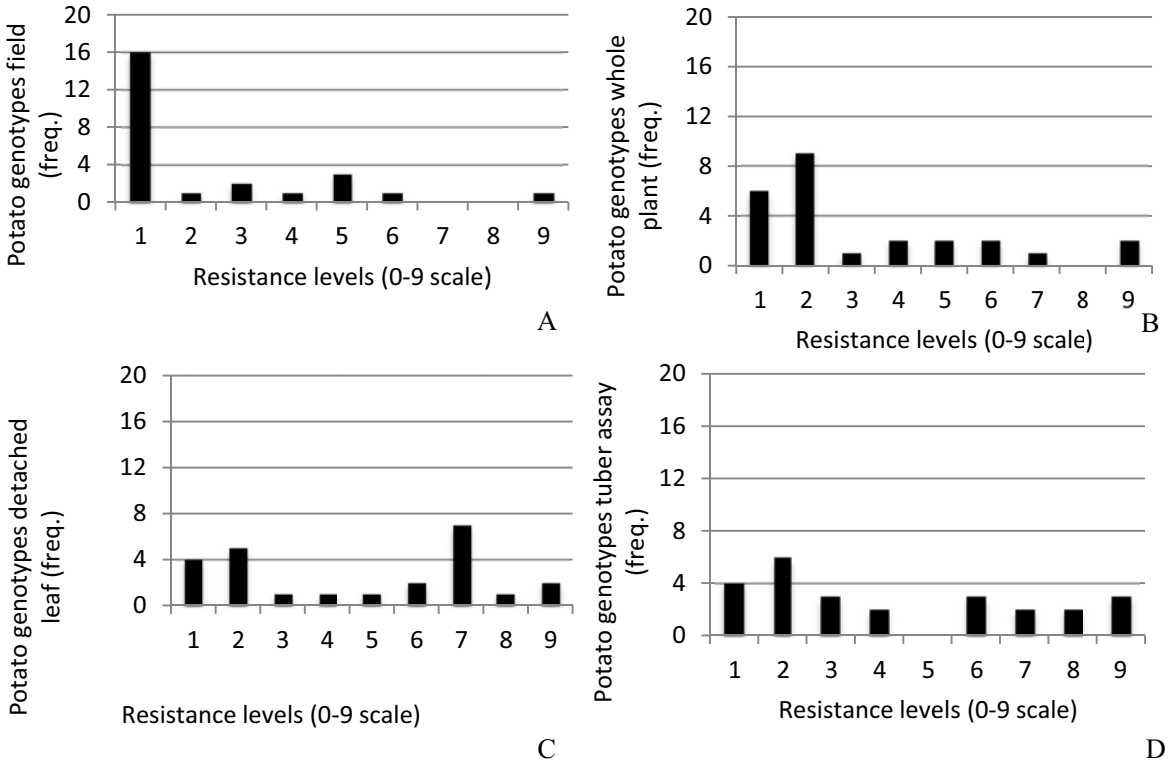

Figure 1. Frequency histogram of 25 potato genotypes evaluated for resistance levels against the late blight pathogen *Phytophthora infestans* in the field (A), on whole-plants in the screen house (B), by detached leaf inoculation (C) and by tuberslice inoculation (D) in Nepal

3.2 Whole-Plant, Detached-Leaf and Tuber-Slice Assays

Results of the whole plant, detached leaf and tuber slice assays were similar to field results in that a number of genotypes were extremely resistant or had even immune phenotypes (Table 1). However, the pattern differed among the assays; the three non-field assays all had fewer plants with a score of 1 or less (Table 2) and also had generally flatter distributions of scale values (Figure 1). Thus, some plants that were immune or nearly immune in the field became infected with the screen house and laboratory assays. Nonetheless, the resistant and susceptible checks were consistent in each assay (Table 1).

3.3 Correlations among Assays

All three assays (whole-plant, detached leaf and tuber slice) were significantly correlated with field data, although the highest correlation was with whole-plants (r = 0.90), followed by the detached leaf (r = 0.63) and finally the tuber assay (r = 0.46) (Figure 2). The contrast among assays noted above could also be observed in this analysis; many plants with immune (or near-immune) reactions in the field had variable reactions in these assays. The lower correlation values are primarily an indication of the degree to which high levels of field resistance was not detected in the assays (Figure 2).

Table 2. Scale values for resistance against *Phytophthora infestans* in 25 potato genotypes evaluated in the field, screen house, with detached leaves and tuber slices in Nepal in 2010

Genotype	Scale values			
	Field	Whole-plant	Detached leaf	Tuber slice
LBr 40 **(C)**	0.0	0.0	0.0	0.4
PRP-266264.01	0.0	0.0	4.6	3.8
PRP-266264.15	0.0	1.0	6.8	2.3
PRP-276264.01	0.1	0.5	3.5	5.9
BSUPO3	0.1	0.5	6.7	8.7
CIP-392657.8	0.1	1.4	0.7	1.5
CIP-384321.15	0.1	0.5	1.0	2.1
PRP- 25861.1	0.1	1.7	2.6	1.1
CIP-393385.39	0.1	1.8	6.6	1.0
PRP-85861.11	0.2	1.5	6.8	0.7
PRP-25861.10	0.2	1.8	0.6	1.2
PRP-85861.8	0.3	3.2	5.0	1.4
PRP-85861.12	0.3	1.7	1.7	0.4
PRP-225861.2	0.3	3.9	1.0	6.0
PRP 35861.2	0.4	1.1	1.1	7.1
PRP-25861.11	0.4	1.1	0.6	0.9
CIP-393280.57	1.7	0.5	1.2	5.9
CIP-389746.2	2.6	4.1	6.0	6.1
CIP-393077.54	2.7	2.4	6.7	7.1
L 235.4	3.5	5.4	DM	8.2
CIP-388580.6	4.0	4.5	6.8	2.1
Janakdev**(C)**	4.4	6.3	7.7	3.3
CIP-394050.110	4.9	5.3	6.6	1.9
CIP-385499.11	5.1	8.1	8.2	7.0
KufriJyoti**(C)**	9.0	9.0	9.0	9.0

C: Control variety, DM = Data missing.

Those values from whole-plants, detached leaves and tubers that are underlined had values of 2 or more (when values are rounded up), while having values of 1 or less in the field.

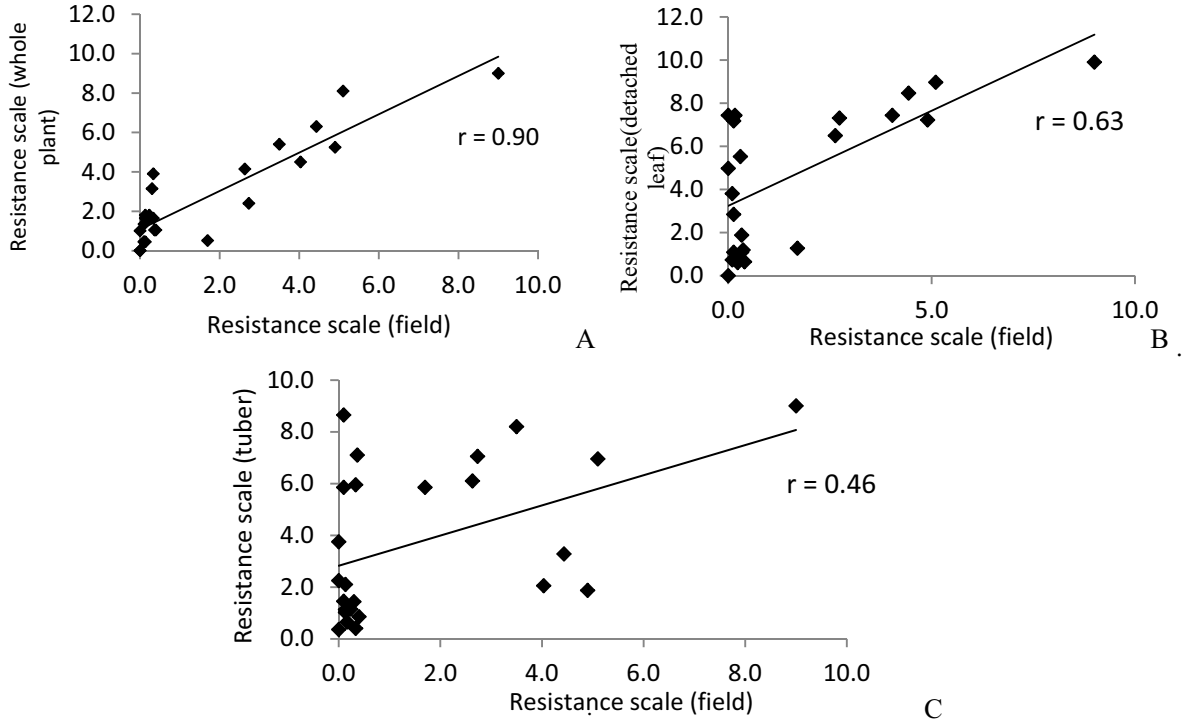

Figure 2. Resistance scale values in 25 potato genotypes against *Phytophthora infestans* as measured in the field correlated with resistance scale values measured in whole plants in a screen house (A), on detached leaves (B) and on tuber slices (C)

4. Discussion

This research demonstrated that several different assay tools can be used to evaluate genotypes of potato for their phenotypic reaction to *P. infestans*, and that in general, these assays will give results which are statistically correlated. Furthermore, the resistant and susceptible controls gave consistent results in all theassays. However, the study also demonstrated that while different tools generally give similar results, the results are not completely consistent and this should be taken into consideration.

Decades of research on potato late blight have demonstrated that highly resistant (immune or nearly immune) phenotypes can frequently indicate an active major R gene, for which compatibility in the pathogen population is absent or, more likely, extremely rare. If an incompatible potato genotype is released for use by farmers in most cases there will be selection of compatible pathogen population and a corresponding "loss" of resistance (Forbes, 2012). For this reason, some researchers have recommended selection of those phenotypes which demonstrate resistance, but are still infected (Forbes & Landeo, 2006).

There were problems with the divergence in results from the resistance assays we tested which would make identification of a quantitative resistance phenotype difficult. In our test, the field evaluation of resistance indicated that 16 of the materials had little or no disease at the end of the season (Table 1). However, in the whole-plant (screenhouse) assay, eight of these 16 genotypes had values of 2 or greater on the resistance scale (when rounded up, Table 2) and thus one might assume that these materials have partial resistance, which could represent a high level of field or quantitative resistance. This also occurred with the detached leaf assay, where nine of the 16 highly resistant genotypes (from the field) had resistance scale values of 2 or more (Table 2); this apparent miss-identification of quantitative resistance in both the whole-plant and detached leaf assays did not always occur with the same genotypes (Table 2). Some genotypes from the 16 highly resistant clones in the field were also identified as partially resistant by the tuberslice test and a few were among the most susceptible, but tuber resistance and foliage resistance have been shown previously to be poorly correlated (Dorrance & Inglis, 1997) and resistance in leaves and tubers may be governed by different genetic systems and/or reflect structural differences in the tissues. Some genotypes showing high level of resistance under field conditions were found most

susceptible in tuber slice assay which also reflected genetic differences as foliage and tuber blight resistance are not always correlated.

Three major components are known to contribute to late blight resistance in tubers; 1) a physical barrier consisting of several layers of phloem cells, known the periderm; 2) the outer cortical cell layers that retard the growth of lesions and can completely block hyphal growth; and 3) medulla storage tissues which can reduce hyphal growth and sporulation of *P. infestans* (Flier,Turkensteen, & Mulder, 1998; Flier, Turkensteen,Van den Bosch, Vereijken, & Mulder, 2001; Pathak & Clarke, 1987). Furthermore, immature tubers are more susceptible to tuber blight than mature tubers (Grinberger, Kadish, & Cohen, 1995), which means that assayed tubers should be of similar age.

It is not possible to know at this time if all 16 genotypes found to have extremely high levels of resistance in the field do in fact indicate the presence of active major genes that would not be durable, but in at least one case we can assume this is the case based on previous experience. Genotype CIP- 393385.39, which had a resistance score of 0.1 in the field in Nepal, was also recently evaluated in Ethiopia where it was found to be highly susceptible (E. Schulte-Geldermann, personal communication). Thus, we assume that should CIP-393385.39 be adopted by farmers in Nepal, it would rapidly select for pathogenic strains in the pathogen population and the host resistance would become ineffective. Unfortunately, similar information is not available for the other 15 materials that were found to be extremely resistant.

Eight of the potato genotypes evaluated in the field had score value between 2 and 5. It cannot be concluded that this resistance is of "horizontal" nature (effective against all pathotypes) but it would appear more probable that this resistance is durable, as we are aware of only a few cases when partial resistance has been clearly associated with short-lived R genes. One of the materials we evaluated, Janakdev, has been widely grown for years in Nepal and its moderate level of resistance appears to be stable. Thus, the remaining seven partially resistant genotypes are good candidates for further evaluation. Those genotypes which were extremely resistant should be considered as having an undetermined level of resistance until they can be confronted with compatible isolates; potato varieties that have "lost" R gene resistance frequently have a moderate level of residual resistance which is still useful.

The reasons of inconsistency between field values and those from the other assays are not known. It appears logical that a multi-cyclic test could give different results than mono- or oligocyclic tests for partial resistance, but this is not the case for extreme resistance. At this time, we do not know why some genotypes found to be extremely resistant in the field became infected in the detached leaf and whole-plant assays. It is possible that the stresses associated with the incubation methodology, or with cutting for the detached leaf test, created a situation highly favorable for the pathogen, unfavorable for the host, or both.

Our work would seem to be consistent with others (Dorrance & Inglis, 1997; Douches et al., 2002) who claimed that screen house and laboratory assays cannot replace the value of evaluating germplasm for foliar resistance to late blight under field conditions. Whole-plant assays gave results relatively similar to the field, but one should be careful in the interpretation of results, particularly for those cultivars with apparently very high levels of quantitative resistance. Nonetheless, the whole-plant assay may serve to screen out highly susceptible potato genotypes.

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Response of Nebraska Horseweed (*Conyza canadensis*) Populations to Dicamba

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Abstract

Dicamba-resistant soybeans are being developed to provide an additional herbicide mechanism-of-action for postemergence weed control in soybean. Numerous broadleaf species, including horseweed, have evolved resistance to glyphosate. It is anticipated that dicamba will be used by farmers as a primary tool to manage these weeds. Studying and understanding variability in horseweed response to dicamba will aid in developing appropriate risk management strategies to extend the utility of the dicamba-resistance technology. Horseweed plants from ten Nebraska populations were treated with one of nine doses of dicamba in greenhouse experiments. At 28 days after treatment (DAT) visual injury estimations were made and plants were harvested to determine dry weight. There was a three-fold difference in the I_{90} (90% visual injury estimate) between the least (638 g ha^{-1}) and most (205 g ha^{-1}) susceptible populations. Two plants from five populations were observed for an additional three months. No plants treated at doses above 280 g ha^{-1} survived to set seeds. These results suggest that maintaining use doses of 560 g ha^{-1} or greater may fully control horseweed populations from Nebraska and minimize the risk of plants surviving to set seed, in addition to practicing other proven herbicide-resistance management strategies.

Keywords: Canada fleabane, dose-response, herbicide resistance, marestail, risk assessment

1. Introduction

In the U.S., 90% of the soybean [*Glycine max* (Merr.) L.] acreage is planted to glyphosate-resistant varieties (Johnson, Strom, & Grillo, 2008). The exclusive use of glyphosate for burndown and postemergence weed control in soybean resulted in the selection of the first glyphosate resistant horseweed population in Delaware in 2000 (VanGessel, 2001). Since 2000, glyphosate-resistant horseweed populations have been reported in 16 U.S. states as well as Brazil, China, Spain, and the Czech Republic (Heap, 2013). Glyphosate-resistant horseweed is problematic in soybean fields because few herbicides that control it are labeled for postemergence use in soybean. In addition, horseweed populations have also evolved resistance to other herbicide modes-of-action, including acetolactate synthase (ALS) inhibitors, cell membrane disrupters, photosystem I inhibitors, and photosystem II inhibitors (Heap, 2013). Horseweed populations that are resistant to multiple herbicide mechanisms of action are particularly difficult to manage (Kruger, Davis, Weller, & Johnson, 2010). In no-tillage cropping systems dicamba and 2,4-D are effective and economical for controlling horseweed prior to planting (VanGessel, 2001), but are not available for use immediately prior to planting nor after soybean has emerged.

Transgenic technologies conferring herbicide-resistance to dicamba or 2,4-D are being developed to complement glyphosate-resistance traits in corn, soybean and cotton (Johnson et al., 2010; Peterson et al., 2009; Simpson et al., 2009). Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a synthetic auxin herbicide that controls a number of important broadleaf weeds in cereal crops. The gene encoding an enzyme from *Pseudomonas malthopilia* that metabolically inactivates dicamba was isolated and inserted into soybean. Plants expressing the trait tolerate 2800 g ha^{-1}, ten times a typical dose of 280 g ha^{-1} commonly used in corn (Behrens et al., 2007). There are 29 weed biotypes that have evolved resistance to synthetic auxin herbicides (Heap, 2013), and only five species are reported to be resistant to dicamba: common lambsquarter (*Chenopodium album* L.) in New Zealand, common hempnettle

(*Galeopsis tetrahit* L.) in Canada, kochia (*Kochia scoparia* L.) in MT, NE, ND and ID, prickly lettuce (*Lactuca serriola* L.) in WA, and wild mustard (*Sinapis arvensis* L.) in Canada and Turkey (Heap, 2013).

With the potential commercialization of dicamba-resistant soybean, agriculture has the opportunity to steward the new technology in a way that will not repeat the lost efficacy resulting from the evolution of glyphosate-resistant, ALS-inhibitor resistant and other herbicide resistant weeds. In a survey sent to weed science scientists, agronomists and farmers asking them to assess the risk likelihood of various weeds evolving resistance to dicamba after commercialization of dicamba-tolerant soybean, 25% rated horseweed as having a high risk and 46% rated horseweed a moderate risk (Crespo, Bernards, & Peterson, 2012). Managing for pesticide resistance ideally is proactive, not reactive. To best implement proactive resistance management, factors such as potential selection pressure resulting from the herbicide use pattern and species variability should be identified, classified and systematically assessed. Given the widespread distribution of glyphosate resistant horseweed populations in eastern Nebraska (Heap, 2013), greenhouse bioassays using dose-response methodology represents a tool to monitor baseline levels of susceptibility and variability in response to dicamba dose across horseweed populations in Nebraska. Therefore, a greenhouse bioassay was conducted to monitor the baseline susceptibility of ten southeastern Nebraska horseweed populations to dicamba dose in terms of biomass production and visual injury. In addition, we collected preliminary information on long term survival and seed production of dicamba-treated horseweed plants.

2. Materials and Methods

2.1 Population Sampling

Seed of ten horseweed populations were collected from seven southeastern Nebraska counties (Butler, Cass, Lancaster, Otoe, Saunders, Seward and York) in September and October, 2009. The seed was collected from roadside populations and populations present in corn and soybean fields. Each horseweed population was a composite of 40 or more plants. Horseweed seed was cleaned and stored at 4°C.

2.2 Plant Growth and Dicamba Application

The experiments were conducted in the greenhouses located on East Campus of the University of Nebraska-Lincoln in Lincoln, Nebraska. Supplemental lighting in the greenhouse provided a 15 h photoperiod. The day/night temperatures were 24 ± 2°C and 19 ± 3°C, respectively. Seed from each horseweed population was planted in potting mix (Miracle-Gro Moisture Control Potting Mix, The Scotts Company LLC, OH, USA) in 50 by 35 by 10 cm black plastic flats. Flats were watered daily to ensure adequate soil moisture. Two weeks after planting three healthy seedlings (three to five leaves) were transplanted into a 10 by 10 by 12.5 cm black plastic pot. Plants were watered as needed. Prior to treatment with herbicide, seedlings were thinned to one plant per pot.

Plants were treated when horseweed rosettes were 8 to 12 cm wide (12 to 16 days after transplanting). Herbicide treatments were applied in a research chamber sprayer (DeVries Mfg. Corp., Hollandale, MN, USA) using a TP8001E flat-fan nozzle tip (Spraying Systems Co., North Avenue, Wheaton, IL, USA), 190 L ha^{-1} carrier volume and a spray pressure of 207 kPa.

2.3 Dose-Response Bioassay

A dicamba dose-response bioassay with nine dicamba doses was conducted for each horseweed population and was repeated twice in time for a total of two runs. The experiment was arranged in a randomized complete block design with seven replications. The dicamba doses were 0, 8, 17, 35, 70, 140, 280, 560 and 1120 g ae ha^{-1} of dicamba (diglycolamine salt of 3,6-dichloro-2-methoxybenzoic acid) (Clarity, 480 g L^{-1}, Herbicide, BASF Corporation, NC, USA).

2.4 Data Collection and Statistical Analysis

Visual injury estimates of treated plants were based on growth suppression and epinastic effects compared to the nontreated control plants. Estimates were recorded at 7, 14, 21 and 28 days after treatment (DAT) on a scale of 0 (no injury) to 100 (dead plants). At 28 DAT, plants were cut at the base of the rosette, oven dried for 2 days in a forced air dryer at 65°C, after which dry weight was recorded for individual plants. Also, two untreated replications were harvested the day of the herbicide application, dried and weighed to calculate the average weight of plants at the time of treatment.

In the second run of the experiment we conducted a preliminary study to evaluate the long term survival and potential to produce seed as affected by dicamba dose. Two replications of five horseweed populations (populations 18, 20, 32, 39 and 44) were grown for 228 days. Plant survival (defined as the plant having green tissue) was recorded at 28, 56, 112 and 168 DAT. At 228 DAT, the seed of each plant was individually harvested.

For each plant with adequate seed production, two subsamples of 100 seeds each were weighed and then averaged. The number of seed per plant was estimated by dividing the total weight of seed by the average 100-seed weight. Two-way ANOVA for the number of seed per treatment for each population was performed using SigmaPlot 11.0 (Systat Software, Inc., IL, USA). Means were separated using an LSD procedure with $\alpha = 0.05$.

Visual injury estimate and dry weight data were analyzed using a nonlinear regression model with the *drc* package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Dose-response models were constructed using a four parameter log-logistic equation:

$$y = c + (d - c / 1 + exp (b (log x - log e))) \quad (1)$$

where y is the response (e.g., visual injury estimate), e is the effective dose to reach the 50% growth reduction (GR_{50}) or injury estimation (I_{50}) and is also the inflection point, b is the slope at e, c is the lower limit and d is the upper limit of the model. The dicamba dose needed to achieve the 50, 80 and 90% dry weight reduction (GR_{50}, GR_{80}, GR_{90}) and visual injury (I_{50}, I_{80}, I_{90}) estimates were calculated. The relative level of resistance were expressed by calculating the R:S (Resistant : Susceptible) ratios between the I or GR values of less susceptible populations and the I or GR values of the most susceptible population.

3. Results

3.1 Effect of Dicamba on Horseweed

The effective dose necessary to achieve the I_{90} varied 3.1 fold between the least and most susceptible horseweed populations (Table 1). Population 44 was the least susceptible and population 32 was the most susceptible to dicamba based on visual injury estimates for both I_{50} and I_{90} (Table 1 and Figure 1). A use dose of 560 g ha^{-1} was calculated to provide greater than 90% injury of all populations evaluated except population 44, for which 90% control required 638 g ha^{-1}.

Table 1. Visual injury estimate regression parameters, I_{50}, I_{80} and I_{90} dicamba doses (g ae ha^{-1}), and standard errors (SE) at 28 DAT for ten horseweed populations from Nebraska. Regression parameters were estimated using a log-logistic equation (Equation 1)

Population	Regression parameters[a]			I_{80} (±SE)		I_{90} (±SE)	
	b	I_{50} (±SE)					
3	-1.1	50	(6)	178	(30)	376	(89)
6	-1.2	41	(7)	129	(27)	252	(76)
7	-1.1	30	(4)	108	(21)	230	(62)
18	-0.9	52	(12)	228	(79)	539	(263)
20	-1.1	31	(4)	112	(21)	236	(64)
32	-1.1	27	(3)	97	(14)	205	(42)
39	-1.3	38	(4)	114	(17)	219	(45)
44	-0.9	61	(16)	268	(99)	638	(335)
52	-1.1	40	(5)	147	(29)	317	(90)
62	-1.0	36	(5)	144	(29)	325	(87)
R:S		2.3		2.8		2.9	

[a] Regression parameters were estimated using a four parameter log-logistic equation (Equation 1), where c represents the lower limit (0 = no injury), d represents the upper limit (100 = plant death), b represents the slope of the line at the inflection point, and e represents the herbicide dose necessary to provide 50% injury (I_{50}).

When the response to dicamba dose was calculated based on dry weight reduction, population 44 was the least susceptible at both GR_{50} and GR_{90} (Table 2). The most susceptible population differed from the visual injury estimates (population 32), and also varied between GR_{50} (population 62) and GR_{90} (population 52). The dicamba doses required to achieve GR_{50} or GR_{90} was less than the doses required for comparable visual injury estimations (I_{50} and I_{90}). However, the variation between most and least susceptible populations was similar for both metrics. There was a four-fold difference for the GR_{50} (population 62 vs population 44) and a 3.8 fold difference for the GR_{90} (population 52 vs population 44). A dicamba use dose of 560 g ha^{-1} provided greater than 90% reduction in dry weight for all populations (Table 2).

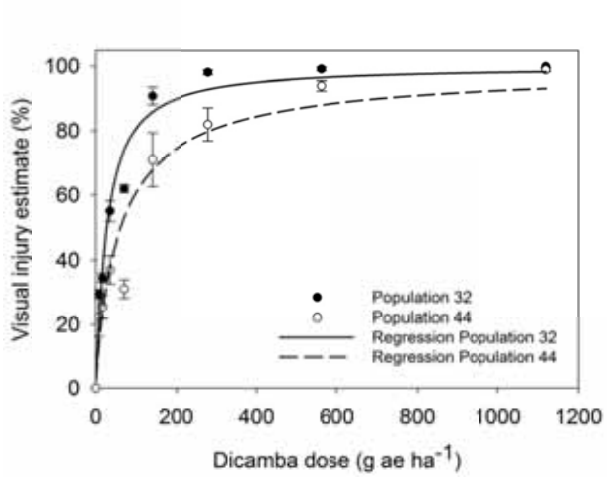

Figure 1. Effect of dicamba dose on visual injury estimate 28 DAT for the least (44) and most susceptible (32) horseweed populations. Data were fit using a log-logistic equation (Equation 1). Regression parameters are given in Table 1

3.2 Effect of Dicamba on Horseweed Survival and Seed Production

In our study, two replications of plants from five populations were allowed to grow up to 228 DAT to assess the effect of dicamba dose on survival and seed production (Tables 3). At 228 DAT all populations had individual plants that survived a dicamba dose of 70 g ha^{-1} or greater (Table 3). Plants in populations 18 and 44 survived dicamba doses of 280 g ha^{-1} and 140 g ha^{-1}, respectively (Table 3). Plants that survived to 228 DAT also produced seed with the exception of population 32 which had a plant survive 70 g ha^{-1}, but the highest dose at which it set seed was 17 g ha^{-1}. Thus there was greater than a 10 fold difference in dicamba dose at which different populations produced seed. Population 18 produced seed at 280 g ha^{-1} compared to populations 32's maximum dose of 17 g ha^{-1} (Table 3). Seed production ranged from 3400 to 30 680 seeds per plant (Table 3).

Table 2. Dry weight regression parameters, GR_{50}, GR_{80} and GR_{90} dicamba doses (g ae ha^{-1}), and standard errors (SE) at 28 DAT for ten horseweed populations from Nebraska. Regression parameters were estimated using a log-logistic equation (Equation 1)

Population	Regression parameters [a]					GR_{80} (±SE)		GR_{90} (±SE)	
	d	*c*	*b*	GR_{50} (±SE)					
3	0.9	0.3	1.2	26	(8)	84	(37)	166	(103)
6	1.8	0.5	1.2	21	(6)	70	(25)	142	(72)
7	1.7	0.3	0.9	23	(5)	99	(28)	235	(96)
18	1.1	0.3	1.0	27	(7)	109	(43)	248	(142)
20	1.0	0.2	0.8	14	(5)	86	(33)	245	(145)
32	1.5	0.2	0.7	10	(4)	66	(24)	207	(121)
39	1.7	0.3	0.9	16	(4)	74	(22)	180	(79)
44	1.5	0.2	0.9	36	(8)	175	(53)	444	(194)
52	1.4	0.3	1.1	17	(3)	57	(13)	116	(40)
62	2.0	0.3	0.7	9	(5)	72	(39)	247	(212)
R:S				4.0		3.1		3.8	

[a] Regression parameters were estimated using a four parameter log-logistic equation (Equation 1), where, where *c* represents the lower limit (minimum dry weight for each population), *d* represents the upper limit (maximum dry weight for each population), *b* represents the slope of the line at the inflection point, and *e* represents the herbicide dose necessary to provide 50% reduction in dry matter (GR_{50}).

Table 3. Number of seeds per plant and survival [a] of five horseweed populations as affected by dicamba dose (g ae ha^{-1}) at 228 DAT

Dicamba dose	Population									
	18 [b]		20		32		39		44	
0	‡19 450	a	‡9810	ab	‡16 430	a	‡30 680	a	‡18 540	ab
8	‡10 990	ab	‡15 640	a	‡11 340	b	‡16 060	b	‡28 110	a
17	‡13 640	ab	‡4300	bc	‡4720	c	‡25 310	ab	‡11 730	bc
35	‡6850	bc	†5190	bc	*0	d	‡10 130	bc	‡6580	bc
70	‡8900	ab	†3440	bc	†0	d	†4960	c	‡7610	bc
140	†4130	bc	*0	c	*0	d	*0	c	†12 050	bc
280	†5770	bc	*0	c	*0	d	*0	c	*0	c
560	*0	c	*0	c	*0	d	*0	c	*0	c
1,120	*0	c	*0	c	*0	d	*0	c	*0	c

[a] Percent plant survival (of two replications) at 228 DAT: Seed numbers preceded by "‡" had 100% survival; seed numbers preceded by "†" had 50% survival; seed numbers preceded by "*" had 0% survival.

[b] Mean values followed by the same letter in the same column are not significantly different ($p < 0.05$).

4. Discussion

Manufacturers usually prescribe herbicide dosages large enough to ensure effective weed control over a broad range of species, management, and environmental conditions (Devlin, Long, & Maddux, 1991), provided weeds are treated while below labeled sizes. But a common scenario is that farmers delay the herbicide application with the objective of controlling more weeds and reducing cost with a single application. The herbicide applied to larger weeds may result in an herbicide dose which is less than the labeled field dose (Terra, Martin, & Lindquist, 2007). The recommended dosage for small, actively growing weeds with dicamba (i.e. Clarity®) is 280 to 560 g ha^{-1}. Applying the labeled dosage should control most of the plants and minimize the risk that plants will survive to produce seed. If weeds are killed prior to producing seed, mutations that confer herbicide resistance are not important.

A previous study found a three- to four-fold range in horseweed tolerance to the diglycolamine salt of dicamba in Indiana (Kruger et al., 2010) which is similar to the results reported in our study in Nebraska. Kruger et al. (2010) reported that at least 300 g ha^{-1} of dicamba should be applied for horseweed control under field conditions. Everitt and Keeling (2007) reported that dicamba doses of 140 and 280 g ha^{-1} provided 93 and 98% control at 28 DAT, respectively, on horseweed populations from Texas treated in the rosette stage. In contrast, Wiese et al. (Wiese, Salisbury, & Bean, 1995) reported only 57 and 75% control of horseweed treated at the rosette stage with labeled doses of 280 and 560 g ha^{-1} of dicamba when plants were drought-stressed at the time of application.

Bell, Nalewaja and Schooler (1972) applied sublethal doses of 2,4-D to four consecutive generations of kochia which resulted in low level, multigenic resistance and a two-fold difference in susceptibility between the least and most susceptible lines. Similarly, Kruger et al. (2010) suggested horseweed populations may have the propensity to evolve to low-level 2,4-D resistance. The interaction of plant size and 2,4-D tolerance levels could enable less susceptible horseweed plants to survive and reproduce in the field following 2,4-D applications (Kruger et al., 2010). Neve and Powles (2004) demonstrated the potential for this to happen by crossing *Lolium rigidum* individuals that survived sublethal doses of diclofop-methyl, and over a period of three generations of selection with increasing herbicide doses developed a population resistant to the standard herbicide use dose. Gressel (2011) argued from a different perspective that low pesticide doses may enhance mutation doses and may hasten the development of resistant populations. Although our study did not address genetic differences among the horseweed populations evaluated, it is not unreasonable to predict that horseweed plants treated with sublethal doses of dicamba may survive and cross with other less susceptible plants in the population. Depending on the genetic mechanisms that confer this lack of susceptibility, populations with elevated tolerance to dicamba may evolve under repeated selection.

The plants that survived and produced seeds did so under controlled environmental conditions, with a single plant per pot and without competition from a crop or other weeds (Table 3). In field conditions, crop shading and plant

density (i.e. crop and weeds) may influence survival and seed production. Bhowmik and Bekech (1993) showed that a single fall emerging horseweed plant can produce nearly 200 000 seeds in no-tillage corn (*Zea mays* L.) stubble, grown at a horseweed density of 10 plants m^{-2}, but 50% less seed at a horseweed density of 200 plants m^{-2}. In general, the amount of seed produced by weeds that escape or survive herbicide applications is less than that of untreated weeds. For example, velvetleaf (*Abutilon theophrasti* Medic.) that escaped atrazine treatment produced 50% less seed than untreated plants (Schmenk & Kells, 1998). When velvetleaf was treated with dicamba, the number of capsules per plant did not vary until the dicamba dose was 318 g ha^{-1} or greater and then was closely related to biomass accumulation (Murphy & Lindquist, 2002).

In summary, ten horseweed populations from southeastern Nebraska were screened with nine dicamba doses. The I_{90} exceeded 280 g dicamba ha^{-1} for five of the ten populations. Plants from two populations produced seed at a dose of 140 g ha^{-1} or greater. Nevertheless, based on R:S ratios and recommended dicamba field use doses, none of the horseweed populations in this baseline study should be considered to be resistant to dicamba. However, there were individuals in the study that survived and produced seed when treated with doses of dicamba that may be considered "reduced doses" (140 g ha^{-1} and less). As such, to minimize the risk of horseweed populations with reduced susceptibility to dicamba being selected in southeastern Nebraska, especially in horseweed populations already resistant to glyphosate, dicamba use doses of 560 g ha^{-1} should be used and horseweed should be treated while in the rosette stage. Reduced doses or large plants at the time of application will increase the probability of individuals with decreased susceptibility surviving and producing seed that carries similar traits. In addition, rotating or tank-mixing different herbicides that are effective on horseweed with dicamba, and employing non-chemical control strategies such as tillage and crop rotation are essential to preserve the maximum utility of dicamba-resistant soybean for many years.

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Biochemical Investigation of Leaf Development in *Capsicum frutescens*

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Abstract

The objective of this research was to investigate the developmental regulation of cell wall components and development of leaves of *Capsicum frutescens*. In order to this, a growth curve was constructed of lengths and widths of fifty *Capsicum frutescens*leaves measured until growth had stopped (20 days). From the curve, four developmental stages were selected and harvested for further study. A range of proteins with molecular weights from 13.4 to 44 kDa were detected in selected samples by SDS-PAGE. A total of four protein bands C, D, K and L with molecular weights 36.2, 27.7, 16.8 and 16.0 kDa respectively were developmentally regulated during the leaf development. This data provide the basis for further more detailed molecular analyses of leaf development in this research.

Keywords: SDS-PAGE, protein, *Capsicum frutescens*, leaf development

1. Introduction

The *Capsicum* genus belongs to the family Solanaceae. Species of Capsicum are used as condiments, spices, ornamentals and in pharmaceutical therapies. *Capsicums* are native to South America and are cultivated in warm, dry climatic conditions, but cannot tolerate frost (Barceloux, 2008; Lozzio et al., 2008). In general, Capsicum *annuum* is mild in taste and is used in dishes and salads. *C. frutescens* and *C. chinese* are hot and pungent owing to their unique alkaloid content. So, these species are used as hot spices in dishes and curries. *Capsicum* pungency is measured in terms of Scoville scale (Prasad et al., 2006). Some species like *C. annuum*. 'MorokAmuba' well known as an ornamental plant in addition to edible spice (Sanatombi et al., 2010). *C.frutescens* is a perennial shrub which grows to a height of 1.83 cm, have the leaves; hermaphrodite flowers and long fruits which are nutritionally and economically important. The most specialised organs are flowers that bear sepals, petals, stamens, carpels that are modified according to the climatic conditions. Various organs usually thorns, bracts, scales are derived from leaves. So, an understanding of leaf development is very important with regards to photosynthesis and respiration and many areas of plant growth and development (Byrne, 2005; Tsukaya, 2002). The cell wall provides strength, shape and allows growth to the cell. Plant cell growth occurs by the expansion of cell walls by a process named controlled polymer 'creep' (Cosgrove, 2005). The cell wall is exterior to the plasma membrane and is a part of the apoplast which is self-contagious and is present between cuticle and plasma membrane (Taiz & Zeiger, 2002). The plant cell wall is complex and is an amalgam of carbohydrates, proteins, lignin, water and substances such as cutin, suberin and other inorganic compounds depending on the cell type, plant species and neighbour cell types. Biotic and abiotic stresses influence the structure and composition of the cell wall. Such variations can be observed in terms of growth and development, environmental sensing and signalling, plant defence, intercellular communication and selective exchange interfaces (Cosgrove, 2005). Capsicum species have high nutritional value and are an excellent source of vitamins e.g. C (ascorbic acid), A, B-complex and E plus minerals such as iron (Fe), molybdenum (Md), manganese (Mn), folic acid, potassium (K) and thiamine. The Beta-carotenoids and vitamins C and A present in chillies are also powerful antioxidants which destroy free radicals (Simonne et al., 1997; Kothari et al., 2010). The vascular bundles play a key role in leaf and whole plant development and are scattered throughout the mesophyll tissue. Bundle sheath cells surround the phloem and xylem. Internally xylem is differentiated into tracheids, trachea, xylem paranchyma and sclerenchyma tissues which together regulate water and mineral transport. Phloem is a complex tissue of vascular bundles and its anatomy comprising of sieve tubes, companion

cells, phloem parenchyma and blast fibres (Gamage & Jesson, 2007). During leaf development process, 'regulated changes in extensibility (relaxation)' of the cell wall offers a balanced increase in the cell size in terms of length, width and girth of the leaves keeping the turgor pressure constant (Cosgrove, 2000; Fleming, 2002).

The objective of this study was to biochemical investigate the developmental regulation of cell wall components and development of leaves of *Capsicum frutescens*.

2. Material and Methods

2.1 Plant Materials

The chilli pepper plants (*Capsicum frutescens*) used in this investigation were grown under greenhouse conditions consisting of 16 hours of light and 8 hours of dark at temperatures of between 15 to 25°C from April to August 2011.

2.2 Growth Curve and Chilli Leaf Harvesting

The lengths and widths of fifty *Capsicum frutescens* (chilli) young leaves were measured and recorded on a daily basis (the chilli fruits were measured from). The chilli leaves were measured starting from lengths of 30, 31, and 32 mm and widths of 11, 12 and 13 mm until they reached their full length (growth had stopped or slowed significantly). Two growth curves were then constructed using average lengths and widths of the chilli leaves and used to select four different developmental stages of growth for further study. The chilli leaves from the selected stages were then harvested and 30mg of chilli leaves from each stage was placed into Eppendorf tubes, labelled and stored at -20°C.

2.3 Extraction of Cell Wall Proteins from Chilli Leaves

200 µl of extraction buffer (Appendix 1) was added to the Eppendorf tubes containing 30 mg of chilli leaves and homogenised (by using Eppendorf grinder). The samples were then centrifuged at 13,000 rpm for 5 minutes and the supernatant transferred into a fresh sterile Eppendorf tube and the pellet discarded. This step was repeated. 800 µl of absolute ethanol (80% v/v) was added to each of the 200 µl samples to precipitate out carbohydrates (cellulose, hemicelluloses, pectin) and heavily glycosylated glycoproteins extensions (hydroxyproline-rich glycoproteins, HRGP) and arabinogalactin proteins (AGP's)]. The sample was then incubated overnight at 4°C. The precipitated sample was centrifuged again at 13, 000 rpm for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 100 µl of sterile distilled water (SDW). The protein extract was stored at -20°C, prior to future usage.

2.4 Protein Assay

2 µl of each of the protein samples (stage 1-4) were placed into a 1.5 ml Eppendorf tube with 798 µl of SDW to make a 400X dilution. 200 µl of BIORAD dye reagent was added to each tube and mixed by gentle inversion of the Eppendorf tubes. 800 µl of SDW and 200 µl of BIORAD dye reagent was put into another Eppendorf tube and used as a control. After 10 minutes, the samples were transferred to cuvettes and the optical density of each of the samples was measured using a spectrophotometer. The absorbance for the samples was read at 595 nm, using the control as a blank. The absorbance of each protein samples was then compared to a standard absorbance curve for Bovine Serum Albumin (BSA) (Appendix 3) to determine the protein concentration for each sample (Bio-Rad laboratories, 2005).

2.5 SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The electrophoresis cell was constructed using two 10 x 10 cm glass plates which were washed with ethanol and three spacers which were placed around three slides of the plates, 1-2 mm from the edge. The plates were then clipped together. To prevent the leakage of separating gel, the cell was sealed with 5X tank buffer (appendix 2). The 4x separating gel solution was prepared (Appendix 2) and gently swirled to avoid excess aeration (which will inhibit polymerization). The separating gel solution was then immediately pipette into the electrophoresis cell, until the solution was 2 cm from the top of the glass plate. A layer of Butan-2-ol saturated with water was then pipette on to the top of the separating gel solution to create a level gel surface and to prevent oxygen diffusing into the separating gel solution inhibiting polymerisation. The gel was then left to polymerise for approximately 25-30 minutes. When the gel has completely polymerized, the butan-2-ol layer was poured off and the top of the gel was washed with SDW. The stacking gel solution was prepared (Appendix 2) and gently swirled. The stacking gel was then immediately pippeted onto the top of the stacking gel solution, ensuring that no air bubbles were trapped and leaving at least 1 cm between the bottom of the comb and the top of the separating gel. The stacking gel and separating gel were poured consecutively until these solutions reached the top of the glass plate. The comb was then placed into the solution was then left with the comb in place to

polymerise for approximately 30-45 minutes. Once the stacking gel had polymerised, the top comb and the bottom spacer were removed and the electrophoresis cell was clipped into the electrophoresis tank. The bottom compartment was then filled with tank buffer (Appendix 2) and the electrophoresis cell was lowered into the bottom tank buffer, ensuring that no air bubbles were trapped at the bottom of the gel as this interferes with electrophoresis process. The top compartment was then filled with tank buffer so that it covered the top of the gel and the top electrode. To prepare the sample, 20 µg of sample buffer (Appendix 2) was added to 20 µg of each protein sample. The samples were then activated by heating them for 10 minutes at 80°C in an Eppendorf heater to denature the proteins. 20 µg of the protein marker, Dalton VII was also prepared in the same way as the protein samples. A Hamilton syringe was used to load the samples and the Dalton VII marker into the wells in the gel. The tank was connected to a power of 13 pack and the gel was run at 200 V for approximately 1- 2 hours until the bromo-phenol blue band had reached the bottom of the gel (approximately 0.5-0.8 cm from the bottom of the gel). The power was then switched off and the tank and the tank buffer from top and bottom compartments were poured away (Skidmore, 2003). The standard fixing solution (Appendix 6) was added to the gel and left for 25 minutes. Then removed and replaced with reconstituted sterling fixative and the gel were fixed for an additional 5 minutes. The gel was then washed in de-ionised water twice for 15 minutes each time. The gel was then immersed in silver staining solution and shacked gently for 10-15 minutes while the gel developed. When the gel had reached the desired intensity the development was stopped by placing the gel in a 5% acetic acid solution for 3-5 minutes. The gel was then placed in de-ionised water, measured and photographed.

3. Results

3.1 Growth Curve for Capsicum Frutescens Leaves

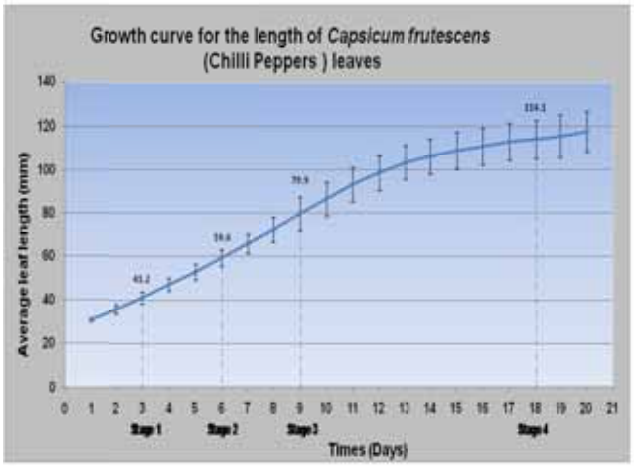
As shown in Graphs 1 and 2 (plotted for average lengths and widths of 50 chilli leaves) asigmaoidal growth curve is clearly observed. The standard deviations for leaf length are calculated. From the growth curve, four developmental stages were selected for the further experiments:

1) Days 3 (40 ± 2.73 mm)- stage 1,

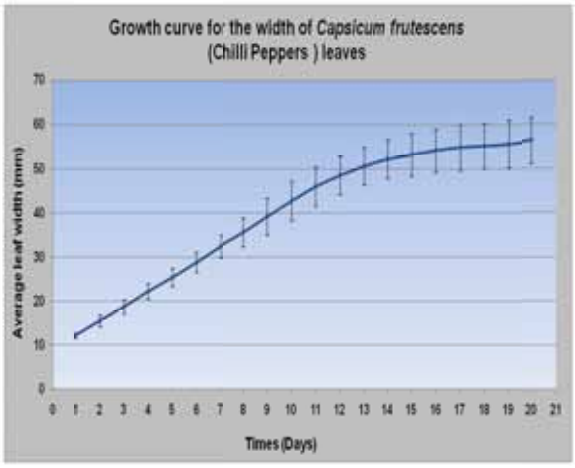
2) Days 6 (60 ± 3.9 mm)- stage 2,

3) Days 9 (80 ± 7.58 mm)- stage 3 and,

4) Days 18 (114.1 ± 8.74 mm) - stage 4.



Graph 1. Sigmoidal growth curve representations the average length of chilli leaves with the four developmental stages, 95% confidence intervals are indicated by bars above and below the curve



Graph 2. Sigmoidal growth curve representations the average width of chilli leaves with the four developmental stages, 95% confidence intervals are indicated by bars above and below the curve

Table 1. Stages of *Capsicum frutescens* leaf development selected for further study

Stage	No. of days of growth (from, 30, 31, 32)	Mean (mm)	Standard Deviation (±)	95% Confidence Intervals (mm)
1	3	40	2.73	±38-42
2	6	60	3.9	±58-62
3	9	80	7.58	±78-82
4	18	114.1	8.74	±110-120

3.2 Protein Assay

A Bovine Serum Albumin standard curve was plotted to determine the protein concentrations of various samples pertaining to stages 1 to 4. From the four stages, absorbance values were read at the wavelength 595 nm. The samples were then diluted to a concentration of 1 µg/µl for the further studies.

Table 2. Protein concentration of samples from the absorbance readings plotted against BSA standard curve

Tube No.	**Sample stage**	**Absorbance (595 nm)**	**Mean**	**Protein concentration from graph (µg / µl)**	**Protein concentration of sample (µg / µl)**
1	1	0.274 0.290	0.282	4	2
2	2	0.454 0.448	0.451	10	4
3	3	0.672 0.662	0.667	17.5	7
4	4	0.457 0.451	0.454	10	4

3.3 SDS-PAGE Results of C. frutescens Leaf Cell Wall Extracts

All together a total of ten SDS-PAGE gels were run. The first four attempts produced poor results. In each gel, 20 µg of samples was loaded along with 20 µl of sample buffer. The initial gels were unsuccessful. So, the samples were spun to a produce clear protein sample. For the effective results, a glass dish was used for staining and all solutions and buffers were made up using steriliseddeionised water. Gel photographs were then taken.

3.3.1 SDS-PAGE Molecular Weights Calculation

At the four developmental stages, protein bands molecular weights were determined by measuring the distance travelled by proteins from the top of separating gel buffer to middle of protein band. Relative mobility was calculated as shown below.

$$\text{Relative Mobility} = \frac{\text{Distance travelled by the protein}}{\text{Distance moved by dye front}}$$

A calibration curve was then drawn for relative mobility of the marker bands against log10 values of known molecular weights from the Dalton VII marker. Several bands were developmentally regulated. The protein bands molecular weights were in the range 13.4 KDa to 44.0 KDa. Altogether, 14 protein bands are observed, only ten of which were expressed in all the four stages with the molecular weights 13.4, 14.0, 17.6, 18.0 19.8, 21.0,22.5,25.6, 39.0 and 44.0 KDa respectively. Ten protein bands **A**, **B**, **E**, **F**, **G**, **H**, **I**, **J**, **M** and **N** were detected in all 4 samples. But, protein bands **C** (36.2 KDa)**, D** (27.7 KDa), **K** (16.8 KDa) and **L** (16.0) were not observed in sample 4, bands **L** (16.0 KDa) not detected in sample 2, 3 and 4 (Table 3). This infers that the protein **L** was developmentally regulated. If any protein missing in the earlier or the later developmental stages,

is said to be developmentally regulated during leaf developmental process. Proteins **A** (44.0 KDa), **B** (39.0 KDa), **E** (25.6 KDa), **F** (22.5), **G** (21.0 KDa), **H** (19.8 KDa), **I** (18.0 KDa) **J** (17.6 KDa), **M** (14.0 KDa) and **N** (13.4 KDa) were abundant for all the stages for the developmental process. These variations in expression are clearly indicative of the developmental regulation of these proteins during the leaf development (from stages 1-4) (see Figures 1-4). These variations are due to housekeeping genes as they produce proteins which are necessary for the metabolic functionality of the cell. Proteins accordingly vary with respect to the house keeping genes presence. Examples of housekeeping gens are actin, ubiquitin, GAPDH (Glyceraldehyde, 3-phosphate dehydrogenase) which is vital for glycolytic pathway and albumin that transports compounds throughout the body. Housekeeping genes are vital for structural proteins coding to makeup cytoskeleton like beta-actin and tubulin. These genes are collectively expressed at all the known conditions at a constant level relatively.

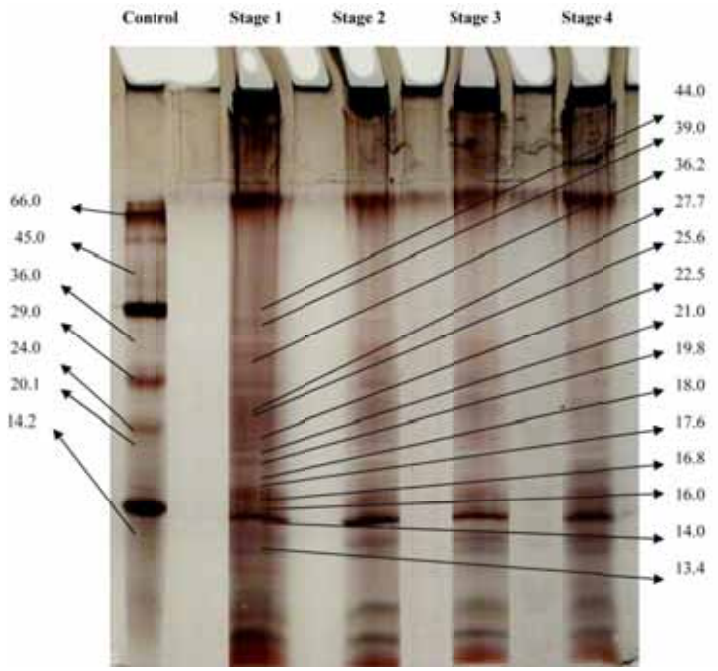


Figure 1. Molecular weights (stage 1): 13.4, 14.0, 16.0, 16.8, 17.6, 18.0, 9.8, 21.0, 22.5, 25.6, 27.7, 36.2, 39.0, 44.0 KDa

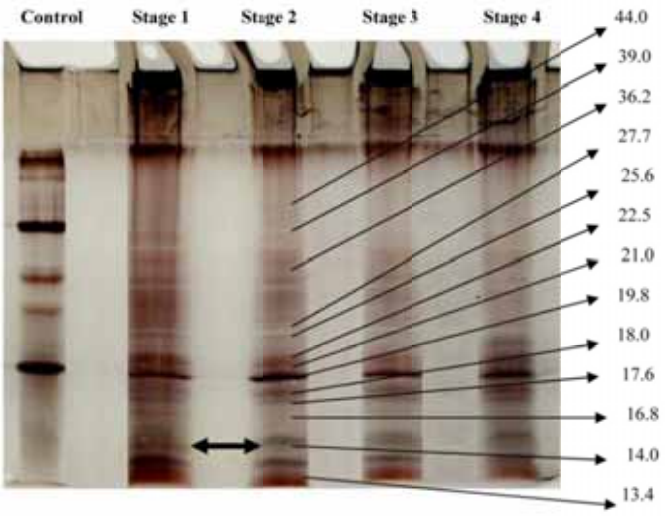


Figure 2. Molecular weights (stage 1): 13.4, 14.0, 16.0, 16.8, 17.6, 18.0, 19.8, 21.0, 22.5, 25.6, 27.7, 36.2, 39.0, 44.0 KDa

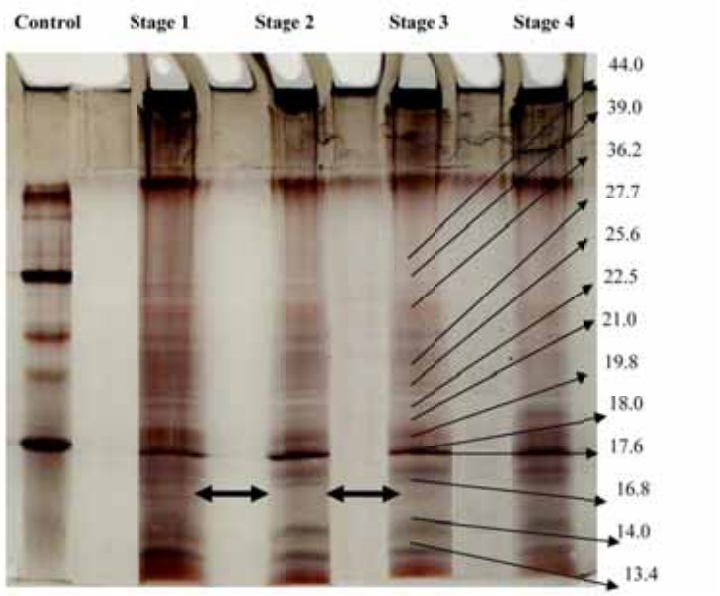


Figure 3. Molecular weights (stage 1): 13.4, 14.0, 16.0, 16.8, 17.6, 18.0, 19.8, 21.0, 22.5, 25.6, 27.7, 36.2, 39.0, 44.0 KDa

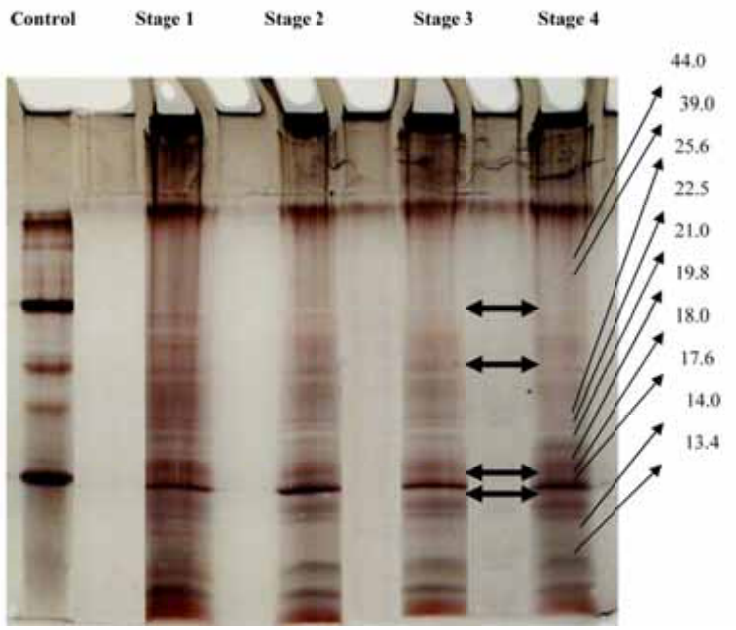


Figure 4. Molecular weights (stage 4): 13.4, 14.0, 17.6, 18.0, 19.8, 21.0, 22.5, 25.6, 39.0, 44.0 KDa

Table 3. Protein bands present in the gel at the four stages of development. '(X)' indicates the presence of a protein band, '(-)' indicates the absence of a protein band

Protein Band	Molecular Weight (KDa)	Development Stage			
		Stage 1	Stage 2	Stage 3	Stage 4
A	44.0	X	X	X	X
B	39.0	X	X	X	X
C	36.2	X	X	X	-
D	27.7	X	X	X	-
E	25.6	X	X	X	X
F	22.5	X	X	X	X
G	21.0	X	X	X	X
H	19.8	X	X	X	X
I	18.0	X	X	X	X
J	17.6	X	X	X	X
K	16.8	X	X	X	-
L	16.0	X	-	-	-
M	14.0	X	X	X	X
N	13.4	X	X	X	X
Number of bands in each stage stages		14	13	13	10

4. Discussion

Leaf is a principle organ of photosynthesis, respiration and is divided into different portions like blade and petiole-stalk. Leaf is responsible for photosynthesis (food manufacture) and respiration (usable energy production). Leaves are the repositories of food and water (Tsukaya, 2002). In response to environmental conditions, leaves themselves modified to various kind of forms to sustain. Leaves are used in multidiscipline fields like food, medicine, fossil fuel and many (Ravishankar et al., 2003; Lozzioet al., 2008). *Capsicum* is a crop having economic importance around the globe especially in the countries like India, Mexico and China. From the SDS-PAGE results, 14 bands with molecular weights in the range 13.4 to 44.0 kDa were detected. Ten protein bands with molecular weights A (44.0), B (39.0), E (25.6), F (22.5), G (21.0), H (19.8), I (18.0), J (17.6), M (14.0) and N (13.4) kDa were found to be present in all the four developmental stages and this conveys all these proteins are vitally important throughout the leaf development in all the stages. Protein bands of molecular weights C (36.2), D (27.7), K (16.8) and L (16.0) were not observed in stage 4 and this infers that this protein is required in initial stage of leaf development but not to later stages. Protein band of molecular weight 16.0 kDa was not observed in stage 2, 3 and 4 inferring that this protein is developmentally regulated. These results convey that the proteins were developmentally regulated. A total of ten SDS-PAGE gels were run. Some of the gels were initially run with 4.1 ml and 0.6 ml volume of Acrylamide in separating and stacking gel respectively used. As a result the gels were broken up. Hence the volume of acrylamide increased to 4.6 ml and 0.8 ml of separating and stacking gels by decreasing SDW from 3.3 ml to 2.8 ml in separating and 1.7 ml to 1.5 ml SDW in stacking gel. This helped to obtain a thicker gel that cope up with the samples. Initial gels were not properly washed with standard fixatives, deionised water with surfactant, so gels were not clear. And majorly, air bubbles were formed inside gel matrix that ceased the smooth flow of the samples from cathode to the anode and fetched improper and unclear bands (Butler, 2005; Wilson & Walker, 2005).

5. Conclusion

*Capsicum frutescens*is an important agro-economic crop which could benefit from the research to improve quality, yields and production levels by using the various plant biotechnological methods. *Solanaceae*members such as *Lycopersicum esculentum* (tomato), *Nicotiana tobaccum* (tobacco) and *Solanum tuberosum* (potato) are the model systems have been subjected to biochemical and immunological investigations (Ochoa-Alejo & Ramirez-Malagon, 2001). Advanced techniques such as genetic transformation, genetic modification can improve the crop qualities and these crop cells are capable of regenerating organs by cell, tissue and organ

cultures and eventually to a whole plant in vitro. According to a study done by Ochao-Alejo and Ramirez-Malagon, conferring resistance to pests and diseases to the plants is highly difficult by recombinant DNA techniques. Till now, much progress has been done in terms of genetic improvement of *Capsicum.* Still a long way is ahead to improve the quality traits. Many efforts are being put to combat various kinds of diseases especially viral induced diseases. But these efforts are still not satisfactory. Various strategies have been employed to protect plants against viruses such as protein mediated resistance and satellite RNA mediated resistance. To block the progress of virus infection, protein based approaches are reliable. In RNA based resistance, viral DNA is degraded by using plants post-transcriptional gene silencing mechanism. Advanced technique like designing artificial microRNA (miRNA) is used against pathogenic viruses. Artificial miRNA down regulates the gene expression in plants. So this technique is exploited to confer resistance in plants to combat against pathogenic virus (Kothari et al., 2010). It could be quite inevitable that all the new cell wall proteins and their genes must be identified and characterised. But the difficulty here is their functions and molecular interactions. Prior to their function, knowing the protein position is a million dollars question. Inculcating mutants by antisense RNA technology is exciting but is a difficult experiment and is raising many concerns regarding ethics. So these concerns must be answered in the upcoming days (Showalter, 1993).

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Appendix 1. Cell wall extraction buffers

Stock solutions

A - 0.5 M EDTA (500 ml)

93 g of dissodium EDTA

Dissolved slowly in 400 ml of SDW

Adjust pH to 8.0 with approximately 10 g of NaOH pellets

B - 2M NaCI (100 ml)

11.2 g NaCI

Dissolved in 100 ml SDW

Autoclave

C - 1M Tris-HCl pH 8.0 (100 ml)

12.11 g Tris base

Dissolved in 80 ml SDW

Adjust pH with Concentrated HCI

Autoclave

Protein extraction buffer

A-10 mM EDTA

B-10 mMNaCl

C- 0.2 M Tris-HCl (pH 8.0)

For preparing 1 ml protein extraction buffer

200 µl 1 M Tris-HCl (pH 8.0)

5 µl 2 M NaCl

20 µl 0.5 M EDTA

775 µl SDW

1 ml Extraction buffer

900 µl Proteinextraction buffer

100 µl 10% Nonidet

1 µl M DTT

Appendix 2. SDS PAGE solutions

Separating gel buffer (250 ml)

45.38 g Tris base

0.67 g EDTA

Dissolve in 125 ml of SDW

Adjust pH to 8.8 with concentrated HCI

Dissolve 1 g SDS (Sodium Dodecyl Sulphate) in solution

Make up volume to 250 ml with SDW

(Adjust pH before adding SDS as SDS compound will damage the pH electrode)

Stacking gel buffer (50 ml)

3.0 g Tris base

0.33 g EDTA

Dissolve in 25 ml SDW

pH to 6.8 with HCl

Add 0.2 g SDS

Make up volume to 50 ml with SDW

Tank buffer (5 x concentrate) (1000 ml)

30 g Tris base

144 g Glycine

5 g SDS

3.72 g EDTA

Make up volume to 1 liter

Do not adjust pH

For use dilute volume (1:4 dilutions with SDW)

Sample buffer (50 ml)

1.2 g Tris base

0.149 g EDTA

Make up volume to 20 ml

Adjust pH to 6.8 with HCl

15 g Sucrose

2 ml Mercaptoethanol

2.5 g Bromophenol blue

4 g SDS

Make up volume to 50 ml with SDW

10% Ammonium persulphate

0.1 g dissolved in 1 ml SDW

4 x Separating gel solution

2.5 ml separating gel buffer

4.6 ml 30% acrylamide, 0.8% bis-acrylamide

2.8 ml SDW

10 µl TEMED (NNNN-Tetraethyimethylenediamide)

100 µl 10% Ammonium persulphate solution

Added in order and used immediately

4 x Stacking gel solution

1 ml Stacking gel buffer

0.8 ml 30% acrylamide, 0.8% bisacrylamide

1.5 ml SDW

5 µl TEMED

25 µl 10% Ammonium persulphate solution, added in order and used immediately

Appendix 3. Bovine Serum Albumin (BSA) protein assay

Tube	Final BSA protein concentration (µg/µl)	Volume of Stock (µg)	BIORAD dye Reagent (µl)	Volume of SDW (µl)	Absorbance (595nm)	Mean
1	1	2	200	1798	0.116 0.122	0.119
2	5	10	200	1790	0.318 0.33	0.324
3	10	20	200	1780	0. 459 0.451	0.455
4	20	40	200	1760	0.760 0.752	0.756
5	25	50	200	1750	0.841 0.853	0.847
Control	0	0	200	1800	0 0	0

Stock Solution (µg/µl)

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Definition of Zones With Different Levels of Productivity Within an Agricultural Field Using Fuzzy Modeling

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Abstract

Zoning of agricultural fields is an important task for utilization of precision farming technology. A method based on fuzzy indicator model theory for definition of zones with different levels of productivity is considered. Fuzzy indicator model for identification of zones with different levels of productivity is based on two general types of fuzzy indicators: the individual fuzzy indicator (IFI) type and the combined fuzzy indicator (CFI) type. IFIs are defined as a number in the range from 0 to 1, which reflect an expert concept and are modeled by an appropriate membership function. CFI is defined using fuzzy aggregated operations. The theoretical considerations are illustrated with this example based on data collected from a precision agriculture study in central Texas, USA. Soil samples were collected at different points, taking into account the actual longitude and latitude for each of these points. Because the experimental data (as in many cases) contained information only about a limited number of parameters, the calculations were restricted in this study. In this study, the parameters grain yield, total carbon (C), total nitrogen (N), and available phosphorus (P) were utilized. Using the author's computer program, fuzzy indicators IFI/CFI was calculated for each zone separately. Utilizing results of calculations maps of zones with different levels of productivity were built.

Keywords: fuzzy set theory, fuzzy indicator, zoning of agricultural fields, precision farming

1. Introduction

Within-field variability is a well-known phenomenon in agriculture and is central to the precision farming concept. One way of dealing with this problem is to subdivide a field into a few contiguous homogenous zones, often referred to as management zones (MZs) (Handbook of precision agriculture-principles and applications, 2006). However, decisions must be made as to how these management zones will be delineated. The evaluation of MZs delineation is the subject of many scientific research studies.

The delineation of management zones could be based on factors such as soil and field characteristics (Fridgen, 2000; Fridgen et al., 2004), digital elevation model (Pilesjo et al., 2000) and yield maps (Stafford et al., 1999). Another method is based on the use of GIS software. Yield values are calculated on a cell-by-cell basis and a map of average yield values is created (Mitchell, 1999).

The most developed approach is based on some sort of clustering methods. Clustering using the fuzzy *k*-means algorithm (fuzzy *c*-means) was described by Tou and Gonzalez (1974) and Fraisse et al. (1999). Yakushev et al. (2007) discussed a method for recognizing relatively homogeneous zones based on limit theorems of probability theory.

In recent years, some progress in the study of within-field variability has been achieved by application of a fuzzy indicator model (Kurtener et al., 2008; Torbert et al., 2009; Krueger et al., 2010). Using this model, it is possible to achieve agricultural field zoning on the bases of the combination of several soil and crop characteristics. This paper reports on the development of a fuzzy indicator model for definition of zones with different levels of productivity within an agricultural field. The theoretical considerations are illustrated with this example based on data collected from a precision agriculture study in central Texas, USA.

2. Method

In the framework of fuzzy sets theory, it is possible to develop fuzzy indicator model, which would be useful to evaluate within-field variation of crop and soil data. In particular, fuzzy indicator model for identification of zones with different levels of productivity is based on two general types of fuzzy indicators: the individual fuzzy indicator (IFI) type and the combined fuzzy indicator (CFI) type. IFIs are defined as numbers in the range from 0 to 1, which reflect an expert concept and are modeled by an appropriate membership function. CFI is defined by fuzzy aggregated operations. The basis of fuzzy indicator model and its applications are considered in several publications (Kurtener et al., 2008; Torbert et al., 2009; Krueger et al., 2010).

The structure of the fuzzy indicator model used in this study is shown in Figure 1. All individual fuzzy indicators (IFI) are built for each zone separately. In this study it is modeled by the trapezoidal-shaped built-in membership function because this function gives opportunity to model optimal area and to take into account the asymmetry of the transition zones.

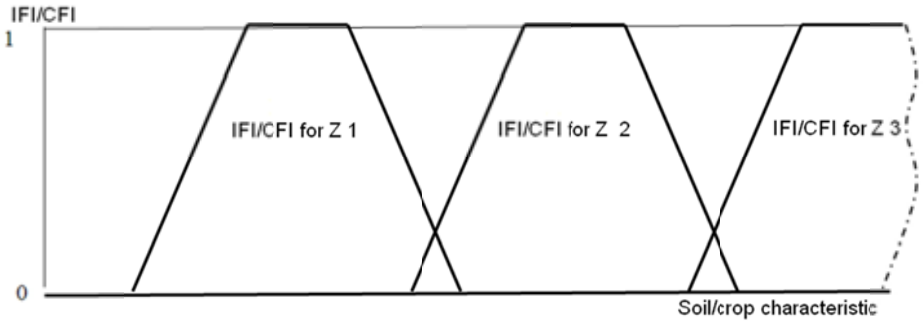

Figure 1. The structure of the fuzzy indicator model

In general, an algorithm for identification of zones with different levels of productivity includes four main parts: 1) Structuring Phase (perceiving the problem, input and output data, obtaining missed data using crisp models); 2) Fuzzy Modeling Phase (identifying fuzzy indicators, definition of membership functions, selecting fuzzy operations); 3) Programming Phase (selection of suitable existed software or designing a new one), and 4) Calculation and Visualization Phase (creation of thematic maps, interpretation of results obtained).

The calculations of fuzzy indicators were conducted utilizing the author's program, which included several scripts written on MATLAB 6.5 (http://www.mathworks.com/). For visualization, it could be applied to different GIS (Geographic Information System) software. In this study, we used the software product Sufer-8 (http://www.goldensoftware.com).

The fuzzy indicators model is an appropriate method for definition of zones with different levels of productivity within an agricultural field. Results of this model are illustrated with the following example.

3. Results

In this example, we used data from an experiment carried out on the north agricultural field located in Bell County, TX on the Elm Creek watershed (Torbert et al., 2000). The soils within the study site consisted of a Heiden clay (fine, montmorillonitic, thermic Udic Chromusterts), a Houston Black clay (fine, montmorillonitic, thermic Udic Pellusterts) and a Ferris clay (fine, montmorillonitic, thermic Udorthertic Chromusterts). Soil samples were collected at different points, taking into account the actual longitude and latitude for each of these points.

Preliminary analysis of the experimental data showed that the experimental field was relatively homogeneous. Nevertheless, we can distinguish two zones with medium productivity (Z 1) and good productivity (Z 2).

Because the experimental data contained information only about the parameters grain yield, total carbon (C), total nitrogen (N) and available phosphorus (P), we were forced to restrict our calculations. The ranges of these parameters within the field and for each zone were made according to expert opinions and are give in Table 1.

Table 1. The range of parameters

	Total C, %	Total N, %	Available P, %	Yield, t/ha
The range of parameters within the field	1.95 - 3.04	0.089 - 0.134	0.027 - 0.043	6.40 - 8.51
The range of parameters within Z 1	1.5 – 2.4	0.05 - 0.1	0.015 – 0.03	6.28 – 7.53
The range of parameters within Z 2	2.41 – 3.5	0.11 – 0.27	0.031 - 0.045	7.60 – 8.79

In this example, we used the trapezoidal-shaped built-in membership function. This function is characterized by four reference points: x_{low1}, x_{opt1}, x_{opt2}, and x_{low2}. Mathematically the trapezoidal-shaped built-in membership function is described as follows:

- If $x < x_{low1}$, then IFI = 0,
- If $x_{low1} < x < x_{opt1}$, then $0 < IFI < 1$,
- If $x_{opt1} < x < x_{opt2}$, then IFI = 1,
- If $x_{opt2} < x < x_{low2}$, then $1 < IFI < 0$,
- If $x > x_{low2}$, then IFI = 0.

For example, reference points for the total carbon in areas with good productivity (Z 2) are: x_{low1} = 2.3%, x_{opt1} = 2.5%, x_{opt2} = 3.4%, and x_{low2} = 3.6%.

Using the author's computer program, fuzzy indicators IFI/CFI were calculated for each zone separately. For illustration the results of calculations of fuzzy indicator on total carbon (IFI_C) for both zones Z 1 and Z 2 are presented in Table 2.

Table 2. The results of the calculation of IFI_C

Longitude	Latitude	Total C	IFI_C	
		%	Z 1	Z 2
97.265707	31.025029	2.7	0	1
-97.264706	31.024772	2.76	0	1
-97.263738	31.024488	2.51	0	1
-97.264099	31.023627	2.32	0.08	0.55
-97.265099	31.023911	2.55	0	1
-97.266069	31.024141	3.04	0	1
-97.26643	31.023307	1.95	1	0
-97.265461	31.023023	2.32	0.08	0.55
-97.26676	31.022418	2	1	0
-97.267151	31.021638	2.04	0.98	0
-97.266057	31.021326	2.81	0	1
-97.265791	31.022134	2.78	0	1
-97.264398	31.022711	2.16	0.68	0.15
-97.264791	31.021823	2.47	0	0.925
-97.265088	31.021043	2.51	0	1
-97.263429	31.022427	2.1	0.875	0
-97.263759	31.021566	2.32	0.08	0.55
-97.26415	31.020786	2.21	0.45125	0.275
-97.26279	31.021309	2.47	0	0.925

Table 2 shows the calculated values of IFI_C for zones Z 1 and Z 2 for the input data (longitude, latitude, total C). It should be noted that the suggested method postulates that for each point in an agricultural field, different levels of productivity exist, but in different levels or grades. The value of the grade is estimated with fuzzy indicators, which range from 0 to 1. For example, at the point in the agricultural field with longitude = -97.265461 and latitude = 31.023023 (see row 4 in Table 2) there are two zones with different levels of productivity (Z 1 and Z 2) where the grade was calculated to be 0.08 for zone Z 1 and 0.55 for zone Z 2. Because the value for Z 2 (0.55) in much greater than Z 1 (0.08), then this point falls within zone Z 2. If any point, in which fuzzy indicators is equal to 0.5 (for zone Z 1) and 0.5 (for zone Z 2), then the state of this point will be uncertain.

Utilizing results of calculations we can build maps of zones with different levels of productivity. For illustration some results of visualization are shown in Figure 2 through Figure 5.

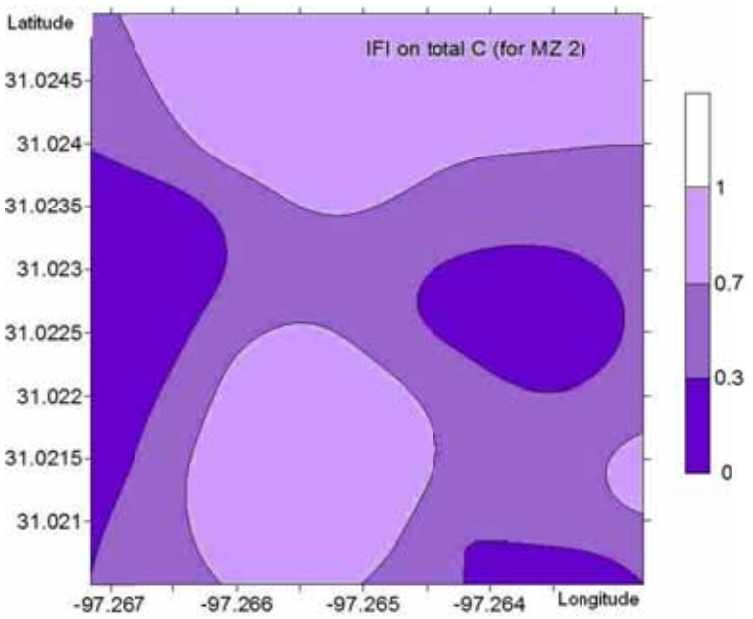

Figure 2. Zone with a good level of productivity defined on the basis of values of fuzzy indicator IFI_C

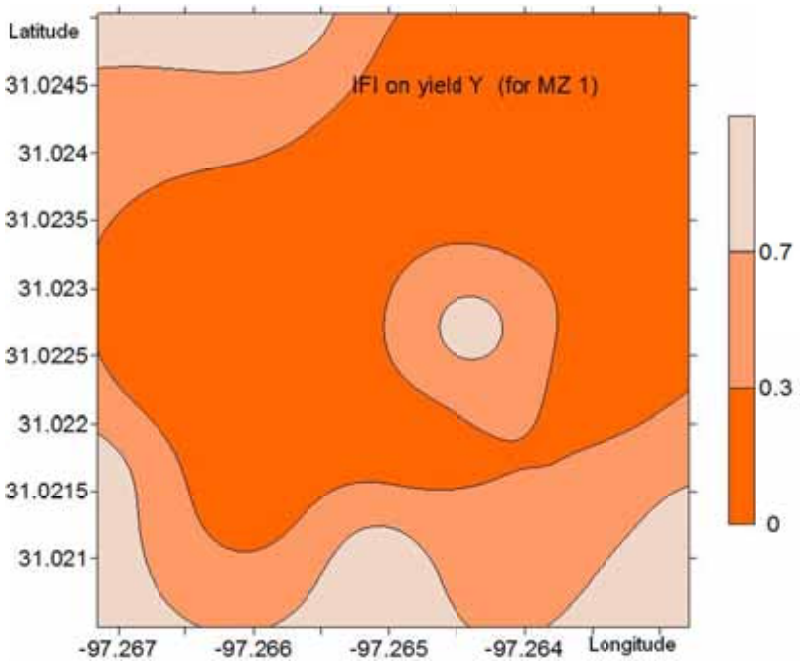

Figure 3. Zone with a medium level of productivity defined on the basis of values of fuzzy indicator IFI_Y

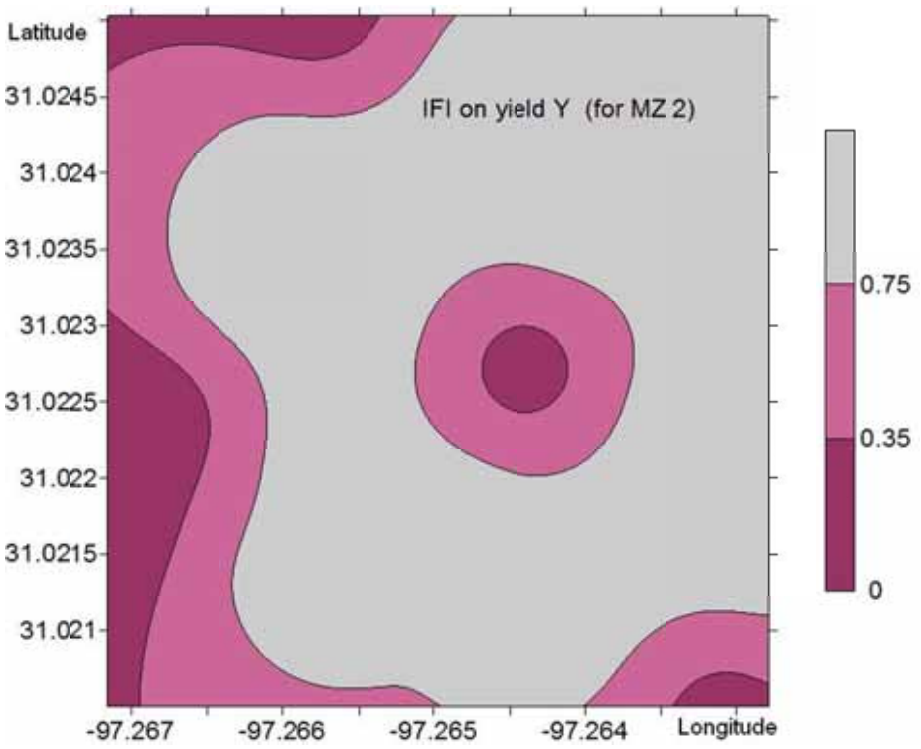

Figure 4. Zone with a good level of productivity defined on the basis of values of fuzzy indicator IFI_Y

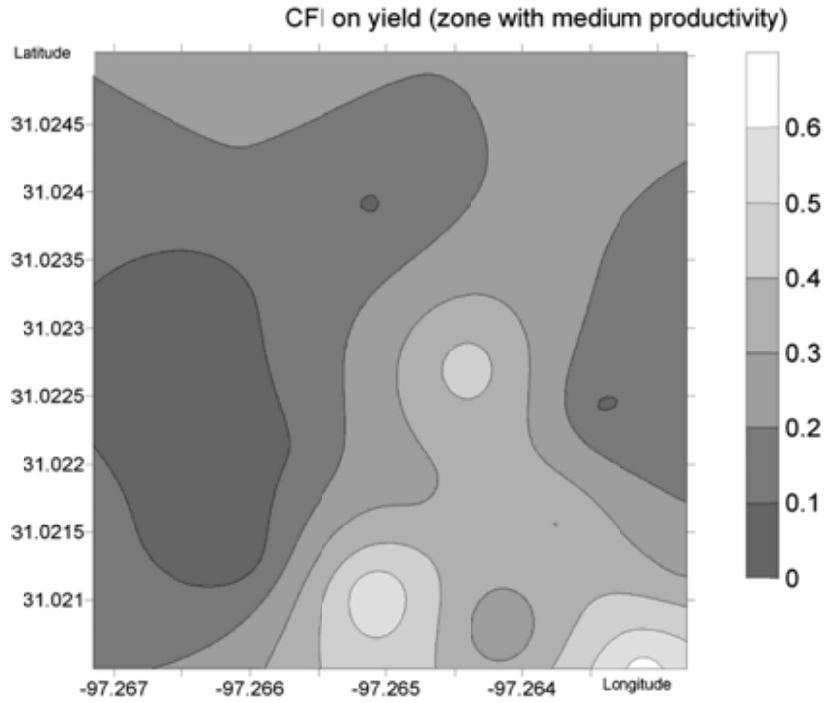

Figure 5. Zone with a medium level of productivity defined on the basis of values of fuzzy indicator CFI

In these figures, the zoning maps for the agricultural field can be observed within which the zones have different significance, which change from 0 to 1. Generally, each map can be divided into 3 areas: a) an area zone with a strong level where the actual range is IFI/CFI > 0.5; b) an area zone within which the presence of the zone is medium, with a range of 0.2 < IFI/CFI < 0.5; and c) an area zone with a weak response, with a range of 0< IFI/CFI < 0.2 (which practically does not exist).

For example in Figure 5, the map based on values of fuzzy indicator CFI for zone with a medium level of productivity contains only the area within which the presence of the zone is medium ($0.2 < CFI < 0.5$) and the area within which the zone is weak ($0 < CFI < 0.2$) practically does not exist.

4. Conclusion

The backbone of this research is the development of an appropriate method for definition of zones with different levels of productivity within an agricultural field. This method is based on fuzzy indicator model, which could be very useful for analysis of within-field variability. To illustrate the proposed method, a series of calculations were made. As input data we used data from an experiment, conducted on the North agricultural field located in Bell County, TX on the Elm Creek watershed (Torbert et al., 2000).

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Potato Production in Kenya: Farming Systems and Production Constraints

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Abstract

Potato (*Solanum tuberosum* L.) is a major food and cash crop in the Kenyan highlands, widely grown by small-scale farmers. Farmer practices and constraints in potato production differ from region to region. A survey was conducted in three major potato producing districts namely Bomet, Molo and Meru Central with the following objectives: 1) to document farmers' practices, key potato production and marketing constraints, 2) to determine farmers' potato cultivar and trait preferences and 3) to assess the prevalence and farmers' management of bacterial wilt. The survey was carried out between November 2011 and March 2012. During the survey, a semi-structured questionnaire was administered to 253 individual farmers. The results show that the average household farm sizes are less than 2.4 hectares in all the districts. Majority of farmers allocate more than 25% of their farms to potatoes. Potato is produced both for food and cash by 90% of respondents in all districts. In Bomet district the red-skinned Dutch Robyjn is widely grown. In Molo district, the white- skinned Cangi is prominent while in Meru Central, the red-skinned Asante is predominantly grown by farmers. Cultivar preferences are mostly dictated by availability of markets, yield potential and taste. The major potato production constraints are diseases with bacterial wilt being the most prominent.

Keywords: bacterial wilt, farming systems, potato production constraints

1. Introduction

Potato (*Solanum tuberosum* L.) plays a major role in food security in Kenya and contributes to poverty alleviation through income generation and employment creation. Despite its importance, the potato sector is plagued by numerous problems such as a lack of clean seeds, lack of proper pest and disease management, a disorganised marketing system and a lack of clear policies on packaging (Riungu, 2011).

The shortage of clean (disease free) planting materials has led to low yields, poor quality produce, and spread of pests and diseases (GIZ-PSDA Kenya, 2011; Riungu, 2011). Kenya produces less than 1% of the national certified seed demand. Because of shortage of clean planting materials, farmers are forced to plant seeds from informal sources such as farm-saved (self supply), local markets or neighbours. The informal system leads to the use of poor quality seeds and often accelerates the spread of seed-borne diseases such as bacterial wilt (Kinyua et al., 2001; Ng'ang'a et al., 2003). According to some studies, bacterial wilt has affected 77% of potato farms (Kaguongo et al., 2010). Because of the high prevalence of this disease, a strict rotation programme is required in the production of the crop; few farmers can rotate for the recommended one and a half years due to paucity of land (Riungu, 2011).

Control of bacterial wilt on potatoes is difficult and no single control method has been found to be totally effective (Champoiseau et al., 2009). The common approach in the management of the disease is an integrated combination of measures such as phytosanitation (use of disease-free seeds and quarantine), cultural practices (crop rotation, intercropping and delayed planting), chemical control and biological control (Martin & French, 1985; Champoiseau et al., 2010). However, most of these measures have been found to be ineffective, impractical and/or

expensive (Lemaga, 1997; Otipa et al., 2003; Kaguongo et al., 2008, 2010; Riungu, 2011). Host resistance could therefore offer a more lasting solution.

Disease resistance, in addition to other good traits, may increase the chances of a cultivar being adopted by farmers as this may reduce production costs. The various end-uses of potatoes require specific tuber characteristics and cultivars. In a previous study, it was found that attributes considered in ranking a potato cultivar by farmers are high yield potential, late blight resistance, taste, maturity period, market demand, bacterial wilt resistance, tuber size, and drought tolerance in that order (Kaguongo et al., 2008). In another study, it was found that farmers prefer cultivars for home consumption to be tasty, high yielding and resistant to late blight while the cultivars should have high market demand and be high yielding if they are destined for the market (McArthur, 1989). Tuber quality characteristics such as skin colour, tuber size, tuber shape and time to maturity are often key factors in cultivar acceptability based on local consumer preferences and criteria for potato processing (McArthur, 1989). Red-skinned cultivars, which are considered to boil quickly and mash easily are favoured for home consumption while white cultivars are preferred for making chips and french fries (McArthur, 1989). Different markets prefer different skin colour, tuber shape and sizes. For example, for making french fries, most processors in Kenya prefer the long white-skinned cultivars while the round red-skinned cultivars are preferred for making chips (Walingo et al., 1998). In addition, red-skinned cultivars have a greater demand in the fresh market probably because they do not turn green when exposed to the light as quickly as white-skinned cultivars. In Kenya, red-skinned cultivars were found to be more popular than the white-skinned ones in Meru Central district while the opposite was found in Nyandarua district (Kaguongo et al., 2008). Early maturity is important for food security and it enables households to generate income early to meet financial obligations. It is also an important trait in potato growing areas with a high demand for land as early harvesting allows more crop cycles in a year. In addition, the short rainy season is often erratic and an early maturing cultivar stands a better chance of carrying the crop to full maturity.

Over time some potato cultivars have been rejected and replaced by others in Kenya; low yield and susceptibility to diseases were cited as the major weaknesses. For instance, Kerr's Pink was removed from its dominant position in Meru Central by Ngure; the latter has been replaced by Asante and Tigoni Red (Durr & Lorenzl, 1980; Crissman et al., 1993). Desiree has been largely abandoned due to low yield, poor market, poor taste and susceptibility to late blight (McArthur, 1989).

For a cultivar to be readily adopted, it must have farmer-preferred traits in addition to disease resistance. Without farmer participation either through participatory rural appraisal, participatory variety selection or participatory plant breeding (PPB), breeders often fail to target farmer-preferred traits (Witcombe et al., 1996) leading to low variety adoption rate (Fukuda & Saad, 2001). The initial stage of PPB involves identification of the end-users preferences and production environments. To achieve this, participatory rural appraisal (PRA) can be employed (Witcombe et al., 2005). During the PRA, the breeder is able to identify and understand both the target environment and farmers. It creates a conducive environment where farmers and breeders exchange ideas and start working towards a common goal (Fukuda & Saad, 2001).

Against this background, a study was undertaken with the following objectives: to document farmers' practices, key marketing and potato production constraints and determine farmers' potato cultivar preferences, the prevalence of bacterial wilt in the major potato growing areas and establish farmers' management of bacterial wilt.

2. Materials and Methods

2.1 Survey Sites and Descriptions

A survey was carried out in three major potato producing counties in Kenya namely, Meru, Bomet, and Nakuru between November 2011 and March 2012. These counties were selected because farmers rank potatoes as their most important commercial crop (Kaguongo et al., 2010). In addition, Nakuru and Meru are among the five leading potato producing counties in Kenya (Ng'ang'a et al., 2003). Bomet county was chosen because its potatoes have a unique demand for processing into chips. Bomet and Nakuru are located northwest of Nairobi while Meru is northeast of Nairobi (Figure 1). In each county, sampling was done at several administrative levels: one district was selected per county, two divisions in each district were selected and all wards (in each division) where potato is a major crop were selected. In Meru County, Meru Central district was selected while in Bomet County, Bomet district was selected. Molo district was selected from Nakuru County.

Bomet district is located in the Rift Valley Province. It has two divisions i.e. Bomet Central and Longisa. The district is home to the Kipsigis subgroup of the Kalenjin community. It is about 300 km northwest of Nairobi and has intensively cultivated steep slopes. The area has a mean monthly temperature of 18ºC with an annual rainfall ranging between 1100 mm and 1500 mm (Jaetzold et al., 2006a).

Meru Central district is located in Eastern province and represents potato growing areas in the Mt Kenya region. The district is the ancestral home to the Meru community. The district lies to the east of Mt Kenya whose peak cuts through the southwest border of the district. In the district, potatoes are mainly produced in the Kibirichia and Abothuguchi West divisions located on the northern slopes of Mt Kenya. These divisions are characterized by annual precipitation ranging between 1400 and 2600 mm and monthly temperature averaging 18ºC (Jaetzold et al., 2006b)

Molo district is located in the Rift Valley province. It comprises two divisions; Molo and Elburgon. Molo is a cosmopolitan district with most of the inhabitants being immigrants from Central and Nyanza provinces. The main inhabitants are Kikuyu, Kalenjin and Kisii communities. The main economic activities are crop production, dairy and sheep keeping. The main cash crops are pyrethrum, potatoes, barley and maize (Jaetzold et al., 2006a).

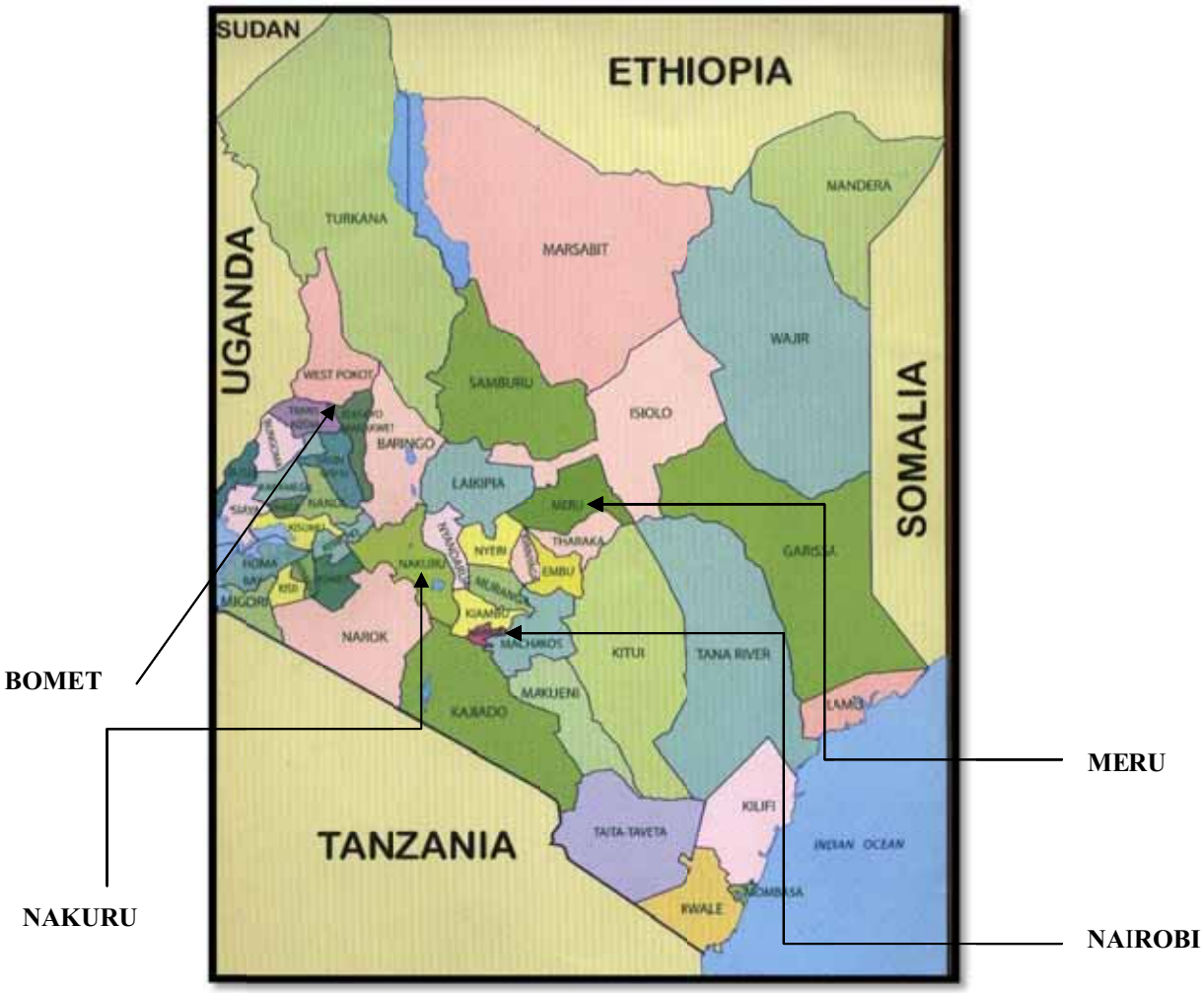

Figure 1. Administrative map of Kenya showing three potato producing counties which were surveyed

2.2 Sampling Method, Data Collection and Analysis

Primary data were collected by administering a semi-structured questionnaire to individual farmers. The questionnaire contained open-ended questions that allowed the respondents to express themselves fully in order to gain as much information as possible. After developing the questionnaire, planning meetings were held with the respective district agricultural officers to agree on the areas to be surveyed and the survey routes to be followed in each district. Following these discussions, the survey routes were mapped and the questionnaire pre-tested on ten households in each district. After pre-testing, changes were effected on the questionnaire and the formal survey commenced.

The survey team consisted of the breeder, a social scientist (both from KARI-Tigoni), an agricultural extension officer and three enumerators (selected from each district). Sampling of the households was both purposeful and systematic; one household (with a current potato crop in the field) within 3 km intervals along selected routes/paths was selected. If no household had a potato crop in the field within the 3 km interval, the next potato farm was sampled.

Interviews were carried out in the field using the questionnaire to capture data on farm size, area under potatoes, potato farming history, cropping system, bacterial wilt management and potato cultivar preferences. The interview was conducted in the local language whenever possible; otherwise it was conducted in Kiswahili, the national language.

The survey team visited the potato plot and scored for bacterial wilt incidence. The incidence was established by measuring the percentage of wilting plants. Prevalence of bacterial wilt was calculated as the number of farms affected by the disease expressed as a percentage of all farms visited in an area.

A global positioning system (GPS-Garmin Inc. Kansas, USA) was used for geo-referencing purposes to supply coordinates (latitudes, longitudes, altitudes) for specific locations. The primary data was analysed using SPSS for Windows Release Version 18.0 (SPSS Inc., 2009). Data analysis was descriptive (means and percentages).

3. Results

3.1 Farmers and Farm Characteristics

A total of 253 farmers were interviewed. In each district, over 60% of the farmers interviewed were men (Table 1). The farms are located between 1933 and 2723 meters above sea level (masl).

Table 1. Descriptions of the three potato growing districts surveyed in Kenya

County	District	Divisions	Altitude (masl)	No of farmers interviewed	% Male farmers
Meru	Meru Central	Abothuguchi west	2126	52	61.8
		Kibirichia	2130	41	70.7
Bomet	Bomet	Longisa	1933	37	70.3
		Bomet Central	2279	42	88.1
Nakuru	Molo	Elburgon	2723	58	65.5
		Molo	2542	23	69.6
Total				253	

Masl= Meters above sea level.

The area surveyed ranged from upper midlands (below 2000 masl) to upper highlands (over 2700 masl) (Table 2). Molo and Elburgon represent the upper highlands while the other divisions represent the upper midlands and lower highlands.

Table 2. Agro-ecological zones of the six potato growing divisions in Kenya surveyed (% of respondents)

Agro-ecological zone	**Altitude (masl)**	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Upper Midlands	less than 2000	0.0	2.7	59.6	0.0	0.0	0.0
Lower Highlands	2001 -2400	100.0	97.3	40.4	95.1	0.0	0.0
Upper Highlands	2401-2700	0.0	0.0	0.0	4.9	50.0	100.0
Upper Highlands	above 2701	0.0	0.0	0.0	0.0	50.0	0.0

In all districts potatoes have been grown for more than nine years. In Meru Central district farmers have been growing potatoes for a longer period than in the other two districts (Table 3). The short potato farming history in Molo district could be attributed to the fact that most farmers are immigrants from other areas, mostly members of Kikuyu community. The recent introduction of potato growing in Bomet district could have been related to the establishment of a company which contracts farmers in this area to plant Dutch Robyjn for processing into chips

and french fries. Bomet district is mainly a tea growing area where the good potato prices in recent years have lured farmers into potato farming.

The average farm sizes range from 0.9 to 2.1 hectares (Table 3). This confirms the general observation that most potatoes in Kenya are predominantly grown by small-scale farmers; the mean farm size are about 2 hectares while potato plots are about 0.5 hectares (Kabira, 1983). These potato growing areas are densely populated and hence the small farm sizes.

A positive correlation (r = 0.66) was observed between farm sizes and the area under potatoes. This means that farmers with bigger farms allocate bigger plots to potatoes. Wakahiu et al. (2007) found a correlation (r = 0.260) between farm sizes and the area under potatoes in a previous study.

Table 3. Average farm size, area under potatoes, years of potato production in three districts in Kenya

District	Divisions	Ave. Farm size (hectares)	Ave. Area under potatoes hectares (% of total farm size)	Ave. years of potato growing
Bomet	Bomet Central	1.70	0.49 (28.8)	9.5
	Longisa	1.66	0.45 (25.5)	9.3
Meru Central	Abothuguchi West	0.97	0.28 (29.0)	16.0
	Kibirichia	1.17	0.49 (41.5)	23. 3
Molo	Elburgon	1.98	0.89 (45.7)	9.6
	Molo	2.10	1.13 (47.9)	9.2

Most potatoes are grown as pure stands in small scale intensive farming systems. Few farmers (5.5%) intercrop potatoes with crops such as maize or beans. The rest grow potatoes in pure stands and practice crop rotation (Table 4). Occasionally, in cases where farm size is very small, potatoes are grown without rotation. In Molo division, about 30% of the farmers surveyed do not practice crop rotation (Table 4).

Table 4. Common rotational sequences (% of respondents) of potato production in three districts in Kenya

Rotation sequence	District and divisions					
	Bomet		Meru Central		Molo	
	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
potato, maize, potato	14.3	2.7	1.9	12.2	34.5	8.7
potato, maize+beans, potato	50.0	37.8	25.0	0.0	17.2	34.8
potato, maize+bean/cabbage,potato	23.8	40.5	13.5	0.0	0.0	0.0
potato, maize/cabbage,potato	0.0	0.0	17.3	9.8	17.2	17.4
potato, cabbage, potato	0.0	0.0	5.8	9.8	5.2	4.3
potato, maize/wheat, potato	0.0	0.0	0.0	14.6	1.7	0.0
potato, maize+bean/wheat, potato	0.0	0.0	0.0	7.3	0.0	0.0
others	9.5	16.2	15.4	26.8	0.0	8.7
No rotation	2.4	2.7	19.2	7.3	15.5	30.4

Maize + beans = maize intercropped with beans; maize + beans/cabbage = maize intercropped with beans or cabbage alone; potato, maize, potato = potatoes followed by maize then potatoes in that sequence; maize/cabbage = maize or cabbage.

Other rotational sequences observed involve minor crops such as carrots, snow peas, millets etc. Over 99% of farmers plant a range of crops on their small farms mainly to cushion themselves against the risk of crop failure (Table 5). Wheat production is specific to Kibirichia while tea is specific to Bomet Central.

Table 5. Crops commonly grown by farmers (% of respondents) in three districts in Kenya

Crops	Districts and Divisions					
	Bomet		Meru Central		Molo	
	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Maize	97.6	89.2	92.3	75.6	91.4	82.6
beans	76.2	91.9	76.9	34.1	44.8	56.5
potatoes	100.0	100.0	100.0	100.0	100.0	100.0
cabbage	31.0	54.1	61.5	56.1	46.6	39.1
Tea	47.6	0.0	15.4	0.0	0.0	0.0
Coffee	0.0	0.0	9.6	0.0	0.0	0.0
wheat	0.0	0.0	0.0	41.5	3.4	0.0
bananas	0.0	0.0	11.5	0.0	0.0	0.0

3.2 Potato Farming System

About 90% of all the farmers interviewed produce potatoes both for cash and food (Figure 2). This possibly explains the allocation of potatoes to large portions of their farms. Ng'ang'a et al. (2003) found that farmers in Nyandarua, Meru Central, Bomet, Nakuru, Nyeri and Keiyo districts grow potatoes mainly for cash, selling over 60% of their produce.

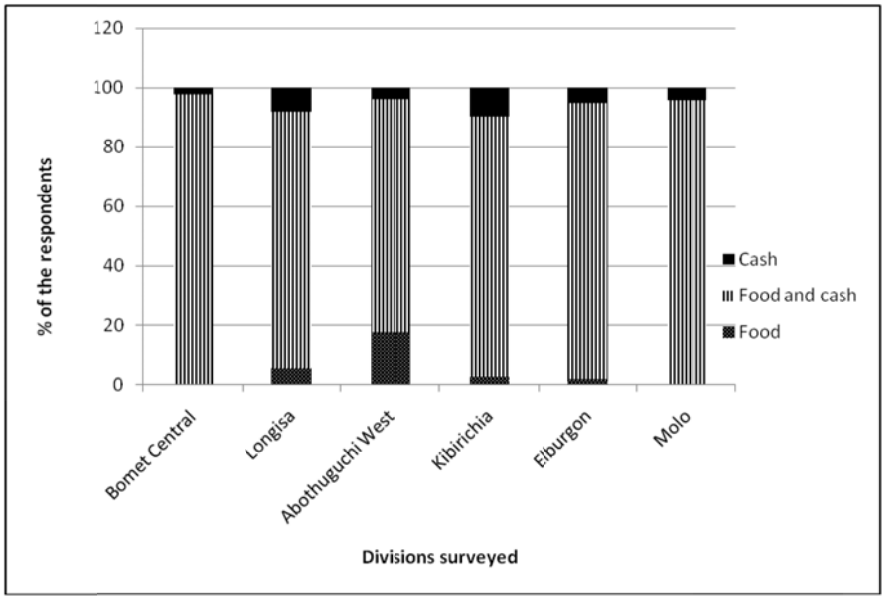

Figure 2. Proportion (%) of farmers that grow potatoes for cash, cash and food, or food in six divisions in Kenya

Farmers generally obtain seed tubers from informal sources (Table 6). The formal seed sources (ADC and KARI Tigoni) are utilized in Bomet district, and to a lesser extent, Elburgon division. The majority of farmers from Abothuguchi West obtain their seeds from the neighbouring Kibirichia division. They argue that potatoes from Kibirichia are rainfed and hence have a lower chance of having bacterial wilt. Farmers from Abothuguchi West believe that seed from their local area had bacterial wilt because it is mainly grown under irrigation.

Table 6. Percentage of farmers obtaining potato seeds from different sources in six divisions in Kenya.

Seed source	Bomet Central	Longisa	Elburgon	Molo	Abothuguchu West	Kibirichia
ADC	4.8	2.7	0.0	0.0	0.0	0.0
ADC, neighbours	2.4	0.0	6.9	0.0	0.0	0.0
KARI Tigoni, own	2.4	8.1	0.0	0.0	0.0	0.0
neighbours	38.1	43.2	34.5	78.3	1.9	9.8
Own (farm-saved)	33.3	21.6	50	17.4	3.8	90.2
own, neighbours	19	16.2	5.2	4.3	1.9	0.0
KARI Tigoni	0.0	8.1	0.0	0.0	1.9	0.0
Local market	0.0	0.0	1.7	0.0	9.6	0.0
ADC,KARI Tigoni	0.0	0.0	1.7	0.0	0.0	0.0
Farmers (Kibirichia)	0.0	0.0	0.0	0.0	76.9	0.0
market, own	0.0	0.0	0.0	0.0	3.8	0.0

ADC= Agricultural Development Corporation

KARI= Kenya Agricultural Research Institute

All farmers sampled from Bomet Central and Longisa divisions grow red-skinned potatoes (Figure 3). Farmers from Elburgon and Molo divisions grow mainly the white-skinned varieties. Most farmers in Kibirichia and Abothuguchi West grow the red-skinned varieties.

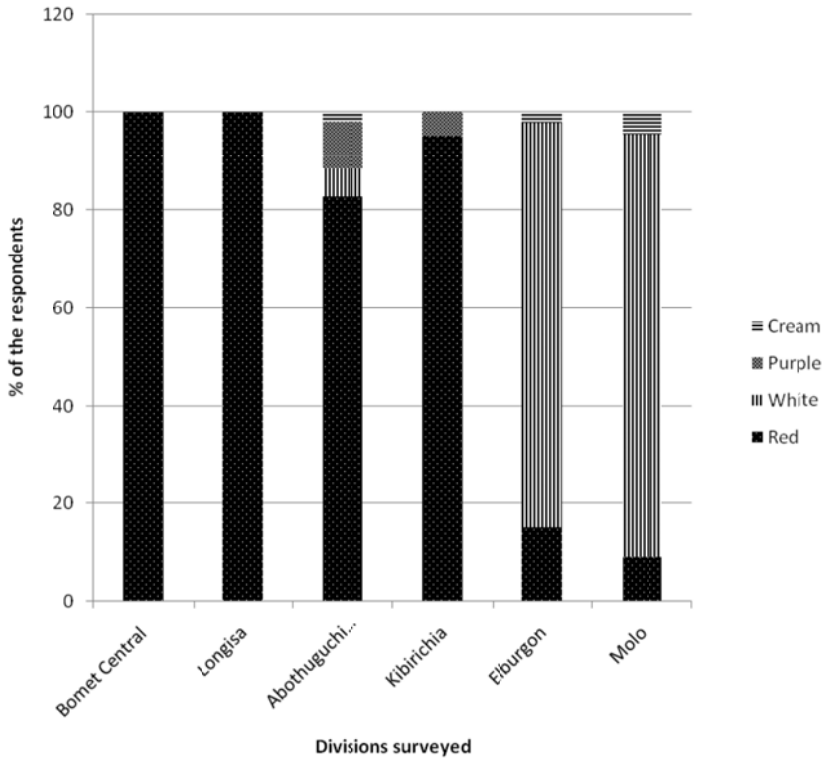

Figure 3. Skin colour of the potato cultivars grown by farmers in six divisions in Kenya

Most farmers across the districts grow white-fleshed potatoes (Figure 4).

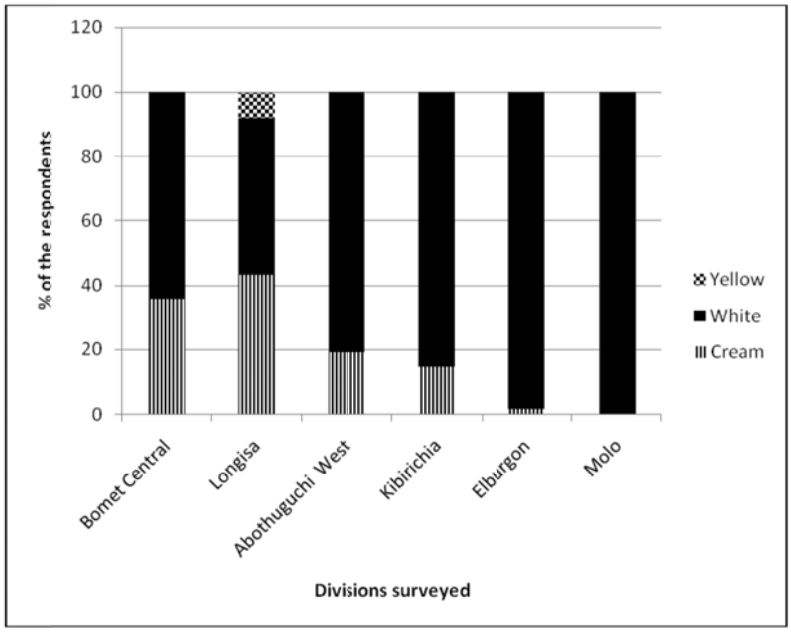

Figure 4. Flesh colour of the potato cultivars grown by farmers in six divisions in Kenya

All farmers in Bomet Central and almost all farmers in Longisa divisions grow the red-skinned Dutch Robyjn (Table 7). Their next popular variety is the red-skinned Desiree. In both Abothuguchi West and Kibirichia, the red-skinned Asante is grown by a majority of farmers followed by the white-skinned Tigoni. The white-skinned Cangi is the most popular in Elburgon and Molo divisions followed by the white-skinned Tigoni.

Table 7. Potato cultivars grown by farmers in six divisions in Kenya (% of the respondents)

Potato Cultivar	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Dutch	100.0	97.3	0.0	0.0	0.0	0.0
Desiree	26.2	16.2	0.0	0.0	1.7	0.0
asante	2.4	2.7	88.5	82.9	10.3	0.0
Tigoni	9.5	5.4	59.6	41.5	44.8	26.1
Kenya Karibu	2.4	2.7	0.0	0.0	8.6	17.4
Cangi	0.0	2.7	0.0	2.4	96.6	100.0
ngure	0.0	0.0	7.7	2.4	0.0	0.0
Kerr's pink	0.0	0.0	7.7	2.4	0.0	0.0
Kibururu	0.0	0.0	7.7	29.3	0.0	0.0
Kombiro	0.0	0.0	1.9	4.9	0.0	0.0
Arka	0.0	0.0	1.9	2.4	0.0	0.0
Komesha	0.0	0.0	0.0	2.4	10.3	17.4
Nyayo	0.0	0.0	0.0	0.0	13.8	8.7
Thimathuti	0.0	0.0	0.0	0.0	3.4	4.3

Market access is the most important factor considered by farmers in Bomet Central and Longisa divisions in deciding which potato cultivar to grow (Table 8). In all the other areas, high yield was the most important factor considered in the cultivar choice. Early maturity was considered an important factor by farmers from Elburgon and Molo divisions as it allows for more crop cycles per year. Early maturity and high yields are the main qualities that have made the local landrace Cangi very popular in these two divisions.

Table 8. Reasons given by potato farmers in deciding the potato cultivar to plant in six divisions in Kenya (% of respondents)

Reasons	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Drought tolerant	3.8	3.1	0.0	6.6	5.6	0.0
Available market	44.3	54.7	1.4	8.2	27	32.7
High yielding	17.7	7.8	59.5	63.9	31	32.7
Good taste	15.2	34.4	13.5	1.6	4.0	8.2
Resists late blight	15.2	0.0	6.8	13.1	5.6	4.1
Only available variety	3.8	0.0	0.0	3.3	0.8	2.0
Matures early	0.0	0.0	14.9	3.3	25.4	20.4
Long post-harvest storage	0.0	0.0	4.1	0.0	0.8	0.0

Generally, there was a high turnover of potato cultivars over the past five years (Table 9). About 34.5% farmers from Bomet Central and 32% from Longisa did not change their favourite potato culyivar. Most farmers in Meru Central district abandoned the red-skinned Kerr's Pink and Ngure cultivars. Farmers from Molo district abandoned the white- skinned Nyayo cultivar.

Table 9. Potato cultivars abandoned by farmers over the past five years in six divisions in Kenya (% of respondents)

Abandoned varieties	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Annett	6.9	0.0	0.0	0.0	0.0	0.0
R.Eburu	8.6	0.0	0.0	1.1	0.0	0.0
Tigoni	13.8	10.0	0.0	1.1	4.9	4.9
Desiree	8.6	20.0	5.3	3.4	13.2	14.6
Nyayo	3.4	4.0	6.3	2.3	24.3	22.0
Asante	1.7	2.0	1.1	1.1	2.1	0.0
Meru	10.3	0.0	0.0	3.4	3.5	4.9
K. Karibu	1.7	0.0	0.0	0.0	2.1	2.4
Arka	3.4	0.0	1.1	0.0	0.0	0.0
Kibururu	3.4	4.0	6.3	8.0	0.7	0.0
Cardinal	1.7	0.0	0.0	0.0	0.0	0.0
Kanongo	1.7	2.0	0.0	0.0	0.7	0.0
Othorongongo	0.0	4.0	0.0	0.0	0.0	0.0
rangimbili	0.0	12.0	0.0	0.0	0.0	0.0
Kienyenji	0.0	8.0	0.0	0.0	0.0	0.0
pmpernel	0.0	2.0	0.0	0.0	0.0	0.0
Kerr's pink	0.0	0.0	31.6	28.7	0.0	0.0
Roslin Tana	0.0	0.0	4.2	0.0	2.1	2.4
Ngure	0.0	0.0	24.2	33.3	0.0	0.0
Dutch	0.0	0.0	3.2	0.0	17.4	12.2
Munyiri	0.0	0.0	4.2	0.0	0.0	0.0
Komesha	0.0	0.0	1.1	2.3	0.7	2.4
Munyonge	0.0	0.0	1.1	0.0	0.0	0.0
Ntuka	0.0	0.0	1.1	0.0	0.0	0.0
Kombiro	0.0	0.0	0.0	3.4	0.0	0.0
Romano	0.0	0.0	0.0	3.4	0.0	0.0
Thimathuti	0.0	0.0	0.0	1.1	4.2	2.4
Karchi	0.0	0.0	0.0	2.3	0.0	0.0
Kiora	0.0	0.0	0.0	1.1	0.0	0.0
Ninty nine	0.0	0.0	0.0	1.1	0.0	0.0
Kihoro	0.0	0.0	0.0	0.0	10.4	14.6
Karoraiguru	0.0	0.0	0.0	0.0	1.4	0.0
Susana	0.0	0.0	0.0	0.0	1.4	0.0
Nderaciana	0.0	0.0	0.0	0.0	0.7	0.0
mwezimoja	0.0	0.0	0.0	0.0	0.7	0.0
Baraka	0.0	0.0	0.0	0.0	0.0	2.4
None	34.5	32.0	9.5	2.3	9.7	14.6

Farmers who changed their popular potato cultivar in Bomet district mainly did so due to lack of market for the cultivars they had been growing (Table 10). Low yield was the main reason behind farmers in Meru Central and Molo districts rejecting some potato cultivars.

Table 10. Reasons given by farmers in six divisions in Kenya for rejecting some potato cultivars five years ago (% of respondents)

Reasons for rejection	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Lack of market	45.2	40.5	0.0	5.5	28.9	44.4
Low yield	4.8	7.1	57.9	63.6	40.8	33.3
Susceptibility to late blight	2.4	0.0	14.0	27.3	3.9	0.0
Bad taste	0.0	9.5	0.0	0.0	0.0	0.0
Late maturity	0.0	2.4	3.5	0.0	7.9	0.0
Lack of seeds	0.0	2.4	7.0	0.0	0.0	0.0
Poor post-harvest storage	0.0	0.0	1.8	0.0	0.0	0.0
none	47.6	38.1	15.8	3.6	18.4	22.2

3.2 Major Potato Marketing Constraints

In all divisions surveyed, produce price fluctuation is the major marketing constraint (Table 11).

Table 11. Major marketing constraints encountered by potato farmers in six divisions in Kenya (% of respondents)

Marketing constraint	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Price fluctuations	45.2	40.5	19.2	17.1	41.4	17.4
Poor roads	33.3	13.5	0.0	12.2	8.6	21.7
Brokers	11.9	5.4	0.0	0.0	12.1	13.0
Extended bag	2.4	21.6	7.7	9.8	36.2	26.1
Lack of market	0.0	0.0	15.4	2.4	0.0	0.0
None	31.0	37.8	63.5	70.7	44.8	47.8

Farmers pack their potatoes in extended bags Figure 5. Traders buy potatoes on per bag basis and not on weight basis. On the market, however, the traders sell the potatoes in smaller containers such as normal sized bags or buckets. Therefore, an extended bag is advantageous to the trader but exploitative to the farmers.

Figure 5. Extended potato bags used to pack potatoes in Kenya

3.3 Potato Production Constraints

Over 75% of the farmers in the surveyed divisions cited diseases as the main potato production constraint (Table 12). The high cost of fungicides and fertilizer was also mentioned as an important constraint. Lack of clean seeds and high seed costs were cited as production constraints by some farmers.

Table 12. Potato production constraints as cited by farmers in six divisions in Kenya (% of respondents)

Production Constraint	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Diseases	92.9	100	96.2	75.6	98.3	91.3
Unpredictable rainfall	26.2	29.7	0.0	9.8	22.4	4.3
High fungicide costs	9.5	8.1	0.0	0.0	10.3	21.7
High fertilizer costs	16.7	21.6	34.6	51.2	27.6	21.7
Lack of clean seeds	11.9	27	5.8	0.0	8.6	8.7
Insect pests	2.4	2.7	7.7	24.4	5.2	4.3
High seed costs	0.0	2.7	19.2	12.2	1.7	0.0

Among the diseases, bacterial wilt is the most common in all divisions surveyed followed by late blight (Table 13). These findings are in agreement with previous studies by Kaguongo et al. (2010) who found that bacterial wilt is common in all potato growing areas of Kenya affecting 77% of potato farms followed by late blight (67%), and viral diseases (12%). Despite 90% of farmers from Kibirichia using their own seed (Table 6), bacterial wilt prevalence is somehow lower than the other areas. This is possibly because they have been educated on selection of clean seed from their own fields and general field hygiene.

Table 13. Major diseases affecting potato production in six divisions in Kenya (% of respondents)

Disease	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
*Bacterial wilt	90.5	75.7	100.0	61.0	98.3	100.0
Late blight	76.2	70.3	75.0	56.1	46.6	52.2
Leaf rust	2.4	0.0	0.0	0.0	0.0	0.0
viruses	0.0	24.3	38.5	24.4	10.3	34.8
leafminer	0.0	0.0	5.8	0.0	0.0	0.0
nematodes	0.0	0.0	0.0	2.4	0.0	0.0
None	0.0	0.0	0.0	19.5	1.7	0.0

* = was directly observed in the fields. The other diseases were reported by the farmers during interview.

About 40 % of all the farms visited in Kibirichia did not have bacterial wilt (Table 14). Most farms across all divisions had bacterial wilt incidence of 50% and below.

Table 14. Bacterial wilt incidence (%) in six divisions in Kenya (% of farms visited)

Bacterial wilt incidence (%)	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
0	9.5	24.3	0.0	39.0	1.7	0.0
1- 10	14.3	10.8	3.8	41.5	1.7	8.7
11-20	19.0	8.1	19.2	12.2	12	0.0
21-30	9.6	51.3	32.7	2.4	1.7	13
31-40	7.2	5.4	32.7	0.0	82.6	56.3
41-50	40.5	0.0	3.8	2.4	0.0	21.7
51-60	0.0	0.0	1.9	0.0	0.0	0.0
61-70	0.0	0.0	1.9	2.4	0.0	0.0
71-80	0.0	0.0	0.0	0.0	0.0	0.0
81-90	0.0	0.0	0.0	0.0	0.0	0.0
90-100	0.0	0.0	3.8	0.0	0.0	0.0

There was a negative Spearman's Rho correlation (r = -0.295) between bacterial wilt incidence and altitude. This is to be expected because disease expression is favoured by high temperatures. However, this does not mean there are no bacterial diseases; in the cold highlands the real danger is latent infection. A negative correlation (r = -0.354) between bacterial wilt incidence and altitude has previously been observed (Wakahiu et al., 2007). According to a previous study, the highest disease incidence was recorded in sites located 1800-2000 masl while the lowest incidence was observed in sites located over 2600 metres above sea level (Ateka et al., 2001).

3.4 Management of Bacterial Wilt

In addition to crop rotation (Table 4), farmers use different methods in managing the disease in the field (Table 15). About 30% of the farmers surveyed in Molo division did nothing extra to control the disease (Table 15).

Table 15. Farmers' management of wilting plants in six divisions in Kenya (% of respondents)

Management of wilting potato plants	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
None	21.4	21.6	11.5	12.2	19	30.4
Spray with fungicides	2.4	0.0	0.0	0.0	1.7	0.0
Rogue and throw in a hole	14.3	2.7	7.7	4.9	0.0	0.0
Rogue, throw in hole and bury	4.8	13.5	17.3	29.3	8.6	8.0
Rogue and throw in a hole and burn, apply ash in the affected area	16.7	35.1	28.8	4.9	15.5	0.0
Rogue and leave on the path	4.8	5.4	5.8	4.9	6.9	13.0
Rogue and throw far away	16.7	5.4	7.7	0.0	41.4	43.5
Rogue and feed cows	0.0	0.0	9.6	2.4	0.0	4.3
Rogue and leave on the field	0.0	0.0	7.7	0.0	0.0	0.0
Rogue, throw in a hole and burn	9.5	0.0	5.8	2.4	5.2	0.0
N/A	9.5	16.2	0.0	39	1.7	0.0

Over 15% of farmers in all divisions except Molo and Kibirichia manage the disease by uprooting and throwing the wilting plants and their tubers in a hole dug outside the field and burning them. They also remove the soil (from where the wilting plant has been uprooted) and throw it in the hole. Subsequently they apply two handfuls of ash in the place where the plant has been uprooted and mix it well with the soil (Table 15). This bacterial wilt management strategy is currently being promoted by KARI. Ashes and lime are known to suppress the bacteria probably by raising the soil pH (Gildemacher et al., 2007). In addition, ashes have the added advantage of containing nutrients such as potassium and phosphorus. There is no rule on the exact amounts to be applied; one handful of lime or two handfuls of ashes can be used as a maximum dose per plant (Gildemacher et al., 2007).

After harvesting, the majority of the farmers in all the divisions surveyed (except Molo) throw the rotten tubers in a hole and bury (Table 16).

Table 16. Farmers' management of rotten tubers after harvesting potatoes in six divisions in Kenya (% of respondents)

Management of rotten tubers	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Throw in a hole	2.4	2.7	15.4	17.1	0	8.7
Leave on the path	7.1	8.1	1.9	0	6.9	4.3
Leave on the surface in the field	19	10.8	11.5	0	13.8	13
Pile outside field and burn	7.1	2.7	11.5	7.3	1.7	0
Throw in a hole and burn	2.4	2.7	0	2.4	0	0
Throw in a hole and bury	52.4	45.9	44.2	29.3	29.3	8.7
Feed cows	0	0	9.6	4.9	8.6	17.4
Throw far away	0	10.8	7.7	2.4	13.8	26.1
Pile outside farm and leave to rot	0	0	0	0	24.1	21.7
N/A	9.5	16.2	0	39	1.7	0

A few farmers feed the rotten tubers to their animals. Once the tubers are fed to the animals, the bacteria find their way into the manure; because most farmers use cattle manure in their fields, the disease is spread even further in the farms.

4. Discussion and Conclusions

The study aimed at collecting information on potato production in Kenya, potato marketing and production constraints, cultivar preferences, and prevalence and management of bacterial wilt in Meru, Bomet, and Nakuru counties. Important information was gathered through individual interviews with farmers.

There is a shortage of clean potato seed in Kenya and farmers depend on informal seed sources which include farm-saved (self supply), local markets or neighbours. Due to limited supply, the certified potato seeds are highly priced (Ayieko & Tschirley, 2006). The informal system leads to use of poor quality seeds which often accelerates the spread of seed-borne diseases (Kinyua et al., 2001; Ng'ang'a et al., 2003).

Despite the problems in potato sector, farmers allocate more than 25% of their farms to potatoes possibly due to its importance as cash and food crop. In Molo and Elburgon, the allocation is more than 45%. Wakahiu et al. (2007) found that farmers in Nyandarua district (another leading potato producer in Kenya) allocate about 50% of their farm to potato production. In Bomet district, farmers allocate less land to potatoes possibly because they grow tea; another lucrative cash crop. In addition, potatoes do not feature prominently in the diets of the local community. In contrast, potatoes are a major component of the diets of the local communities in Meru Central and Molo districts (McArthur, 1989).

Generally, farmers plant potatoes every second rainy season (Table 4). This is probably due to small farm parcels, limited choices of alternative crops as a result of unpredictable weather especially rainfall, and economic considerations due to a short potato growth period. However, this rotation is too short for proper management of soil fertility and plant diseases especially bacterial wilt. Wakahiu et al. (2007) found that 68.8% of farmers in Nyandarua practice a one season rotation. Furthermore, some farmers in the same county plant potatoes for 3-4 seasons consecutively.

In addition to potatoes, farmers grow other crops probably to meet various uses as well as hedge against the risk of crop failure. This was also observed by McArthur (1989) and Kaguongo et al. (2008). Among the crops, maize is grown by majority of farmers in all divisions surveyed. In this study, it was found that taste, yields and availability of market are the major factors determining potato cultivars grown in an area. This is in agreement with previous studies by Wakahiu et al. (2007). In another study, farmers in the main potato growing counties in Kenya ranked high yields as the most important criterion for growing a specific cultivar (Ng'ang'a et al., 2003).

There are regional differences in potato cultivars grown (Table 7). All farmers in Bomet Central and almost all farmers in Longisa divisions grow the red-skinned Dutch Robyjn. Wakahiu et al. (2007), Kaguongo et al. (2008) and Kaguongo et al. (2010) also found that farmers in these divisions grow Dutch Robyjn. This could be due to the specific processing market that farmers in this area supply. Kaguongo et al. (2010) found that the most commonly grown potato cultivar in Kenya was Tigoni (cultivated by 25.7% of farmers) followed by Nyayo (cultivated by 24.8% of potato farmers) and then Thima thuti (22.7% of farmers). In addition, Tigoni was most popular in Nakuru County (grown by 61.9% of potato farmers and occupying 43.2% of potato area while Nyayo was grown by 37.1% of farmers on 16.3% of potato area in the same county. Tigoni and Nyayo are white-skinned and white-fleshed. The two have since been overtaken by Cangi (a white-skinned white-fleshed) farmer selection (Table 7). In Meru Central district, most farmers abandoned the red-skinned Ngure and Kerr's Pink (Table 9) for the equally red-skinned Asante (Table 7). It appears that despite changing the varieties, farmers did not change the skin colour. This indicates that market demand for a certain skin colour strongly affects variety choice.

Among the potato marketing constraints, price fluctuation is the most important (Table 11). Price fluctuations are due to seasonality in potato production leading to glut and lean times. Most farmers produce potatoes twice a year due to bimodal rainfall patterns in most potato growing areas (McArthur, 1989; Kinyae et al., 2004). The potato growers lack the ability to influence selling prices for their produce because of the poor keeping quality of potatoes and lack of adequate on-farm storage facilities. Over 80% of locally marketed potatoes go through brokers who shield the farmers from getting market information and in the process exploit them.

In the potato producing districts most of the access roads are impassable during wet season. This results in high transportation costs of the produce and a lowering of farm-gate prices by the traders as soon as the rains begin.

Among the production constraints, diseases are the most important. Bacterial wilt is the most common disease in all divisions surveyed followed by late blight (Table 13). The high prevalence of bacterial wilt in the potato growing areas can partly be due to planting of seeds from informal sources as well as inadequate rotation. Most farmers use seeds from informal sources (Table 6) partly due to high cost of certified seeds and/or lack of seeds (Ayieko & Tschirley, 2006). The informal system leads to use of poor quality seeds and often accelerates the spread of seed-borne diseases (Ng'ang'a et al., 2003). This, in addition to lack of effective control method making

bacterial wilt a major constraints headache to small scale potato farmers in Kenya. Although most farmers practice some form of crop rotation (Table 4), the cycle is often too short to eliminate bacterial wilt inoculum in the soil. In addition, farmers leave volunteer potato plants thereby rendering rotation irrelevant. According to Gildemacher et al. (2007), a crop rotation sequence where potatoes are grown once in every four seasons is required so long as no other Solanaceous crop is grown. However, in most potato growing areas in Kenya there is not enough land for such a long rotation (Riungu, 2011).

In addition to a suitable crop rotation scheme, removal of volunteers is extremely important (Gildemacher et al., 2007; The Organic Farmer, May 2012).

The PRA study has provided an insight into potato production in the Kenyan highlands. Most of the farmers are small scale and grow other crops in addition to potatoes. Potatoes are grown for both cash and food. There are regional differences in cultivars planted by farmers; cultivar preferences are mostly dictated by availability of market, yields and taste. Bacterial wilt is a major production constraint; this is managed through many cultural methods including crop rotation. However, all these methods have not been effective; there is need to breed for host resistance.

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Application of the DSSAT Model to Simulate Wheat Growth in Eastern China

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Abstract

In order to test the applicability of the DSSAT model in the Chaohu Lake area, an important drinking water catchment of Anhui Province, the model was calibrated based on three year field experiments (2007-2010). Calibrations were based on wheat growth stages, leaf area index (LAI) and yield. The model was used to simulate the effect of different sowing dates and sowing density on wheat yield and the effect of nitrogen fertilizer level on wheat yield and nitrogen losses.

The simulation results for the three years 2007 to 2010 agreed well with the measured data from the wheat growth experiment with Nash-Sutcliffe model efficiencies of 0.95 (growth stages), 0.85 (LAI) and 0.92 (yield). These results indicate that plant growth development and yield can be simulated efficiently for the conditions at the experimental site.

Simulations with different sowing dates and planting densities showed that early sowing dates correspond with relatively low sowing densities while later sowing dates correspond to medium sowing densities. Compared with the usual sowing date and sowing density for wheat in the experimental region the results indicate that there may be some possible yield increase (and saving of sowing material) with lower densities than actually applied.

The simulated N losses according to the model are largely determined by ammonia volatilization and denitrification whereas the simulated losses due to leaching are negligible. A future optimization strategy for fertilization should focus on the type of N fertilizer (urea versus other N fertilizers), the pH value of the soil and tillage and irrigation measures to reduce ammonia volatilization.

Keywords: Chaohu Lake, DSSAT model, wheat growth, yield, simulation, leaf area index

1. Introduction

At the beginning of the 21st century, there are plenty opportunities and challenges towards sustainable development of Chinese agriculture. China faces huge population pressure and limited arable land resources. Therefore, only by improving the productivity of the cultivated land and reasonable fertilization and irrigation measures can increase food production. Simultaneously, agricultural environmental pollution must be mitigated. In order to increase the output to meet the growing demand of the market, many farmers blindly use large amounts of mineral fertilizers without justification. Therefore, very often an unbalanced system exists where unjustifiable high inputs are accompanied by low outputs resulting in low nitrogen use efficiency (Mittal et al., 2007). This often results in heavy pollution of ground and surface waters.

The main crop in Anhui province (Eastern China) is wheat with a production area of 196 million hectares. This is 8% of the total wheat growing area in China and it is the province with the fourth largest wheat growing area in China (Wang et al., 2008).

Lake Chao is the largest water body in Anhui province. It is located between 117°5′ E and 117°16′ E and between 31°25′ N and 31°43′ N (Hong et al., 2007; Zhang et al., 2008). Lake Chao has an area of 776 km² and its volume is approximately 403 million m^3 (Hefei Municipal Government [HMG], 2006). The cultivated area is 4,780 km² which is about 30% of the total basin area (Wu et al., 2008). However, large amounts of nitrogen fertilizer with less than 30% efficiency (i.e. the ratio of the fertilizer applied to the N-uptake of the whole plant) in the production of

wheat result in high residual post harvest amounts of nitrogen in the soil, an important pollution source for the soil, water and air (Wu et al., 2010). Over 50% of the N and P pollution load comes from agricultural non-point source pollution (Xu et al., 2006).

The growth of wheat is affected by many factors, such as genetic parameters, climate, fertilization, management, amongst others. Winter wheat simulation models proved to be an important tool for knowledge acquisition, determination of quantitative relationships, hypothesis testing, dynamic prediction and decision support. Productivity simulations can be used for yield forecast and for the assessment of N-losses of different fertilization methods. In that way, production rules can be developed before the introduction of specific production methods. Consequently the time for the development of new strategies can be shortened considerably. Several authors state that the development and intensive application of simulation models marked the entrance of world agriculture into the information age (Penning de Vries, 1977; Hoogenboom, 1994).

In this investigation the DSSAT 4.0 (Decision Support System for Agro Technology Transfer) model was used. It is an integrated computer system developed by IBSNAT (International Benchmark Sites Network for Agro Technology). With respect to N dynamics and crop growth, it simulates mineralization, denitrification, volatilization, transport of nitrogen and the growth and nitrogen uptake of plants (Uehara et al., 1998). DSSAT integrates a variety of different crop models and it can be used to verify scientific hypotheses, simulate seasonal changes, spatial transformation and the effect of different management measures on the process of crop growth (Gijsman et al., 2002). DSSAT has models for 18 different crops, including the CERES model, the CROPGRO model and the SUBSTOR potato model. All these models require the same input format for the soil data (e.g. texture, soil organic matter, C/N-ratio), weather data (e.g. precipitation, evapotranspiration, irradiation or daily sunshine hours) and management data (e.g. crop data, variety genetic coefficients, tillage dates, N-fertilizer applications, dates of sowing and harvest) (Iglesias, 2006).

The aim of this paper was i) to test the applicability of the DSSAT model against experimental data under the conditions in Eastern China, ii) to simulate wheat growth as an aid for decision-making procedures, such as sowing density and N fertilizer application, iii) to optimize wheat production by proposing N fertilization options to reduce Nitrate losses and simultaneously to optimize yield.

2. Materials and Methods

2.1 The Test Site

The experimental site was selected in the district of Chaohu in Anhui province (China). The location is situated at 117°47′35" E and 31°38′45" N, 17 meters above sea level. The experimental field is flat; the surrounding areas show a maximum slope of 20%. The experimental field is situated approximately 2 km away from the lake and has abundant water resources, convenient transport facilities, and a moderate climate as an effect of the lake. Therefore, it is suitable for farming all the year round. The wheat variety used was Yangmai-13, a wheat variety commonly used in Anhui province. It is an early mature, semi-dwarf variety with a good tolerance to lodging and high and cold temperatures and a moderate resistance to powdery mildew and stripe rust.

2.2 Soil Data

The soil at the experimental field is a typical paddy soil with high clay content. The soil is classified as a Rice Soil according to the Chinese Soil Classification System. The soil characteristics have been analyzed by standard laboratory methods and are given in Table 1.

Table 1. Physical and Chemical Characteristics of the Soil

Soil depth	pH	Total N ($g \cdot kg^{-1}$)	Organic matter ($g \cdot kg^{-1}$)	CEC ($cmol \cdot kg^{-1}$)	Clay (%)	Silt (%)	Sand (%)	Bulk density ($kg \cdot L^{-1}$)
0-20 cm	7.0	1.58	34.07	16.4	60.3	0.0	39.7	1.34
20-40 cm	7.4	0.27	23.11	14.2	57.2	0.0	42.8	1.33
40-60 cm	7.4	0.14	3.28	13.5	58.2	0.1	41.7	1.46
60-80 cm	7.4	0.24	2.67	13.1	58.8	0.0	41.2	1.50
80-100 cm	7.5	0.13	2.71	23.6	60.0	0.1	39.9	1.55

2.3 Experiment Design

The experiment was designed as a randomized block design with six different treatments with three replications each and was conducted in the years 2007/08, 2008/09 and 2009/10 (Table 2). The treatments were zero fertilization (control), conventional fertilization, optimized fertilization, 30% reduction in N fertilizer, and optimized fertilization plus 3000 kg/ha rice straw. The row spacing was 20 cm and the sowing depth was 3-5 cm. The size of each plot was 30 m^2 (4 x 7.5 m). There was no irrigation during the experiment.

The sowing density was 375 plants/m^2 and 300 plants/m^2 for the broadcast and drill seeded treatments, respectively. Sowing method was broadcast (treatments 1, 2, 3, 5) and drill seeding with a line width of 20 cm and a sowing depth 3-5 cm (treatment 4). The type of N fertilizer was N compound fertilizer plus urea (treatment 2), urea (treatment 3 and 4) and urea plus rice straw (treatment 5). 3000 kg/ha rice straw was used in treatment 5 to cover the field immediately after sowing. The straw was crushed to 3-5 cm long pieces.

The sowing density was 375 plants/m^2 and 300 plants/m^2 for the broadcast and drill seeded treatments, respectively. No dressing or seed soaking was applied. Tillage, seed bed preparation, weed and pest control were applied according to general agricultural practice in the region.

The dates of the different Zadoks growth stages, i.e. tillering (Z 23), jointing (Z 30), booting (Z 41), anthesis (Z 60), physiological maturity (Z 91) and harvest maturity (Z 94) were recorded.

The leaf area index (LAI) was determined using the portable leaf area analyzer LI-3000C measuring the leaf area of three subplots of 0.25 m^2 each.

At harvest, the yield was measured by counting the stems per m², randomly selecting 20 stems, counting the number of grains per ear, drying the grains at 80°C for 8 hours, and determining the weight per grain. From these figures the grain yield per hectare was calculated.

Table 2. Fertilization program of the field experiments 2007-2010

Treatment	Total amount of fertilizer (kg/ha）			Pre-sowing fertilization (kg/ha)			Top dressing (kg/ha)			N ratio pre-sowing/ top dressing
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N February	N March	K_2O February	%
1: zero fertilization (control)	0	0	0	0	0	0	0	0	0	-
2:conventional fertilization	206	68	68	137	68	68	41	28	0	2:1
3: optimized fertilization	211	90	136	126	90	95	53	32	41	3:2
4: 30% nitrogen reduction	148	90	136	84	90	95	32	32	41	4:3
5: optimized fertilization plus 3000 kg of rice straw 2007-2008	210	90	130	126	90	94	52	32	31	4:3
5: optimized fertilization plus 3000 kg of rice straw 2008-2010	148	45	104	84	45	73	32	32	31	4:3

2.4 The DSSAT 4.0 Simulation Model

For this study the CERES-Wheat model in DSSAT was selected (John & Retchle, 1991); it simulates plant growth, plant development and yield on a day by day basis (Jones et al., 2003; Tsuji, 2003).

Before using the model to describe the experimental results, the genetic parameters describing the growth of the wheat variety used must be calibrated. There are 7 genetic parameters in DSSAT: P1V (vernalization sensitivity coefficient), P1D (photoperiod sensitivity coefficient), P5 (grain filling phase duration), G1 (kernel number per unit canopy weight at anthesis), G2 (standard kernel size under optimum conditions), G3 (standard, non-stressed dry weight of a single tiller at maturity), and PHINT (phyllocron interval between successive leaf tip appearances) (Jones et al., 2003). The sensitivity of simulated values describing plant development (days to anthesis and maturity stage), crop components (tops weight, grain yield, straw weight and harvest index), and yield structure (grain number per square meters and single grain weight) to changes in the 7 genetic parameters was evaluated by sensitivity analysis according to Hunt et al. (1993) and Mavromatis et al. (2001). The analysis showed that the most sensitive and, therefore, most important parameters were P1D for plant development stages, and G1 and G2 for crop components and yield structure. These parameters were adjusted based on the data for the year 2007/08 and their optimum was finally determined where the root mean square error (RMSE) of the simulated and observed plant development stages, yield/yield components and yield structure was at minimum. This best combination of genetic parameters (P1V = 24, P1D = 70, P5 = 500, G1 = 17, G2 = 39, G3 = 5.0, PHINT = 95) was used to simulate the experimental data. The calibration of the genetic parameters is described in detail by Hunt et al. (1993). The calibration was validated based on crop development and yield data from the years 2008/09 and 2009/10.

2.5 Model Quality Evaluation

To evaluate the quality of the simulations different quality measures were applied. For a quick overview of the modeling quality, graphs of the measured against the simulated values were drawn together with the linear regression, the correlation coefficient and the 1:1 line. Without any model error, the measured and simulated values are identical and all points should lie on the 1:1 line. The points of good quality simulations should lie close to the 1:1 line, the slope of the linear regression should be close to one and the correlation coefficient should be close to one.

Numerical measures of agreement between the measured and simulated values were used as follows: A simple method to quantify the average difference between the measured and simulated values is the bias (Wallach, 2006):

$$bias = \frac{1}{N}\sum_{i=1}^{N}\left(X_i - P_i\right) \tag{1}$$

where N is the number of observations, X_i are the measured values and P_i the simulated (predicted) values. There should be no bias, i.e. no over- or under-prediction of the values on an average. However, a bias close to zero is not sufficient to quantify model quality, because this could be also a result of a good prediction, or large over- and under-prediction may simply cancel each other.

A measure which avoids compensation between over- and under-prediction is the mean absolute error (MAE; Wallach, 2006):

$$MAE = \frac{1}{N}\sum_{i=1}^{N}\left|\left(X_i - P_i\right)\right| \tag{2}$$

The MAE should be close to zero. Both bias and MAE have the same units as the measured and simulated data.

A widely used measure of agreement between measured and simulated values is the root mean squared error (Wallach, 2006; Xiong et al., 2008):

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(X_i - P_i\right)^2} \tag{3}$$

where N is the number of observations, X_i are the measured values and P_i the simulated (predicted) values. RMSE also has the same units as the measured and simulated values. However, large differences are weighed much higher than small differences between measured and simulated values.

A variant of the RMSE is the relative root mean squared error (RRMSE), which is the RMSE divided by the average of the observed values (Wallach, 2006):

$$RRMSE = \frac{\sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(X_i - P_i\right)^2}}{X_{av}} \tag{4}$$

where X_{av} is the average of the measured X_i values. It is a meaningful measure to compare simulation quality of data with highly different averages and it is independent of the units used (e.g. yield in kg/ha and LAI).

To compare completely different data or different models, a widely used measure is the Nash-Sutcliffe modeling efficiency EF (Wallach, 2006):

$$EF = 1 - \frac{\sum_{i=1}^{n}(X_i - P_i)^2}{\sum_{i=1}^{n}(X_i - X_{av})^2} \quad (5)$$

EF calculates the advantage of model results compared to using one average value. If the model gives perfect results, the predicted values P_i will be equal to the measured values X_i and, thus, EF = 1. If the average of the measured values is used as a predictor for every case, EF = 0. A model which is a worse predictor than the average may result in EF < 0. A model with acceptable quality should have EF > 0.5 (Wallach, 2006).

3. Results and Discussion

3.1 Simulation of Wheat Growth Stages

Table 3 shows the result of the comparison of simulated and measured growth stages. There was no effect of fertilization treatment on the measured or simulated growth stages as this is largely genetically determined. The simulated time to the tillering stage was generally 9 to 11 days longer than the measured values for all years. The simulated time to the jointing stage was between 0 and 10 days shorter compared to the measured values. The difference between simulated and measured time to booting in the different years varied between +13 and -7 days, the difference between simulated and measured time to anthesis varied between +5 and -11 days, the difference between simulated and measured time to physiological maturity varied between +11 and -8 days. The simulated time to harvest varied between 0 and -9 days. The average absolute difference between simulated and measured growth stages was 3.7 days (2007/08), 0.3 days (2008/09) and 2.6 days (2009/10). The good quality of the simulation of the wheat growth stages is a prerequisite for a correct simulation of crop growth and, thus, of yield simulations.

Table 3. Simulated and Measured Wheat Growth Stages (days after sowing) for the three experimental periods 2007/2008/2009

Growth stage (Stage[1])	2007/08			2008/09			2009/10		
	DAS[2] simulated	DAS measured	Days difference	DAS simulated	DAS measured	Days ifference	DAS simulated	DAS measured	Days difference
Tillering (21)	65	54	+11	68	59	+9	61	52	+9
Jointing (30)	145	145	0	149	155	-6	119	129	-10
Booting (41)	158	163	-5	163	170	-7	168	155	+13
Anthesis (60)	176	171	+5	181	181	0	153	164	-11
Maturity (91)	207	196	+11	219	217	2	186	194	-8
Harvest (99)	212	212	0	225	225	0	197	206	-9
Average absolute difference (days)			3.7			-0.3			-2.7

[1]: Zadoks Growth stages; [2]: DAS: Days after sowing

3.2 Simulation of Leaf Area Index (LAI)

The simulated and measured LAI was compared at the 5 growth stages tillering, jointing, booting, anthesis and physiological maturity for the treatments 1 to 4 (zero fertilization, conventional fertilization, optimized fertilization and 30% nitrogen reduction; Figure 1). Treatment 5 (optimized with straw addition) showed the same values as treatment 3 and is not shown in the figure.

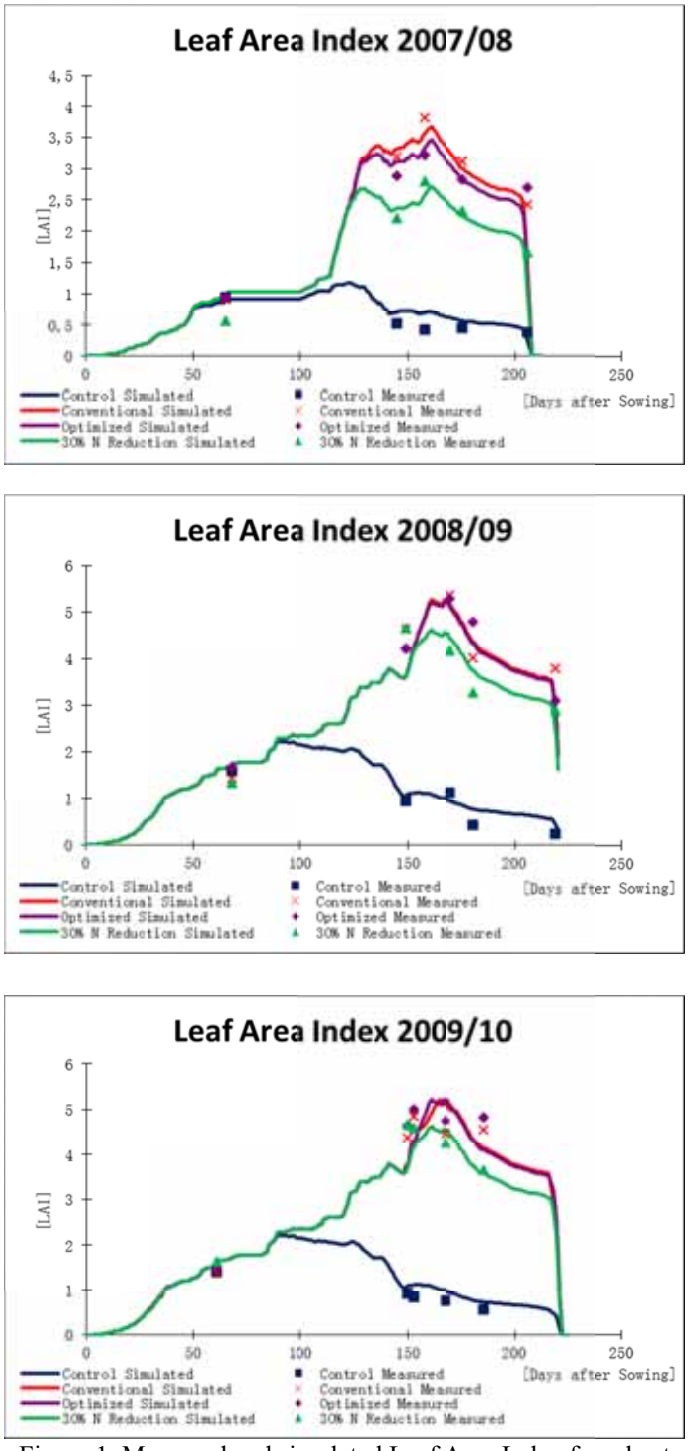

Figure 1. Measured and simulated Leaf Area Index for wheat

The LAI of the conventional and optimized fertilization was very similar and highest throughout the vegetation period, whereas the LAI of the treatment with 30% nitrogen reduction was considerably lower. The LAI of the zero fertilization treatment was much lower than the fertilized ones after about day 100 (Figure 1). Just like in many other studies (Bai et al., 2005), the maximum LAI was measured in the booting stage. There is no effect of fertilization on LAI up to about day 100 (tillering stage). After that, the LAI in the unfertilized treatment is considerably lower, which is the reason for the low yield. After day 150 (booting stage) the LAI of the conventional and optimized fertilization is very similar whereas the 30% N reduction treatment shows a

significant lower LAI. The simulation quality parameters are discussed later. In comparison to the growth stages there is a strong effect of fertilization on the leaf area index as the latter depends on the nutrient status while the former is mainly genetically determined.

3.3 Simulation and Comparison of Yield

The DSSAT model was used to simulate the wheat yield for the different treatments for the years 2007 to 2009 (Table 4). The relative error between the simulated and measured yield ranges between -13.3 and +12.9%, the average error for the different treatments ranges from -8.2 to +6.2%, the average error for the different years ranges from -0.7 to -6.5%. Generally, the error is negative indicating that the simulated yield is slightly lower than the measured yield. A possible reason will be the manual yield measurement which minimizes grain losses. Yield measurements based on plot combines or on field combines under practical conditions will be less and, thus closed to the simulated results.

Table 4. Simulated and measured yield (kg/ha) for the three experimental periods 2007/2008/2009

Treatment	2007/08			2008/09			2009/10			Average error %
	sim.	meas.	error %	sim.	meas.	error %	sim.	meas.	error %	
1:zero fertilization (control)	2283	2334	-2.2	1889	2178	-13.3	1735	1741	-0.34	-5.3
2:conventional fertilization	5528	5723	-3.4	6373	5645	+12.9	5445	4985	+9.23	+6.2
3:optimized fertilization	5459	5945	-8.2	6160	5889	+4.6	5275	5329	-1.01	-1.5
4: 30% nitrogen reduction	4866	5501	-11.5	5491	5489	+0.0	4444	4799	-7.40	-6.3
5:optimized fertilization plus 3000 kg of rice straw	5529	5945	-7.0	5533	6000	-7.8	4485	4967	-9.70	-8.2
Average error %			-6.5			-0.7				-3.0

3.4 Simulation Quality

The overall agreement of the measured and simulated data (graph of the measured against the simulated values) was good with R^2 of 0.98 (growth stages), 0.92 (LAI) and 0.94 (yield) (Figure 3).

The simulation quality for the wheat growth stages (Table 5) was very good for year 2008/09 and 2009/10 with a modeling efficiency of 0.99, whereas in 2007/08 the simulation efficiency was only 0.85. This result is also reflected in the bias, mean absolute error, RMSE and RRMSE. The overall bias was slightly negative (-3.4 days) indicating the model simulated a slightly longer time to reach the different growth stages. The mean absolute error was 10.5 days, the RMSE was 18.4 days and the relative RMSE (RRMSE) was 17.1%.

The quality for the simulation of the LAI showed a similar tendency as the growth stages with respect to modeling efficiency EF: It was highest in the year 2009/10 and lowest in 2007/08. The overall bias was slightly positive (+0.11) indicating that the model simulated a slightly smaller LAI compared to the measured values. The mean absolute error was 0.33, the RMSE was 0.45 and the relative RMSE (RRMSE; 17.1%) was nearly the same as for the wheat growth stages (Table 5).

The wheat yield simulation resulted in modeling efficiencies of 0.93 to 0.91 where the first year was not worse than the subsequent years. The overall bias was positive (+132 kg/ha) indicating that the model simulated a slightly higher yield on an average compared to the measured values. The mean absolute error was 326 kg/ha, the RMSE was 394 kg/ha and the relative RMSE (RRMSE; 8.2%) was only half as much as that of the growth stage and LAI simulation (Table 5).

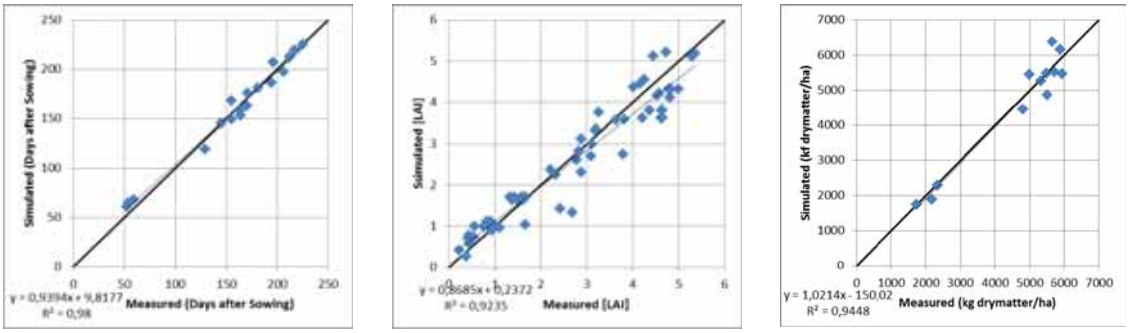

Figure 3. Measured and simulated wheat growth stages (left), leaf area index (center) and wheat yield (right)

Table 5. Model quality for wheat growth stages, leaf area index and yield for 2007-2010

2007-2010 Simulated Wheat Growth Stages (example harvest maturity, days after sowing)						
Year	Average (days)	Bias (days)	MAE[1] (days)	RMSE[2] (days)	RRMSE[3] (%)	EF[4]
2007/2008	212	-16.7	17.3	29.3	29.9	0.85
2008/2009	225	+1.2	5.5	6.4	6.0	0.99
2009/2010	197	+5.3	8.7	9.4	8.3	0.99
All Years	211	-3.4	10.5	18.4	17.1	0.95
2007-2010 Simulated Leaf Area Index						
Year	Average	Bias	MAE	RMSE	RRMSE (%)	EF
2007/2008	1.91	+0.10	0.28	0.44	22.8	0.66
2008/2009	2.94	+0.15	0.41	0.51	17.2	0.79
2009/2010	3.17	+0.10	0.31	0.39	12.4	0.87
All Years	2.67	+0.11	0.33	0.45	16.8	0.85
2007-2010 Simulated Wheat Yield						
Year	Average (kg/ha)	Bias (kg/ha)	MAE (kg/ha)	RMSE (kg/ha)	RRMSE (%)	EF
2007/2008	5090	+357	357	413	8.1	0.91
2008/2009	5040	-49	351	425	8.4	0.91
2009/2010	4364	+87	271	339	7.8	0.93
All Years	4831	+132	326	394	8.2	0.92

[1] MAE: Mean Absolute Error, [2] RMSE: Root Mean Square Error,
[3] RRMSE: Relative Root Mean Square Error, [4] EF: Model Efficiency.

4. Model Application

4.1 Effect of Sowing Date and Sowing Density on Simulated Yield

Table 6 gives the simulated yield with respect to different sowing dates (beginning of October to middle of November) and different sowing densities simulated with the calibrated model for the year 2009/2010 and fertilizer treatment no. 3 (optimized fertilization). The simulated yield ranges from 6400 to 4000 kg dry matter/ha. For early sowing dates (first two weeks in October), a sowing density of 80 to 100 plants/m² gives a maximum yield. Later sowing dates (first two weeks in November) result in highest yields with higher planting densities 120 to 190 plants/m². The absolute highest yield (6404 kg dry matter/ha) was simulated with a sowing date of Oct., 3rd and a planting density 80 plants/m^2. The next highest yield (6059 kg dm/ha) was simulated with a sowing date of Nov., 7th and a planting density 150 plants/m².

The results showed that for climatic condition investigated, early sowing dates correspond with relatively low planting densities (80-100 plants/m²) while later sowing dates correspond to medium planting densities of 190 to 220 plants/m². Compared with the usual sowing date and sowing density for wheat in the region of the experimental field (middle of October, sowing density 350 plants/m²), the results indicate that there may be some possible yield increase (and saving of sowing material) with lower densities than actually applied.

Table 6. Effect of different sowing dates and planting density on wheat yield (kg/ha) for treatment 5 (optimized fertilization plus rice straw) for the year 2009/2010

Sowing date	Planting density (plants/m^2)							
	80	100	120	150	190	220	250	300
10/03/2009	6404	6002	5805	5592	5420	5285	5130	4914
10/08/2009	5162	4855	4746	4554	4376	4256	4146	3997
10/13/2009	6006	5759	5423	5164	4960	4871	4832	4765
10/18/2009	5958	5696	5509	5133	4939	4832	4680	4573
10/23/2009	5755	5531	5385	5129	4778	4642	4580	4415
10/28/2009	5676	5383	5116	4981	4745	4523	4393	4206
11/02/2009	5442	5446	5166	4926	4720	4629	4519	4319
11/07/2009	5327	5774	6049	6059	5873	5706	5620	5435
11/12/2009	5231	5661	5944	5876	5644	5471	5412	5259
11/17/2009	4906	5329	5635	5762	5591	5469	5322	5248

4.2 Simulation of Different N Fertilizer Levels on Simulated Wheat Yield and N Loss

Because plant growth and yield is described correctly by the model, it can be assumed that also the magnitude of the N losses will be simulated correctly. To evaluate the effect of N fertilizer on wheat yield for the conditions studied, simulations were carried out for 5 different fertilizer levels (0 to 280 kg N/ha) (Table 7). The results show that highest yield (7516 kg/ha) is obtained with 280 kg/ha N. The relative increase in yield is decreasing with higher fertilization levels. However, in addition to higher yields, the extra amounts of N increase the N losses considerably and, thus, increase adversary effects on the environment. With increasing fertilizer levels, the simulated losses due to ammonia volatilization increase up to 98 kg N/ha whereas the simulated losses due to denitrification increase only up to 24 kg N/ha. The losses due to leaching are negligible. The total N losses are between 36 and 44% of the whole N fertilizer application.

The reasons for the high ammonia volatilization losses are urea fertilization in combination with high pH values (pH 7-7.5); under such conditions urea is converted to ammonia by hydrolysis, and if the urea is not incorporated into the soil, the ammonia is lost to the air. Other factors are high temperatures and relative high wind velocities due to the open topographical position. Future strategies to minimize ammonia volatilization must concentrate on different N fertilizers or the immediate incorporation of the urea into the soil.

The reason for the denitrification losses are reducing conditions due to the high clay content of the soil resulting in reducing chemical conditions over long time periods.

Table 7. Simulated wheat yield and Nitrogen losses for different levels of N fertilizer application

Nitrogen fertilizer (kg/ha)	Yield (kg/ha)	N leached (kg N/ha)	Denitrification (kg N/ha)	Ammonia volatilization (kg N/ha)	Total N losses (kg N/ha)	N losses (% of fertilizer application)
0	2492	1	8	0	9	--
70	4775	1	12	12	25	36%
140	6259	1	15	35	51	36%
210	7104	1	19	64	84	40%
280	7516	1	24	98	123	44%

5. Conclusions

The simulation results for the three years (2007-2010) of the wheat growth experiment are good with overall model efficiencies of 0.95 (growth stages), 0.85 (LAI) and 0.92 (yield). These results indicate that plant growth development and yield can be simulated efficiently for the conditions at the experimental site. Therefore, the model can be used to find optimum sowing density and sowing date for the conditions investigated. The results indicate that there may be some possible yield increase (and saving of sowing material) with lower densities than actually applied (80 to 100 plants/m^2 for early sowing dates and 120 to 190 plants/m^2 for later sowing dates). These tendencies were similar for the other two years of the experiment. Therefore, the aspect of lower plant densities should be investigated in further field experiments.

Wheat yield increases with the amount of applied N with a decreasing increment. However, the additional N fertilization also results in additional N losses to the environment. The most important source of N losses is ammonia volatilization and, to a much smaller extent, denitrification losses. N losses due to leaching are negligible in this experiment. A future optimization strategy for fertilization should focus on the type of N fertilizer (urea versus other, however more expensive N fertilizers), the pH value of the soil and tillage and irrigation measures to reduce ammonia volatilization.

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The Prediction of Population Dynamics Based on the Spatial Distribution Pattern of Brown Planthopper (*Nilaparvata lugen* Stal.) Using Exponential Smoothing – Local Spatial Statistics

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Abstract

This study aims to predict the population dynamics of Brown Planthopper (BPH) in highly endemic areas of Central Java province, Indonesia. The research was conducted by modifying the method proposed by Legendre and Fortin (1989), through three stages. Those were predicting BPH attacks using Exponential Smoothing Holt Winter, analyzing spatial structure using I, C and Z test on Local Statistic, and making the connectivity inter the periodic predictions of planting season. The results showed that, the studied areas will experience the hotspots phenomenon based on the analysis by the method of Moran's I, Geary's C and Getis Ord Statistic. The analysis of Local Moran's and Getis Ord showed that, four counties namely Boyolali, Klaten, Karanganyar and Sragen experienced a local migration current from region to region around them, whereas other counties are independent. The migration current was influenced by topography, biotic interactions, and anthropogenic factor. Viewed from the spatial scalability in the studied areas, there are four categories of BPH population distribution; point, site, local, and landscape. BPH local migration interregion happened in the County of Klaten, Boyolali, Karanganyar and Sragen. It was caused by some factors: (1) the local climate, (2) the repetition of the use of rice plant variety in a long time, (3) the use of insecticide intensively (3-4 times in one planting period/season), and (4) the irrigation, allowing the spread of BPH larvae and eggs into its surroundings.

Keywords: population dynamic, brown planthoppers, spatial pattern, local spatial statistic

1. Introduction

1.1 Background Research

The population dynamics of Brown Planthopper (*Nilaparvata lugens* Stal.), hereinafter referred to the BPH, can be modeled and predicted using statistics approach (Damos & Soultani, 2012; Hunter & Price, 1998). BPH population dynamics are influenced by three components, of which the source of food, the natural enemies, and the biotic factors such as weather and topography (Hunter & Price, 1998). Those three components can be a limiting factor of the BPH population development in a particular time and zone. The significant indicator that can be used as an indication of the limiting factors in the BPH population dynamics is the concentration of the population following the spatial and temporal patterns (Perry, 1997). Basically, BPH population dynamics models can be used as a signal occurrence identification of factors that affect population fluctuations and cycles, the reconstruction of population distribution, and the empirical test of the natural population dynamics phenomenon (Ellner, Seifu, & Smith, 2002). Population dynamics can be classified into two categories : descriptive quantitative, is the identification of changes in the total population that can be used to measure the trend and predictions for the future and identification of the various physical and biological factors as the population limiting factors in order to develop a strategy of population control (Juliano, 2007).

Several countries have taken the advantage of population dynamics data for predicting the future population tendency. They have also carried out the strategy of BPH population control through the surveillance program of plant pest, which was called as insect of crop. One of the prediction methods used in pest surveillance program is

Holt-Winters Exponential Smoothing, which is later on called as HW (Pal & Gupta, 1994). Based on empirical studies, HW method was chosen for surveillance with some considerations, which are: simpler, adaptive and easy to be developed in the application, more accurate and reliable compared to other methods, and effective for seasonal spatiotemporal data classification on various scalability (Shmueli & Fienberg, 2005; Unkel & Farrington, 2010; Lu, Zeng, & Chen, 2008; Burkom, Muphy, & Shmuelli, 2006). HW method is appropriate for seasonal and trend pattern data, such as BPH surveillance data (Ai, 1999; Suhartono, 2008).

BPH is one kind of a rice plant (*Oryza sativa* L.) pest, which is the most destructive and has a major impact on food tenacity around the world. In Asia, BPH attacks occurred in Bangladesh, Brunei, Burma (Myanmar), China, Hong Kong, India, Japan, Cambodia, Korea, Laos, Malaysia, Nepal, Pakistan, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, and Vietnam (Catindig et al., 2009; Kuno & Dick, 1984). In 2011, BPH attacks in Indonesia covered 228,133 ha, the highest attack intensity occurred in East Java with an area of 147,857 ha and Central Java with an area of 50,390 ha. In Central Java, the minor attack intensity covered 32 474 ha, 3867 ha for middle attack, 804 ha for severe attack, 13,245 ha for puso (Humas, 2012; Deptan, 2012).

Widely BPH invasion was caused by the migration capability in a remote geographical distance following seasonal wind flow (Monsoon). Physiologically, BPH is capable of flying at the speed of 5-11 m/sec and in an altitude of 1000-3000 AD (Wada et al., 2008; Seino et al., 1987). BPH migration and distribution will lead to changes of the complex spatial and temporal structure comprising topography, climate, anthropogenic and biotic interactions factors (Wang, 2009). Distribution of population caused by topography factor is irrigation, climatic factors (duration of solar radiation, temperature, rainfall and humidity) that support the proliferation of BPH (Win et al., 2011). The population distributions caused by anthropogenic factors are the action of fertilization and using insecticides (Win et al., 2011). Furthermore, distribution of population caused by biotic interactions factor, for example regeneration ability in temperate region can only occur for three generations, whereas when migrating to the tropics, it is able to reach 12 generations per year (Bottrell & Schoenly, 2012).

All this time, the distribution of population modeling has been done using descriptive statistical approach which comprises three methods: K-Ripley, Moran's I, and the autocorrelation function (Aukema et al., 2008). Moran's I method can be used to explain the distribution of a group of individuals in one population of hosts or predators and give an overview of the frequency distribution of the population (Ellner et al., 2001; Ellsbury et al., 1997). This study aims to predict the population dynamics based on the spatial distribution patterns of BPH population in the province of Central Java Indonesia which becomes high endemic of BPH. The research was carried out by modifying the method proposed by Legendre and Fortin (1989) as a contribution in this study. Population dynamics prediction method that uses spatial pattern approach was done in three stages: the prediction of BPH attacks using HW, the analysis of the spatial structure using test I, C and Z on Local Statistics and the connectivity inter the periodicity planting season prediction (Legendre & Fortin, 1989).

This paper is organized as follows: Section 1 describes research background. Section 2 describes previous research as a reference of this study. Section 3 describes the theoretical background of the spatial distribution of spatial insect, spatial distribution insect pattern, and local spatial statistics. Section 4 explains the methods and stages of the research. Section 5 describes experiments containing research data, BPH attack prediction, the prediction of spatial distribution, and visualization of the spatial distribution predictions. Section 6 provides the conclusions and the future research.

1.2 The Related Works

Insect population dynamics phenomenon was originated from the existence of geographical factor as the limiting evolution process, like the ocean, mountains, and glaciers that were formed 400 million years ago. The acceleration of insect population dynamics had occurred when human motilities have increased drastically in the last 1000 years. It occurs through the formation of lineages in a new location (the invasion) either naturally or anthropogenically. The invasion of insect is divided into three phases, namely: the arrival phase is the process by which individuals move into new areas outside their native range, the growth phase is the process by which a population grows to a sufficient level so that extinction is highly unlikely, and spreading phase is attacking species range expansion into new areas (Liebhold & Tobin, 2008). Insect population dynamics in various scenarios such as changes in weather conditions, changes in the landscape and the abundance of food resources in the environment can be modeled using spatial and temporal approach (Gruebler, Morand, & Daenzer, 2007).

The insect distribution modeling through several approaches comprising are: non-linear method, the estimation of *disperse index*, the frequency distribution and Geostatistics. In the following decade, Geostatistical methods got more attention from ecologists as the solution in population dynamics (Tobin, Fleischer, & Pitts, 1999).

Geostatistics method use for the development of Integrated Pest Management model using GIS technology (Geographical Information System). This model was applied for several purposes including: modeling the pest and predator insect spatial distribution, detection and monitoring of the pest insect spatial structure in a broader scalability, the determination of high-risk of pest areas based on Agroclimatological information, and compilation of time and intensity of pest emergence prediction. The classification of attack potency was assessed based on the pest spatial and temporal tendencies comprising: the pattern of eating habits, the potency of economic impact, the pattern of population dynamics, the method of spreading and recommendations to handle the attack (Dminić et al., 2010).

Minh et al. (2002) developed an early warning model of BPH potential risks in Trungan, Thotnot District, and Cantho City Vietnam by combining several methods including multivariate regression, interpolation and Geostatistics. The study was conducted using data collected from 120 locations and 10 observation periods. The basic principle of this study was to determine the pattern of the relationship of environmental rainfall factors, maximum and minimum air temperature, air humidity and the prevalence of BPH attacks. This model can provide information about predictions of the spatial distribution occurrence prior to the BPH attack. Another observed factor is the high of water surface, the density of natural enemies and periodicity fertilization.

Song et al. (1994) conducted the use of geographic information systems study to analyze the spread movement of rice plants (*O. sativa*) insect in South Korea. The study was conducted using surveillance data from 152 observation stations of pest plant with data from the period of 1981 to 1991. The analysis of BPH spatial distribution patterns was conducted based on the results of the observation value interpolation process throughout the observation stations. This study shows that the BPH population dynamics are influenced by the temperature and migration variable.

Prasetyo et al. (2012) had conducted the research of BPH endemics determination using GISA, LISA, and Getis Ord methods in Central Java, Indonesia based on the historical data in 2001-2010. The studied areas consist of 7 counties, constituting the high BPH endemics areas. According to that research, it can be seen that the pattern of spatial object connectivity, such as BPH population centralization/clustering, the rainfall, and the geographical position of the area influences the distribution of BPH population in the whole studied areas.

1.3 Insect Spatial Distribution

Spatial distribution is the most dominating characteristic in the life of the insect population. Its attribute is dynamic. As a result, spatial and temporal variability will form different population structure. The knowledge of population structure both spatially and temporally will be able to provide clues to the information, which are: the spread of insect, identification of population dynamics and population density. This information will be useful in pest control framework and understanding of ecological processes that occur on a local scale (Debouzie & Thioulouse, 1986; Pata et al., 2010). The study of the insect population spatial distribution and other biophysical factors may help us to reveal several things related to the life cycle of insect, namely: the characteristics of the environment and the spread of the individual, the development of habitat manipulation strategy especially the beneficial species, and the design of the sampling area determination, assuming that the appropriate design can help to overcome the deviations caused by spatial heterogeneity (Holland et al., 1997).The focus on the determination of the spatial pattern was to see if a population of organisms has a random, homogeneous, or the combination of random and homogeneous distribution. However, this concept is extended to see how great the size of the organisms' population convergence is (Perfecto & Vandermeer, 2008). The spatial pattern is one of the important indicators in identifying organisms that form the dispersion of disease vectors. One type of vector-borne disease will not spread even be endemic if that disease focuses on one location only. Thus, the changes of spatial pattern will affect the changes in spreading and increasingly expanded the organism attacks (Klas, 1965). Generally, the study of spatial patterns for the ecological analysis has four objectives: testing spatial autocorrelation, spatial structure test, the causal variables test, interpolation mapping and the structure of spatial autocorrelation function. Every research goal has different methods and results, as shown in Table 1.

Table 1. The analysis methods of spatial pattern for the ecological analysis based on the research objectives according to Legendre and Fortin (1989)

Objective	Method	Analysis
Testing SpatialAutocorrelation	Moran's I	Correlogram Single Variable
	Geary'c	
	Mantel Test	Mantel Test between variable
	Mantel Correlogram	Multivariate Data
Description Spatial Structure	Correlogram, Variogram	Description Spatial Structure
	Clustering	Description Spatial Structure
Test Causal Model	Partial Mantel Test	
Estimation and Mapping	Trend Surface Analysis	Map Interpolation SingleVariable
Spatial Autocorrelation	Variogram	Kriging Map
Structure Function		

The approach of the spatial patterns allows the predictive modeling and detailed mapping to be compiled in order to get a better understanding of the formation of an endemic pattern of a disease. The method of determining spatial patterns of endemicity can be done by the measurement of the studied area (Chadsuthi et al., 2010). The methods of characterization of the spatial patterns based on the research objective divided two categories: the measurement of population distribution pattern by the NNA and QA method, detecting the spatial pattern of organisms attack by the method of SAA. This can be seen in Table 2 (Chaikaew et al., 2009).

Table 2. The characteristic of spatial pattern according to Chikaew et al. (2009)

Method	Objective
Quadrant Analysis (QA) and Nearest Neighbor Analysis (NNA)	The measurement of spatial pattern for determining population distribution
Spatial Autocorrelation Analysis (SAA)	Detecting spatial patterns of organism attack based on the attributes of the two scenes.

1.4 Local Spatial Statistics

Local spatial statistics was developed to meet the needs of the measurement and analysis of observation data result connectivity in a small area. That information will be useful for: (1) the identification of the population concentration or hotspot, (2) the assessment of the data stationarity structures, and (3) the identification of object distances that is out of reach of the population but has connectivity to population centers (outliers). Local spatial statistics function comprising Getis-Ord Statistics called as G and G*, Local Moran's called as I and Local Geary called as C (Getis & Ord, 1996). Local spatial statistics function, that has oftenly been used in practice, comprises G (d) statistics, Local Moran's and Local Geary (Du & Chen, 2003). Getis-Ord Statistics function can be seen in equation 1 and 2, and Local Geary function in Equation 4.

$$G_i(d) = \frac{\sum_i w_{ij}(d)x_j - W_i\bar{x}(i)}{s(i)\{[(n-1)S_{1i}) - W_i^2]\}^{\frac{1}{2}}}, j \neq i \quad (1)$$

$$G_i^*(d) = \frac{\sum_i w_{ij}(d)x_j - W_i^*\bar{x}^*}{S^*\{[(nS_{1i}^*) - W_i^{*2}]/(n-1)\}^{1/2}}, allj \quad (2)$$

In this equation, n is identified area with the georeference i= 1, 2,…n. Each i value is associated by variable value of the research in studied area represented by x notation. $w_{ij}(d)$ notation is spatial weight vector, with the value defined as the distance among i area. Local Morans'I function according to Du and Chen (2003) is on equation 3.

$$I = (Z_i/S^2)\sum_j^n w_{ij}Z_j \quad (3)$$

$Z_i = x_i - \bar{x}$ and $Z_j = x_j - \bar{x}$ is deviations from the mean value of the observed study variables. w_{ij} notation is a weight matrix element which will be used for determining spatial proximity among areas. S^2 notation is the weighted mean of the deviation around. Local Geary function according to Du and Chen (2003) is on equation 4.

$$C_i = \sum_j^n w_{ij} (Z_i - Z_J)^2 \quad (4)$$

Local Geary was used to calculate the difference between the squared deviations in location *i* and location *j*.

2. Material and Methods

2.1 Research Procedure

The analysis of time series on surveillance data requires a minimum of 30 periods of observation (Ylioja et al., 1999). The criterion of number of observations area of the research that aims for the exploration of the spatial structure is at least 30 regions (Fortin & Dale, 2005). In accordance with this approach, this study used data of 240 observations and 124 observation area. The research was conducted in four steps, as shown in Figure 1, step I is the classification of BPH outbreak data and rainfall data into three planting season, which are : main planting season, gadu planting season and dry planting season. Step II is the prediction of BPH potential attacks using the exponential smoothing holt-winters method. Step III is the modeling and mapping of the spatial distribution using local spatial statistical approaches, including: Local Moran's (I), Local Geary (C) and the Getis-Ord Statistic (G and G *). Step IV is the visualization of the distribution in the form of: choropleth map, G and G * map, Local Moran's and Geary Local Map and Boxplot. Final step is the analysis and interpretation of results based on the type of spatial patterns which consists of the three forms, namely: the pattern of concentration (Cluster Pattern), the pattern in random (Random Pattern) and the pattern of spread (Dispersed Pattern) as in Table 3 (Zhang et al., 2009).

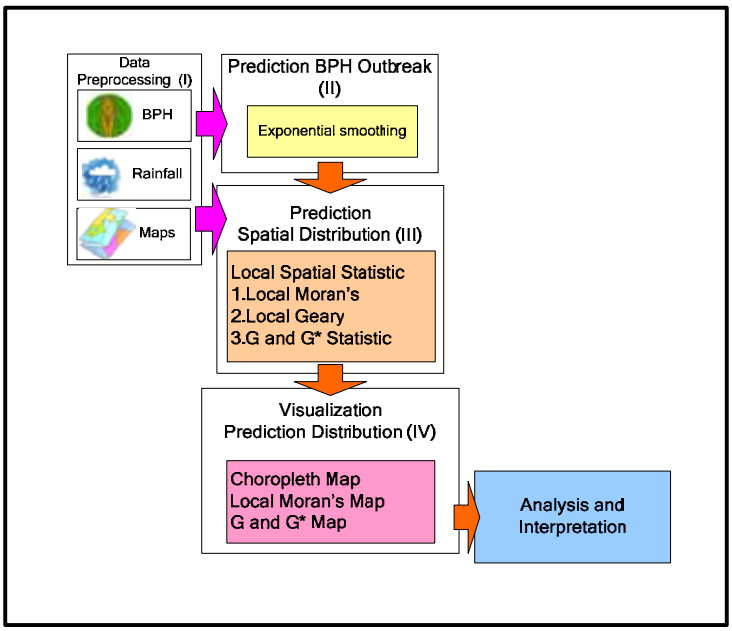

Figure 1. The research stages

Table 3. The Spatial Pattern according to Zhang et al. (2009)

Spatial Pattern	Geary's C	Moran's I
Cluster Pattern	0<C<1	I>E(I)
Random Pattern	C~=1	I~=E(I)
Dispersed Pattern	1<C<2	I<E(I)

Getis-Ord Statistic method in this study is used for detecting and evaluating local spatial autocorrelation on BPH distribution. The high value of Getis-Ord statistic represents the concentration occurence (cluster). The high value is notated as High (hot spots). Whereas the low value of Getis-Ord statistic represents the concentration occurrence (cluster). The low value is notated as Low (cold spots) (Truong & Somenahalli, 2011). Francis et al. (2012) interpreted the value of Z to the criteria if the value of $Z < -1.96$ called as cold spots, if the value of $Z > 1.96$, it is called as hot spots with a confidence level of $p < 0:50$. The analysis of the spatial distribution of BPH is done by considering other environmental factors including anthropogenic, fertilization, predator behavior and climatic factors, rainfall.

2.2 The Research Data

The surveillance data used in this experiment is collected from Pest Plant Diseases Laboratory Observations Region V Surakarta, Central Java, Indonesia. The area of surveillance covering 6 counties is divided into 124 districts as the observation stations. The data periodicity was biweekly taken between 2001 and 2010. The data research consists of two forms, first is data of BPH outbreak and rainfall each of 124 records and second is map data in the form of shape files (ESRI ArcView formats). Cumulative choropleth map of BPH attack occurrence in 2001-2010 in studied area is on Figure 2.

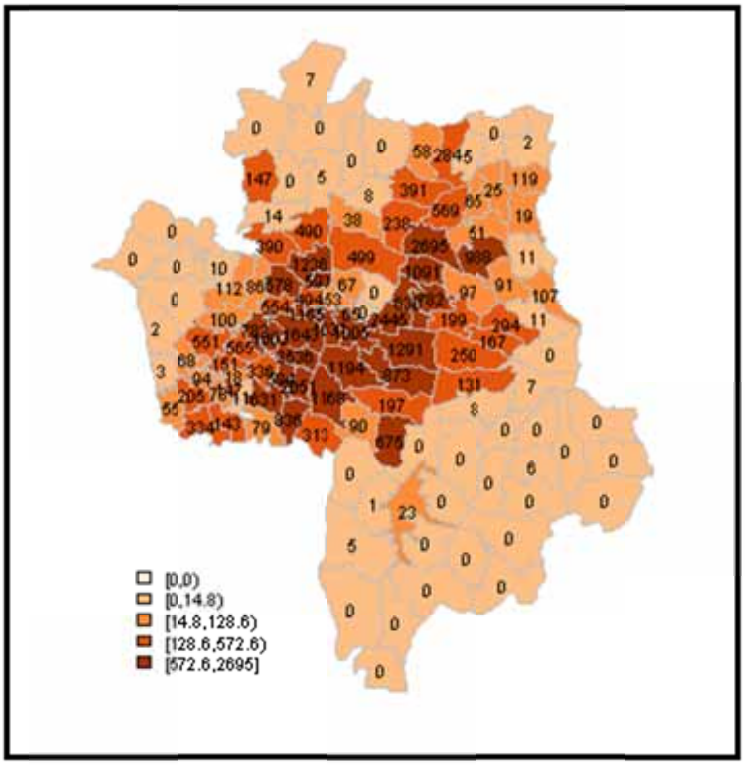

Figure 2. The choropleth map of BPH attack occurrence in 2001-2010 in studied area

From Figure 2, we can see that, the BPH distribution for 10 years is in all counties that have geographically connectivity. The term connectivity is defined as the behavior of local or individual regional movement among habitats in the same periodicity. The pattern of population movement constitutes one indicator to determine the population dynamics of insect. The limiting factor that determines the movement of the local scope includes the form of isolation (distance) and the size of the population (Matter et al., 2005). The cumulative data of attack occurrence will be the basis of predictions of BPH and BPH spatial modeling population dynamics. The prediction and modeling of spatial distribution pattern of BPH used the R (http://cran-project) version 2.14. Prediction version using package tseries and modeling spatial patterns used spdep package, sp, classInt, RColorBrewer, maptools, rgdal and maps.

3. Results

3.1 The Prediction BPH Outbreak

Generally, there are three planting period: the main planting season which takes place in November-February, Gadu planting season that takes place in March to June, and a dry planting season which lasts between the month of July to October (Wijaya, 2000). Based on the BPH attack data in the planting season periodicity between the year of 2001 - 2010, BPH attack patterns have been detected since 2005 and reached its peak in 2009-2010 (Figure 3). Trend information (red line) and the seasonal data pattern (blue line) can be identified to see the trend of the future attacks.

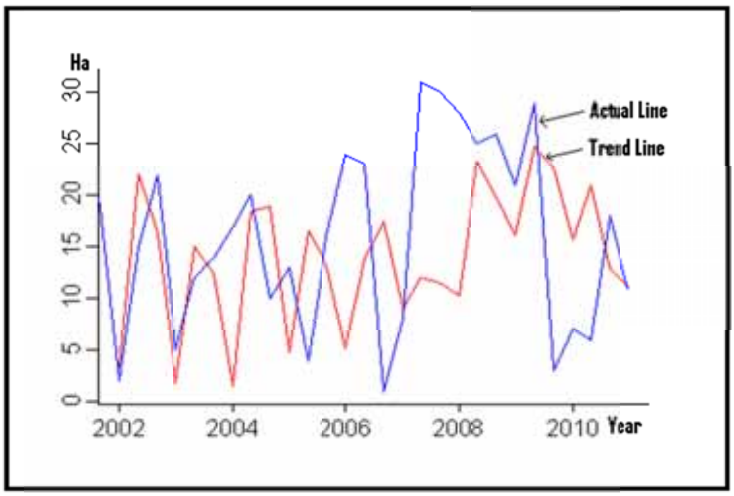

Figure 3. The data and trend line of BPH attack occurrence in studied area of 2001 – 2010 in planting season period

The prediction of BPH attack occurrence was conducted based on the trust significance level of 50, 80, and 95 percents for next six seasons. The result of the prediction of the BPH attack occurrence can be seen on Figure 4. The validation of prediction result was tested using means of error (ME), which was 8.8909453, root means square error (RMSE), which was 14.8319699, means absolute error (MAE), which was 12.1873029, means percentage error (MPE), which was -0.7532082, means absolute percentage error (MAPE), which was 135.8722813, and means absolute scaled error (MASE), which was 1.4062273.

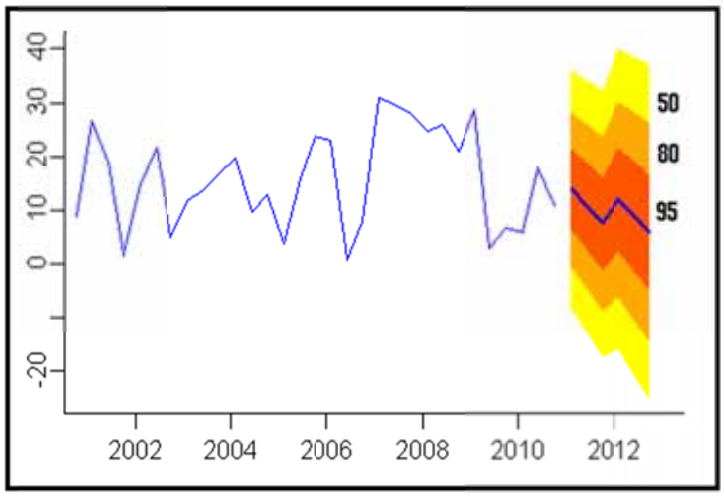

Figure 4. The prediction of BPH attack occurrence in six seasons in studied area on the trust level of 50%, 80% and 95%

3.2 The Prediction of Spatial Distribution

The prediction of BPH population distribution per county based on the spatial pattern according to the method of Zhang et al. (2009) is in Table 4.

Table 4. The comparison of the prediction of six period-Moran's I and Geary's C spatial pattern per county according to Zhang et al. (2009)

County	(I)	Spatial Pattern	(C)	Spatial Pattern
Boyolali	0.5238903	Cluster	0.5757535	Cluster
Klaten	0.1844786	Cluster	0.6559216	Cluster
Sukoharjo	0.0695636	Cluster	0.7633815	Cluster
Karanganyar	-0.221604	Cluster	1.1410184	Dispersed
Wonogiri	-0.036473	Cluster	0.9275305	Cluster
Sragen	0.0711412	Cluster	0.7198907	Cluster
Surakarta	-0.404762	Dispersed	1.1904762	Dispersed

Based on Table 4, the potential occurrence of local migration and population distribution from one area into surrounding areas through a variety of media that have connectivity can be identified. However, the high endemicity has not occured yet because of the humidity factor, as an indicator of low population increase. The low humidity was caused by the low rainfall in the studied area. The analysis of Getis-Ord Statistic is used to detect local spatial association indicated by the formation of hotspots. Hotspots represent the concentration of the number of occurrences of high BPH attacks. On the other hand, concentration of low value, called as coldspots, represents the concentration of a low number of BPH attacks occurrence (Table 5).

Table 5. Hotspots detection using the analysis of Getis-Ord Statistic

County	Getis-Ord Statistic	Spatial Pattern
Boyolali	0.60852186	Hotspots
Klaten	0.21746730	Hotspots
Sukoharjo	0.37549060	Hotspots
Karanganyar	0.05891069	Hotspots
Wonogiri	0	-
Sragen	0.32170523	Hotspots
Surakarta	1.00	Hotspots

4. Discussion

4.1 The Visualization of Spatial Distribution Prediction

In the analysis of Local Moran's and Getis Ord map, four counties were selected because they showed high spatial connectivity in terms of local migration current from one area to its surrounding based on BPH attack widespread (in hectares). Those counties were Boyolali, Klaten, Karanganyar and Sragen. The other counties showed that BPH attacks did not have spatial connectivity, or in other words, they were independent. The area on the Local Moran's map, which is worth High-High (HH), is called as positive autocorrelation and indicated by the red colour. The HH area has the high BPH value, surrounded by its nearby areas which have high value as well. It is indicated that there is the local movement and migration of BPH population to nearby areas in HH area. On the other hand, the area on Local Moran's map, indicated by the white colour, has High-Low (HL) value. It means that, the area with high value is surrounded by its neighbors which have low value. This low value area is potential for the occurrence of BPH population distribution from the high value area, called as negative autocorrelation. While the area in yellow on the Local Moran's map is worth insignificant, it means that, it is not significant toward the distribution of BPH population in the nearby areas.

Getis-Ord and G-Stat tests produced Getis-Ord and G-Stat map. The subdistrict which has Z positive value is called as positive autocorrelation (HH), while the subdistrict with Z negative value is called as negative autocorrelation (LL). It is indicated that, there will be the occurrence of the movement of BPH population distribution to the nearby areas in the area with positive autocorrelation. The higher the Z value is, the higher BPH population will be. Consequently, it forms population clustering in that area. Based on the Local Moran's map in Boyolali, it is predicted that, there will be 3 subdistricts with HH value; those are Sambi, Ngemplak and Banyudono. These areas are called as positive autocorrelation and it is indicated that, there will be BPH population distribution to nearby areas with HL values (Figure 5).

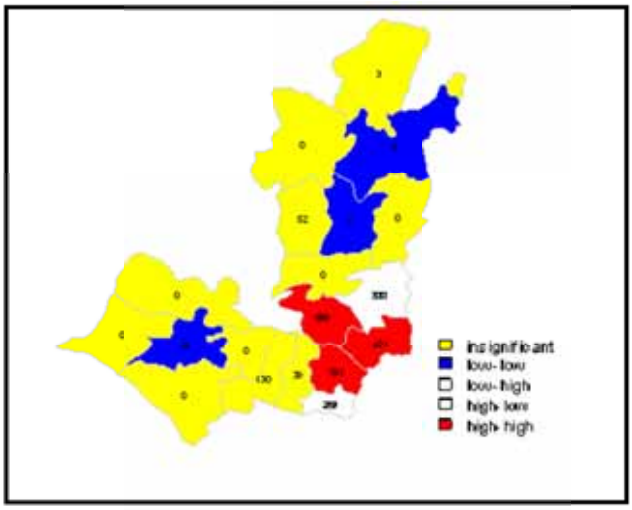

Figure 5. The prediction of local BPH migration in Boyolali based on the Local Moran's map

The map of Getis-Ord and G-Stat showed that, 7 subdistricts in Boyolali (Sambi, Ngemplak Banyudono, Simo, Nogosari, Teras and Sawit) constitute positive autocorrelation areas (HH). It is indicated that, there is the distribution of BPH population from subdistricts with high Z value to nearby areas with low Z value, and vise versa shown on Figure (6 a-b).

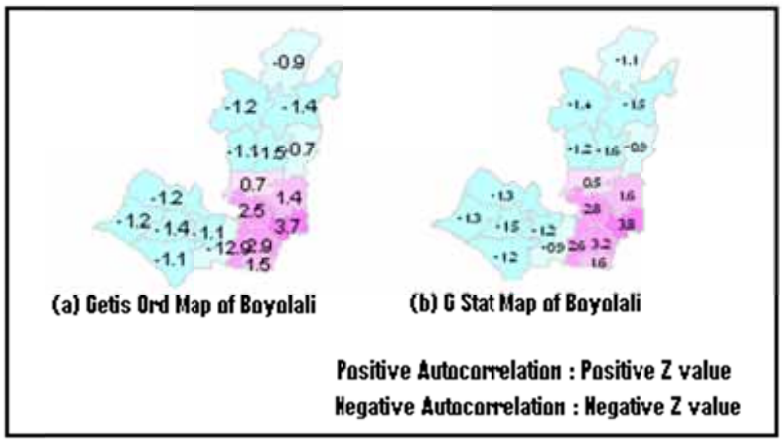

Figure 6. The prediction of BPH distribution based on the map of Getis Ord (a) and G* Statistic (b) in Boyolali

The Local Moran's map showed that, 3 subdistricts in Klaten (Juwiring, Pedan, & Karangdowo) constitute positive autocorrelation areas. It is indicated that, there is BPH population distribution to the nearby areas, in these areas. Although the areas with negative autocorrelation have the lower BPH population, they are still potential to turn into positive autocorrelation from the areas with high values (Figure 7).

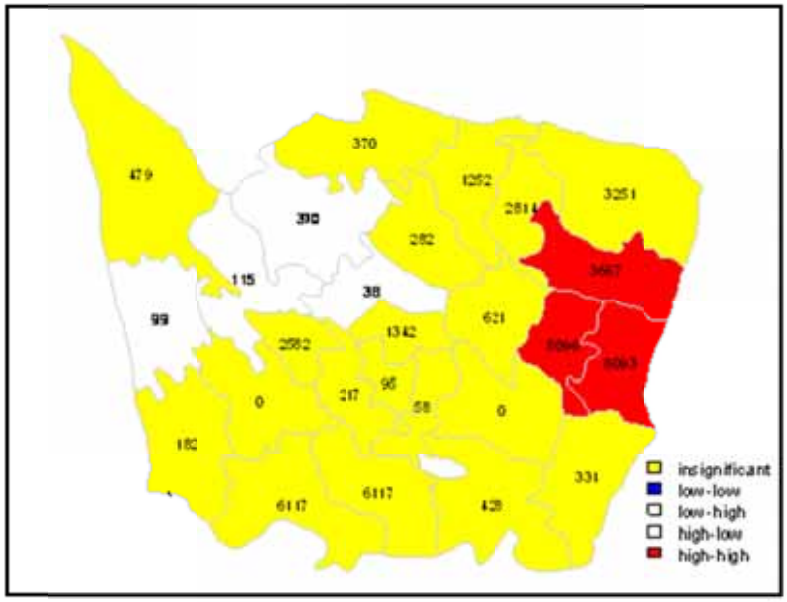

Figure 7. The prediction of BPH local migration in Klaten based on the Local Moran's map

Getis-Ord and G-Stat tests showed that, there are Z positive values on 10 subdistricts (Won sari, Juwiring, Karangdowo, Cawas, Pedan, Ceper, Delanggu, Prambanan, Gantiwarno and Jogonalan). The subdistricts with Z positive value are called as positive autocorrelation (High-High) and Z negative are negative autocorrelation (Low – Low). It is indicated that, there is BPH population distribution in the areas with positive autocorrelation. The negative autocorrelation areas are not significant toward BPH distribution (Figure 8a-b).

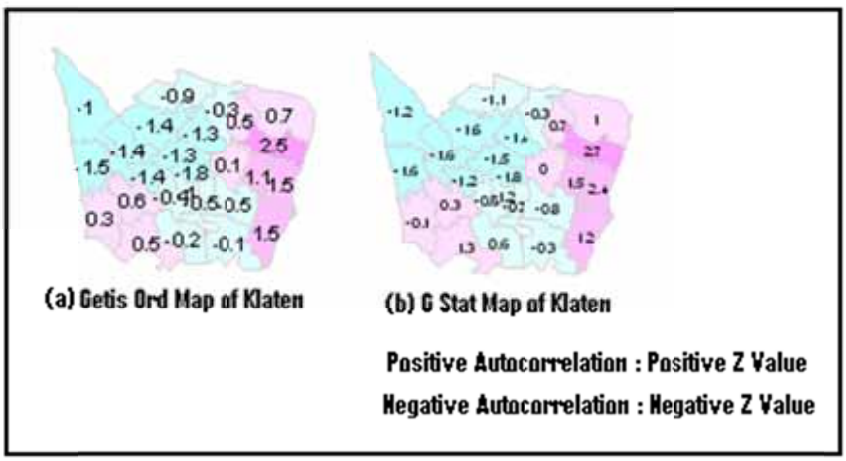

Figure 8.The prediction of BPH distribution based on Getis Ord map and G statistic map in Klaten

The Local Moran's map showed that, 3 subdistricts in Karanganyar (Tasikmadu, Jaten and Karanganyar) constitute positive autocorrelation areas. It is indicated that, there is BPH population distribution to the nearby areas in these areas. The areas with negative autocorrelation are turning into positive autocorrelation as the result of BPH population movement from the nearby areas that have positive autocorrelation values (Figure 9).

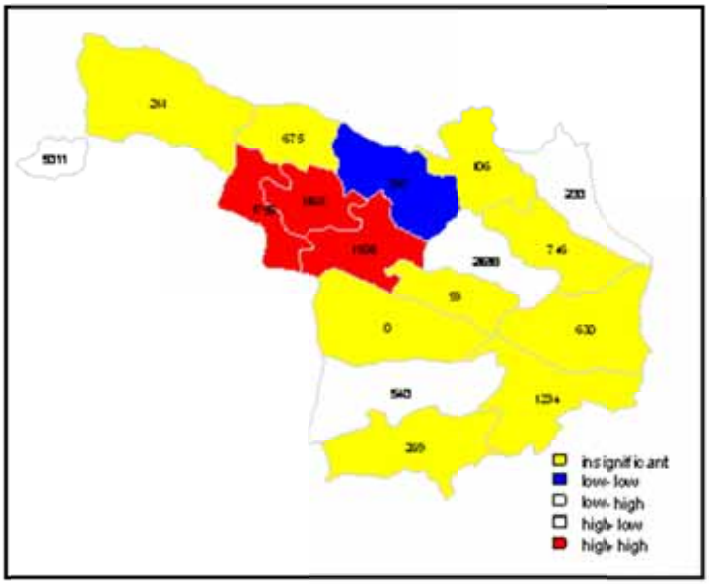

Figure 9. The prediction of BPH local migration in Karanganyar based on the Local Moran's map

Getis-Ord and G-Stat tests showed that, there was positive autocorrelation (High – High) in the county of Karanganyar (Gondangrejo, Kebakkramat, Mojogedang, Tasikmadu, Karanganyar, Jaten, Matesih and Colomadu). It is indicated that, there is BPH population distribution from the areas with high Z value to the low one, and vise versa (Figure 10 a-b).

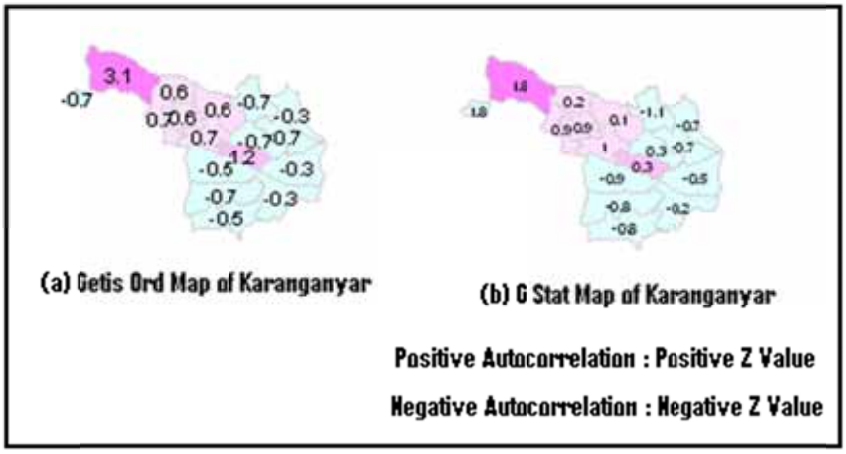

Figure 10. The prediction of BPH distribution based on Getis Ord map and G Statistic map in Karanganyar

The Local Moran's map showed that, Kalijambe and Plupuh subdistrict are positive autocorrelation areas. It is indicated that, there are population distribution and BPH attack clustering in these areas. On the other hand, the areas on other subdistricts indicated insignificance. It means that, they are not significant toward BPH population distribution (Figure 11).

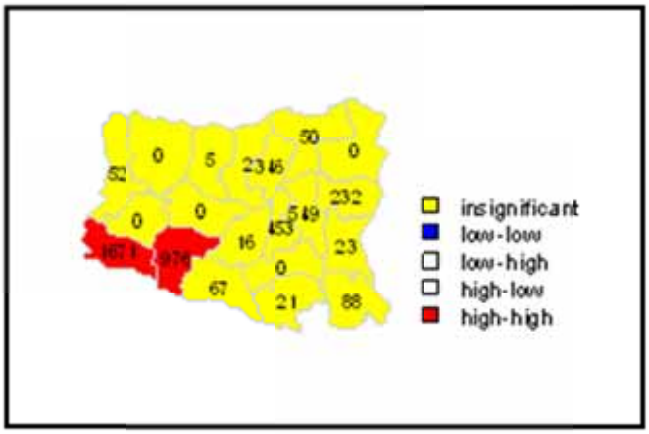

Figure 11. The prediction of BPH local migration in Sragen based on the Local Moran's map

The map on Getis-Ord and G-Stat tests showed that, there are positive autocorrelation (High – High) in 6 subdistricts (Miri, Gemolong, Kalijambe, Plupuh, Masaran and Sidoharjo). It is indicated that, there is BPH population distribution from subdistricts with high Z value to the areas with low Z value, or vise versa (Figure 12 a-b).

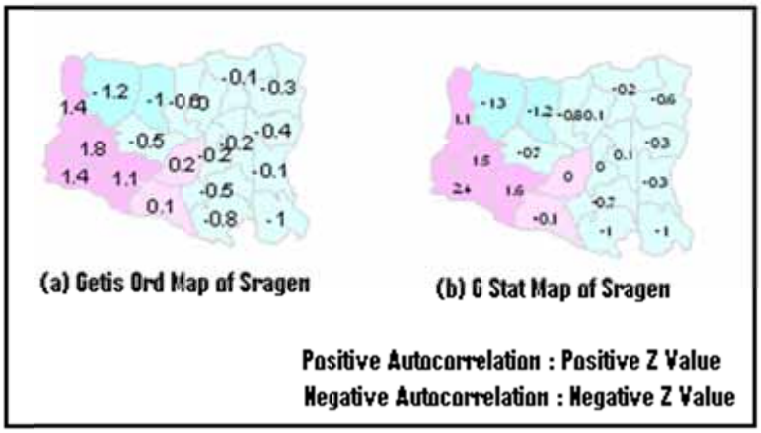

Figure 12. The prediction of BPH distribution based on Getis Ord map and G statistic map in Sragen

Figure (5) until Figure (8) showed the prediction of the BPH population distribution dynamics through local migration process of a group of large areas of high BPH attack towards the surrounding region having large areas of lower attack. Factors supporting the local migration process is spatial component connectivity in the region have similar environmental characteristics. The first factor is the spatial topography component such as irrigation network, climatic factors (duration of solar radiation, temperature, rainfall and humidity) that are almost the same. The second factor is the component of biotic interaction. That is the ability to regenerate in the new area. The third factor is the component of anthropogenic including fertilization and insecticide, which are evenly distributed throughout the studied area. This model can provide detailed information such as: (1) map of incidence, (2) map the dynamics of pest populations, and (3) the prediction of potential pest attack. The limiting factor of BPH distribution in the studied area is the distance. Based on the distribution scalability, it was categorized into four: (1) distribution distance *point* < 101, (2) *sites*, the distribution distance 103-101, (3) *local*, distance distribution of 104-103, and (4) *landscape*, distance distribution 2 x 105-104 (Hortal et al., 2010).

4.2 The Basic Factors of BPH Population Distribution in the Studied Areas

Generally, BPH population explosion in one field was influenced by 4 components: (1) the irrigation systems, allowing the farmers to plant more than twice a year, (2) intensively solar radiation on the field, (3) the repetition of the use of rice plant variety in a long time, and (4) intensively insecticide uses. While, the dynamic of BPH

population, supporting the migration process from one rice field to another was influenced by 4 factors: (1) local climate and weather, (2) provision of foods, (3) competitor insects, and (4) predator organisms (Win et al., 2011). The result of the research showed the increase of population distribution and an abundance of BPH from one rice field to its surroundings (Figures 5, 6, and 7). The climate factor, especially air temperature, air humidity and rainfall, played an important role in the phenomenon of BPH population dynamics and endemics (Olanrewaju, 1998). The studied areas had the temperature of 25-30°C. At these temperatures, there were some local ecological processes. They were (1) the occurrence of optimally insects distribution and abundance into its surroundings, (2) faster life cycles, the high regeneration pace during the season (Dyck et al., 1977), (3) the occurrence of high WBC attack (Kisimoto & Dyck, 1976). The humidity in Asia is generally influenced by Monsoon and El Nino/La Nina phenomenon, i.e. the increase and decrease and the frequency of rains significantly (Jagtap & Chan, 2000). The humidity in studied areas lies between 75-85%, which constitutes the optimal humidity for the growth of BPH population in between 70-85% (Dyck et al., 1977). The areas of Boyolali, Klaten, Karanganyar, and Sragen have the high rainfall and air humidity. As a result, it can be supporting factor BPH regeneration acceleration cycles. BPH larvae and eggs produced in a great amount will be washed away and brought by the water stream through the irrigation networking, broadly distributed to the surrounding ricefields. BPH larvae and eggs will sticks to rice plants and weeds until the condition of the environment, such as the decrease of rainfall, air humidity stability and optimally air temperature allowing BPH larvae and eggs to grow (Prasetyo et al., 2012). The data of the rainfall prediction in Boyolali, Klaten, Karanganyar, and Sragen can be seen in Figure 13. Based on that figure, it can be seen that in three predicted periods, 2011-2013, there were the increase of the rainfall pattern in the whole BPH clustering areas compared to the data in 2001-2010. Those two areas were potential in distributing BPH to its surroundings although both of them have the lower rainfall, and the surrounding county areas will have high BPH endemicity, characterized by the formed hotspot in LISA and GISA analysis (Prasetyo et al., 2012).

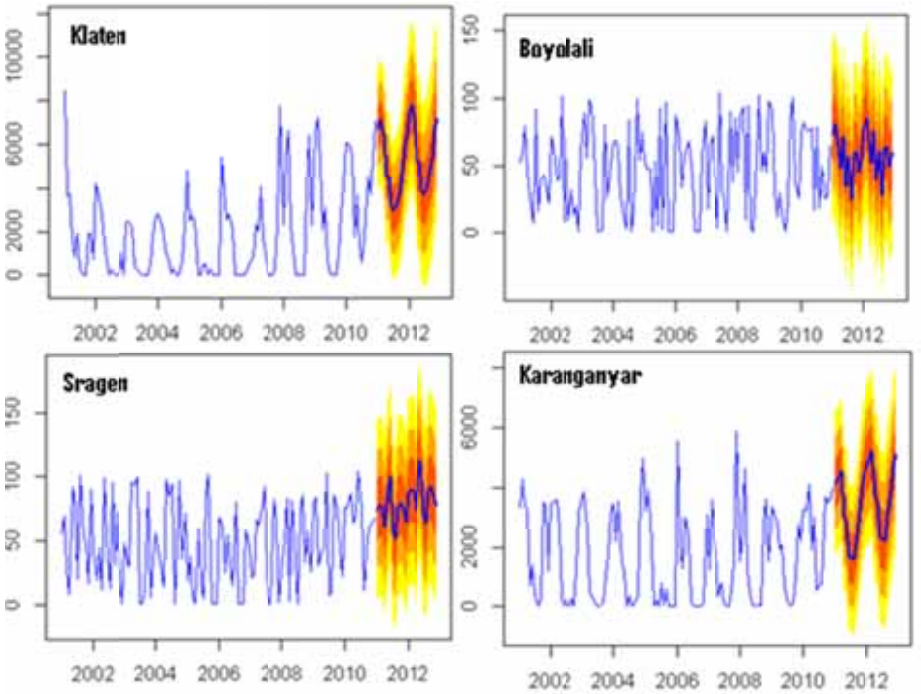

Figure 13. The prediction of the Rainfall in Boyolali, Klaten, Karanganyar, and Sragen Areas

5. Conclusion and Future Work

BPH population dynamics can be predicted by using Exponential - Smoothing Holt Winters and Local Spatial Statistics. The results showed that, the entire studied area (7 counties) showed hotspot phenomenon as much as 6 analyzed counties with the methods of Moran's I and 5 analyzed counties with Geary's C method. The comparison using Getis Ord Statistic method showed that, the entire counties experienced the hotspot phenomenon. The analysis of LISA and Getis Ord Statistic showed the areas that become the center of the attack and the potential distribution area of BPH population. The analysis of Local Moran's and Getis Ord showed four counties with high

spatial connectivity in terms of local migration current from one area to surrounding based on the BPH attack widespread data (in hectares). The counties were Boyolali, Klaten, Karanganyar and Sragen. The other counties did not have spatial connectivity, or they were independent each other.

Some factors that support local migration process based on spatial component connectivity are topography, biotic and anthropogenic interactions. From the spatial scalability in the studied area point of view, there were four categories of BPH population distribution: point, site, local, and lanscape. Looking at it from the spatial connectivity concept, BPH local migration interregion happened in the County of Klaten, Boyolali, Karanganyar and Sragen. It was caused by some factors: (1) the local climate (the rainfall, the temperature and the air humidity), (2) the repetition of the use of rice plant variety in a long time, (3) the use of insecticide intensively (3-4 times in one planting period/season), and (4) the irrigation networking, allowing the spread of BPH larvae and eggs into its surroundings.

In our future works, we will conducts the spatial modeling and BPH migration prediction based on the spatial connectivity on regional scalability using spatial statistic approach. The prediction of BPH population migration will be done by using the seasonal BPH data and climate data classification according to local season.

Acknowledgements

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Effects of Cow Dung Treated to Various Management Practices and Nitrogen Levels on Maize Grain Yield in the Northern Guinea Savanna of Nigeria

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Abstract

This study consisted of collection and incubation of cow dung, followed by evaluation of the incubated cow dung in field experiments in years 2003 to 2004 at the Institute of Agricultural Research, Ahmadu Bello University and Samaru College of Agriculture farms, all located in Samaru, Zaria. The objectives of this study are to determine the effects of subjecting cow dung to different management practices and nitrogen fertilizer on maize grain yield. The study was a factorial experiment, with 3 cow dung management practices, 4 storage durations in the field and 2 levels of nitrogen. There was a control treatment, where no cow dung or nitrogen fertilizer was applied. These gave a total of 25 treatment combinations, laid out in a randomized complete block design, replicated three times. The results showed that, the best cow dung management practice that gave the highest maize grain yields in the two farms was the surface heaped covered in April, nitrogen amended treatment. The non N amended treatments were not able to significantly increase the maize grain yields than the untreated control.

Keywords: cow dung, management practices, urea fertilizer, maize, yield, northern guinea savanna and Nigeria

1. Introduction

Maize is the third most important cereal crop in the world after rice and wheat (Food Agricultural Organization [FAO], 1997), and the second most important cereal crop in the farming systems of the Guinea savanna of Nigeria (Tarfa et al., 2003). Among these three crops, maize have been found to have the highest average yield per hectare and a good source of energy for human and animal and has been discovered to be very easy to process and readily digestible (Okoruwa & Kling, 1996). In Nigeria, maize is a very important cereal crop in the farming systems of the Guinea savanna and about 4.5 million tones was produced in the country from 3.5 million hectares of land in 1983 with the Guinea savanna accounting for 70 percent of this total production (Enwenzor et al., 1989). In 1984, land area under maize cultivation in Nigeria was estimated to be about 653,000 ha and it rose to about 5.4m ha in 1994 and lately decreased to 4.5 m ha in 2004 (Federal Ministry of Agriculture [FMA], 2005). A number of factors could have been responsible for the decrease in production and productivity.

The moist savanna (Guinea savanna) region of sub-Sahara Africa (SSA) with 42% of the SSA human population has been recognized to have the potential for increased crop and livestock production (McIntire, Bourzart, & Pingali, 1992; Winrock, 1992; Jabbar, 1996). Increasing agricultural productivity in the region without due attention to natural resource management or the fragile soil resource of the region could impose negative consequences. It is estimated that as much as 85% of the land in this region is threatened by degradation (International Food Policy Research Institute [IFPRI], 1995).

The current global drive for sustainable agricultural systems that optimize use of low inputs, require close monitoring of soil quality (FAO, 1989). To achieve this, integrated soil fertility management systems, by combining the use of chemical amendment, biological and local organic resources, such as crop residues, green

manure, biological N- fixation and agro-forestry for low activity clays of the savanna soil have been suggested (Kang & Wilson, 1987). The critical factor for the success of improved farming systems seem to be the efficient recycling of organic materials (Kang & Duguma, 1985).

The recent increases in cost of inorganic fertilizers, has triggered scientific interest towards the evaluation of organic fertilizers based on locally available resources, including crop residues, animal manure and green manures (Reijntjes, Haverkrot, &Waters-Bayer, 1992). Focus on soil fertility research has shifted towards the combined application of organic matter and mineral fertilizers as a way to arrest the on going soil fertility decline in sub Saharan Africa (Vanlauwe, Wendt, & Diels, 2001c). The organic sources can reduce the dependency on costly fertilizers by providing nutrients that are either prevented from being lost (recycling) or more truly added to the system (biological N-fixation). When applied repeatedly, the organic matter leads to build-up of soil organic matter, thus providing a capital of nutrients that are slowly released (Giller et al., 1997) and at the same time increasing the soils buffering capacity for water, cations and acidity (de Ridder & van Keulen, 1990).

Animal manure (called manure) according to Defoer, Budelman, Toulmin and Carter (2000) is an organic fertilizer consisting of partly decomposed mixture of dung and urine. Manure is recognized as a key resource in sustaining soil fertility in the tropics, supplying the soil with a range of macro- and micro- nutrients and organic matter. According to Camberato, Lippert, Chastain, and Plank (1996) and Fulhage (2000) the nutrients content of manure varies widely with animal species, age, ration quality and feed consumption, as well as with different methods of storage, handling methods, housing type, temperature and moisture content, treatment and land application. The beneficial role of animal manure in crop production has long been recognized (Schlecht, Mahler, Sangare, Susenbth, & Becker, 1995; Karanja, Kapkiyai, Bunyasi, & Murage, 1997; Harris, Lyord, Hofni-Collins, Barrett, & Brown, 1997). The utilization of cattle manure as a soil amendment is an integral part of the Nigerian guinea savanna farmers (Harris & Yusuf, 2001; Iwuafor et al., 2002). However, the information that is lacking to most of the farmers is the methods of manure management practices for optimal quality before field application and time of application of animal manure for optimum crop production. Also, Iwuafor et al. (2002) observed that, the results of trials conducted in the northern guinea savanna showed the need to investigate the high variability in manure quality across different farmers/sites, and to look for ways to avoid losses during manure storage, or at least to establish ranges of N contents for manures with different origins and storage methods. Therefore, the objectives of this study are to determine the effects of cow dung subjected to different management practices and Urea fertilizer on maize grain yield in the Northern Guinea Savanna of Nigeria.

2. Materials and Methods

2.1 Location and Description of Experimental Site

The field studies were carried out at Samaru at two different locations within the same zone at the IAR Research Farms and the Samaru College of Agriculture (SCA) Farm, Samaru, which are both located at Latitude 11° 11′ N and Longitude 7° 33′ E in the Northern Guinea Savanna zone of Nigeria.

Samaru has mean annual rainfall of about 1050 mm, spanning the periods from May to September, while the dry season starts from October to April with a mean daily temperature of 24°C (Kowal & Knabe, 1972). The hottest months are those that precede the rains (March to April) and coldest months occur in November to January, October and February are considered as transition months. The global radiation is evenly distributed throughout the year, ranging from 440 cal. cm^2 day^{-1} in August to 550 cal. cm^2 day^{-1} in April to May (Kowal, 1972).

2.2 Cow Dung Collection and Subjected to Management Practices

The study consisted of collection and incubation of cow dung and subsequent evaluation using field experiments. The cow dung that was used for these experiments were collected from the National Animal Production Research Institute (NAPRI), Shika-Zaria in years 2003 and 2004. The cow dung collected was subjected to different management practices as described in Figure 1.

Fresh cow dung was collected early in the morning from pens and piled into a heap. The cow dung was then mixed thoroughly with a shovel with the aim of harmonizing it. After mixing it thoroughly, it was then subjected to the various management schedules as follows: (i) cow dung placed in a pit of 2 x 2 m and 75 cm deep and covered (PC) with a polythene sheet, (ii) cow dung heaped on the ground surface and covered (SHC) with a polythene sheet, and (iii) cow dung heaped on the ground surface and left uncovered (SHU). The collection of the cow dung and its distribution to the 3 different management practices was repeated for the next 2-3 days as described above until enough cow dung was gathered. The cow dung was then allowed to decompose for four weeks (one month, composting) without any disturbance before it was removed and stored in the field.

This experiment started in February, 2003 with the collection of cow dung and allowing it to decompose (composting) for 4 weeks which means the field storage (exposure) of the cow dung was from March to May (12 weeks of field storage before application to the soil as amendment). The same cow dung treatment as described for February above was repeated in March against April to May (8 weeks of field storage before application to the soil as amendment), April against May (4 weeks of field storage before application to the soil as amendment) and May against June (0 week) where cow dung was collected at the termination of composting and applied to the field immediately, without field storage (the moisture content was taken into consideration). The same procedure was repeated in the second year (2004).

Weeks	Treatments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Duration of Storage
Month		January				February				March				April				May				
PC	Treatment 1					Composting				Field Storage												12wks
	Treatment 2									Composting				Field Storage								8wks
	Treatment 3													Composting				Field Storage				4wks
	Treatment 4																	Composting				0 wk
SHC	Treatment 1					Composting				Field Storage												12wks
	Treatment 2									Composting				Field Storage								8wks
	Treatment 3													Composting				Field Storage				4wks
	Treatment 4																	Composting				0 wk
SHU	Treatment 1					Composting				Field Storage												12wks
	Treatment 2									Composting				Field Storage								8 wks
	Treatment 3													Composting				Field Storage				4 wks
	Treatment 4																	Composting				0 WK

Figure 1. Diagrammatic presentation of experimental set up

2.3 Cow Dung and Soil Sampling and Preparation

Cow dung samples were taken after subjecting the cow dung to the three different management practices i.e. (PC, SHC and SHU) but before taking them to the field for storage. This set of cow dung after collection was air dried and stored for analysis. The second sampling of the cow dung was done at the end of field storage, before application and incorporation into the soil in the field (at this stage, the cow dung treatments must have been exposed at the field in storage after the 1 month of composting for different time durations of 12 weeks, 8 weeks, 4 weeks and 0 week). These were all carefully processed and kept for analysis and for use in the field.

Before the commencement of the experiment surface soil sample (0 to 20 cm depth) was collected from the field where the field experiment was conducted at IAR and SCA farms. The soil was air-dried and sieved to pass through the 2 mm sieve and kept for analysis.

2.4 Cow Dung and Soil Analysis

The surface soil samples for field studies were analyzed by the following methods: particle size distribution using the standard hydrometer method (Klute, 1986). The soil pH was determined in water and 0.01 M $CaCl_2$ with a pH glass electrode using a soil: solution ratio of 1 : 2.5. Organic Carbon was determined by wet oxidation method of Walkley-Black (Nelson & Sommers, 1982).

Exchangeable bases were determined by extraction with neutral 1 N NH_4O AC saturation method. Potassium and Sodium in the extract were determined by the flame photometer, while Ca and Mg were determined by atomic absorption spectrophotometer (Juo, 1979). Available P was extracted by the Bray 1 method. The P concentration in the extract was determined colorimetrically using the spectronic 70 spectrophotometer. Total N was determined by the Kjeldahl procedure (Bremner & Mulvaney, 1982; Bremner, 1982).

2.5 Field Experiments

The field experiments were conducted at two locations. The first trial was carried out at the IAR Farm, Samaru in the year 2003 season. The second trial was established at the SCA Farm, Samaru in 2004 season. In all the experiments, the same treatment combinations, experimental design, observations and procedures were maintained.

The experiment was a factorial experiment with 3 factors, laid out in a randomized complete block design replicated three times. The treatments were: 3 cow dung management practices, 4 different storage times after 1 month incubation (composting) before application to the field, 2 levels of N (3 x 4 x 2). There was a control treatment where no cow dung or nitrogen fertilizer was applied. These gave a total of 25 treatment combinations.

The land was plowed and harrowed and the field was mapped out into plots in the first year of the experiment. The plot sizes were 4 x 5 m (20 m^2) and each plot was separated from the other by one meter. The plots were then immediately ridged manually at 75 cm between ridges with the hand hoe to incorporate the cow dung. Cow dung subjected to different management practices which had been conveyed and stored in the field at different times (March for 12 weeks, April for 8 weeks, May for 4 weeks and June for 0 week) were applied manually at 5.0 t ha^{-1} on dry matter weight basis.

In both years of the experimentation, maize (Var. Oba super II) dressed with Fernasand D was sown at two seeds per hole, at a spacing of 25 cm within the row. The seedlings were later thinned to one plant per hill at two weeks after planting.

A blanket application of P was applied as single super phosphate (SSP) at the rate of 60 kg P_2O_5 ha^{-1} and at 45 kg N ha^{-1} as urea was applied in two split equal doses to the appropriate plots. The first application was done immediately after the first weeding (3 WAP). The second dose was applied at the time of second weeding (6 WAP). In each case the fertilizer was applied by single band about 5 cm deep, made along the ridge, 5-8 cm away from the plant stand and covered immediately.

The weeding operation was carried out at the third and sixth weeks after planting. Remolding was carried out at 8-9 WAP to ensure proper weed control and a clean field at the time of harvesting.

The net plots (four inner rows) were harvested when the crop was fully matured and dry. Ears for each net plot were de-husked and the fresh weight of the cobs was taken immediately. After sun drying, the cobs were shelled using the manual Sheller. The dry grain weight for each treatment was recorded.

2.6 Statistical Analysis

The data collected from the field studies were subjected to analysis of variance (ANOVA) using the SAS package (SAS Inst., 1999). Significant means were separated using the Duncan's Multiple Range Test (DMRT) at 5% level of probability.

3. Results and Discussion

Some selected physical and chemical properties of the two sites are shown in Table 1, while the NPK content of the cow dung used in the field experiments are presented in Table 2.

Table 1. Some physical and chemical properties of the soil of the first and second experimental sites at commencement of study

Parameters	IAR Farm	SCA Farm
Sand (g kg^{-1})	640	360
Silt (g kg^{-1})	210	540
Clay (g kg^{-1})	150	100
Texture	Sandy loam	Silt loam
pH 1:2.5 (H_2O)	5.90	5.90
pH 1:2.5 ($CaCl_2$)	5.10	5.20
Organic Carbon (g kg^{-1})	7.40	4.40
Total N (g kg^{-1})	0.53	0.70
C/N ratio	14.00	6.29
Bray 1 P (mg kg^{-1})	7.00	2.00
Exchangeable Calcium (cmol kg^{-1})	2.00	1.60
Exchangeable Magnesium (cmol kg^{-1})	0.80	1.00
Exchangeable Potassium (cmol kg^{-1})	1.84	0.49
Exchangeable Sodium (cmol kg^{-1})	1.87	1.13

IAR = Institute for Agricultural Research; SCA = Samaru College of Agriculture.

Table 2. Total NPK content of cow dung used for greenhouse and field studies

Management practices (one month incubation)	Time of manure exposure in the field before use (weeks)	2003 SEASON						2004 SEASON					
		N (%)		P (%)		K (%)		N (%)		P (%)		K (%)	
		a	b	a	b	a	b	a	b	a	b	a	b
SHUM	12	1.05	1.40	0.75	0.25	5.48	1.65	0.88	1.00	0.67	0.64	3.60	2.78
SHUA	8	1.40	1.40	0.39	0.39	1.43	1.65	1.23	1.08	0.60	0.67	3.30	3.08
SHUY	4	1.40	1.58	0.60	0.50	1.35	1.35	1.23	1.20	0.60	0.91	2.55	6.08
SHUJ	0	1.75	1.75	0.53	0.75	1.25	2.25	1.20	1.23	0.67	0.71	3.68	2.63
SHCM	12	1.23	1.40	0.75	0.67	1.35	1.75	1.05	1.08	0.39	0.51	1.73	2.10
SHCA	8	1.40	1.05	0.83	0.39	1.50	1.28	1.23	1.10	0.46	0.60	0.98	4.65
SHCY	4	1.23	1.23	0.67	0.32	1.65	1.43	1.23	1.58	0.53	0.71	1.88	3.53
SHCJ	0	2.10	1.93	0.91	0.75	1.50	3.08	1.23	1.25	0.53	0.60	0.98	2.55
PCM	12	1.75	1.45	0.79	0.39	3.15	1.98	1.05	1.05	0.80	0.60	4.88	1.58
PCA	8	1.75	1.05	0.60	0.32	5.25	1.20	1.58	1.58	0.53	0.53	4.28	1.80
PCY	4	1.58	1.75	0.67	0.49	3.68	1.58	1.98	1.05	0.53	0.71	3.15	2.63
PCJ	0	1.75	1.58	0.83	0.53	4.28	1.58	1.70	1.70	0.58	0.53	3.60	1.73
CONTROL	-	1.58		0.75		1.65		1.55		0.73		1.50	

a = At termination of 1 month incubation; b = At time of application for field trial.

SHUM = Surface heaped uncovered March SHCM = Surface heaped covered March

PCM = Pit covered March SHUA = Surface heaped uncovered April

SHCA = Surface heaped covered April PCA = Pit covered April

SHUY = Surface heaped uncovered May SHCY = Surface heaped covered May

PCY = Pit covered May SHUJ = Surface heaped uncovered June

SHCJ = Surface heaped covered June PCJ = Pit covered June

3.1 Maize Grain Yields

The effects of cow dung management practices, duration of storage before field application and N levels on maize grain yield for 2003 and 2004 seasons are shown in Table 3. There were significant ($P < 0.05$) differences among the treatments in the two farms. Results of treatments were consistent for the two years e.g. all the N amended treatments (+N) consistently gave higher maize grain yield values than the zero N amended (oN) treatments. The values for the control also consistently gave lower values compared to the N amended treatments. Where treatments were amended with nitrogen, the surface heaped covered April treatment (SHCA) consistently gave higher maize grain yields in the two years. Among treatments that were not amended with N, the surface heaped uncovered May (SHUY) gave higher grain yields in the two years (farms).

Table 3. Effects of manure management practices, time of application and nitrogen levels on maize grain yield (kg ha^{-1}) in IAR and SCA farms

Treatments	IAR farm		SCA farm	
	2003		2004	
	oN	+N	oN	+N
SHU				
SHUM	1120.8c-f	1925.0a-e	241.7i	1308.3c-g
SHUA	904.2ef	2341.7ab	500.0hi	2158.3ab
SHUY	1645.8a-e	2195.8abc	800.0e-i	1633.3a-d
SHUJ	1341.7b-f	1966.7a-e	225.0i	1083.3d-h
SHC				
SHCM	959.2def	1629.2a-e	243.3i	1316.7c-g
SHCA	1270.8b-f	2545.8a	691.7f-i	2308.3a
SHCY	1312.5b-f	1987.5a-e	441.7hi	1466.7b-e
SHCJ	1395.8a-e	1875.0a-e	525.0hi	1050.0d-h
PC				
PCM	1079.2c-f	2090.8a-e	508.3hi	1950.0abc
PCA	1412.5a-e	2112.5a-e	766.7e-i	1766.7a-d
PCY	1387.5a-e	2120.8a-d	608.3ghi	1416.7c-f
PCJ	1345.8b-f	1904.2a-e	208.3i	1108.3d-h
Control	211.7f		275.li	
SE+	348.65		230.96	

Means with the same letter(s) within the same group are not significantly different at 5% level of significance

SHUM = Surface heaped uncovered March, SHCM = Surface heaped covered March,
PCM = Pit covered March, SHUA = Surface heaped uncovered April,
SHCA = Surface heaped covered April, PCA = Pit covered April,
SHUY = Surface heaped uncovered May SHCY = Surface heaped covered May
PCY = Pit covered May SHUJ = Surface heaped uncovered June
SHCJ = Surface heaped covered June PCJ = Pit covered June

oN = Direct evaluation (non N amended), +N = 45 kg N ha^{-1} (N amended).

But looking at the nutrient content of the cow dung used in Table 2, particularly the N content; it was expected that the June treatments which had the highest N content irrespective of the management practice at the time of application of cow dung to the field to give the highest grain yield. This was not so probably because of the nature of the nutrient release pattern, which did not coincide with the period of highest nutrient demand by the maize crop

to produce the optimum grain yield. The management practices must have enhanced nutrient availability with the period of highest nutrient demand by the maize crop through the incubation process which must have enhanced nutrient availability to coincide with the period of highest nutrient demand by the maize crop at the SHCA treatment that gave the highest grain yield. Many scientists have advanced reasons why such discrepancies in the manure do exist. Myers, Palm, Guevas, Gunatilleke and Brossard (1994) reported that good manure should synchronize mineral nitrogen release and plant demand such that the peak mineral nitrogen release coincides with peak plant biomass development and hence peak nitrogen requirements. Also, Lekasi, Ndung'u and Kifuko (2005) reported that it is advantageous if the organic materials added to the soil mineralize nutrients slowly and the rate of nutrient mineralization increased as the plant growth progressed. He further explained that, good soil releases adequate nutrients for optimum plant growth as they mature. Closer synchronization of nutrients demand ensures efficient utilization of organic inputs applied to the soil, he added. In other words, high content of nutrients in the cow dung was not an indication for high performance in crop production. Organic materials that mineralize too readily, subject mineralized nutrients to losses through processes such as leaching and volatilization on the other hand, organic materials that releases nutrients later in the season will not benefit the plant or crop as it would have matured with inadequate availability of nutrients during the critical growing stages. The overall amounts of nutrients released from organic amendments for crop uptake depends on the quality, the rate of application, the nutrient release pattern and the environmental conditions (Mugwira & Mukurumbira, 1986; Murwira & Kirchmann, 1993).

All the N amended treatments gave significantly ($P < 0.05$) higher grain yields than the control treatment, while most of the non N amended treatments were statistically at par with the control treatment. This showed that, the application of cow dung alone that have been subjected to different management practices, was not enough to give a significant difference on maize grain yield. This agreed with the work of Uyovbisere and Elemo (2002) who stated that organic matter cannot be used alone, but with some level of inorganic fertilizer. It has been recognized that the combined application of organic matter and inorganic fertilizer is required to increase crop production and arrest soil nutrient depletion in West Africa (FAO, 1999; Giller, 2002; Iwuafor et al., 2002). Tanimu, Iwuafor, Odunze and Tian (2007) reported higher doses of N fertilizers increased grain yield and yield related components of maize.

4. Conclusion

Based on the results of this study, the surface heaped covered April (SHCA). N amended treatment consistently gave the highest maize grain yield in the two farms than all other treatments. The non N amended treatments was not able to significantly increase the maize grain yields than the untreated control. It is therefore concluded that for high maize productivity in this zone the surface heaped covered April treatment, amended with N at 45 kg N ha^{-1} is recommended for use.

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Dissipation of Propamidine Fungicide Residues in Greenhouse Tomato

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Abstract

A method of reverse phase high performance liquid chromatography (RP-HPLC) was established to analyze the dissipation of propamidine residue in tomato. Residue of propamidine was extracted from tomato using methanol buffered and determined by RP-HPLC with UV detection at 262 nm. The results showed that the average recoveries of the samples fortified with propamidine at the concentration range of 25 to 300 mg kg^{-1} ranged from 87.972 to 106.341% with a relative standard deviation ranged between 0.169 to 3.503%. Initial deposit ranged from 2.45 to 5.70 mg kg^{-1}. The dissipation of propamidine in tomato followed the first order kinetic equation. The dissipation rate constants in tomato treated with recommended and double recommended dose applied at 4 times and 2 times ranged from 0.110 to 0.151 days, and the corresponding half-lives from 4.589 to 6.300 days. At the day 14 after the last application the residue concentrations of propamidine in tomato ranged from 0.42 to 0.54 mg kg^{-1} from the two blocks for all treatments. These propamidine residues dissipated below the limit of detection of 0.07 mg kg^{-1} 28 days after the last treatment. The results presented in this work and the low toxicity of propamidine for environment proved that propamidine will not pose any residual toxicity problem after 14 days of application and tomato fruits could be used safely for human consumption.

Keywords: propamidine fungicide, RP-HPLC, residue, dissipation, tomato

1. Introduction

The use of pesticides in agriculture is necessary to combat a variety of pests that could destroy crops and to improve the quality of the food produced. Agricultural use of pesticides plays a beneficial role in providing a plentiful, low cost supply of high quality fruits and vegetables. On the other hand, as a consequence of this use, the presence of residues in food that was critical elements of overall population health is unavoidable and pesticide residues in food is of great importance in the evaluation of food quality (Goto et al., 2003). The cultivation of tomato especially in greenhouse conditions demands frequent application of a large number of pesticides to control a variety of insects and diseases, but over time both insects and diseases have developed resistance to such pesticides. Grey mould is one of the most serious vegetable diseases in greenhouses in China (Ji et al., 1998). With the intensive use of pesticides in greenhouse crops, residues may be accumulated at levels higher than those permitted by the China pesiticide management legislation or international Maximum Residue Levels (MRLs).

Propamidine is a novel, systemic plant fungicide which is mainly used to control various diseases caused by botrytis fungi on fruits and vegetables under field and greenhouse conditions, spraying of propamidine fungicide at 90-180 g a.i. ha^{-1} had higher control effectiveness to disease than fungicides such as procymidone, pyrimethanil and dimethachlon at 450 g a.i. ha^{-1} (Chen et al., 2005). It was reported that propamidine is of low toxicity, safe to human being and environment. However no published data are available concerning the residues of propamidine in plant extracts. Therefore to guarantee the use of propamidine in field according to good agricultural practices and to protect consumer health residual analysis study of propamidine in tomato become indispensable to know the residual level, the rate of dissipation and the half lives ($t_{1/2}$) of propamidine in tomato. Hence, an ultrasonication-assisted solvent extraction method using reverse phase high performance liquid chromatography was determined and validated in this study to perform propamidine fungicide residues determination in tomato for understanding the behaviour of propamidine fungicide residue in tomato fruit grown under greenhouse conditions.

2. Materials and Methods

2.1 Solvents, Reagent and Pesticide

The organic solvent, methanol used was HPLC grade and was purchased from Laiyang Shuangshuang Chemical Co., Ltd (China), sodium dodecyl sulfate (SDS), phosphoric acid, de-ionized water and the propamidine, TC > 95% were provided by Research and Development Center of Biorational Pesticide (RDCBP).

De-ionized water and methanol were degassed by ultrasonic cleaner bath. All samples and solvents were filtered through Millipore membrane filters (0.45 μm pore size) before injection on the column. The analytical stock solutions of the pesticide were prepared in methanol and stored in a volumetric flask maintained at 4°C.

2.2 Field Trial

Tomato variety Jinpeng No.1 was grown during the summer 2012 in a commercial Chinese greenhouse at the Xi Xiao-zai village (Shaanxi, China) located at fifteen kilometer in the west of Northwest A & F University. A randomized complete block design with 2 blocks, each block containing 5 plots; keeping a distance of 70 cm between rows and 30 cm within rows was performed for experiment. Propamidine was not applied to the test plots during this experiment. Irrigation and all cultural practices were carried out as local practices (OCDE, 2009). After formation of fruits the plots were treated with the commercial formulation of propamidine (TC > 95%) with a hand sprayer at the recommended rate 90 g a.i. ha^{-1}, double recommended rate 180 g a.i. ha^{-1}, and zero dose 0 g a.i. ha^{-1} sprayed with water as a control treatment (Tao et al., 2010; Zhou et al., 2004).

2.3 Sampling and Storage

About 1kg of tomato fruits was randomly collected into plastic polyethylene bags from each plot as representative samples. The fruits also were randomly sampled from the plants approximately 0 days (2 hours), 1, 3, 7, 14 and 28 days after last application of propamidine. The Samples were immediately transported to the laboratory and homogenized. Two representative subsamples of 4 g were taken for each plot. One subsample was prepared for chromatographic analysis and the other was placed into glass containers and frozen at -40°C, temperature at which enzymatic degradation of pesticide residues is usually extremely slow (CAC/GL 40, 1993; FAO, 1986) prior to use; the frozen samples were thawed at 4°C overnight.

2.4 Instrumental

The Experiments were conducted on Schimadzu Liquid Chromatograph- system equipped with a LC-6AD pump, a SCL-10A vp controller, a detector SPD-10A uv, and 10 μm Hypersil BDS C_{18} Column (4.6 mm× 250 mm). Quantitative and qualitative analysis were conducted using the optimized chromatographic condition performed based on the report of Yuan et al. (2007) but the mobile phase was modified by adding the Phosphoric acid buffer.

2.5 Preparations of Standard Solutions

Pesticide stock standard solution (1000 μg ml^{-1}) of propamidine fungicide ($C_{17}H_{20}N_4O_2.2HCl$) was prepared by dissolving 0.0250 g of the pesticide in 25 mL of methanol and stored in volumetric flask 25 mL in freezer at 4ºC. Working standard solutions (50 - 600 μg mL^{-1}) were prepared by appropriate dilutions of the stock standard solution immediately before used. Matrix- extract standard solutions are the standards prepared in blank matrix. After extraction procedures, the extract was evaporated and reconstituted with standard solution (Kumar, 2010) to give standards of the required concentration.

2.6 Preparation of Fortified Sample

Tomato fruits free of propamidine were first washed and triturated using mortar and pestle. Sample fortification was made by adding 2 mL of appropriate concentration of the propamidine working standard solution to a 4 g sample, letting it stands for a few minutes before extraction to allow the spiked solution to penetrate into the matrix.

2.7 Sample Extraction

Sample extraction was based on ultrasonication-assisted solvent extraction method of Devanand et al. (2005); Shen et al. (2005) and Vagi et al. (2007). A volume of 10 mL of extraction solvent was added to a 4 g tomato sample and sonicated at room temperature in an ultrasonic cleaner bath for 15 min. This step was repeated two times for a total of 20 mL of extraction solvent. The extract was then separated by centrifugation at 8000 rpm for 15 minutes at 20ºC using a high speed refrigerated centrifuge and the supernatant obtained was collected into an Erlenmeyer flask. The obtained organic subtract was subject to another extraction-centrifugation with 10 mL of extraction solvent. The extract from the first and the second extraction were collected in the same flask and the

combined extract was passed through a 0.45 μm Teflon filter for chromatographic analysis. The same procedure was used for propamidine extraction in tomato fortified and real sample.

2.8 Validation Method

The validation procedure was based on the investigation of the following parameters: Selectivity, analytical curve and linearity, recovery and accuracy, precision (repeatability), matrix effect, stability, detection limit and quantification limit.

The selectivity was carried out by comparing representative chromatograms of standard working solution, blank extract and fortified sample. Calibration curves were carried out with calibration solutions at 5 concentration levels (50, 100, 200, 400, and 600 μg mL^{-1}) prepared in methanol and in methanol containing matrix extract. The solutions were injected in RP-HPLC system in triplicate and chromatograms were recorded. Calibration curves were obtained by plotting average peak area versus concentrations.

The linearity based on the construction of the analytical curves was obtained using both analytical standard solutions. Accuracy of a method is defined as the closeness of a measured value to the true value. The accuracy of the method was tested (% recovery and % RSD of individual measurements) by analyzing tomato samples without the interest pesticide residue. Tomato samples were fortified at five concentration levels: 25, 50, 100, 200 and 300 mg kg^{-1}. Each concentration level was extracted and analyzed three times. Repeatability of the instrument was evaluated by calculating the relative standard deviation for six injections (Putheti & Leburu, 2008; Rahman et al., 2010) of propamidine working standard solution (200 μg mL^{-1}) in the chromatographic system.

Matrix effect was performed by comparison between calibration curves prepared in pure methanol and methanol containing tomato extract prepared as described above. Calculation was made using the following equation used by Cardoso et al. (2011).

$$Matrix\ effect\ (\%) = \frac{Slope\ (x_1) - Slope\ (x_2)}{Slope\ (x_2)} \times 100$$

Where x_1 = slope of the curve obtained by injection of the analytical solutions prepared in the extract and x_2 = slope of the curve obtained by injection of the analytical solution prepared in methanol.

The stability of propamidine in solvent and solvent containing extract of tomato was carried out by evaluation of the percentage deviation in chromatogram peak response. Propamidine working standard solution (200 μg mL^{-1}) and tomato fortified sample solution concentration level 100 mg kg^{-1} freshly prepared were injected in RP-HPLC system to check the freshly chromatogram peak area. Both solutions were further stored in a freezer at 4ºC, room temperature at 25 ± 2ºC and oven at 54ºC. Finally, After 3 days and 14 days of storage the standard working solution and sample solution were reanalyzed and the percentage deviation in peak response was evaluated.

Limit of detection (LOD) and limit of quantification (LOQ) of the method were determined using signal-to-noise ratio method (U.S. FDA, 1996; Walfish, 2006; Assis et al., 2011); in which the lowest concentration detected or measured should be the one where the peak height is 3 and 10 times respectively, the peak height of the equipment noise at the retention time of the peak of propamidine.

2.9 Statistical Analysis

The dissipation kinetic of the propamidine in tomato was determined by plotting residue concentration against time and the maximum squares of correlation coefficients found were used to determine the equations of best fit curves. For all the samples studied, exponential relationships were found to apply, corresponding to first order rate equation. Confirmation of the first order kinetics was further made graphically from the linearity of the plots of Log Concentration against time. The rate equation was calculated from the first order rate equation:

$$C_t = C_0 e^{-kt}$$

Where, C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration and k is the rate constant in $days^{-1}$.

The half-life ($t_{1/2}$) was determined from the *k* value for each experiment, being as calculate by Wang et al. (2007) and Liang et al. (2011).

$$t_{1/2} = \frac{ln(2)}{k}$$

3. Results and Discussion

3.1 Chromatographic Conditions

Several tests were carried out based on the report of Yuan et al. (2007) to determine the instrumental condition. The best response analytical signals of propamidine fungicide was achieved with a mobile phase consisting of 0.1% phosphoric acid, methanol/de-ionized water (V/V, 80:20) solution containing 3.0 mmol/L SDS. For the HPLC system the best results of the analysis were obtained with a 10 µm Hypersil BDS C_{18} column (4.6 mm × 250 mm) at the flow rate of 1.0 mL min^{-1} and the column temperature of 25ºC; injection volume was 20 µL and the UV detector wavelength was set at 262 nm using an isocratic elution system.

3.2 Validation Method

The selectivity was evaluated by comparing representative chromatograms of standard working solution (100 mg L^{-1}), blank extract and fortified sample (100 mg kg^{-1}). The results are shown in figure 1.

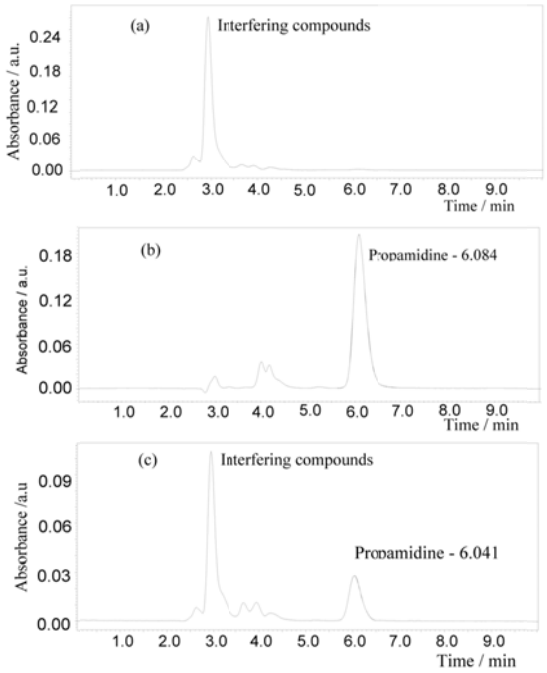

Figure 1. Chromatogram showing the retention times of propamidine in selectivity study. Chromatogram Figure 1 a, b, c presenting respectively the chromatogram of tomato blank sample, propamidine from solvent standard solution (100 mg L^{-1}), and propamidine from tomato fortified (100 mg kg^{-1}) sample solution

The absence of signal at the retention time of propamidine indicates that no interfering compounds were present. The external calibration curve (Figure 2) was obtained by plotting average peak area versus concentrations of interest pesticide.

The values obtained for the analytical curves with the solutions prepared in solvent (methanol) and in solvent containing matrix extract demonstrated satisfactory linearity with linear regression equation y = 51987x + 311379, y = 50737x + 530132 and correlation coefficients 0.9999, 0.9997 for the pesticide in solvent and solvent with matrix extract respectively. The comparison test of the intercept performed with zero showed that the intercept is not significantly different from zero at α = 5% probability in the two cases with T_{cal} for equation in standard solution (SC) and standard containing matrix extract (SMC) respectively, SC T_{cal} = 3.0647 and SMC T_{cal}: = 2.92321< T_{tab} = 3.1824.

For quantification of propamidine, since the intercept was not significantly different from zero at α = 5% probability and the calibration function y = a + bx didn't give satisfactory recovery the slope of regression line was recalculated by forcing the calibration curve through the origin. The linear regression obtained y = 52721x with R^2 ≥ 0.999 was used for quantification of propamidine in fortified and real sample.

The accuracy of the method was tested by analyzing tomato samples free of propamidine fortified at five concentration levels ranged from 25 to 300 mg kg^{-1}. Each concentration level was extracted and analyzed three times and the results are shown in table 1.

Table 1. Accuracy data of the method (n=3)

Accuracy of the test method (Recovery studies)					
Fortification level (mg/kg)	Amount		% Recovery	Average recovery	RSD (%)
	Added (mg)	Found (mg)			
	0.100	0.105	105.368		
25	0.100	0.105	104.874	106.030	1.503
	0.100	0.108	107.848		
	0.200	0.204	101.997		
50	0.200	0.200	100.224	101.947	1.666
	0.200	0.207	103.619		
	0.400	0.432	108.026		
100	0.400	0.433	108.301	106.341	2.972
	0.400	0.411	102.694		
	0.800	0.741	92.599		
200	0.800	0.787	98.391	94.566	3.503
	0.800	0.742	92.708		
	1.200	1.057	88.095		
300	1.200	1.056	88.008	87.972	0.169
	1.200	1.054	87.813		

Recovery values of propamidine from tomatoes fortified sample were 87.972 to 106.341% with RSD (0.169 - 3.503%) less than 10% (European Commission, Directorate General Health and Consumer Protection [EC DGHCP], 2010) indicates that the method was accurate.

Precision was studied by performing repeatability studies expressed as RSD. For retention time and peak area the values of RSD were respectively 0.094 and 4.982%. The RDS of repeatability lower than 10% (EC DGHCP, 2010) indicates that the method was precise. Matrix effect was performed by comparison between calibration curves prepared in pure methanol and in tomato matrix extract. The signal suppression/enhancement (C %) found in this experiment was -2.43%, located in the range -20% < C % < 20% (Cardoso, 2011) indicating that the matrix effect was not significant. For stability, the values of percentage deviation in the peak response from initial data were located in the acceptance criteria ranged from -20% to 10% (VICH, 2011) so propamidine was found to be stable in methanol and in tomato extract solution in freezer, room temperature and oven condition for the period of 14 days (period of essay). Instrumental LOD based on S/N of 3:1 and LOQ based on S/N of 10:1 were 0.07 mg kg^{-1} and 0.2 mg kg^{-1} respectively.

3.3 Dissipation of Propamidine in Tomato

3.3.1 Propamidine Residue Levels in Fresh Harvested Tomato

Field tomatoes were treated with propamidine commercial formulation 4 times and 2 times at the recommended 90 g a.i. ha^{-1} and double recommended rate 180 g a.i. ha^{-1} at 7 day intervals. Tomatoes samples were collected at 0 day (2 h), 1, 3, 7, 14 and 28 days post spraying. Table 2 shows the results of analysis using RP-HPLC system.

The initial residue concentration of propamidine in tomato sample collected from block 1 and block 2 were respectively: for the plot treated at normal rate at 4 times (3.17; 2.73 mg kg^{-1}); double dose at 4 times (5.70, 5.47 mg kg^{-1}); plot treated at normal rate at 2 times (2.92, 2.45 mg kg^{-1}) and plot treated at double rate at 2 times (3.06, 3.37 mg kg^{-1}). We have not found residues of propamidine on sample from control plot.

Residues concentration decreased rapidly on the first days. At the day 14 after the last application the residue concentrations of propamidine in tomato ranged from 0.42 to 0.54 mg kg^{-1} from the two blocks for all treatments. There was no significant difference in dissipation pattern between the two rates of applications ($P > 0.05$) suggesting that the dissipation of propamidine was independent of the dose and number of application. Similar results were reported by Saimandir and Gopal (2012) with indoxacarb on eggplant fruits. The residues were non detectable at day 28.

Table 2. Residues values (mg kg^{-1} ± SD) of propamidine found in greenhouse tomatoes at various interval times (days) after application at recommended dose 90 g a.i. $ha^{-1}$and double recommended dose 180 g a.i. ha^{-1} (n=2)

Block	Dose g a.i.ha^{-1}	Application number	Residues (mg kg^{-1} ± SD) at days after last application					
			0	1	3	7	14	28
Block 1 (Treatments)	90	4	3.17 ± 0.76	2.04 ± 0.04	1.78 ± 0.11	0.85 ± 0.07	0.50 ± 0.05	nd
	180	4	5.70 ± 0.37	2.33 ± 0.07	2.00 ± 0.06	1.37 ± 0.09	0.54 ± 0.02	nd
	90	2	2.92 ± 0.33	2.03 ± 0.04	1.84 ± 0.11	0.57 ± 0.18	0.47 ± 0.001	nd
	180	2	3.06 ± 0.14	1.71 ± 0.05	1.59 ± 0.08	0.52 ± 0.01	0.42 ± 0.05	nd
Block 2 (Treatments)	90	4	2.73 ± 0.09	1.86 ± 0.01	1.83 ± 0.13	0.81 ± 0.13	0.47 ± 0.03	nd
	180	4	5.47 ± 0.31	2.72 ± 0.02	2.37 ± 0.03	1.38 ± 0.05	0.51 ± 0.05	nd
	90	2	2.45 ± 0.03	1.81 ± 0.07	1.54 ± 0.01	0.91 ± 0.07	0.50 ± 0.06	nd
	180	2	3.37 ± 0.01	1.84 ± 0.02	1.18 ± 0.08	0.87 ± 0.15	0.48 ± 0.04	nd

nd: non detectable.

3.3.2 Dissipation of propamidine in tomato

Figure 3 shows the dissipation curve of propamidine in tomato from different plots sprayed at recommended rate (90 g a.i. ha^{-1}) and double recommended rate (180 g a.i. ha^{-1}).

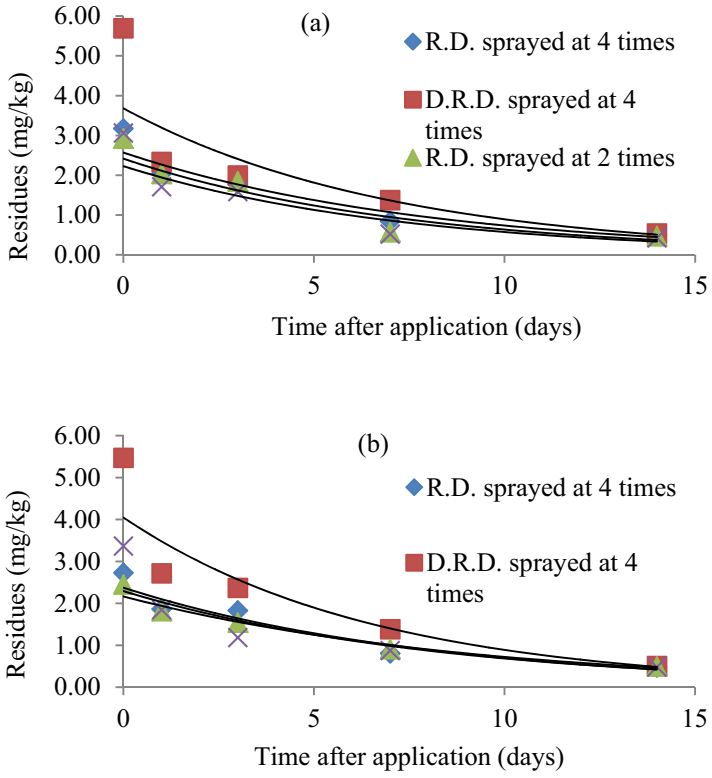

Figure 3. Dissipation curve of propamidine in tomato after repeated applications at different doses; the curves in Figure 3 a & b presents the dynamic curves of propamidine on block 1 and block 2 for recommended rate 90 g a.i. ha^{-1} and double recommended dose 180 g a.i. ha^{-1}.

R.D., recommended dose; D.R.D., double recommended dose

The results presented in Table 2 and Figure 3 had shown a rapid decrease of residues concentration after the first time of sampling. Decrease in levels of residues of propamidine was found as an exponential decrease in the residue concentrations over the period of time and followed first-order rate of dissipation. The dynamics could be described by the following equation $C_t = C_0e^{-kt}$. Table 3 shows the first-order kinetics equation, half-life and other parameters for propamidine dissipation in tomato.

Table 3. Half-life and other statistical parameters for propamidine dissipation in the tomato in green houses conditions after 4 and 2 times' application at the dose 90 -180 g a.i. ha^{-1}; (n=2)

block	Dose g a.i.ha^{-1}	Application number	Kinetics Equation	Determination Coefficient (R^2)	Constant k ($days^{-1}$)	Half-life (days)
Block 1	90	4	$y = 2.570e^{-0.125t}$	0.942	0.125	5.544
(Treatments)	180	4	$y = 3.684e^{-0.142t}$	0.888	0.142	4.880
	90	2	$y = 2.415e^{-0.134t}$	0.862	0.134	5.172
	180	2	$y = 2.228e^{-0.136t}$	0.846	0.136	5.096
Block 2	90	4	$y = 2.374e^{-0.123t}$	0.950	0.123	5.634
(Treatments)	180	4	$y = 4.048e^{-0.151t}$	0.949	0.151	4.589
	90	2	$y = 2.164e^{-0.110t}$	0.979	0.110	6.300
	180	2	$y = 2.292e^{-0.121t}$	0.872	0.121	5.727

The first-order kinetic equations determination coefficients (R^2) were ranged from 0.846 to 0.979. The study revealed that propamidine dissipation rate in tomato was independent of initial deposit. The theoretical half-live of propamidine at the recommended and double recommended dose showed less variations on the trial for both cases (4 times and 2 times applications). The half life values ranged from 4.589 to 6.300 days, Hu et al. (2005) have reported similar half-life time ($T_{1/2}$: 6.37 days) with 2- allyphenol a new fungicide used on tomato against grey mould caused by *botrytis cinerea* in tomato.

The decline of the residue may be attributed to growth dilution between application and sampling, volatilization that occurs during the first days following application, transfer of propamidine from plant to soil due to the systemic propriety of propamidine, sunlight UV radiation, or other complex conditions. Further studies are required to assess the breakdown products, exposure risk, and the environmental fate of propamidine.

4. Conclusion

The present study revealed that the method is suitable for the determination of propamidine fungicide residues in tomato. The system was linear in the concentration range of 0.05 to 0.6 mg ml^{-1}; interfering peaks at elution times and significant matrix effects were not observed for the interest pesticide. Intermediary precision, recovery and accuracy have proved that the method is precise and accurate, also the limit of detection and quantification were satisfactory. Concerning residues dissipation study, initial deposit of double dose sprayed at 4 times were 5.70 mg kg^{-1}; 5.47 mg kg^{-1} respectively in block 1 and 2. After 14 days of application the initial deposit dissipates respectively to 0.54 mg kg^{-1} (90.55%) and 0.51 mg kg^{-1} (90.74%). The longest half -life was 6.300 days. Hence the MRL of propamidine TC > 95% has not set up by China pesiticide management legislation and the FAO/WHO.

According to the results presented in this work the method could be useful for the establishment of MRL of propamidine and routine residues analysis to ensure food safety routine residue analytical methods.

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Interactions of Fish Sector and Non-Fish Sector in DSGE Model

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Abstract

This paper presents a three sector models showing that the shock of preference of non-fish has only effect on non-fish production, and fish production increase of labor can both influence fish and non-fish sector. But the influence is higher in the non-fish sector. Labor shock can increase the investment and labor in the fish sector and decrease investment and labor in the non-fish sector. This may be caused by high labor share and changes in work in the fish sector.

Keywords: shocks, fish sector, non-fish sector

1. Introduction

Two definitive characteristics of China economy, as a whole, after performing the reform and opening-up policy in 1978, are the fast and stable growth in GDP and strong investment. As a part of total investment, fish investment also has experienced fast increase, even with a sharper increase. But statistic data shows that total catch displays the other direction before 1999, forcing an upward trend thereafter, a slight move downward follow end by a rapid decrease. To understand these economic phenomena, it is crucial to answer two questions: (1) what is the key factor(s), causing the violent fluctuation of fish catch? (2) While the whole economy is stable, what are effects of the fish industry on the whole economy?

In this paper, we address these problems with a quantitative model. We develop and estimate using a Bayesian likelihood approach, a dynamic stochastic general equilibrium model of the China economy that models the price and the production of the fish market. We do so with two goals in mind. Firstly, we need to understand changes in preference in fish and non-fish production, and labor, respectively. Secondly, to estimate the spillovers from the fish industry to the wider economy, the model should reproduce some key features of the data.

Our analysis combines two elements: (1) a multi-sector structure with fish and non-fish goods; (2) a rich set of shocks, which are essential for the model specification

In most articles, only one sector is often discussed; the fish industry, to the best of my knowledge is often ignored. However, there are many papers that consider the uncertain factors. Uncertainty can at least be classed into four kind, process uncertainty, observation uncertainty, model uncertainty and environmental uncertainty. Clark (2010), Anderson and Seijo (2010), and Conrad (2010) have dealt with uncertainty in detail in their studies. Since in our paper we merge three sectors, some additional stochastic factors are therefore allowed; preference uncertainty, labor uncertainty, etc.

Section 2 lays out the model. Section 3 calibrates parameter estimates, where we use the model to discuss related issues to the role of fish industry in the wider economy. Section 4, concludes.

2. The Model

The model features sector heterogeneity. On the demand side, households maximize the utility. They can freely allocated work hours between fish industry and non-fish industry (denoted as a common industry). They consume fish and non-fish items with taken prices; they own capital of the economy and supply funds to either fish firm or non-fish firms.

On the supply side, there are two sectors. To maximize the profit, fish firms hire workers, use fund and sell the produce. The common firms have no difference except that they have no constrains from nature, because production of fish firms is strongly influenced by fish's natural growth process.

2.1 Households

There is a continuum of measure 1 of agents. It is a representative household that maximizes the expected present value of lifetime utility as given by:

$$E_{t=0}^{\infty}\beta^{t}(z_t \log(c_t - \varepsilon^0 c_{t-1}) + j_t \log(f_t - \varepsilon^1 f_{t-1}) + \tau_t /(1+\eta)(n_{ct}^{1+\xi} + n_{ft}^{1+\xi})^{\frac{1+\eta}{1+\xi}})$$

$$\text{s.t.} \quad p_{ct}c_t + p_{ft}f_t + k_{ct} + k_{ft} = (1-\delta+r)(k_{ct-1} + k_{ft-1}) + w_{ct}n_{ct} + w_{ft}n_{ft}$$

$$\ln z_t = \rho_z \ln z_{t-1} + u_{zt}, u_{zt} \sim N(0, \sigma_z)$$

$$\ln j_t = \rho_j \ln j_{t-1} + u_{jt}, u_{jt} \sim N(0, \sigma_j)$$

$$\ln \tau_t = \rho_\tau \ln \tau_{t-1} + u_{\pi}, u_{\pi} \sim N(0, \sigma_\tau)$$

Variables c, f, n_c and n_f represent non-fish consumption, fish consumption, hours working in non-fish firms and hours working in fish firms. The discount factor is β. And symbol E_t denotes the expectation operator based on information of time t. Random variables z_t j_t and τ_t capture shocks to inter-temporal preference, to the demand for fish and to the supply of labor respectively. These shocks follow stationary autoregressive processes of order one:

The parameter ε measures the degree of habit formation in consumption. If ε^0 and ε^1 equal zero, hours worked across the two sectors are perfect substitutes, both sectors pay the same wage in equilibrium. Positive values of ε^0 and ε^1 allow capturing some degree of sector specificity. The value of η measures the inverse elasticity of the labor supply.

Households maximize their lifetime utility subject to the budget constraint. Agents select consumption plan c_t and f_t, capital in non-fish sector k_{ct}, capital in fish sector k_{ft}, working hours n_{ct} and n_{ft} to maximize their utility subject to the constraint above. We assume that No free capital market exists. Households cannot borrow money from other parts.

2.2 Non-Fish Firms

Non-fish firms produce non-fish item and also employ labor and capital. Firms pay wages to households and repay the returns to capital owners and the households.

The firm's problem is to maximize the profit as follows:

$$Max\{p_{ct}y_t - w_{ct}n_{ct} - r_c k_{ct-1}\}$$

$$\text{s.t.} \ y_t = (a_{ct}n_{ct})^{1-n} k_{ct-1}^{\ n}$$

The production function y_t is Cobb-Douglas types. The price of non-fish items is p_{ct}. The value of a_{ct} represents the technology progress.

2.3 Fish Firms

Fish firms are deeply influenced by man-made factors, labor, capital, technology, and also by natural factors such as climate, temperature, etc.

The firms' problem is to maximize the profit as follows:

$$Max\{p_{ft}h_t - w_{ft}n_{ft} - r_f k_{ft}\}$$
$$\text{s.t. } h_t = (a_{ft}n_{ft})^{\alpha} k_{ft}^{\beta} x_t^{1-\alpha-\beta}$$
$$x_{t+1} - x_t = g_t x_t (1 - x_t / k_t) - h_t$$

Fish firms make use of the Cobb-Douglas production function, where the value of a_{ft} represents the technology progress and the value of x_t is stock. The fish nature growth function forms the logistic model where the fish price is p_{ft} .

3. Quantitative Analysis

3.1 Calibration

The model is calibrated by the year frequency data in order to match properties of the China's fish sector and other sectors. The discount factor is 0.9, so that the average annual rate of return is 20%. This is not uncommon in China, based on the following two reasons. One reason is the fast growth speed of China. The other reason is that except the government controll firms, the private firms seldom can obtain credits from the formal finance institution. So the high return rate is established by the illegal finance trades. The share of labor income in common firm is set at 0.25, and the fish firm 0.35 which can be explained that Chinese products are so cheap and there is cheap labor. The depreciation rate is also very high at 10% and can be justified by the infrastructure fixed fee. The lack of job mobility results from the Hukou system which specifies that anyone who is not of local birth has no rights to work in the special local firms, especially in the public firms. And workers who want to change jobs from one sector to another find it difficult. According to a Chinese idiom, 'A carrot occupies a pit until it dies'.

The model is computed using Bayes theorem. We first transform the data into suitable computation. Then we chose the appropriate prior distributions for the parameters and estimate posterior distribution with Monte Carlo methods.

Table 1. Prior and posterior distribution of the structural parameters

parameter	Prior distribution			Posterior distribution			
	distr	mean	st.dev	mean	2.5 percent	median	97.5 percent
ε^0	Gamma	0.5	0.075	0.6535	0.6096	0.6548	0.6986
ε^1	Gamma	0.5	0.075	0.524	0.494	0.5236	0.6494
η	Gamma	0.8	0.1	0.8739	0.7137	0.816	0.9177
ξ	Normal	1	0.1	0.5259	0.3141	0.5226	0.7325

Table 2. Prior and posterior distribution of the shock process

parameter	Prior distribution			Posterior distribution			
	distr	mean	st.dev	mean	2.5percent	median	97.5percent
ρ_z	Normal	0.8	0.1	1.059	0.8694	1.0623	1.2640
ρ_j	Normal	0.8	0.2	1.759	1.6011	1.762	1.912
ρ_τ	Normal	0.8	0.1	0.972	0.941	0.9726	1.0054
σ_z	Gamma	0.01	0.002	0.64	0.569	0.641	0.7171
σ_j	Gamma	0.01	0.002	0.847	0.765	0.847	0.931
σ_τ	Gamma	0.01	0.002	0.0007	-0.0167	0.0072	0.0298

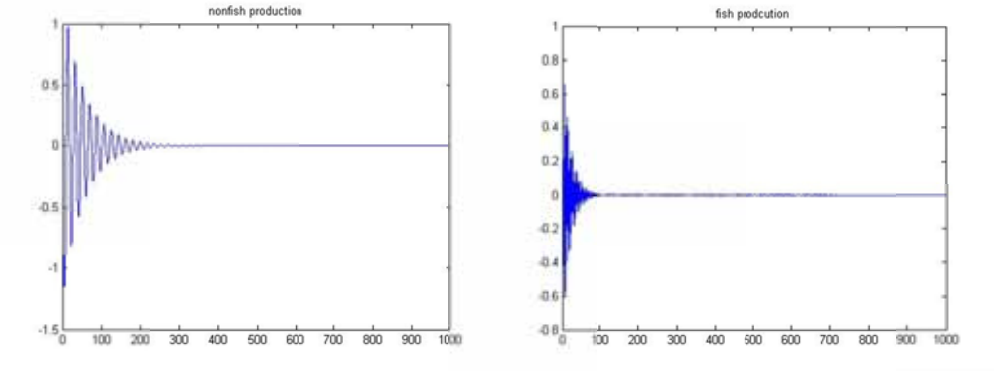

Figure 1. Impulses from common consumption preference

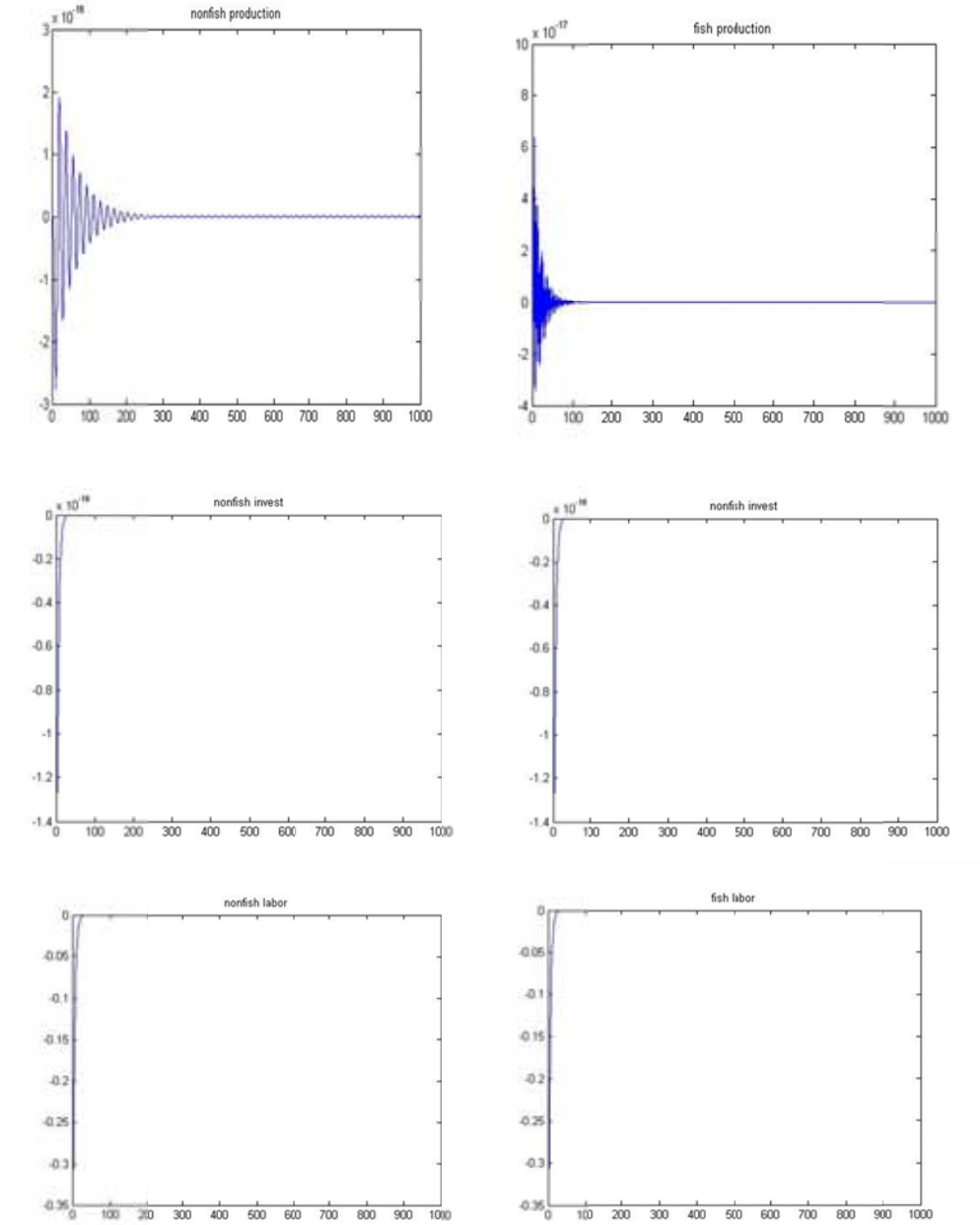

Figure 2. Impulses from fish consumption preference

3.2 Impulse Response

The exogenous shocks analyzed in the simulation experiment are shock of common item preference, shock of fish item preference and shock of supply of labor. Followed by a standard deviation of common item preference as shown in Figure 1, the only change is the common item production which shows the period changes and the vibration range which becomes smaller. As household's utility function has a positive relationship with consumption. So a rational consumer should reduce the current consumption sharply to increase the next consumption extremely. Although shock does not have influence on the investment and labor supply, investment is influenced by the return rate and labor-supply by the disutility of labor and wage. And both have no relationship with item preference in our model. The fish preference shock is similar to the shock of preference of common item. There are extreme differences in the labor supply, as shown in Figure 2. With increase in labor supply, the production of fish and non-fish all get to grow although the growth of fish production is relatively small. The answer may be due to the small share labor in fish sector. But labor and investment will increase in the fish sector but decrease in the non-fish sector. This can be explained by the fact that in the fish sector labor can have a high share of 0.35 and 0.25 in the non-fish sector.

4. Conclusion

This paper develops a three sector DSGE model, and shows that the fish sector's growth contributes to the fish preference and non-fish preference and cannot have effects on the fish industry. However, the non-fish preference has a significant influence in the non-fish sector, with much more change range. As the labor supply grows, the production of the two sectors also shows the periodical vibration, but the range of non-fish sector is consistently fiercer. Without doubt, labor in fish sector should be increased for higher labor share. But the increase in the investment of fish sector is a surprising outcome. It may be as a result of the byproduct of the changed working force.

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Assessment of Comparative Virulence and Resistance in Soybean Using Field Isolates of Soybean Rust

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Abstract

A major impediment to breeding for resistance to Asian soybean rust (*Phakopsora pachyrhizi*) is the lack of stable sources of resistance, due to high variability in the pathogen. The objectives of this study were to assess comparative virulence of five diverse field isolates from major soybean producing areas in Uganda, and identify lines with resistance to isolates of soybean rust in seedling and adult plants under screen house and field conditions respectively. When inoculated with the five field isolates, all twelve lines evaluated showed diverse and mixed reactions, suggesting each location differed in soybean rust races and/or virulence. Experimental sites growing many diverse soybean lines yearly had the greatest diversity of soybean rust. The effectiveness of specific resistance genes was restricted to certain locations and gene R*pp2* previously resistant was ineffective producing a susceptible tan reaction at the seedling stage. A positive correlation between mean lesion density at the seedling stage and adult plant severity indicated that using field isolates to screen for seedling resistance can be a useful breeding approach to extrapolate resistance in adult plants. Overall, these results emphasise the relevance of using field isolates from the target areas to evaluate lines for soybean rust resistance.

Keywords: *Phakopsora pachyrhizi*, comparative virulence, stable resistance

1. Introduction

Asian soybean rust (*Phakopsora pachyrhizi* Sydow) is a major threat to soybean production worldwide. The pathogen is an obligate parasite that causes multi-cyclic infections during the growing season. The uredinal stage produces urediniospores responsible for disease development and subsequent yield losses are due to premature defoliation and reduction in photosynthesis that adversely affects grain filling (Kumudini et al., 2010; Miles, Frederick, & Hartman, 2003). Since the disease was first detected in Japan, researchers have focused their efforts on several ways to manage it. Eradication or elimination efforts of the source of soybean rust inoculum are unlikely to succeed due to the wide host range including legumes which are an integral part of most cropping systems (Goellner et al., 2010; Miles et al., 2003; Miles, Pastor-Corrales, Hartman, & Frederick, 2007; Slaminko, Miles, Frederick, Bonde, & Hartman, 2008). Chemical control, though effective, poses a greater challenge since its effectiveness depends on frequent symptom monitoring and timely routine fungicide application (Yorinori et al., 2005). Genetic resistance is currently the most economic and strategically important means of managing rust soybean being pursued by several soybean breeding programmes (Arias et al., 2008). Resistance to rust is conferred mainly by R genes (major genes) and is dependent on specific prevalent soybean rust isolates. Major resistance genes R*pp*1, R*pp2*, R*pp3*, R*pp4*, R*pp5* and R*pp?* (Hyuuga), have been identified to show resistance to specific races of soybean rust (Bromfield & Hartwig, 1980; Garcia et al., 2008; Hartwig, 1986, McLean & Byth, 1980; Monteros, Missaoui, Phillips, Walker, & Boerma, 2007).

Several studies investigating the effectiveness of single soybean rust resistance genes have used single spore isolates with evaluations done under controlled conditions at seedling stage (Li, 2009; Paul & Hartman, 2009; Twizeyimana et al., 2009). However, in nature the soybean rust pathogens often exist as mixtures with either homogeneous or heterogeneous virulence among sub-populations with varying aggressiveness. Single spore isolation does not always capture the variability present within a field isolate (Freire et al., 2008; Paul & Hartman, 2009). Given the high virulence diversity within and among *P. pachyrhizi* isolates, use of single pure isolates in determining soybean rust resistance will not necessarily allow extrapolation to the field level. Moreover, differences have been observed in the capacity of soybean to express resistance depending on the stage of growth

and environment. Seedling evaluation for soybean rust resistance does not always guarantee adult plant resistance (Miles et al., 2008; Ribeiro et al., 2007). Seedling resistance is, however, still important given that soybean rust attacks soybean at any phenological stage of development. Having both seedling and adult plant resistance guarantees complete protection of soybean irrespective of the time when the disease manifests.

In Uganda, previous race characterisation studies using race differentials and molecular analysis showed low racial diversity with three races found in most soybean growing areas (Lamo, 2004). However, the scenario is likely to have changed since two cultivars with specific resistance deployed in 2005 have shown increased susceptibility to soybean rust (Tukamuhabwa et al., 2009). In addition, no cultivar in Uganda utilises any of the available characterised classical resistance genes. The effective use of these exotic gene sources depends on knowledge of how they respond to local soybean rust populations. Differential response of the host genotypes to local rust populations can further help understand the virulence patterns of Asian soybean rust in Uganda. This information would help facilitate utilisation and development of the stable host resistance. Therefore the objectives of this research were to i) assess comparative soybean rust virulence patterns of natural pathogen populations in the different major soybean growing areas and ii) identify soybean lines with stable seedling and adult stage resistance when challenged by geographically diverse field isolates.

2. Materials and Methods

2.1 Soybean Lines

Twelve soybean lines including two susceptible checks (Wondersoya and Nam 2) were used in this study. Their pedigree, origin and source are presented in Table 1. Sources of resistance PI 230970 (R*pp2*), Ankur (R*pp3*), PI 459025 (R*pp4*), G000138-29, Maksoy 1N were previously characterised for rust resistance for three seasons at Namulonge (Oloka, Tukamuhabwa, Sengooba, & Shanmugasundram, 2008). In addition, two high potential materials with resistance genes PI 459024, UG 5; three released cultivars Maksoy 2N, Namsoy 4M and Maksoy 3N. This set of soybean lines was also selected on the basis of a relatively similar growth cycle under the local field conditions to reduce the effect of crop phenology on disease severity during evaluation.

Table 1. A set of 12 putative differential soybean lines used to assess resistance to five field isolates using seedling and adult plants

No	Genotype	Pedigree	Reason for selection	Origin	Source
1	PI230970	G8586	Resistance gene (R*pp2*)	Japan	AVRDC, Taiwan
2	Ankur	G7955	Resistance gene (*Rpp3*)	India	AVRDC, Taiwan
3	PI459025	G10428	Resistance gene (*Rpp4*)	China	AVRDC, Taiwan
4	PI 459024	G10427	Resistant	China	AVRDC, Taiwan
5	G000138-29	(CH#1 x Anoka) x (Clarke 63 x 64.4)	Resistant	China	AVRDC, Taiwan
6	UG 5	-	Resistant	Uganda	MAK, Uganda
7	Maksoy 1N	TGx1835-10E	Resistant	Nigeria	IITA, Nigeria
8	Maksoy 2N	Duiker x GC000138-29	Resistant	Uganda	MAK, Uganda
9	Maksoy 3N	Duiker x TGx 1835-10E	Resistant	Uganda	MAK, Uganda
10	Namsoy 4M	Nam 2 x GC000138-29	Resistant	Uganda	NARO, Uganda
11	Nam 2	87D-668	Susceptible	Nigeria	NARO, Uganda
12	Wondersoya	-	Susceptible	Nigeria	IITA, Nigeria

NARO - National Agricultural Research Organisation - Uganda.

MAK - Makerere University.

IITA -International Institute of Tropical Agriculture.

AVRDC - World Vegetable Centre.

2.2 Field Procedures for Assessment of Adult Plant Resistance

The layout for the field experiment was a randomised complete block design with three replications at the five sites of Makerere University Agricultural Research Institute-Kabanyolo (MUARIK), National Crops Resources Research Institute (NaCRRI), Iki-Iki (IKI), Nakabango (NAK) and Kasese (KAS) in 2010 and 2011 leading to 10 season-location environments. These sites represent areas of high soybean production in Uganda with endemic seasonal soybean rust epidemics. At each site each entry was sown at the same time with 25-30 seeds in 2 metre rows replicated three times in a randomised complete block design. Entries were randomised across sites within a given year with a 60 cm x 5 cm inter- and intra-row spacing. Spreader rows of a susceptible cultivar Nam 2 were planted at the same time every after five rows around each replicate.

2.3 Field Data Collection and Analyses

Reaction types and sporulation were evaluated at the R6 stage (Fehr et al., 1971) when symptoms were clearly seen on susceptible checks using three trifoliate leaves of the mid-canopy. Disease reaction types were recorded as Red Brown (RB), Tan (T) and Mixed (MX). Red brown lesion colour was considered a resistant reaction whereas tan lesions expressed susceptibility (Miles et al., 2003). Mixed reactions had both red brown and tan lesion of the same leaf or different plants of the same soybean line. Field sporulation levels were rated on a 1 to 5 scale (Pham et al. 2009) using the susceptible cultivar Wondersoya as a control. Severity scale was based on 1 to 9 scale where 1- no lesions; 2 = 1-30; 3 = 31-75; 4 = 76-150; 5 = 151-300; 6 = 301-750; 7 = 751-1500; 8 = 1501-3000 and 9 = >3000 lesions (Miles et al., 2008). Prior to analysis severity and sporulation scores were subjected to transformation by square root and arcsine methods respectively to normalise them. Back transformed data was presented as the final results. All analyses were done using GENSTAT software 13th Edition and means compared using standard error (Payne et al., 2010). Interactions between lines and isolates for sporulation and disease severity were analysed using ANOVA.

2.4 Screen House Procedures for Assessment of Seedling Resistance

Two sets of three plants for each of the twelve genotypes were grown in wide trays in the screen house for inoculation in a split plot design. The main plot factor was the five isolates and subplot factor genotype. Using a handheld Liliput® vacuum composite soybean rust field isolates were harvested in June 2011 from random soybean leaves at the R6 stage at each of the five sites (used for the field trial). Isolates from NAK, MUARIK, IKI were inoculated on the same day and those from KAS and NaCRRI a week later. For each isolate, freshly harvested field spores were mixed with distilled deionised water containing the surfactant Tween-20 at 0.5 ml/l. Urediniospore suspensions were diluted to a concentration of 50 000 spores per millimetre using a Neubauer haemocytometer. Prior to inoculating each set of entries, germination ability of the spores was tested on water agar to ensure infectivity. In each set, three trifoliates one from each plant were artificially inoculated with 1.5 ml of spore suspension on the abaxial leaf surface using a Canyon® (Model 5A, England) hand sprayer. After inoculation plants were covered with polythene bags for 24 hours at 22°C-24°C to maintain high relative humidity necessary for infection. Trays containing plants with each isolates were spatially separated within the screen house to avoid any possibility of cross contamination. After 24 hours polythene bags were removed for the duration of the experiment.

2.5 Screen House Data Collection and Analysis

Using 20X magnification lenses, the soybean lines were monitored for lesion colour, incubation period and latent period. Days to appearance of symptoms from inoculation day were recorded as incubation period and days to urediniospore production as latent period. Lesion density (cm^{-2}) and the frequency of sporulating lesions were recorded after 16 days from the middle leaflet and analysed using analysis of variance, means were compared using standard error. Starting at seven days after inoculation, data on lesion density were collected four times at three day intervals (up to 16 days) to plot the areas under disease progress curve (AUDPC) using the following formula:

$$\text{AUDPC} = \sum_{i=1}^{k} 1/2\,[(s_i + s_{i+1})(t_{i+1} - t_i)] \quad (1)$$

Where s_i is the rust severity at time i, t_i is the number of days after the first observation on assessment date i and k is the number of successive observations (Campbell & Madden, 1990).

3. Results

3.1 Reaction Type

Based on reaction types, soybean lines responded differentially to the five field isolates during screen house inoculation (Table 2). Soybean lines PI 230970 (R*pp2*), Nam 2 and Wondersoya produced consistently tan lesions

typical of a susceptible reaction in all locations. However, the two classical resistance gene sources Ankur (R*pp3*) and PI 459025 (R*pp4*), local genotypes UG 5 and Maksoy 3N responded with a red brown phenotype at all locations indicating presence of resistance genes. Mixed reaction responses were observed between lines PI 459024, GC000138-29, Maksoy 1N and Maksoy 2N in all the five isolates tested. All field isolates showed at least one mixed reaction in the twelve soybean lines evaluated. Immune response was not observed in any genotype.

Table 2. Reaction types shown by 12 soybean lines against Ugandan field rust pathogen populations

Source of Isolate	PI 230970	Ankur	PI 459025	PI 459024	UG 5	GC00138-29	Namsoy 4M	Maksoy 1N	Maksoy 2N	Maksoy 3N	Nam 2	Wondersoya
MUAIRK	T	RB	RB	MX	RB	MX	MX	MX	T	RB	T	T
NAK	T	RB	RB	RB	RB	RB	MX	T	T	RB	T	T
IKI	T	RB	RB	RB	RB	RB	MX	MX	T	RB	T	T
NaCRRI	T	RB	RB	RB	RB	MX	MX	MX	MX	RB	T	T
KAS	T	RB	RB	RB	RB	RB	MX	T	MX	RB	T	T

T-Tan, RB red brown, MX-mixed.

3.2 Severity and Sporulation

Table 3 Analysis of variance of soybean rust severity and sporulation of 12 soybean lines against five field isolates at the seedling and adult plant stage

Source of variance	df	Mean Squares		Mean Squares	
		Severity	Probability	Sporulation frequency	Probabilit
Seedling resistance					
Sets	1	1.37	0.130	0.04	0.351
Isolate	4	14.44	<.001	0.16	0.015
Isolate x Sets	4	0.54	0.456	0.03	0.685
Genotype	11	2.44	<.001	2.86	<.001
Isolate x Genotype	44	1.19	0.006	0.13	<.001
Error	59	0.58		0.05	
Adult plant resistance					
Year (season)	1	1.43	<.001	0.63	0.001
Isolate[a]	4	0.86	<.001	1.90	<.001
Isolate x Year (season)	4	2.18	<.001	1.11	<.001
Rep [Isolate x Year (season)]	20	0.02	0.569	0.05	0.930
Genotype	11	2.00	<.001	2.52	<.001
Isolate x Genotype	44	0.20	<.001	0.32	<.001
Genotype x Year (season)	11	0.35	<.001	0.32	<.001
Isolate x Year (season) x Genotype	44	0.19	<.001	0.15	<.001
Pooled Error	160	0.03		0.06	

[a] For adult plant resistance use of the term ‘isolate’ refers to the location where the field evaluation was done.

In the screen house experiment, analysis of variance indicated that there were significant isolate, genotype and isolate x genotype differences for severity and sporulation for seedling resistance. However, differences in the isolates had the strongest effects for disease severity whereas genotype had the strongest effects on sporulation rate (Table 3). In the field evaluations for adult plant resistance, all sources of variation were significant with isolate, genotype and isolate x year (season) contributing largely to the differences in severity and sporulation. The year (season) and isolate x year (season) effects were, however, more pronounced for severity than sporulation.

In the field, the overall mean severity score was greater in 2010 with more soybean lines producing mixed reaction across test locations than in 2011. However, in 2011 IKI had the lowest mean severity score which was 3.2 less than previous year (Table 4). Susceptible lines Nam 2 and Wondersoya had severity scores consistently greater than the location means with predominantly tan and mixed reaction types. Conversely, Ankur (R*pp3*), PI 459025 (R*pp4*) and Maksoy 3N had red brown lesions across lesions with mean severity generally lower than the mean averages of the locations. There was no relationship between lesion colour and severity during the two seasons of evaluation in the five test locations.

Table 4. Soybean lines used for adult plants resistance assessment in the field, their severity score (1-9), reaction type (in parentheses) and location of isolate origin

Location	Field, 2010					Field, 2011				
	IKI	KAS	MUARIK	NaCRRI	NAK	IKI	KAS	MUARIK	NaCRRI	NAK
Entry										
PI 230970 (R*pp2*)	6.0(RB)	5.3(RB)	3.3(RB)	3.3(RB)	3.3(RB)	2.0(RB)	3.6(RB)	3.3(MX)	3.6(T)	4.0(T)
Ankur (R*pp3*)	6.0(RB)	4.0(RB)	2.3(RB)	2.0(RB)	3.0(RB)	2.0(RB)	2.3(RB)	2.0(RB)	2.6(RB)	3.0(RB)
PI 459025 (R*pp4*)	5.6(RB)	4.0(RB)	2.3(RB)	1.0(RB)	2.0(RB)	3.0(RB)	4.0(RB)	3.3(RB)	2.0(RB)	2.0(RB)
PI 459024	4.0(RB)	5.3(MX)	3.0(RB)	3.3(RB)	3.3(RB)	4.0(MX)	5.3(RB)	2.0(RB)	2.0(RB)	4.0(MX)
GC000138-29	4.0(RB)	3.0(MX)	2.3(RB)	2.0(RB)	2.3(RB)	2.3(RB)	4.0(RB)	3.3(RB)	2.0(RB)	4.0(MX)
UG 5	5.0(RB)	2.6(MX)	2.0(RB)	1.0(RB)	2.3(RB)	2.0(RB)	2.6(RB)	2.6(RB)	4.0(RB)	2.0(RB)
Maksoy 1N	6.6(RB)	5.0(T)	6.0(RB)	5.6(MX)	5.0(T)	2.0(RB)	4.0(RB)	7.3(RB)	3.3(RB)	2.6(RB)
Maksoy 2N	5.6(RB)	6.6(MX)	3.3(RB)	3.6(MX)	4.6(RB)	2.6(MX)	2.0(RB)	6.6(RB)	4.3(T)	4.0(T)
Maksoy 3N	5.0(RB)	3.6(RB)	2.6(RB)	3.0(RB)	3.3(RB)	2.0(RB)	2.0(RB)	2.6(RB)	2.0(RB)	2.0(RB)
Namsoy4M	6.3(RB)	5.3(MX)	4.6(MX)	4.6(MX)	4.3(RB)	3.0(RB)	4.0(MX)	5.3(MX)	4.6(RB)	3.3(RB)
Nam 2	7.6(RB)	4.6(MX)	6.3(MX)	6.6(MX)	6.0(MX)	2.0(RB)	5.6(MX)	6.6(T)	6.6(T)	4.3(T)
Wondersoya	6.6(MX)	8.3(T)	6.3(MX)	5.6(MX)	6.0(MX)	5.0(MX)	7.3(T)	6.6(T)	4.6(T)	5.3(T)
Mean	5.7	4.8	3.7	3.5	3.8	2.7	3.9	4.3	3.5	3.4
SE ±	0.29	0.45	0.48	0.53	0.40	0.28	0.46	0.58	0.41	0.29

RB-red brown; MX-mixed; T-tan.

In the screen house experiments, evaluating the frequency of sporulation per square centimetre, KAS isolate had the greatest mean sporulation frequency (Table 5), 11.1% more than the least sporulating isolate from NAK. Isolates from KAS and NaCRRI resulted in sporulation in all soybean lines whereas NAK did not show sporulation in three lines (Table 5).

Table 5. Sporulation frequency averaged of 12 soybean lines in the screen house for seedling resistance experiment using Ugandan field isolates from five different sites

Genotype	Percentage sporulation frequency of isolate				
	IKI	KAS	MUARIK	NaCRRI	NAK
PI230970 (R*pp2*)	100	89.99	100	85.06	95.66
Ankur (R*pp3*)	73.58	38.46	0	1.31	13.39
PI 459025(R*pp4*)	31.50	30.76	10.87	39.53	10.19
PI 459024	83.97	47.86	80.38	11.31	35.47
GC000138-29	0	45.96	0	16.92	0
UG 5	0	13.21	42.48	18.45	0
Maksoy 1N	100	93.98	99.00	83.75	100
Maksoy 2N	100	94.49	100	98.98	100
Maksoy 3N	2.86	18.45	8.71	11.69	0
Namsoy4M	37.01	80.54	100	58.91	67.20
Nam 2	85.34	96.25	100	99.49	94.31
Wondersoya	100	99.49	100	97.94	100
Mean	59.52	62.45	61.78	51.94	51.35
SE±	12.18	9.55	13.04	11.38	13.04

SE± standard error of the mean.

Table 6. Summary of means of soybean rust infection parameters from inoculation averaged across five Ugandan field isolates

Genotype	Incubation period (IP)	Latent Period (LP)	Number of lesions/square centimetre (LS)	Frequency of sporulating lesions (FS)[1]	AUDPC
PI 230970 (R*pp2*)	5.2	7.2	22.8	94.14	57.7
Ankur (R*pp3*)	4.8	7.6	21.1	25.35	52.2
PI 459025 (R*pp4*)	4.6	7.5	21.9	24.57	55.7
PI 459024	5.1	9.1	19.8	51.80	44.3
GC000138-29	5.7	7.8	21.2	12.58	49.0
UG 5	5.2	8.2	21.7	14.83	56.3
Maksoy 1N	4.8	9.8	29.7	95.34	74.9
Maksoy 2N	5.1	10.2	37.0	98.98	85.8
Maksoy 3N	5.2	7.2	19.5	8.34	46.8
Namsoy4M	4.9	10.4	31.7	68.73	70.0
Nam 2	4.9	10.2	30.6	95.08	78.5
Wondersoya	5.1	9.9	33.7	99.49	81.4
Mean	4.98	8.75	25.9	57.43	62.7
SE±	0.08	0.37	1.78	11.09	4.21

SE± standard error of the mean;

[1] Expressed as a percentage.

Analysis of variance indicated that there were significant ($P \leq 0.01$; ANOVA not shown) differences in incubation and latent period, lesion density, percentage frequency of sporulating lesions and AUDPC for lesion density of lines under screen house conditions. In general, most local cultivars showed longer latent periods that the exotic lines in response to the five isolates tested (Table 6). Namsoy 4M had typically the longest latent period compared to PI 230970 (R*pp2*) and Maksoy 3N with the least. Despite the long latent period of Namsoy 4M, lesions density

was the third highest in all the test lines. Contrary, Maksoy 3N had the shortest latent period and lowest number lesions per square centimetre, 6.4 lower than the overall mean. PI 230970 (R*pp2*) with a classical resistance gene and Maksoy 2N were equally highly sporulating and comparable to the susceptible check lines. Maksoy 3N, GC000138-29 and UG 5 had light sporulation with less than 15% of the uredinia sporulating per square centimetre. Maksoy 2N had the highest AUDPC followed by Wondersoya which differed significantly from all the exotic sources of resistance and cultivar Maksoy 3N (Table 6).

3.3 Correlations Among Soybean Rust Infection Parameters

Using genotype averages across isolates a significant ($P \leq 0.001$) positive correlation was observed between percentage frequency of sporulating lesions and AUDPC (Table 7). Similarly, latent period and number of lesions density were significantly positively correlated with AUDPC. It was noted that incubation period had a negative non-significant correlation with other soybean rust resistance parameters evaluated. Mean disease severity of the adult soybean lines for the two years and seedling lesion density were positively correlated ($r = 0.813$, $P < 0.001$).

Table 7. Correlations among infection parameters evaluated during the seedling stage using five diverse field isolates

	IP	LP	LS	FS
LP	-0.224ns			
LS	-0.209ns	0.857***		
FS	-0.232ns	0.702**	0.799**	
AUDPC	-0.279ns	0.801**	0.972***	0.819***

***$P \leq 0.001$; **$P \leq 0.01$; ns-not significant;

IP-Incubation Period; LP-Latent period; LS-Number of lesions per square centimetre; FS-F frequency of sporulation lesions; AUDPC-Area under disease progress curve.

4. Discussion

The knowledge of rust virulence in soybean rust populations and how soybean lines react to field isolates in different regions is important for successful breeding and deployment of resistance genes (Miles et al., 2011). The reaction types obtained from the five isolates may indicate that each isolate is distinct which is suggestive of the existence of different race populations or virulence patterns (Yamanaka et al., 2010). It was, however, surprising that PI 230970 (R*pp2*) with a classical resistance produced tan lesions. This genotype was recommended for inclusion in the local germplasm after evaluations between 2005 and 2006 at NaCRRI in Uganda (Oloka et al., 2008). This could suggest resistance breakdown and underscores the importance of evaluating for resistance in the target geographic locations due to the differences in diversity and virulence of the rust pathogen.

Mixed lesions were observed on at least one genotype in all locations, which is indicative of a mixture of races with heterogeneous virulence (Miles et al., 2008). This was, however, more pronounced in MUARIK and NaCRRI which have the largest area of different experimental soybean lines every year. Increased virulence diversity could be an evolutionary consequence prompted by deployment of a wide assortment of lines in these two locations.

The significance of isolate-by-genotype interaction for severity and sporulation frequency implies that ranking of lines changes markedly with isolates. This presents a great challenge when breeding for resistance using specific gene resistance to manage soybean rust due to great differences exhibited by the lines. Furthermore, this underscores the importance of evaluating candidate lines using rust populations present in the target areas. In the field, the isolate x year (season) had substantial impact to disease severity greater than sporulation (Table 3). Though the two resistance indices are not completely independent of each other this could suggest greater effect of environment on severity.

This study purposefully used field isolates to understand comparative virulence and identify stable sources of soybean rust resistance. The greater preponderance of mixed reactions in the field attributed to heterogeneous race composition coupled with environmental factors that influenced the amount of inoculum and disease progress during the seasons (Miles et al., 2007, 2008). However, the significance of isolate, genotype and isolate x genotype interaction factors using severity and sporulation rate indices (Table 3) and strong positive correlation of disease severity and lesion density both during seedling and adult plant stages suggests that these are related. Similarly, isolates from KAS, MUARIK and IKI were the most aggressive in both mean seedling and adult plant assessments

compared to those from NaCRRI and NAK. The seedling resistance tests using field isolates can therefore be used to extrapolate resistance under field conditions with better accuracy. On the contrary, seedling and adult plant were observed not to be necessarily correlated during resistance evaluations in Brazil (Ribeiro et al., 2007). Differences obtained in seedling and adult plant resistance done in the US and Paraguay respectively were attributed to differences in virulence of the isolates used and longer, multiple cycles of exposure in the field (Miles et al., 2008). Our study, however, used the same field isolate of similar composition and virulence for both seedling and adult plant resistance hence the comparable results.

The positive correlation between AUDPC and sporulation relate to rapid advance of the disease caused by increased sporulation which results in more secondary infections. It was also observed that AUDPC was directly related to lesion number which is an important disease resistance index. A positive correlation between latent period and AUPDC suggested that lines that expressed disease urediniospores early have slower disease progress. This could imply that the resistance mechanism present in these lines responds rapidly once the rust pathogen is established in the host cells compared to those with a longer latent period.

Overall, the results indicate that soybean rust breeding programmes utilising specific resistance are challenged due to the restricted locations for which such resistance applies. The longevity of R*pp2* was about 5 years, which is relatively short and further limits specific resistance. Great soybean diversity was observed in sites which grown several varieties every season. Latent period, lesion density and proportion of sporulating lesions are important disease resistance parameters that can be used to extrapolate disease progress. Prospecting and exploration for other sources of resistance and strategies such as partial resistance and tolerance is highly recommended.

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