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Full Length Research Paper

Effects of moisture content and loading orientation on some mechanical properties of the african oil bean seed (*Pentaclethra Macrophylla* Benth)

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The seeds were quasi-statically loaded to determine rupture force, toughness, yield force, rupture stress, modulus of stiffness and elasticity. Compression tests were carried out on seeds at moisture content of 15.76, 19.81 and 34.43% (wb), seed orientation in both the longitudinal and transverse axis at a loading rate of 25 mm/min. Values of the rupture force, toughness, rupture stress, yield force and modulus of stiffness of the seeds decreased linearly from 362.04 to 168.82 N, 1.783 to 0.623 J, 7.4 to 3.15 N/mm^2, 213.42 to 89.68 N and 39.6 to 21.97 N in transverse axis and from 276.64 to 195.26 N, 1.355 to 0.641 J, 16.12 to 6.23 N/mm^2, 211.58 to 124.72 N and 38.74 to 24.77 N in longitudinal axis with increase in moisture content from 15.76 to 34.43 (%wb). Values of modulus of elasticity determined using hertz theory of parallel plate contact increased from 1.529 to 3.52 MPa with increase in moisture content from 15.76 to 19.81 (%wb) but decreased to 1.563 MPa with increase in moisture content. Highest value for rupture force (362.04 N) and toughness (1.783 J) of the seeds were obtained at a moisture content of 15.76% wb. The seeds are more flexible in longitudinal orientation and the rupture force requires less energy under longitudinal loading than transverse loading.

Key words: African oil bean seeds, mechanical properties, compressive tests, moisture content, loading orientation.

INTRODUCTION

African oil bean seed (*Pentaclethra macrophylla* Benth) belongs to the Leguminosae family and the sub-family of Mimosoideae (Keay, 1989) with no known varietal characterization. It is a tropical tree crop found mostly in the southern and middle belt regions of Nigeria and in other coastal parts of West and Central Africa. The seed is planted or retained along the edges of home gardens and farms mainly for its seed from which edible oil can be extracted (Oboh, 2007). African oil bean seed has been cultivated in Nigeria since 1937 (Ladipo, 1984) and for many years in other West African countries where its seed is relished as a food. Some parts of the plant also have medicinal values.

The seeds of the African oil bean are edible when boiled and fermented especially among the eastern part of Nigeria. The seed when cooked, processed and fermented is called 'ugba' (in Igbo language of Nigeria) and is used for the preparation of many delicious

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Plate 1. African oil bean seed.

delicacies like African salad, soups and sausages for eating with different staples (Enujiugha, 2003). The seed is a source of edible oil; it contains more than 52% oil in its cotyledons (Enujiugha and Agbede, 2000). Their edible seeds require tedious but careful processing and fermentation before they can be eaten as food supplement. The seeds are eaten alone with other ingredients like stockfish, garden egg, sliced tapioca or they can be mixed with vegetables popularly known as "African salad" among the Eastern part of Nigeria (Okafor and Fernandez, 1987).

Mechanical properties of agricultural materials affects their processing, handling storage and consumption. These properties are important in the design of planters, harvesters and in postharvest operations such as cleaning, conveying and storage (Balasubramanian et al., 2012; Asoegwu et al., 2006). Presently, all the post harvest handling and processing practices are done manually and it is necessary to design tools, equipment and machines for the above processes (Asoegwu et al., 2006). Design of these equipments without putting these properties into consideration may yield poor results (Polat et al., 2007).

Some physical properties of the African oil bean seed were studied by (Asoegwu et al., 2006) but no information appears to have been reported on the mechanical properties of the seeds and their relationship with moisture content.

The objective of this research is to study the effects of moisture content and loading orientation on some mechanical properties of the African oil bean seeds.

MATERIALS AND METHODS

Sample preparation

Samples of the African oil bean seeds were obtained from a local market in Ojoo, Ibadan. The seeds were manually cleaned to remove defective ones and other foreign materials before conducting the tests as presented in Plate 1.

The initial moisture content of the African oil bean was determined using the moisture analyzer and it was found to be 8.25% wb. The samples were divided into three parts and a method for adjusting the seeds moisture content without damage in its morphology was developed to prepare samples for subsequent tests. The seeds were oven dried at 105°C for 16 h until there was no change in its mass. The desired moisture levels were obtained by soaking each of the divided samples in water for different time intervals. In this method, the initial moisture content (8.25% wb) was used with the value of quantity of water absorbed by the seed using the mass balance equation, moisture content value obtained are 15.76, 19.81 and 34.43%.

The conditioned seed were put in cellophane bags and stored in a refrigerator at 5°C for one week to have uniform moisture distribution throughout samples. The required seed quantity was taken out of the refrigerator and allowed to equilibrate to room temperature before each test (Kara et al., 2010).

Compression tests

Quasi-static compression tests were performed with an Instron Universal Testing Machine (Model M500) with a 25 KN compression load cell and Integrator. Tests were conducted at room temperature of 30°C at the five moisture level. In this study a total of 90 seeds were compressed. The three perpendicular dimensions of the seeds were measured with a micrometer and recorded before each test. The individual seeds were loaded between parallel plates of the machine and compressed until rupture occurred as is denoted by a rupture point in the force deformation curve. To determine the effect of the orientation of loading, the grain was positioned horizontally with the major axis of the grain being normal to the direction of loading, or lengthwise as shown in Figure 1. For vertical loading the major axis of the grain was parallel to the direction of loading as shown in Figure 1b. The deformation (strain) was taken as the change in the original dimension of the grain. The energy required for causing rupture (failure) in the grain was determined by calculating the area under the force-deformation curve up to grain rupture. The results and the force-deformation curves obtained at each loading orientation and moisture level were analyzed for the following:

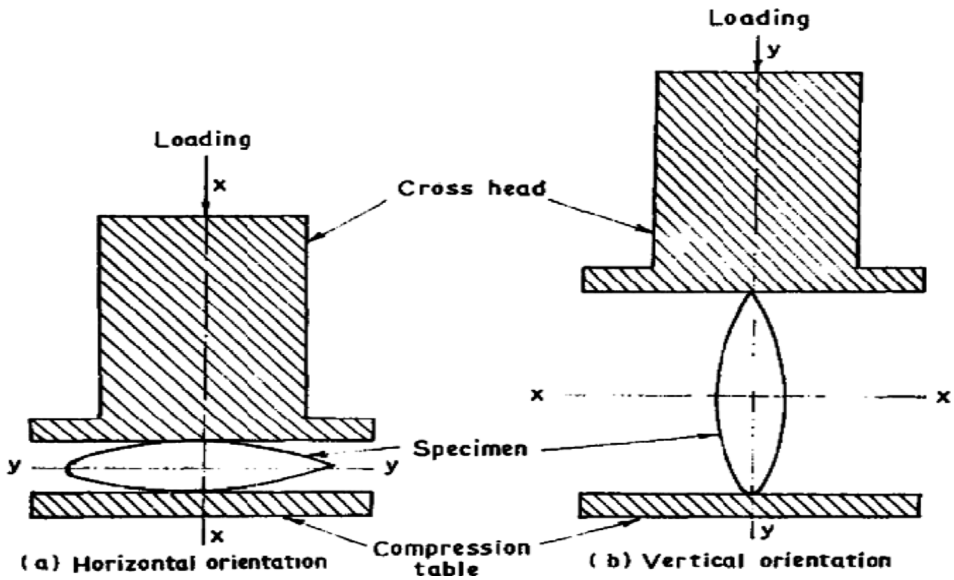

Figure 1. Orientation of African oil bean seed under compressive loading. Source: Saiedirad et al. (2007).

a. Rupture force
b. Yield force
c. Rupture stress
d. Toughness
e. Modulus of Stiffness
f. Modulus of elasticity

The rupture force was taken as the point on the force-deformation curve, at which the compressed shell became completely broken and torn with the kernel exposed and the rupture stress was obtained as the ratio of rupture force and cross sectional area of the seeds.

The modulus of stiffness was taken as the ratio of the average maximum force to the average maximum deformation of the shell; it was calculated from the force-deformation data of the shell following the method employed by Aviara and Ajikashile (2010).

The modulus of elasticity, which is defined by Hertz's expression for modulus of elasticity, was calculated using a method employed by Adgidzi et al. (2006). The values of Forces 'F' and Deformation 'D' for each shell were gotten from the force-deformation data. The value of Poisson ratio was taken to be 0.35 (Mohesnin, 1970):

$$E = \frac{0.531F(1-\mu^2)}{D_2^3}\left(\frac{1}{R}+\frac{1}{R_1}\right)^{\frac{1}{2}} \quad (1)$$

$$R = 0.5H \quad (2)$$

and

$$R_1 = \frac{H^2+\frac{L^2}{4}}{2H} \quad (3)$$

Where: E is the modulus of elasticity, μ is the poisson ratio (0.35), D is the deformation, R and R_1 are radius of curvature of seed samples, F is the rupture force, H is the minor diameter and L is the major diameter.

RESULTS AND DISCUSSION

Rupture force (F)

The rupture force was taken as the point on the force-deformation curve, at which the compressed shell became completely broken and turned with the kernel exposed. Tested and calculation values are plotted and Figure 2 shows that as moisture content reduced, rupture force increased. The average force required to initiate seed rupture decreased from 362.04 to 168.82 N and 276.64 to 195.26 N in the transverse and longitudinal axis with an increase in moisture content from 15.76 to 34.43 (%wb) respectively.

A similar trend was reported by Fathollahzedah and Rajabipour (2008) and Kiani Deh Kiani et al. (2008) in the compression of red beans grains and barberry respectively and they both attributed this to the fact that as the moisture content reduced, the tissue of the nuts became tougher. Saiedired et al (2007) in their work on the mechanical properties of Cummin seeds also observed that the force required to initiate seed rupture decreased from 58.2 to 28.8 N as moisture content increased from 5.7 to 15 (%db) and they also attributed this to the fact that at higher moisture content, the seeds were softer and required less force. The relationship between rupture force, loading orientation and moisture content may be expressed by the following regression equations:

$$F_T = -10.601M - 535.62 \ (R^2 = 0.9947) \quad (4)$$

$$F_L = -4.476M + 350.21 \ (R^2 = 0.9936) \quad (5)$$

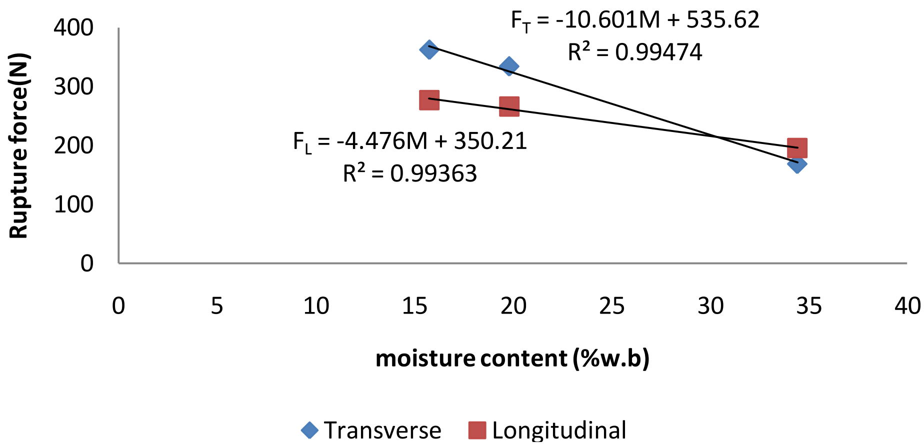

Figure 2. Effect of moisture content on rupture force of African oil bean seed under longitudinal and transverse loading orientation.

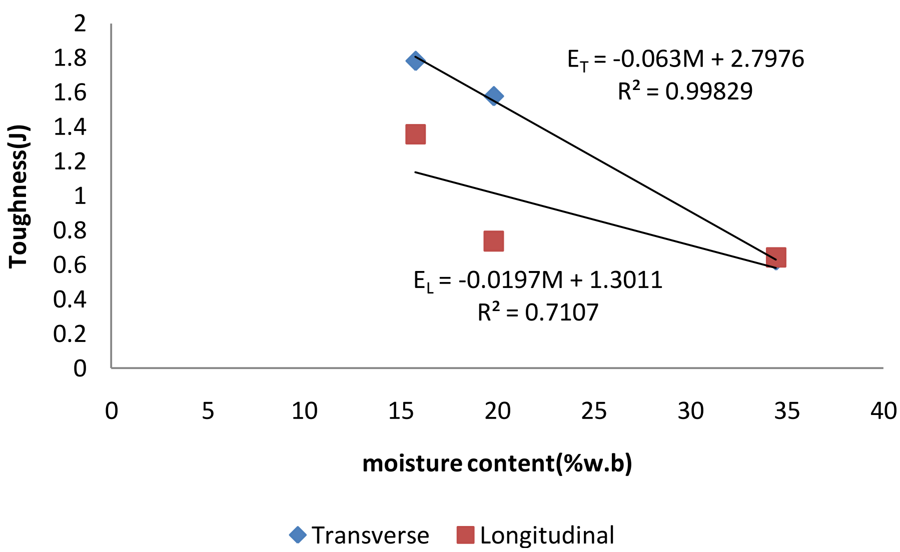

Figure 3. Effect of moisture content on toughness of African oil bean seed under longitudinal and transverse loading orientation.

Toughness (E)

Toughness was taken as the energy absorbed by the shell at which the shell got completely broken. Values obtained as shown in Figure 3 showed that as moisture content reduces the value for toughness increased. The toughness averagely decreased from 1.783 to 0.623 J and 1.355 to 0.641 J in the transverse and longitudinal orientations respectively with the toughness being more under transverse orientation. A similar trend was observed in the compression of Hemp seed by Taheri-Garavand et al. (2010) and also by Maghsoudi et al. (2012) in the determination of the rupture energy of the *Badami* variety of unsplit *Pitaschio* nut and they attributed this to the hardness of the shell at lower moisture content making it absorb more energy at lower moisture contents during compression.

The relationship between toughness, loading orientation and moisture content may be expressed by the following regression equations:

$$E_T = -0.063M + 2.7976 \ (R^2 = 0.9983) \quad (6)$$

$$E_L = -0.0197M + 1.3011 \ (R^2 = 0.7107) \quad (7)$$

Rupture stress (S_T)

The rupture stress decreased from 7.4 to 3.15 N/mm^2 and from 16.12 to 6.23 N/mm^2 as moisture content

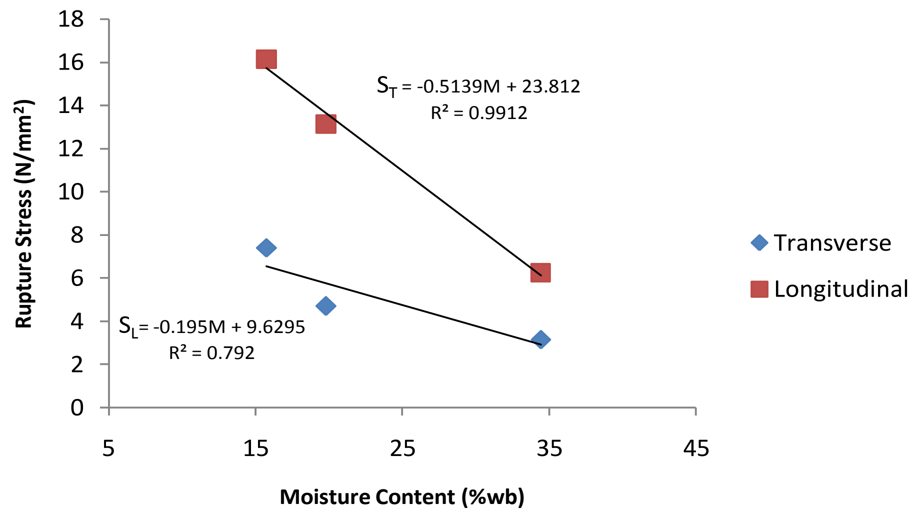

Figure 4. Effect of moisture content on rupture stress of African oil bean seed under longitudinal and transverse loading orientation.

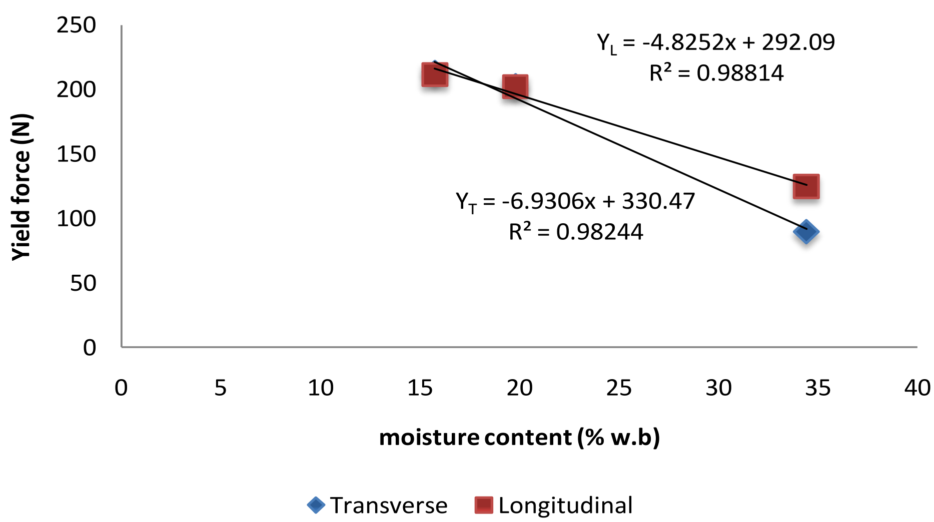

Figure 5. Effect of moisture content on yield force of African oil bean seed under longitudinal and transverse loading orientation.

increases from 15.76 to 34.43 (%wb) in the transverse and longitudinal orientations respectively; the rupture stress was higher under transverse loading than longitudinal loading. Similar trends were reported by Fadeyibi and Osunde (2012) and Fadeyibi (2012) in the compression of Rubber seed, and Haddad et al. (2001) and Kang et al. (1992) for wheat kernels in their works on the mechanical properties of wheat kernels. Figure 4 shows the linear relationship between the rupture stress, loading orientation and moisture content.

$$S_T = -0.5139M + 23.812 \quad (R^2 = 0.9912) \qquad (8)$$

$$S_L = -0.195M + 9.6295 \quad (R^2 = 0.792) \qquad (9)$$

Yield force (Y)

The yield force was taken as the point on the force-deformation curve, at which the visible failure of the nutshell became initiated and the shell just began to tear. The variation of the yield force of African oil bean seed with moisture content when subjected to compression in the transverse and longitudinal orientation as shown in Figure 5 shows that the yield force averagely decreased from 213.42 to 89.68 N and 211.58 to 124.72 N in the transverse and longitudinal direction respectively. This indicates that the minimum force needed in the compressive cracking of African oil bean shell to initiate the failure of the shell at the macroscopic level is

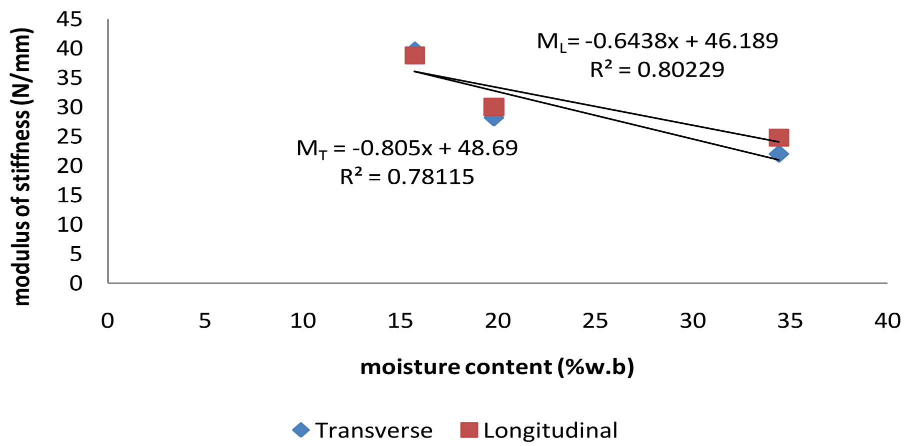

Figure 6. Effect of moisture content on modulus of stiffness (N/mm) of African oil bean seed under longitudinal and transverse loading orientation.

Table 1. Effect of moisture content on modulus of elasticity of African oil bean seed under longitudinal and transverse loading orientation.

Loading orientation	Moisture content (%w.b)	Rupture force N	Modulus of elasticity $P_a \times 10^6$
	15.76	362.04	1.529
Transverse	19.81	333.92	3.52
	34.43	168.82	1.563
	15.76	276.64	5.253
Longitudinal	19.81	265.4	1.405
	34.43	195.26	2.194

moisture dependent. A similar trend was observed by Aviara and Ajikashile (2011) and they attributed this to the fact that at higher moisture content, the seeds were softer and required less force. Also Tavakoli et al. (2009a) and Tavakoli et al. (2009b), attributed the same effect to the elastic behavior of Agricultural material at lower ranges of moisture content. The relationship between the yield force, loading orientation and moisture content may be expressed by the following regression equations:

$$Y_T = -3.1416M + 244.9\ (R^2 = 0.9982) \quad (10)$$

$$Y_L = -4.8252M + 292.09\ (R^2 = 0.9881) \quad (11)$$

Modulus of stiffness (M)

The variation of the modulus of elasticity of the African oil bean seed with moisture content when subjected to compression in the transverse and longitudinal orientation as shown in Figure 6 shows that the modulus of stiffness decreased as moisture content increased. There was an average decrease from 39.60 to 21.97 N and 38.74 to 24.77 N in the transverse and longitudinal direction respectively. Aviara and Ajikashile (2011) reported a similar trend in the compression of *Conophor* nut. The relationship between the yield force, loading orientation and moisture content may be expressed by the following regression equations:

$$M_T = -0.805M + 48.69\ (R^2 = 0.7812) \quad (12)$$

$$M_L = -0.6438M + 46.189\ (R^2 = 0.8023) \quad (13)$$

Modulus of elasticity

The variation of the modulus of elasticity of African oil bean seed with moisture content when subjected to compression in the transverse and longitudinal orientation shows that it behaved differently under different loading orientations. The modulus of elasticity averagely increased from 1.529 to 3.528×10^6 P_a as moisture content increased from 15.76 to 19.81 (w.b) but decreased to 1.563×10^6 P_a with further increase in moisture content in the transverse axis as presented in Table 1. However, in the longitudinal axis, there was a reverse trend. The modulus of elasticity decreased from

5.253 to 1.405×10^6 P_a as moisture content increased from 15.76 to 19.81 (w.b) but increased to 2.194×10^6 P_a) with further increase in moisture content. A similar trend was observed by Adgidzi et al. (2006), that the modulus of elasticity of most soft biomaterials increases with increase in strain (Mohsenin, 1980). It means that for the same material, there will be different values of elasticity depending on the point at which it was calculated.

Conclusions

The study of some mechanical properties of the African oil bean seed at various moisture contents and loading orientation suggested the following conclusions:

1. The force required to initiate seed rupture decreased with an increase in moisture content. The rupture force was higher under transverse loading than at longitudinal loading.
2. The rupture energy decreased with an increase in moisture content. The rupture energy was higher under transverse loading than at longitudinal loading. The African oil bean seeds are more flexible in the horizontal loading direction and the rupture under horizontal loading demanded less energy than under vertical loading. This may be due to decreasing contact area of seed with loading.
3. The yield force decreased as moisture content increased from 15.76 to 34.43 (%wb) in the transverse and longitudinal axes respectively. This indicates that the minimum force needed in the compressive cracking of African oil bean shell to initiate the failure of the shell at the macroscopic level is moisture dependent.
4. The rupture stress and modulus of stiffness of the African oil bean seed decreased as moisture content increased.
5. The modulus of elasticity of the African oil bean seeds behaved differently under different loading orientations.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

On-farm reproductive performance and adaptability evaluation of Dorper sheep crosses (DorperXAdilo) in different husbandry system, southern Ethiopia

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A flock monitoring study was conducted in Wolaita zone, Damot gale and Damote sore districts and Siltie zone, Mirab Azernet district with the objectives of evaluating the reproductive and productive performance of Dorper sheep and to determine the current production systems. Data was collected from November 2013 to April according to Kaufmann case histories format and were subjected to general linear models (GLM) procedure of SAS. The fixed factors considered were breed, districts, season, birth type and lambing year. Mean birth weight, weaning weight, weaning age, market age, market weight, litter size, age at first lambing and sexual maturity for Dorper sheep were 2.25±1.72 kg, 17.30±0.98 kg, 3.16±0.55 months, 12.66±1.39 months, 30.66±3.26 kg, 1.48±0.71, 11.81±1.37 months and 5±0.74 months, respectively. Location, season, birth type, parity, sex and blood group had significantly (P<0.05) affected weaning weight. Season had influence on weaning age. Pre-weaning mortality rate of Dorper sheep was 2.93% and lower in Wolaita than Siltie zone. In Siltie, rather than Wolaita zone Dorper sheep was housing in the separate house. Housing Dorper sheep in separate house was good practice, so sharing the practice for the others through training, experience share and field trip should be needed. Further study is needed to characterize meat quality and carcass yield percentages from locally available feeds of Dorper sheep in the area.

Key words: Adaptability, crosses, Dorper, husbandry, performance, Ethiopia.

INTRODUCTION

Ethiopia has around 26 million sheep (CSA, 2013) that may be grouped into about 14 traditional sheep populations (Gizaw et al., 2008). Most of the sheep population of the country is kept by smallholder farmers and sheep production in the country is traditional (EARO, 2001). Moreover, sheep production in Ethiopia has great potential to contributing more to the livelihoods of the people in low-input, smallholder farmers and pastoralists under traditional and extensive production systems (Kosgey and Okeyo, 2007).

However, comparing the presence of large and diverse sheep genetic resources similar to other tropical untries, the productivity of indigenous sheep is very low mainly due to low genetic potential for functional traits as

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compared to improved tropical and temperate breeds (Tsegaye et al., 2013). Due to these reasons tropical countries have been implementing crossbreed of indigenous animals with improved exotic genotypes to improve the genetic potential of indigenous animals. Within the aim of improving indigenous sheep productivity, in Ethiopia crossbreeding has been undertaken employing several exotic breed. However, efforts made so far did not bring significant change in developing countries in the tropics.

Dorper, improved exotic sheep breed well perform and thrive in all agro ecological climate. Recently, the Dorper was imported from South Africa to evaluate the breed as a potential performance to Areka Agricultural Research Center took place in Autumn of 2008, with first round 3 rams and second round 38 rams were imported (AARC, 2012). Dorper sheep breed until now not been evaluated under Ethiopia production systems in above discussed districts. Therefore, the objectives of the research were: to evaluate the reproductive and productive performance of Dorper sheep under farmer's management conditions in different agro ecological zones.

MATERIALS AND METHODS

Description of the study area and study breeds

Siltie zone is one of the 14 zones of the Southern nation nationality and people's regional government. It is located at 173 km from Addis Ababa the capital city of Ethiopia and 177 km from the regional town Hawassa. It has eight woreda, of which Mirab Azernet was purposively selected based on Dorper sheep distribution. The Woreda is located in 2889 masl, 7.43 to 7.66°N latitude and 37.86 to 37.90°E longitudes. It has two different agro-climatic conditions, Dega and Woina-dega and consisting 37 and 63%, respectively. The annual average minimum and maximum temperature range from 14 to 17°C, respectively and the average annual rainfall ranges from 1200 mm (Melesse et al., 2013). There are three distinct seasons; big rainy season (June, July, August and September); dry season (January, February and March); small rainy season (October, November and December) (MAWAO, 2013; NMA, 2012).

Wolaita zone located at 330 km to the south-west of Addis Ababa and 160 km from Hawassa, the regional capital city. Its altitude ranges from 1200 to 2950 masl. It has twelve woreda, of which, two woreda (Damot Gale and Damot Sore) were purposively selected based on Dorper sheep distribution. Damot Gale and Damot Sore woredas are located in between 6°51" and 7°35" North Longitude; and 37°46" and 38°1", respectively. Agro ecology of the areas is 62% highland (Dega), 38% midland (Weina Dega) and 58% highland (Dega), 42% midland (Weina Dega), respectively. The average temperature varies from minimum 13.6°C to maximum 25.1 and 14°C to maximum 21°C, respectively. The annual average rainfall in Damot Gale and Damot Sore is 1175 and 1200 mm, respectively. In a bimodal pattern with three distinct seasons; dry (November to February), small rains from March to June and big rains from July to October in both districts (NMA, 2012, WZFED, 2013). For the study crossed Dorper sheep breed (DorperX Adilo, dominant indigenous breed in the area).

Distribution of improved breeds in the study areas

Improved breeds of sheep (Dorper) were introduced over the years in order to increase lamb production and to show carcass yield percentages within Adilo local sheep. As this result, Development agents (DAs) collect money from model farmers based on their interest.

Sampling technique

All farmers who received improved breed (Dorper) sheep were a sample frame. A total of 65 households (48 from Mirab Azernet district, Siltie zone, 9 from Damot Gale and 8 from Damot Sore district, Wolaita zone) were monitored and considered purposively for the household survey in the current study based on Dorper sheep breed distribution.

Breed management

Sheep in the study areas are kept in combination with other species of animals, usually with cattle and equines in Wolaita (Shigdaf et al., 2013). All the farmers construct house for their sheep.

Flock monitoring

Flocks of 65 households were monitored between November 2013 and April 2014. Prior to sampling, previous survey results and secondary data from the Office of Agriculture and Rural Development (OoARD) on overall agricultural production, socioeconomics and crop-livestock integrations were reviewed, and experts of animal husbandry consulted. Field visit was also made to gather pre- information and select the study Kebeles, villages and thereby the households.

For each Kebele, flock density one enumerator was recruited from the respective locality (5 for the whole study (2 enumerators for Wolaita zone and 3 enumerators for Silte zone). All the data collectors are diploma completed, unemployed and able to speak local language and Amharic. Training and demonstration was undertaken before commencement of the study. All animals were identified and numbered at the start of the study. Reproductive data (age at first lambing, lambing interval, and litter size), productive (birth weight and weaning weight) and mortality data were recorded (Appendix 1 and 2). Within 24 h of the new born; date of birth, birth weight, type of birth, sex of lamb and ewe/dam parity were taken. Weaning weight was recorded on 90^{th} day. Weights were taken by using balance scale.

Case histories

To grasp adequate information on the parameters like age at first lambing, lambing interval, lamb mortality, litter size, case histories of breeding females was taken according to Kaufmann (2005, Appendix 3). This is because the monitoring time was still short to record these events. The breeding females whose histories were recorded were those that gave birth at least once. Case histories were done giving priority for older females assuming that they were more informative. Twenty seven case histories were recorded on breeding ewes.

Data management and statistical analysis

Reproductive and growth data were subjected to GLM procedure of Statistical Analysis System SPSS. Statistical significance for quantitative data was done using F test. Fixed effects fitted in the model included the effects of location (2), sex (2), parity (1- >3), birth type (Single, twin, triple, multiple) and season of birth (dry,

Table 1. Distributed Dorper sheep breed in number, where it distributed and blood groups of the breed.

Parity	Blood group and sex						Delivered to	Year
	75%	Sex		50%	Sex			
		M	F		M	F		
1st	12	7	5	86	61	25	Azernet and wolaita	2010/2011
2nd				75	51	24	Wolaita	
3rd				8	3	5	Mirab Azernet	
4th				31	13	18	others	2011/2012

Male, male; F, female and others; distribution of Dorper sheep to other zones. Source: (AARC, 2012)

small rainy and big rainy season). The statistical model is explained as follows:

$Y_{inlmjyo} = \mu + L_i + X_j + P_l + B_m + S_n + e_{ijlmnyo}$

Y_{inlmjo} = Weights and weaning of the n^{th} lamb; μ = the overall mean; L_i = the fixed effect of the i^{th} location; X_j = the fixed effect of j^{th} sex; P_l = the fixed effect of l^{th} parity; Bm = the fixed effect of m^{th} type of birth; S_n= the fixed effect of n^{th} season; ei_{nlmjo}= the random error,

RESULTS

Reproductive performance of Dorper sheep

Birth weight, weaning weight, weaning age, market weight and market age were varied among the two zones. Weaning weight and market age mainly dependent on location, birth type, parity and blood group and observed differences were significant (P< 0. 05) level of significance. The least square means (kg) of birth weight, weaning weight, weaning age (month), market age (month) and weight(kg) of Dorper sheep lamb was 2.25, 17.30, 3.16, 12.66 and 30.66, respectively (Table 1). Weaning weight, market age and market weight were affected by birth type and blood group. Location had significance on weaning weight and market age, whereas season had significance on weaning weight and market weight. Weaning weight also affected by parity and sex of lamb.

Housing systems

Sheep are housed in different ways. The majority of the respondent in Mirab Azernet (95.8%), house their sheep in the separate house and housing sheep in main house was also reported by some farmers in the same woreda. However, the all respondents of Damot Gale and Damot Sore woreda (100%) house their sheep in the main house with families.

Adaptability of Dorper sheep

Survival rate

Based on the data obtained within six months from flock monitoring, the average pre weaning mortality of the Dorper sheep lamb was 2.93%. As the result indicated that the pre weaning mortality rate was highest in Siltie (2.20) and female (2.20) than in Wolaita (0.73) and male (0.73). Unlike other improved sheep breed, Dorper sheep breed has good adaptation under different climatic conditions. Thus farmers should be encouraged to use the breed for crossbreeding with the native or local breeds.

Fast growth

Moreover, fast growth of Dorper sheep as indicated 44.4, 37.5 and 29.2% in Damot Gale, Damot Sore and Mirab Azernet, respectively, followed by easy to manage, 33.3, 25.0 and 39.6% in Damot Gale, Damot Sore and Mirab Azernet made reasons to future expand of improved sheep breed.

Feeding habit

Discussion with key informants and flock holders in two zones showed that farmers appreciate the capability of Dorper to produce lamb, as meat better than Adilo sheep, with minimum input. The fact is that, Dorper sheep are able to consume diverse feed source and plant species that cannot be easily consumed by local sheep: Like Crop residues (straw of wheat, teff, barley), *chat* leftover and pods and broken seeds of haricot bean), was mentioned as the amazing desirable feature of Dorper sheep breed.

DISCUSSION

Age at first lambing

A total of 27 ewes were assessed for their life histories in the study area. Age at first lambing (AFL) varied by location and season, although observed differences were not significant (P>0. 05) level of significance (Table 2). Age at first mating is influenced by genetics. The current finding of least square means age at first lambing of

Table 2. Least square means and standard errors for age at first lambing, sexual maturity (in month) and litter size of ewe Dorper sheep.

Source of variation	N	Age at first lambing LSM(±SE)	Sexual maturity LSM (±SE)	Litter size LSM (±SE)
Overall	27	11. 81 ±1.37	5. 00±0.74	1.48±0.71
Location		NS	NS	NS
Siltie	18	11.77±0.51	5.16±0.27	1.50±0.26
Wolaita	9	11.89±0.70	4.66±0.38	1.44±0.36
Season		NS	NS	NS
Dry season	10	12.20±0.60	4.90±0.32	1.20±0.31
Small rainy season	5	11.60± 0.77	5.40±0.42	1.40±0.40
Big rainy season	12	11.58±0.00	4.91±0.29	1.75±0.54
Blood group		**	NS	NS
50% cross of Dorper	13	11.07±0.53[a]	4.84±0.28	4.88±0.27
25% cross of Dorper	14	12.50±0.65[b]	5.14±0.36	4.96±0.26

Means within each subclass with different superscript (a,b) letters differ significantly (P<0.05); NS, non significant.

Dorper sheep, 11.81 months was comparable with the previous reports of 12 months (Tsegaye et al., 2013) in Ethiopia and 11.5 months of ewe sheep of Dorper (Fourie et al., 2009). There is micro difference existed due to difference in production systems and climate.

Sexual maturity

Sexual maturity was recorded. The current finding of least square means sexual maturity of Dorper sheep, 5 months was comparable with the previous reports of Budai et al. (2013) and Cloete et al. (2000).

Birth and weaning weight

Location, season, sex, birth type, parity and blood group had no influence on birth weight and observed no differences were significant (P> 0. 05) level of significance. The mean birth weight obtained in this study, 2.25 kg was comparable with Berhanu and Aynalem (2009) of indigenous sheep. However, Gavojdian et al. (2013) reported 3 and 3.5 kg for crossed and pure Dorper sheep. This reflects that the variation of blood group and production systems. Births in the late dry and small rainy seasons could be disadvantageous due to poor quality and limited quantity feed, especially as little supplementary feeding is practiced in the studied areas. Mean weaning weight of Dorper sheep found in the present study, 17.30 kg was comparable to 18.2 kg for Dorper sheep (Cloete et al., 2000). The present result was lower than previous result of Daniel and Held (2005) reported weaning weight of 33. 4 kg under intensive production systems, this variation is due to difference in production system.

Market weight and age

Since Dorper sheep breed growth fast, it attains optimum market weight in short period of time in mid attitudes and semi intensive production systems. Majority of the farmer's sale their animals early before attaining optimum market weight was reported by Getahun (2008). The mean market weight and market age of current study of Dorper sheep breed was 30.68 kg and 12.66 months, respectively. The current finding of market weight was lower than the report of 36 for female crossed and 70 for male pure Dorper sheep (Fourie et al., 2009). Market weight and market age were affected by birth type and blood group. Parity and location also had significant on market age of lamb. Season had significant on market weight. In Siltie zone, Dorper lamb reached market age at 12.87 months, which was significantly higher (P<0.05) than those in Wolaita zone, 12.05 months.

Housing systems

Housing system for sheep depends on the management systems. In the Mrab Azernet district, the system of house used separate house for their sheep is different from that of Wolaita of Damot Gale and Damot Sore districts, used main house. Discussion with key informants and field observation in the area revealed that the materials used for housing also vary according to the economic status of the family. Dorper sheep are kept in house during night to protect them from predators, theft and abrupt climatic changes.

Adaptability of Dorper sheep

Dorper sheep are able to consume diverse feed source and (straw of wheat, teff, barley), *chat* leftover and pods and broken seeds of haricot bean), was mentioned as the amazing desirable feeding habit of Dorper sheep breed, uncommon on Adilo sheep and comparable with the study of (Ermias, 2014). As this result, the breed gain adaptation through feeding mechanisms. And pre weaning mortality of the breed was higher than the previous study of 13.5 of Adilo sheep (Belete, 2009). The difference is due to Dorper sheep breed consume all feed source unlike that of Adilo indigenous sheep breed.

CONCLUSIONS AND RECOMMENDATIONS

Dorper sheep crosses had better reproductive performance than indigenous sheep breed of Adilo especially in weaning weight and market weight even though it has similar sexual maturity and litter size. The high survival rate of the breed indicates high adaptation through the unique feeding habit in addition to less disease load. The study also pointed out the non genetic factors has significant effect, especially housing Dorper sheep in separate house was good practice.

In view of the above, the following recommendations were suggested;

1. Housing Dorper sheep in separate house was good practice, so sharing the practice for the others through training, experience share and field trip should be needed
2. In Siltie zone, there should be adequate supply of improved 50% cross of Dorper sheep breed with least cost to farmers because of in the area there was awareness on the breed.
3. Further study is needed to measure body weight, identifying economically important diseases, characterize meat quality and carcass yield percentages from locally available feeds of Dorper sheep in the area.

Conflict of Interest

The authors have not declared any conflict of interest.

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Appendixes

Appendix I: Flock monitoring format

Flock dynamics follow-up format									
Breed/class	**Inventory (Number)**	**Monitoring 1 Date**						**Monitoring 2 date**	**Monitoring 3 date**
		Birth			**Purchased (number)**	**Sold (number)**	**Died (number)**		
		Single	**twin**	**triple**					
Local									
Ewe									
pregnant									
suckling									
dry									
ram									
lambs									
<3month									
3-6month									
7-12mont									
Exotic									
Ewe									
pregnant									
suckling									
dry									
ram									
lambs									
<3month									
3-6mont									
7-12mont									
Crosses 50/75%									
Ewe									
pregnant									
suckling									
dry									
ram									
lambs									
<3month									

Appendix I: Contd.

3-6mont
7-12mont

Appendex 2: Lamb weight

Lamb weight monitoring format

Farmer name: ____
Wereda:____
Zone: ____

Ewe name	Breed	Parity	Lambing date	Birth Type	sex	Weight							
						date	kg	date	kg	date	kg	date	kg
Ewe #1													
Ewe #2													
Ewe #3													
Ewe #4													
Ewe #5													
Ewe #6													
Ewe #7													

Appendex 2. Lamb weight

Ewe #8

Ewe #9

Ewe #10

Type : single, twin, triple

Appendix 3. Life histories questionnaires for breeding females

1. Name of the owner
2. Name of the ewe
3. Does she have ID?
4. Tag No. of the ewe
5. When did you get? How (purchased, from research centers)?
6. Age of the ewe when acquired
7. Was she born in your flock or did you get from somewhere else?
8. How many lambs did this ewe deliver up to now? (The complete number, alive as well as dead ones) a) at the first___________________________________
   b) At the second_________________________________
   c) At the third _________________________________
9. At what age did she give birth for the first time? (Age in months)
10. The month and year of first lambing, second, third___________________________________
11. Does the animal have any problems like udder abnormalities, mastitis, poor milk let down of else? If yes, for which problem you could account for?
12. Does this animal have any abortions?
    a. If yes, how many and before which lamb did they occur?
    b. Where those early or late abortions?
13. Did this animal ever show difficulties to conceive? a. yes b. no
14. If yes, what do you think was the reason?
15. Is the ewe now pregnant and since how many months?

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Assessment of the severity and impact of drought spells on rainfed cereals in Morocco

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Drought is a major factor affecting cereal production in most the rainfed areas of West Asia and North Africa. Recent increases in drought frequency in Morocco have resulted in the yields of field crops being extremely variable and generally low. The objective of this study is to assess drought severity in the main cereal production areas of Morocco and to evaluate its effects on grain yield. Also the study seeks to evaluate if the standardized precipitation index (SPI) may be used as a tool to predict drought and crop yield early in the season. Data analysis showed that for the period 1988 to 2008, yields fluctuated from 150 to 3000 kg/ha with a coefficient of variation of between 30 and 50% in the north and 60 and 70% in the south. Based on the SPI, the regions studied experienced, on average, a drought once every 2.6 years. However, very severe droughts were observed only once in 7 years. The SPIs computed for the periods October to June and January to March were highly correlated. Moreover, there was a high positive correlation between the yield and the SPI calculated for the period January to March. The coefficients of determination varied between around 0.20 and 0.62 for bread and durum wheats, and between 0.28 and 0.69 for barley. It is concluded that soil moisture levels during the tillering and stem elongation periods of the cereals are the most important determinants of yield. Hence an SPI computed for the period January to March can be used to predict drought severity and yields early in the season.

Key words: Rainfall, drought, cereals, yield, standardized precipitation index, predictions

INTRODUCTION

Morocco is characterized by low rainfall and high fluctuations in the amount of precipitation. Average rainfall decreases from north to the south and from west to the east of the country.

Agriculture in Morocco is highly dependent on climatic conditions as shown by the high correlation observed between precipitation levels and agricultural value-added (Ouraich and Tyner, 2012). Moreover, the effects of drought on agriculture are always measured in relation to their impact on cereal production, because these crops are highly sensitive to climatic conditions, as they are mainly grown under rainfed conditions. Moreover, they represent 55% of the total value-added of crop production and occupy 65% of the agricultural area. However,

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because of recurrent droughts during the period 1980 to 2011 (ONICL, 2012) the national average production of cereals was affected and, hence, imports were, on average, 2.6 million tonnes, which represented half of the Moroccan production. The effect of drought will be exacerbated by climate change because of its effects on rainfall and temperature. Recent studies (Gommes et al., 2009) have shown that by the end of the 21st century, rainfall, in Morocco is likely to decrease by 20 to 40% and the temperature will increase by 3 to 6°C, depending on the region.

Yields of rainfed cereals are affected by rainfall amounts and their distribution during the season. This effect can vary depending on the soil characteristics, cultivation practices, and the genotype. However, in the Mediterranean farming systems of the Western Australian wheat belt, crop yields are influenced primarily by the amount and distribution of rainfall and the soil's capacity to hold moisture (Lawes et al., 2009). In fact, Christopher et al. (2008) indicated that the soil depth associated with the soil's capacity to retain moisture in the presence or absence of rainwater, determined wheat productivity. Busscher et al. (2001) stated that corn yield was limited by deep compacted strata because these reduced root exploration within the soil profile. Richards (2008) specified that the increase in depth explored by the roots and the root density of the cereal allow increased water and nutrient absorption from the soil, which increases productivity.

In Morocco, there are three main periods of drought; the early drought that occurs in the autumn and usually decreases the stand establishment of the crop; the mid-season drought that can reduce the onset of kernels, and the late or terminal drought that usually negatively affects grain filling. Under these conditions of water scarcity, one of the potential strategies to increase and stabilize yields is to capture more rainwater for use in transpiration, to use the fixed carbon dioxide more effectively in producing biomass, and to convert more of the biomass into grain (Passioura, 1977, 2006). Despite the importance of both the source (biomass production) and sink (grain production) in the elaboration of cereal yields illustrated by this strategy, it was argued that only a relatively small fraction of the whole growing period is actually critical to the determination of yield (Otegui and Slafer, 2004). This is, in general, the period when the number of grains per unit land area is largely determined in response to the growing/partitioning conditions of the crops.

Drought indices are used to characterize drought and to provide spatial and temporal representations of its occurrence (Pashiardis and Michaelides, 2008) and severity. However, some of these indices are inaccurate and others are difficult to calculate. The Palmer Drought Stress Index (PDSI), for example, developed by Palmer (1965) is very effective in the evaluation of agricultural drought because it uses not only historical precipitation data; but also those of temperature and available water. The disadvantages of using this index is that it does not allow the detection of drought occurrence on time and it is less adapted to areas characterized by frequent extreme situations of the climate, such as the ones we usually observe in the dry areas of WANA (Karrou, 2006). In this study, we opted to use the standardized precipitation index (SPI) to evaluate drought severity and the effects of this climatic hazard on cereal production during the last two decades. This index was chosen because of its simplicity, the possibility to describe drought on different time scales, and its standardization, which ensures independence from the geographical position of the measuring stations. (Pashiardis and Michaelides, 2008). The SPI is calculated from the monthly precipitation data. It was developed by Mckee et al. (1993) to quantify the precipitation deficit for multiple time scales that reflect the impact of drought on the availability of the different water resources, including rainfall. The SPI was also selected because precipitation is not normally distributed; therefore, absolute rainfall values are usually more poorly correlated with yields than when the rainfall values are standardized (Teigen and Thomas, 1995). Yamoah et al. (2000) demonstrated for maize that up to 64% of the yield variability was explained by the SPI in a curvilinear relationship. According to these authors, the objective in relating SPI as a function of yield is to advise farmers on how to adjust their cropping plans ahead of time to maximize returns or reduce costs.

The objectives of this study are to evaluate how SPIs, calculated for the main periods of cereal growth and development, are linked to yield and determine if this index can be used to predict drought and help decision-makers better select the mitigation and preparedness measures to cope with this climatic hazard.

METHODOLOGY

Data collection

Long-term data on the average grain yields (1988 to 2008) of bread and durum wheats and barley in the major cereal production provinces of Morocco were provided by the Department of Statistics of the Ministry of Agriculture. These provinces represent the major agro-ecosystems existing in the country and their locations follow a rainfall gradient from the north (high rainfall) to the south (low rainfall) (Map 1). The wetter regions are represented in our study by Kenitra, Meknes, and Fes provinces, with average rainfalls for the last 20 years of 522, 450, and 442 mm, respectively. The relatively drier provinces, El Jadida, Safi, and Khouribga, had average precipitations of 387, 370 and 324 mm, respectively. Kenitra (high rainfall) and El Jadida (low rainfall) regions have some irrigated areas and hence the crops may have received some supplemental irrigation in the spring. Fes, Meknes, and Kenitra are located in the north of Morocco, El Jadida and Safi in the south, and Khouribga in the south east.

Rainfall data for the period 1988 to 2008 were provided by the National Directorate of Meteorology of Morocco. These data were collected from the synoptic weather stations of Fes (33° 97' N, 4° 98' S), Meknes (33° 88' N, 5° 53' S), Kenitra (34° 30' N, 6° 60' S),

Map 1. Map of Morocco showing the locations of the different regions studied.

El Jadida (33° 23' N, 8° 52' S), Safi (32° 28' N, 9° 23' S), and Khouribga (32° 87' N, 6° 97' S). The data show that the inter-year variations were very high for all the regions. The coefficient of variation is 42% for El Jadida, 38% for Kenitra, 33% for Fes, 31% for Meknes, 39% for Khouribga, and 47% for Safi.

Drought evaluation and statistical analyses

To evaluate drought severity in the selected regions, the SPI is calculated using the equation:

$$SPI = (Pi-Pm)/\sigma$$

Where Pi is the precipitation of year i at a given time scale and Pm is the mean precipitation of a long series of data. In our study, we used only 21 years because of the lack of data for the weather stations considered. σ is the standard deviation.

The SPI was computed for different time scales (3 and 9 months) using a series of monthly rainfall data covering the period of 1988 to 2008. These SPIs were determined for the three major growth periods of cereals: SPI October to December (SPI-OD), which corresponds to the germination and seedlings emergence (stand establishment) phases; SPI January to March (SPI-JM) when tillers and spikes are formed; and SPI April to June (SPI-AJ), which coincides with the grain filling time of cereals. We also calculated SPI October to June (SPI-OJ) because this 9 months period covers the whole life cycle of the cereal crops. These crops are usually planted in November/December and harvested in May/June depending on the species, the rainfall conditions of the year, and the region. Positive SPI values signify greater than median precipitation, while negative ones mean less. An SPI of zero indicates average conditions. A value greater than +2 indicates extremely humid conditions while one less than -2 generally indicates extremely dry conditions (Yamoah et al., 2000). SPIs of +0.99 and -0.99 are considered mild stress situations for cropping systems adapted to the region (McKee et al., 1993).

To evaluate the impact of drought severity on cereal yields, a simple regression analysis was performed between SPI levels and the yields of the bread wheat, durum wheat and barley. The

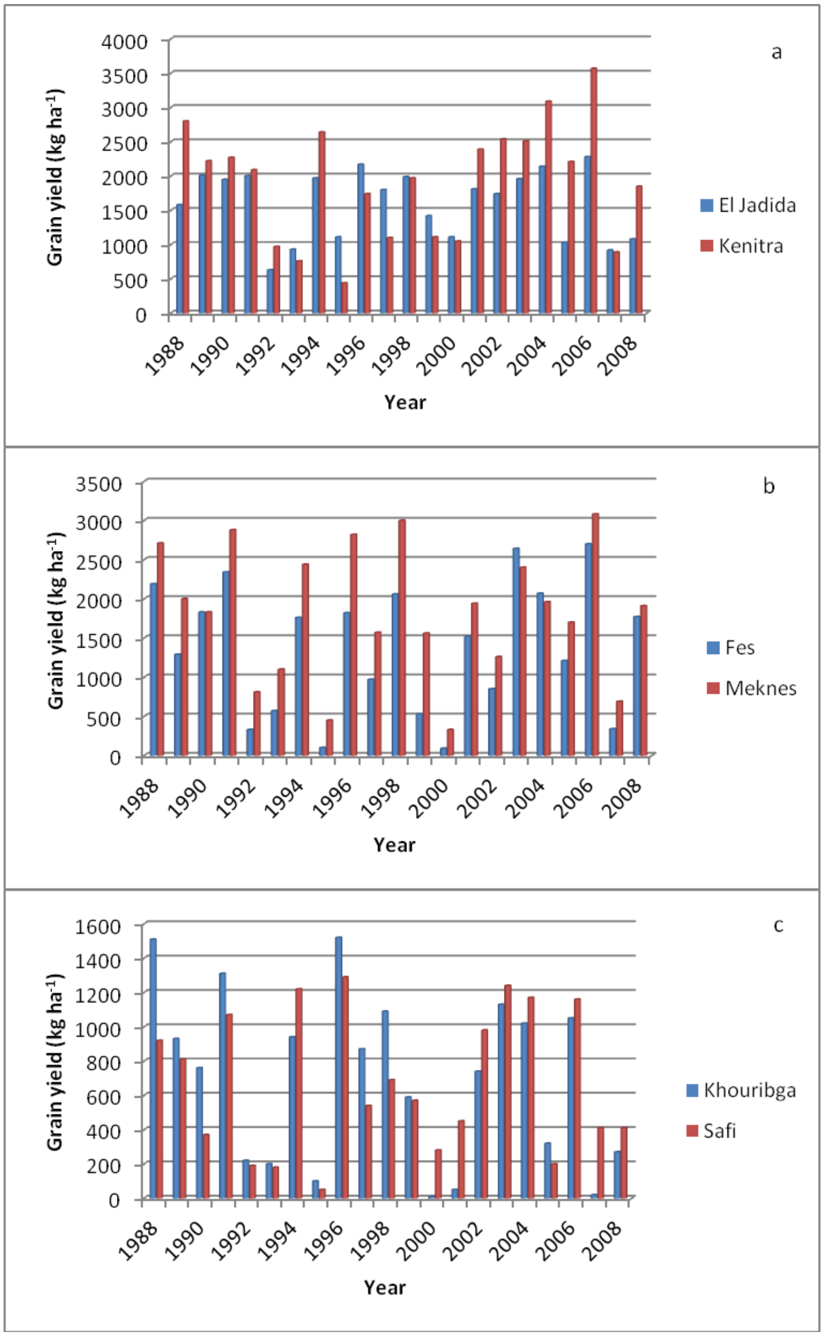

Figure 1. Variation of yield of bread wheat in six regions of Morocco from 1988 to 2008.

coefficients of determination (R^2) were computed for a series of yields for SPIs calculated for the periods October to December, January to March, April to June, and October to June.

RESULTS

Yield

Figures 1 to 3 present the changes in yields for wheat and barley in different regions from 1988 to 2008. The data show that, in general, the yield is low and variable in time and space and follows the trend of the rainfall. The coefficient of variation was very high, ranging from 30 to 55% in El Jadida and Kenitra, 45 to 65% in Fes and Meknes and 60 to 72% in Khouribga and Safi (the driest areas). On average, the yields of barley were lower than those of the bread and durum wheats.

In El Jadida and Kenitra there were the lowest yields

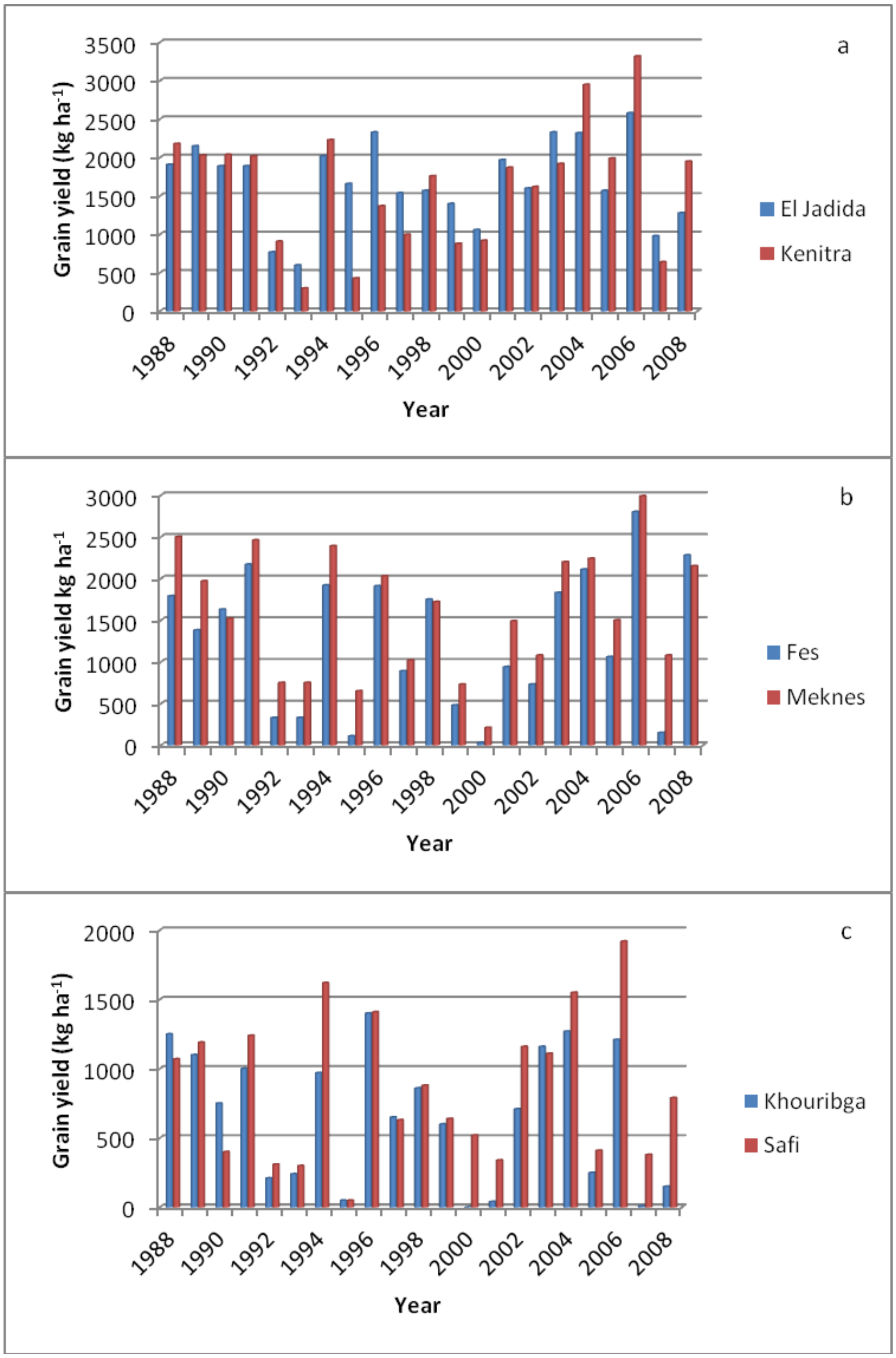

Figure 2. Variation of yield of durum wheat in six regions of Morocco from 1988 to 2008.

for all three cereal species in 1992, 1993, 2000, and 2007. They were, respectively in El Jadida, 630, 930, 1110 and 920 kg/ha for bread wheat, 770, 600, 1060 and 980 kg/ha for durum wheat and 450, 400, 270 and 600 kg/ha for barley. However, the yield of barley was very low – 100 kg/ha in 1995. For Kenitra, the analysis showed similar trend for 1992, 1993, 1995 and 2007 with respective yields of 970, 760, 440 and 890 for bread wheat, 910, 300, 430 and 640 kg/ha for durum wheat and 930, 350, 320 and 640 kg/ha for barley.

In El Jadida, high yields were realized for bread wheat in 1996 and 2006, for durum wheat in 1996, 2003, 2004, and 2006, and for barley in 1991 and 2006. In Kenitra high yields were obtained for bread and durum wheats in

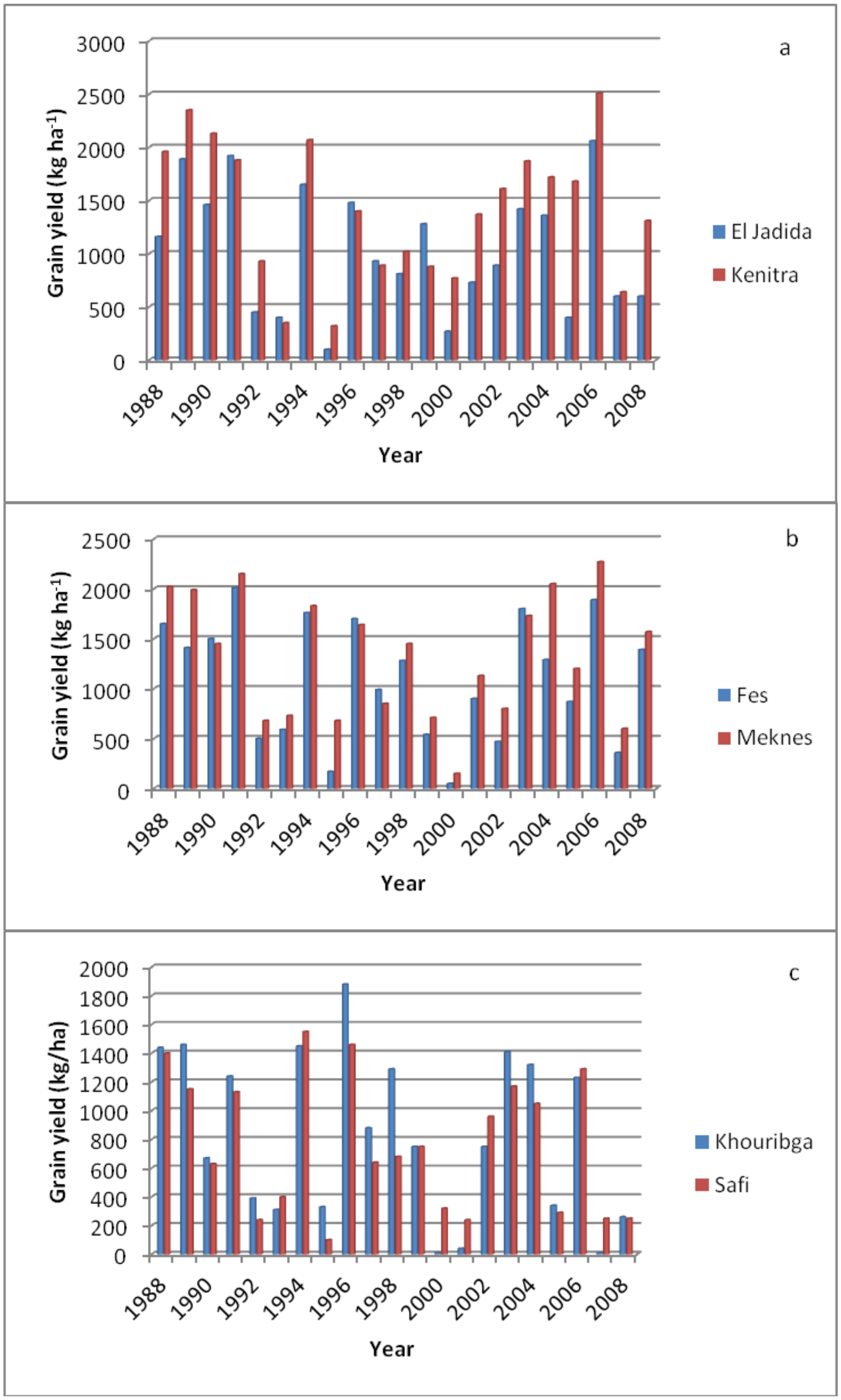

Figure 3. Variation of yield of barley in six regions of Morocco from 1988 to 2008.

2004 and 2006 and for barley in 1989 and 2006. The high values for durum wheat varied from 2000 to 2500 kg/ha in El Jadida and from 2500 to 3000 kg/ha for bread wheat in the case of Kenitra.

In Fes, low yields of bread wheat were obtained in 1992 (330 kg/ha), 1995 (100 kg/ha), 2000 (90 kg/ha), and 2007 (340 kg/ha). In Meknes the figures were 810 kg/ha in 1992, 450 kg/ha in 1995 and 330 kg/ha in 2000 and

690 kg/ha in 2007 (690 kg/ha). In Fes, yields of bread wheat were also low in 1993 (590 kg/ha) and 1999 (530). High yields of bread wheat were registered in Fes in 1991 (2340 kg/ha), 2003 (2640 kg/ha), and 2006 (2700 kg/ha) and in Meknes in 1991 (2880 kg/ha), 1996 (2820 kg/ha), 1998 (3000 kg/ha), and 2006 (3080 kg/ha).

For durum wheat, productivity in Fes was low in 1992 (330 kg/ha), 1993 (330 kg/ha), 1995 (110 kg/ha), 1999 (480 kg/ha), 2000 (30 kg/ha), and 2007 (150 kg/ha). The corresponding yields in Meknes were in 1992 (750 kg/ha), 1993 (750 kg/ha), 1995 (650 kg/ha), 1999 (730 kg/ha), 2000 (210 kg/ha), and 2007 (1080 kg/ha). In Meknes, 1997 was also a dry year and the yield of durum wheat was 1020 kg/ha. The highest yields of durum wheat were obtained in 2006 – 2800 kg/ha in Fes and 2990 kg/ha in Meknes.

For barley, low yields were registered in Fes in 1992 (500 kg/ha), 1993 (590 kg/ha), 1995 (170 kg/ha), 1999 (540 kg/ha), 2000 (50 kg/ha), 2002 (470 kg/ha), and 2007 (360 kg/ha). In Meknes low yields were recorded in 1992 (680 kg/ha), 1993 (730 kg/ha), 1995 (680 kg/ha), 1999 (710 kg/ha), 2000 (150 kg/ha), and 2007 (600 kg/ha).

In Fes, the high yields of barley were 2010 kg/ha in 1991 and 1890 kg/ha in 2006. In Meknes, high yields of barley were achieved in1988 (2020 kg/ha), 1989 (1990 kg/ha), 1991 (2150 kg/ha), 2004 (2050 kg/ha), and 2006 (2270 kg/ha).

For bread wheat, the average yield for Khouribga was 698 kg/ha and for Safi, 676 kg/ha. In Khouribga the years of low yields were 1992 (220 kg/ha), 1993 (200 kg/ha), 1995 (100 kg/ha), 2000 (10 kg/ha), 2001 (50 kg/ha), 2005 (320 kg/ha), and 2007 (20 kg/ha). In Safi low yields of bread wheat were obtained in 1990 (370 kg/ha), 1992 (190 kg/ha), 1993 (180 kg/ha), 1995 (50 kg/ha), 2000 (280 kg/ha), and 2005 (200 kg/ha). High yields of bread wheat were recorded in Khouribga in 1988 (1510 kg/ha), 1991 (1310 kg/ha), and 1996 (1520 kg/ha). In Safi high yields of bread wheat were obtained in 1994 (1220 kg/ha), 1996 (1290 kg/ha), 2003 (1240 kg/ha), 2004 (1170 kg/ha), and 2006 (1160 kg/ha).

For durum wheat, the average yield for Khouribga was 661 kg/ha and for Safi, 853kg/ha. Low durum wheat yields were obtained in Khouribga in 1992 (210 kg/ha), 1993 (240 kg/ha), 1995 (50 kg/ha), 2000 (0 kg/ha), 2001 (40 kg/ha), 2005 (250 kg/ha), 2007 (10 kg/ha), and 2008 (150 kg/ha). In Safi, the low yields of durum wheat were 400 kg/ha in 1990, 310 kg/ha in 1992, 300 kg/ha in 1993, 50 kg/ha in 1995, 340 kg/ha in 2001, and 380 kg/ha in 2007. The high yields that were obtained in Khouribga were 1250 kg/ha in 1988, 1100 kg/ha in 1989, 1000 kg/ha in 1991, 1400 kg/ha in 1996, 1160 kg/ha in 2003, 1270 kg/ha in 2004, and 1210 kg/ha in 2006. In Safi, the high yields were 1620 kg/ha in 1994, 1410 kg/ha in 1996, 1550 kg/ha in 2004, and 1920 kg/ha in 2006.

The average yield of barley in Khouribga was 831 kg/ha and in Safi, 760 kg/ha. The low yields of barley in Khouribga were 390 kg/ha in 1992, 310 kg/ha in 1993, 330 kg/ha in 1995, 10 kg/ha in 2000, 40 kg/ha in 2001, 340 kg/ha in 2005, 10 kg/ha in 2007, and 260 kg/ha in 2008. Low yields of barley were obtained in Safi in 1992 (240 kg/ha), 1993 (400 kg/ha), 1995 (100 kg/ha), 2000 (320 kg/ha), 2001 (240 kg/ha), 2005 (390 kg/ha), 2007 (250 kg/ha), and 2008 (250 kg/ha).

The high yields of barley were obtained in 1988 (1440 kg/ha), 1989 (1460 kg/ha), 1994 (1450 kg/ha), 1996 (1800 kg/ha) and 2003 (1410 kg/ha) in Khouribga. For Safi, the high yields of were 1400 kg/ha in 1988, 1550 kg/ha in 1994, and 1460 kg/ ha in 1996.

Standardized precipitation index

Data analysis showed a high correlation (r = 0.67) between SPI-JM (January to March) and SPI-OJ (October to June). However, the relationship between SPI-AJ (April to June) and SPI-OJ was lower (r = 0.33). All the correlations between the other SPIs were not significant; the coefficients of correlation between SPI-OD (October to December) and SPI-JM was 0.01, between SPI-OD and SPI-AJ, 0.24, and between SPI-OD and SPI-OJ was 0.12. The correlation between SPI-JM and SPI-AJ was zero (r = 0).

To evaluate drought severity during the entire cereal growing season (October to June), we used the SPI-OJ (Table 1). SPIs with negative values were obtained in all regions, except those indicated between parentheses after the date, in 1992, 1993, 1995, 1999, 2000 (Safi), 2001 (Kenitra and Fes), 2002, 2005, 2007 (Fes), and 2008. The values for these listed 10 years are underlined and bold in Table 1.

Negative values were also registered in Khouribga in 1989 (-0.10), Fes in 1994 (-0.21), El Jadida (-0.28) and Safi (-0.15) in 1998.

The highest SPIs (more than + 1.00) were obtained in all regions in 1996, being +2.19 in El Jadida, +1.89 in Kenitra, +1.21 in Fes, +1.98 in Meknes, +2.26 in Khouribga, and +2.58 in Safi. High values were also registered in 1997 in El Jadida (+1.73), Kenitra (+1.14), Meknes (+1.01) and Khouribga. Other years were also very wet; among these are 1988 in Khouribga (+1.06) and Safi (+1.33), 1990 in Fes (+1.28) and Meknes (+1.47), 1994 in Khouribga (+1.04), 2003 in Kenitra (+1.47), 2004 in El Jadida (+1.3), Kenitra (+1.10) and Fes (+1.31), and 2006 in Fes (+1.34) and Meknes (+1.09).

Relationship between SPI and yield

The results of the regression analysis relating yield as a dependent variable to SPI-OD and SPI-AJ as independent variables showed that the values of R^2 were very low and the relationships were not significant. The observed variations of yields were explained mainly by the variations in SPI-JM and SPI-OJ.

Table 1. Variation in SPI-OJ in different regions from 1988 to 2008.

Year	Region with access to supplemental irrigation		Northern region under rainfed conditions		Southern region under rainfed conditions	
	El Jadida	Kenitra	Fes	Meknes	Khouribga	Safi
1988	0.71	0.51	0.31	0.66	1.06	1.33
1989	0.09	0.28	0.03	0.31	-0.1	0.56
1990	0.48	0.2	1.28	1.47	0.46	0.71
1991	0.32	0.6	0.91	0.74	0.52	0.35
1992*	**-0.99**	**-1.63**	**-0.54**	**-0.57**	**-1.06**	**-1.38**
1993*	**-1.17**	**-0.98**	**-0.85**	**-0.81**	**-0.7**	**-0.68**
1994	0.23	0.26	-0.21	0.27	1.04	0.18
1995*	**-2.22**	**-1.7**	**-2.46**	**-1.52**	**-0.76**	**-1.63**
1996	2.19	1.89	1.21	1.98	2.26	2.58
1997	1.73	1.14	0.6	1.01	1.75	0.95
1998	-0.28	0.85	0.23	0.56	0.3	-0.15
1999*	**-0.03**	**-0.86**	**-1.55**	**-1.54**	**-0.6**	**-0.82**
2000*	**-0.87**	**-0.72**	**-1.13**	**-0.98**	**-1**	0.02
2001*	**-0.53**	0.45	0.2	**-0.45**	**-0.74**	**-0.81**
2002*	**-0.35**	**-0.34**	**-0.65**	**-0.74**	**-0.21**	**-0.27**
2003	0.86	1.47	0.99	0.94	0.54	0.5
2004	1.3	1.1	1.31	0.38	0.3	0.83
2005*	**-0.95**	**-0.83**	**-0.89**	**-1.15**	**-0.96**	**-1.12**
2006	0.17	0.43	1.34	1.09	0.46	0.59
2007*	**-0.65**	**-1.24**	0.09	**-0.57**	**-1.66**	**-1.09**
2008*	**-0.06**	**-0.87**	**-0.2**	**-1.09**	**-1.16**	**-0.7**

* Years when drought was registered in all regions according to the SPI classification (negative numbers).

Table 2. Coefficients of determination (R^2) showing the relationship between grain yield of bread wheat and SPI at different time scales.

Region	SPI-OD	SPI-JM	SPI-AJ	SPI-OJ
El Jadida	0.05	0.36	0.04	0.48
Kenitra	0.00	0.20	0.00	0.34
Fes	0.00	0.51	0.01	0.62
Meknes	0.02	0.51	0.01	0.50
Khouribga	0.01	0.62	0.00	0.74
Safi	0.00	0.42	0.07	0.52

For bread wheat (Table 2) in the drier areas, the relationships between yield and SPI-JM were positive and significant. The value of R^2 was 0.62 for Khouribga and 0.42 for Safi. For SPI-OJ, the R^2 value was 0.74 for Khouribga and 0.52 for Safi. For Fes the R^2 value was 0.51 for Meknes 0.51, for El Jadida 0.36, and Kenitra 0.20 when the SPI-JM was considered. For SPI-OJ the R^2 value was 0.58 for Fes, 0.50 for Meknes, 0.58 for El Jadida, and 0.28 for Kenitra.

For durum wheat (Table 3), the R^2 value linking the yield and SPI-JM was 0.56 in Khouribga, 0.36 in Safi, 0.55 in Fes, 0.39 in Meknes, 0.30 in El Jadida, and 0.17 in Kenitra. For SPI-OJ, the coefficient of determination was 0.65 in Khouribga, 0.38 in Safi, 0.52 in Fes, 0.36 in Meknes, 0.40 in El Jadida, and 0.23 in Kenitra.

For barley (Table 4), similar results were observed. The R^2 value between yield and SPI-JM was 0.63 for Khouribga, 0.48 for Safi, 0.69 for Fes, 0.38 for Meknes, 0.43 for El Jadida, and 0.28 for Kenitra. In the case of SPI-OJ, it was 0.70 in Khouribga, 0.53 in Safi, 0.62 in Fes, 0.36 in Meknes, 0.45 in Al Jadida, and 0.27 in Kenitra.

Table 3. Coefficients of determination (R^2) showing the relationship between grain yield of durum wheat and SPI at different time scales.

Region	SPI-OD	SPI-JM	SPI-AJ	SPI-OJ
El Jadida	0.04	0.30	0.00	0.34
Kenitra	0.06	0.17	0.00	0.30
Fes	0.01	0.55	0.00	0.55
Meknes	0.00	0.39	0.00	0.39
Khouribga	0.01	0.56	0.00	0.65
Safi	0.01	0.36	0.10	0.44

Table 4. Coefficients of determination (R^2) showing the relationship between grain yield of barley and SPI at different time scales.

Region	SPI-OD	SPI-JM	SPI-AJ	SPI-OJ
El Jadida	0.00	0.43	0.09	0.42
Kenitra	0.00	0.28	0.01	0.28
Fes	0.00	0.69	0.01	0.60
Meknes	0.00	0.38	0.00	0.42
Khouribga	0.01	0.63	0.00	0.69
Safi	0.01	0.48	0.08	0.61

DISCUSSION

The cropping systems of Morocco are dominated by cereals, which are mainly grown under rainfed conditions. These are characterized by frequent droughts that limit production. In fact, data analysis showed that, during the last two decades, the average grain yields of all cereals were very low and the inter-annual variation was very high; productivity varied from 150 to 3000 kg/ha. Chafai et al. (2008) reported a wheat yield range for the whole country of Morocco from 500 to 1500 kg/ha, with a coefficient of variation of 40%, and regional and species differences. In Kenitra, yields tended to be higher than in El Jadida for bread wheat and barley. In respect of durum wheat, the difference between the two regions was not clear. The higher performance of cereals in Kenitra can be explained by the fact that this zone is located further north in the country where rainfall is usually higher. In Meknes, the yields of the three species were, in general, higher than in Fes. In the case of the drier regions, the yields were the highest in Safi for durum wheat and in Khouribga for barley. Khouribga is known for its barley production because of the importance of livestock production in the region and in Safi the farmers practice fallow, which conserves water for the following crop (usually wheat).

The yield gap analysis conducted on wheat in the rainfed areas of WANA (Pala et al., 2011) showed that the differences between the farmers' yields and those that are potentially achievable are high. This yield gap results from the inappropriate crop and land management practices used by most of the farmers, with some differences between wheat and barley. In the case of barley, the low levels of grain yields can be explained by the fact that this crop is usually grown in monoculture in marginal lands (shallow, degraded, saline, sandy soils, etc.) with minimum inputs (fertilizers, herbicides, etc.). These low levels of yields are also a result of growing old varieties or landraces that have low harvest indices. Nonetheless, with their high vegetative biomass production, they are the more preferred genotypes by farmers because they are used as forage for livestock. Wheat, however, receives relatively better management. This crop is usually grown after food legumes or fallow and benefits from the residual soil moisture and nitrogen. Moreover, the farmers, especially in more favorable areas, apply some fertilizers and use certified seeds and herbicides. The higher yield of wheat as compared to barley can be explained also by the higher genetic gain in wheat, as more varieties with better harvest indices have been released and are being used by farmers. Sanchez-Garcia et al. (2013) showed that in Spain from 1930 to 2000, the genetic gain in terms of yield in wheat was 0.88%.

In general, analysis of the data showed high inter-season grain yield variations and the trend followed that of the rainfall pattern. Low yield values were obtained for all species in 1992, 1993, 1995 (except for Al Jadida and Kenitra), 1999, 2000 and 2007, and in Meknes for bread wheat in 1993. Low yields were also registered in 1999 for all species in Fes and Meknes. In the particularly wet years of 1996 and 2006, high productivities were obtained.

In 1996 high yields were obtained everywhere for all crops, except in Fes and Meknes for all species. Also in 2006 high yields were obtained in all regions and for all species except for durum wheat and barley in Khouribga and for barley in Safi.

The yield levels obtained in each region are very much affected by the rainfall gradients that characterize Morocco. In the country, the average rainfall amount decreases from north to south and from west to east. Fes, Meknes, and Kenitra, Located in the northern part of the country, usually receive more rains and tend to produce greater yields. However, in the southern parts, such as Khouribga, El Jadida, and Safi, precipitation is lower, with Safi being the driest, and hence crops yields are less. Nevertheless, yields in Kenitra and El Jadida tended to be relatively high because these regions are located in irrigated areas and some fields may have received supplemental irrigation in dry years. Research conducted on the response of wheat to water applications (ICARDA, 2013) in the rainfed area of the Tadla region of Morocco showed that, on average, supplemental irrigation during spring increased wheat yield by around 20% and saved more than 1000 m^3 of irrigation water as compared to the farmers' flood irrigation technique.

The general feature of drought in Morocco before 1987 was demonstrated by studies of the Direction de la météorologie national (DMN, 1997). These studies showed that the country experienced 10 episodes of major moderate to severe droughts between 1900 and 1987. Moreover, Jlibene (2011) states that drought has become more frequent during the last three decades. It has increased from one dry year to 15 normal ones before 1980s to a frequency of one dry year to each three year period. However, this author does not specify the intensity of the drought. In our case, rainfall data analysis, using the meteorological drought index SPI-OJ, demonstrated that during the last two decades, all the regions studied experienced, on average, droughts with different intensities (SPI varying between -0.5 and -2.5) with a frequency of one year of drought for every 2.6 year period. The frequency of moderate to severe drought (SPI less than -1) as defined by Mckee et al. (1993) was, however, only one year of drought for each seven year period. In all regions, 1992 and 1995 tended to be the drier years. Other years were also very dry in some regions, such as 2000 in Fes and Khouribga and 2007 in Kenitra, Khouribga, and Safi.

Because whenever there is drought in Morocco it tends to be general, it is difficult to cope with this natural catastrophe in rainfed agriculture. In fact, cereal yields under these conditions are generally low and there is no possibility of compensation among regions in terms of production. Moreover, access to supplemental irrigation is very limited. Under these conditions, the common solution adopted by the State to meet the immediate needs of the population is to import commodities to fill the gaps. Unfortunately, relying on imports only is a crisis management approach which is very costly and poses the problem of some dependence on other countries. A potential solution to the low yields in Morocco arising from recurrent drought is the adoption of a risk management approach, such as taking more advantage of good (wet) years and producing more using appropriate agronomic packages. More production, however, means the development of more grains storage facilities.

Early warning of drought occurrence and the prediction of yields early during the cereal cropping season is one of the components of a drought preparedness approach. SPI is an index that can be used for this purpose. Our study showed that there is no correlation between SPIs computed for the stand establishment and grain filling periods of cereals. This result confirms that of El Mourid and Watts (1989) who did not find any link between autumn rainfall and that of spring. The only positive and significant correlations observed in our study are the ones that exist between the SPI-JM and SPI-OJ and between these indices and yield (high R^2). These results contradict the findings of Yacoubi et al. (1998) and Barakat and Handoufe (1998) who showed, in rainfed areas of Morocco, a strong relationship between the rainfall deficit index of October, November, and December and the cereal production deficit of the growing season.

The no correlation between yield and SPI-OD in our study can be explained by the fact that most of the farmers are waiting until it rains enough to be able to prepare a good seedbed and ensure a good germination of seeds and emergence of seedlings. They also usually increase the seed rate to compensate for low stand establishment under deficit soil moisture conditions following planting. The non-significant relationship between yield and SPI-AJ might result from the increase of carbon assimilates translocated from the stems and sheaths to the grains under drought conditions during spring. Reynolds et al. (2005) stated that reserves accumulated in the stem by anthesis may play a crucial role in grain filling under terminal stress conditions when photosynthesis is impaired.

The importance of rainfall or soil moisture conditions in the mid-season growth period can be explained by the fact that the most determinant yield components in cereals, the number of spikes and number of kernels, are formed during the January to March period. Consequently, any water stress during this period has a negative effect on the development and growth of tillers and spikes. Otegui and Slafer (2004) indicated that only a relatively small fraction of the whole growing period of cereals is actually critical to the determination of yield. This corresponds, in general, to the stem elongation period where the number of grains per unit land area is largely determined in response to the growing/partitioning conditions of the crops. The importance of the availability of moisture in the soil during the stem elongation period was confirmed by supplemental irrigation studies. Boutfirass (1990) demonstrated that supplemental irrigation during tillering-stem elongation increases wheat yield. Also Karrou and Boutfirass (2007) found that the availability of the soil moisture required for growth and development during the stem elongation period is a pre-requisite for wheat yield increase and stability in rainfed areas. Because of the relatively high correlation between SPI-JM and SPI-OJ and between SPI-JM and yield, the former index (SPI-JM) can provide an early prediction of the severity of the whole season drought and also the yields.

This index can be considered as a drought preparedness tool that can help the farmers better schedule and manage supplemental irrigation, fertilizers application and weed control, accordingly. The tool can be also used by policy-makers to better plan the allocation of water in agriculture, forage availability for the livestock safeguard, seed production, and wheat imports. Nevertheless, to better detect the development of droughts and monitor their intensity and duration, SPI should be used in coordination with other tools such as remote sensing (NDVI-based indices) to cover vast geographic regions; and this will further improve the timely predictions of drought onset that can trigger appropriate responses by the policymakers (Sarkar, 2011).

From this study, we can conclude that rainfall conditions during the tillering and stem elongation periods of cereal growths are the most important determinants of yield and, hence, the SPI computed for the period of January–March can be used to predict drought severity and yields early in the season.

Conflict of Interest

There are no conflict of interest regarding this publication.

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Production and reproduction performance of rural poultry in lowland and midland agro-ecological zones of central Tigray, Northern Ethiopia

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The study was conducted in lowland and midland agro-ecological zones of central Tigray, in northern Ethiopia with the objective of characterization of village poultry marketing system under rural household management. A total of 160 households and 50% of them were female headed households. Data were collected using semi structured questionnaire and monitoring individual households. Chi-square test was employed for ordinal and nominal data. ANOVA was also employed for continuous data. Average age at first mating of cockerels was 26 and average age at first egg of local pullets was 27.2 weeks. Average egg production per year was 43.4 eggs for local hens, 81.4 eggs for cross breed hens and 144.3 eggs for exotic hens. Average number of eggs set for incubation per broody hen was 10.2±0.23 eggs with hatchability of 82.5 and 88.85% in lowland and midland agro-ecologies, respectively. The average survival rate of chicks was 61.95% in lowland and 69.4% in midland agro-ecology. Average weight of cocks, hens, cockerels and pullets was 1.69, 1.37, 1.024 and 1.02 kg, respectively in lowland and 1.81, 1.356, 1.119 and 1.064 kg, respectively in midland. Average mortality of chickens was 10 per year and it was significantly higher (P<0001) in lowland (12.96) than in midland (7.05). Relatively local chickens in midland agro-ecology have better performance. Egg production, hatchability, survival of chicks and mortality of chickens vary with agro-ecology.

Key words: Mortality, hatchability, predators, disease.

INTRODUCTION

Poultry production is an important sector in Ethiopia where chickens and their products are important sources of food and income. Ethiopian chickens are estimated to be over 56 million, and almost every family in the rural areas of the country practice traditional chicken production system (ILCA, 1993; Solomon, 2003). Poultry production systems in Ethiopia show a clear distinction between traditional, low input systems on the one hand and modern production systems using relatively advanced technology on the other hand (Alemu, 1995).

The chickens in free-range traditional poultry production systems are a function of natural selection which are mainly local or indigenous breeds. As a result the performance of chickens under rural conditions

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remain generally poor as evidenced by highly pronounced broodiness, slow growth rates, small body size and low production of meat and eggs (Kitalyi, 1998; Sonaiya, 2000; Gausi et al., 2004).

Even with its challenges, traditional poultry production, which is still important in low-income food-deficit countries, is an appropriate system to supply the fast-growing human population with high quality protein (Tadelle et al., 2003a). Moreover, indigenous chickens are known for their merits such as broodiness behavior with high fertility and hatchability, disease resistance thermo tolerant, good egg and meat flavor, hard eggshells, productivity at zero or minimal feed supplementation and high dressing percentage (Abera, 2000) that matches with the poor family poultry production systems. However, the indigenous chickens have been neglected in areas of scientific research on identifying distinct line breeds and its characterization, production performance, potential improvement and system development efforts.

Objective

1. To assess flock composition and flock dynamics of rural poultry production in male and female headed households in the lowland and midland agro-ecological zones in central Tigray.
2. To explore the production performance of rural chickens
3. To identify the major constraints and opportunities of rural poultry production in male and female headed households in the lowland and midland agro-ecological zones in central Tigray.

MATERIALS AND METHODS

The study was conducted in central Tigray, Northern Ethiopia which is located between 13°15' and 14°39' North latitude, and between 38° 34' and 39°25' East longitude. Two sample districts, namely Adwa and Merebleke, were selected using systematic random sampling method. The study area (central zone of Tigray) was stratified into two agro-ecologies as midland and lowland based on their altitude and as customarily used by the local administration and bureau of agriculture. A total of 160 sample farmers, 80 from each district, 40 male and 40 female headed households were selected randomly using lottery method from those households reared at least one chicken in the year. Data like production and reproduction performance, hatchability, poultry loss and survival rate of chickens were collected using repeated farm recording methods and pre-tested formal semi-structured questionnaire. In addition four focus group discussions with an average group size of 16 individuals were conducted with key-informants (model farmers, elders, women association leaders, experts from Agriculture and Rural Development and Relief Society of Tigray office, administrative bodies, youths and extension workers) in both agro-ecological zones. Tape recorder was used to record the forwarded ideas during the group discussion. Statistical analysis were made using JMP5 (SAS, 2002). Descriptive statistics such as mean, range and percentile were used. Chi- square test was employed for ordinal and nominal data such as egg production, chicken loss and hatchability. ANOVA was also employed for continuous data type like body weight and sexual maturity.

RESULTS AND DISCUSSION

Sexual maturity

Average age at first mating of cockerels was 26 weeks for local, 24.9 weeks for cross and 25.2 weeks for exotic breeds and there was no significant difference between lowland and midland agro-ecology (Table 1). A bit faster age of sexual maturity of cockerels (24.6 weeks) was reported by Fisseha et al. (2010) in North West Ethiopia, similarly Halima et al. (2007), reported that Pullets and cocks reached sexual maturity at an age ranging from 20 to 24 weeks Western Gojam. Kugonza et al. (2008) also reported that sexual maturity of male Chickens in Eastern Uganda was 5.5 months (22 weeks).

Average age at first egg was 27.2 weeks for local breeds ranged from 24 to 28 weeks, 25.7 for cross breeds ranged from 24 to 27 weeks and 25.4 for exotic breads ranged from 24 to 27 weeks. There was significant difference ($P<0.05$) on sexual maturity of both exotic, cross and local pullets between lowland and midland agroecology. Maturity of chickens was late in lowland than in midland agroecology. This might be attributed to the management practice like feeding, housing and health care of the farmers. Relatively better feeding and housing management was observed in midland agro-ecology. Sexual maturity of chickens always depends on chicken management and overall production systems of the households mainly on feeding and disease management practices.

This result was similar with 6.8 months reported by Tadelle et al. (2003b) and 6.5 months (26 weeks) reported by Kugonza et al. (2008) in Eastern Uganda but somewhat longer than the reported 5.9 months by Bogale (2008) in Fogera woreda and 168 days (24 weeks) by Benabdeljelil et al. (2001) in Morocco.

About 64.4% of the respondents had their own breeding cock and 71.1% of which were local breeds, 18.3% cross and 10.6% were exotic breeds (Rhode Island Red). Regarding source of cocks, 58.3% home grown, 31% purchased from market or neighboring farmers and the rest 10.7% received from GOs and NGOs (Table 2).

Egg production

With the same management practices of the households average number of eggs laid per hen per clutch was 13.6 for local hens ranged from 9 to 18 eggs, 25.7 for cross breed hens ranged from 15 to 35 eggs and 44.4 for exotic breeds ranged from 30 to 65 eggs (Table 3). Egg production of exotic breed and cross breed chickens was

Table 1. Sexual maturity of chickens in male and female headed households in lowland and midland agroecology of central Tigray.

Variables	Lowland		Midland		P value
	MHH	FHH	MHH	FHH	
Age at first mating in weeks (Mean±SE)					
Local	26±0.17[a]	25.8±0.18[a]	25.8±0.18[a]	26.2±0.17[a]	0.3175
Cross	24.8±0.21[ab]	25.3±0.21[a]	24.5±0.21[b]	24.7±0.21[b]	0.0548
Exotic (RIR)	25.8±0.32[a]	25±0.32[ab]	24.8±0.32[b]	25.2±0.32[ab]	0.1599
Age at first egg in weeks (Mean±SE)					
Local	27.4±0.11[a]	27.5±0.13[a]	26.8±0.13[b]	27±0.1[b]	0.0001
Cross	25.5±0.18[b]	26.1±0.18[a]	25.5±0.18[b]	25.7±0.18[ab]	0.0305
Exotic (RIR)	25.7±0.24[a]	25.9±0.24[a]	24.9±0.24[b]	25.3±0.24[ab]	0.0261

Least square means with different superscripts within the row are significantly different (P<0.05); MHH = Male headed households; FHH = Female headed households.

Table 2. Ownership of breeding cocks in male and female headed households in lowland and midland agroecological zones of central Tigray.

Variables	Lowland		Midland		X^2 value	P value
	MHH (%)	FHH (%)	MHH (%)	FHH (%)		
Owner ship of breeding cock						
Yes	57.5	67.5	60	72.5	2.5	0.4745
No	42.5	32.5	40	27.5		
Source of cocks						
Home grown	73.9	63	50	48.3		
Market purchase	13.05	22.2	41.7	44.8	8.56	0.1997
Received from GOs or NGOs	13.05	14.8	8.3	6.9		
Breed of cocks						
Local	69.6	64.3	70.8	79.3		
Cross	17.4	21.4	20.8	13.8	2.26	0.8938
Exotic (RIR)	13	14.3	8.4	6.9		

MHH = male headed households; FHH = Female headed households; n = number.

significantly higher (P<0.01) in midland than lowland. This difference could be due to the less resistance of these chickens to high temperature (40°C) in lowland which may affect their productivity. In addition the management level of the farmers may create difference in the production potential of the chickens, for example the management level and egg production of the households were positively correlated (r=0.53; n=160). This indicates that the low production and productivity of the chickens in the area is attributed to the poor management practice of the farmers. Similarly, Mwalusanya et al. (2004) reported that, the low productivity of chickens in Tanzania was partly due to the prevailing poor management practices, in particular the lack of proper health care, poor nutrition and housing.

The average number of clutches per year per hen was 3.2 for local hens ranged from 2 to 5 with an average clutch length of 21.6 days ranged from 15 to 28 days, 3.1 for cross breed hens ranged from 2 to 4 with an average clutch length of 31.6 days ranged from 18 to 40 days and 3.2 for exotic breeds with average clutch length 44.4 days.

Relatively small number of clutch per year (2 to 3) but longer clutch size (69 days) was reported by Kugonza et al. (2008) in Eastern Uganda. In addition 4 cycles of broodiness were recorded per year in hens with an average duration of 12 to 15 days per clutch in Kashmir (Iqbal and Pampori, 2008).

Clutch length in cross breed hens was significantly longer (P<0.001) in midland (34.6 days) than lowland agro-ecology (28.7 days). This result might be attributed to the difference in management practice of the farmers living in lowland and midland agro-ecology. As explained by the key informants in the group discussion, clutch

Table 3. Egg production performance of chickens male and female headed households in lowland and midland agroecological zones of central Tigray.

Variables	Lowland		Midland		P value
	MHH	FHH	MHH	FHH	
Average clutch number/year (Mean±SE)					
Local	3.2±0.06^{a}	3.15±0.07^{a}	3.2±0.07^{a}	3.2±0.06^{a}	0.9123
Cross	3.2±0.18^{ab}	2.7±0.18^{b}	3.1±0.18^{ab}	3.3±0.18^{a}	0.1478
Exotic (RIR)	3±0.11^{b}	3.2±0.11^{ab}	3.3±0.11^{a}	3.26±0.11^{ab}	0.1920
Clutch length in days (Mean+SE)					
Local	21.1±.35^{b}	22.3±0.39^{a}	21.7±0.4^{ab}	21.5±0.33^{ab}	0.1621
Cross	28.5±1.1^{b}	28.9±1.1^{b}	34.7±1.1^{a}	34.5±1.1^{a}	<0.0001
Exotic	43.2±1.4^{a}	42.7±1.4^{a}	44.9±1.4^{a}	46.6±1.4^{a}	0.2220
Egg production/clutch/hen (Mean±SE)					
Local	13.4±0.25^{ab}	14.1±0.28^{a}	13.7±0.28^{ab}	13.3±0.24^{b}	0.1361
Cross	22.4±1.1^{b}	24.6±1.1^{b}	31.5±1.1^{a}	31.2±1.1^{a}	<0.0001
Exotic (RIR)	40.3±1.4^{bc}	36.7±1.4^{c}	44.0±1.4^{ab}	46.3±1.4^{a}	<0.0001
Average egg production/year/hen (Mean±SE)					
Local	43±1.2^{a}	44.3±1.3^{a}	43.7±1.3^{a}	42.7±1.1^{a}	0.8254
Cross	71.7±4.4^{b}	65.3±4.4^{b}	96.3±4.4^{a}	100.8±4.4^{a}	<0.0001
Exotic (RIR)	120±5.1^{b}	117.2±5.1^{b}	146±5.1^{a}	150.3±5.1^{a}	<0.0001

Least square means with different superscripts within the row are significantly different (P<0.05); MHH = Male headed households; FHH = Female headed households.

number and clutch length of exotic breed hens were hardly identified by the farmers because, it was very difficult for the farmers to know whether the interruption of egg production is due to nature of the hen or shortage of feed because exotic breeds are sensitive to feed shortage. Average egg production per year per hen was 43.4 eggs for local hens, 81.4 eggs for cross breed hens and 144.3 eggs for exotic hens.

Egg production of exotic breed and cross breed chickens was significantly (P<0.01) higher in midland than in lowland. This could be due to the management level of the farmers and the high temperature in lowland by itself might have a negative effect on the production performance of the exotic hens. In line with this a study conducted at the College of Agriculture, Alemaya, has indicated that the average annual egg production of a native chicken was 40 eggs under farmer's management (Tadelle et al., 2000) but higher egg production, 54.3 eggs/year/hen was reported by Abraham and Yayneshet (2010) for local hens and 185 eggs for exotic (Rhode Island Red) breeds similarly large number of eggs (78 eggs/hen/year) was reported by Benabdeljelil et al. (2001) for local hens in Morocco. From the result of this study, we can conclude that exotic and cross breed chickens can produce large number of eggs than local breeds mainly in midland agro-ecology in the presence of adequate amount of feed.

Hatchability and survival rate of chicks

In both agroecologies the average number of eggs set for incubation per broody hen was 10.2 eggs with hatchability of 85.8% for local eggs and 78.97% for cross breed eggs (Table 4). The hatchability of local and cross breed eggs was 82.5 and 72.5% in lowland areas and 88.9 and 85.5% in midland areas. This might be attributed to the high temperature in lowland that may affect the quality of the eggs and in addition broody hens would be restless during high temperature. This is in line with the reported 82.6% hatchability for local eggs in Bure wereda (Fisseha et al., 2010), 89.1% in Southern Ethiopia (Mekonnen, 2007) in addition, 90% of egg hatchability in Eastern Uganda (Kugonza et al., 2008), and 83.6% hatchability in Tanzania was reported by Mwalusanya et al. (2004) but higher than the reported 70.5% hatching rate (Tadelle et al., 2003b) and 78.6% hatchability of local eggs reported by Abraham and Yayneshet (2010) for Northern Ethiopia, 61.8% hatchability in Botswana (Aganga et al., 2000) and the hatchability ranged 77 to 81% in Kashmir (Iqbal and Pampori, 2008). This variation might be due to the

Table 4. Hatchability and survival of chicks in male and female headed households in lowland and midland agroecological zones of central Tigray.

Variables	Lowland		Midland		X^2 value	P value
	MHH (%)	FHH (%)	MHH (%)	FHH (%)		
Average eggs set for incubation (Mean±SE)						
Local	10.2±0.21[a]	10.3±0.24[a]	10.2±0.24[a]	10.2±0.2[a]		0.9706
Cross	8.1±0.29[ab]	7.3±0.29[b]	8.9±0.29[a]	8.7±0.29[a]		0.0017
Hatchability						
Local	82.1	82.96	88.3	89.4	37.74	<0.0001
Cross	72.8	72.1	86.7	84.2	21.06	0.0002
Survival of chicks to 8 weeks of age						
Local	62.5	61.4	70.2	68.6	8.39	0.0172
Cross	60.7	55.4	69.6	69.2	10.06	0.0071

Least square means with different superscripts within the row are significantly different (P<0.05); MHH = Male headed households; FHH = Female headed households.

difference in management practices of the poultry producers in the different climatic zones. Chicks reached grower stage 8 weeks (survival rate) were 65.8 and 63.7% for local and cross breed chicks, respectively. There was significant difference (P<0.05) in survival of local and cross breed chicks between lowland and midland agro-ecology. This could be due to the difference in disease prevalence rate and management practice of the farmers in the area. This is lower than the reported 75% of the chicks survived the brooding period in Sudan (Khalafalla et al., 2001), but higher than the reported 60.5% of birds reached grower stage in Bure wereda (Fisseha et al., 2010), 51.3% average survival rate of chicks in Ethiopia (Tadelle et al., 2003b) and about 44.2 % mortality of chicks (55.8 % survived) reported by Abraham and Yayneshet (2010) for Northern Ethiopia. In addition, the overall mean chick survival rate to 10 weeks of age in Tanzania was 59.7% (Mwalusanya et al., 2004).

Body weight of indigenous chickens

The average weight of mature males (cocks) was significantly higher (P<0.05) in midland (1.812) kg than in lowland (1.694) agro-ecology (Table 5). But similar body weight of hens (1.37 kg and 1.356 kg), cockerels (1.024 kg and 1.119 kg) and pullets (1.021 kg and 1.064 kg) was recorded in lowland and midland agroecology, respectively. The substantial differences in body weight observed for the different classes could be attributed to non genetic factors like supplementary feeding, watering and health care. The average weight of mature males (cocks) in this study is higher than the average weight (1.5 kg) of the indigenous chicken of the Central Highlands of Ethiopia (Alemu and Tadelle, 1997) and lower than the mean weight (2049.07 g) of indigenous chicken in Northwest Ethiopia (Halima et al., 2007). Moreover the reported mean weight of mature male (1.6 kg) and female (1.3 kg) chicken in Southern Ethiopia was lower than this result but the mean weight of grower male (1.05 kg) and female (1.04 kg) chicken in that area (Mekonnen, 2007) was similar to this result.

Poultry loss

In fact feed shortage, accidents and theft could play a considerable role in poultry loss but high chicken mortality has always occurred at time of disease outbreak and predators in both agroecological zones as mentioned by the key informants in group discussion. According to the interviewed farmers in the study area hatchability was high but eventually they left with two or three birds reached matured age. When farmers were asked to rank the major causes of high mortality in their locality 90% of male and 77.5% of female headed households in lowland and 72.5% of male and 57.5% of female headed households in midland agroecology ranked disease as first major cause of chicken loss whereas the rest 10% of male and 22.5% of female headed households in lowland and 27.5% of male and 42.5% of female headed households ranked predators as first cause of chicken loss (Table 6). Disease followed by predators as major causes of chicken loss in the study area is in agreement with that reported by Halima et al. (2007) in North West Ethiopia and Abdelqader et al. (2007) in Jordan. Similarly in Morocco as reported by Benabdeljelil et al. (2001) high mortality was recorded as a result of diseases and predators (mortality rates reached up to 77%) and in Uganda predators and diseases were responsible for the

Table 5. Body weight of indigenous chickens in lowland and midland agroecology.

Body weight in (kg)	Lowland (Mean±SE)	Midland (Mean±SE)	P value
Grower male (cockerel)	1.024±0.03[a]	1.119±0.03[a]	0.0511
Grower female (pullet)	1.021±0.03[a]	1.064±0.03[a]	0.3441
Mature male (cock)	1.694±0.03[b]	1.812±0.03[a]	0.0167
Mature female (hen)	1.370 ±0.04[a]	1.356±0.04[a]	0.8220

Least square means with different superscripts within the row are significantly different (P<0.05).

Table 6. Chicken mortality in lowland and midland agroecological zones of central Tigray.

Chicken age class and breed	Lowland		Midland		X^2 value	P value
	MHH (%)	FHH (%)	MHH (%)	FHH (%)		
Local breed chickens mortality/year	8	10.5	5.2	5.5	49.47	<0.0001
Cross breed chickens mortality/year	6.6	8.9	5.2	5.9	8.19	0.0422
Exotic breed chickens mortality/year	6.7	9.2	5.3	6.8	16.14	0.0011
Overall chicken mortality/year	11	14.95	6.8	7.4	66.16	<0.0001
Cause of mortality						
Disease	90	77.5	72.5	57.5	11.85	0.0079
Predators	10	22.5	27.5	42.5		

MHH = male headed households; FHH = female headed households; n = number.

high mortality of chicks (Illango et al., 2002.). Muchadeyi et al. (2004) also reported that Predation and diseases were said to be the major causes of mortality in Rushinga District of Zimbabwe.

Average annual mortality of chickens was 10. Mortality was significantly high (P<0001) in lowland (12.96) than in midland (7.05). This could be attributed to the high prevalence of disease in lowland areas and poor management practices of the farmers mainly their housing system which was easy for predators attack. There was also significant difference (P<0.05) between male headed and female headed households in chicken mortality. This of might be attributed to the difference in management system like housing, feed supplementation and cleaning rate chicken house. The study also revealed that average annual mortality of chicks was 3.98 for local, 3.7 for cross breed and 3.2 for exotic breed chicks (Table 6). Mortality of exotic breed chicks was relatively lower than the local and cross breeds. This could be attributed to the vaccination given to the chicks before the time of distribution which may help them in acquiring resistance against prevalent disease in the area hence, exotic breed chicks were hatched in hatchery machine and distributed to beneficiaries after 5 or 7 days old by governmental or none governmental organizations. Average mortality of growers (birds with 2 to 6 months of age) was 1.97 per year for local birds, 2.3 for cross breeds and 2.2 for exotic breeds.

Annual matured chicken (birds > 6 months) mortality was 1.3 for local birds, 1.2 for cross breed birds and 2.1 for exotic breed chicken. In general average mortality of local, cross and exotic breed chickens was 9.25, 7.8 and 7.8 chickens/year in lowland and 5.3, 5.5 and 6.1 chickens/year in midland agroecology, respectively. According to the interviewed farmers high mortality always occurred at the end of dry season mainly from March to June. High temperature and moisture in this season may create a favorable condition to bacterial and/or viral disease outbreak resulted in high chicken mortality. Similarly Swatson et al. (2001) reported that poor protection from adverse climatic conditions (very hot and cold weather) increased the severity of disease outbreaks resulting in losses of up to 70% of the flock at 12 weeks of age.

Predators

Predators were the major causes of year round losses of chickens in both agroecological zones. About 42.5% of female headed households in midland agro-ecology indicated that highest loss of chicken was from predation (Table 7). The most common predators mentioned by the farmers were Wild cat, Hawk, Genet, Snake and fox in their order of importance. Although all those predators were mentioned by the farmers as main causes of chicken loss, their order of importance varies with season and agro-ecology. For example hawks were the problem

Table 7. Rank of predators in order of their importance in lowland and midland agroecology.

Predators	Rank	Lowland		Midland		Overall
		MHH (%)	FHH (%)	MHH (%)	FHH (%)	
Wild cat	1st	27.5	17.5	52.5	60	30
	2nd	22.5	30	32.5	22.5	27.8
	3rd	30	37.5	10	7.5	27.8
Hawk	1st	17.5	20	30	22.5	21.6
	2nd	45	50	52.5	40	48.8
	3rd	27.5	22.5	15	7.5	22.5
Genet	1st	47.5	52.5	5	10	38.7
	2nd	17.5	17.5	5	27.5	14.3
	3rd	17.5	10	40	55	20.3
Fox	1st	0	0	12.5	7.5	3.1
	2nd	0	0	10	7.5	2.5
	3rd	0	0	22.5	12.5	5.6
Snake	1st	7.5	10	0	0	6.6
	2nd	15	2.5	0	2.5	6.6
	3rd	25	30	12.5	17.5	23.8

MHH = male headed households; FHH = female headed households; n = number.

of households living in open and more plane areas both in lowland and midland agro-ecology mainly in dry season and were mentioned as important predator by 21.6% while wild cat and genet were more prevalence predators caused high loss of chickens at the end of rainy season, mentioned by 22.5 and 50% of the households in lowland and 56.25 and 7.5% of the households in midland agroecology, respectively. Snakes were common predators in lowland areas whereas foxes were a problem of those households living on hillside and nearby to enclosure areas in midland agro-ecology. According to the respondents hawks attack chicks in the dry season but other predators attack all age class of the chickens mainly during rainy season because the predators can hide themselves around the backyard in the bush or shrub. In line with this, losses of chickens in Nigeria were attributed mainly to predators (Sonaiya, 2006). Similarly in morocco causes of mortality in poultry other than diseases were predators and accidents (Faouzi et al., 2002).

When farmers were allowed to prioritize the more affected breed type by predators, 45 and 40% of the households in low land and midland agro-ecology respectively said that all breeds were affected equally whereas 55 and 60% of the households in lowland and in midland agro-ecology respectively mentioned exotic breed as more attacked or sensitive group. According to these farmers unlike local chickens exotic breeds are not fast and active to escape away from predator's attack. Farmers in the study area always tried to prevent their chickens from predator attack using different mechanisms like killing the predator using foxhound, but also constructing houses and keeping the chickens in house could be a solution to reduce chicken loss due to predators.

Diseases

Seasonal and recurrent disease outbreak was the major cause of poultry loss in both agroecological zones of the study area. The study revealed that 87.5% of the households in lowland and 68.75% in midland experienced with chicken disease in the past one year (2011 - 2012). According to the animal health experts (veterinarians) in Agriculture and Rural Development office of Adwa woreda and Mereb-leke district, even though many bacterial and viral diseases like Salmilosis, Fowl typhoid and fowl pox were important diseases in the area, ND was the most devastating disease and considered to be a major constraint to the development of poultry production in the area. Similarly Halima et al. (2007) reported that, the major causes of death for local chicken ecotypes in North-West Amhara were seasonal outbreaks of chicken diseases, specifically Newcastle disease. Newcastle disease (ND), called *Fengil* in Ethiopia, is reported to be the most important cause of economic loss in poultry production (e-Newsletter, 2006).

Table 8. Occurrence of disease, season of high disease prevalence and more affected breed and age group of chickens in lowland and midland agroecology.

Variable	Lowland		Midland		X^2 value	P value
	MHH (%)	FHH (%)	MHH (%)	FHH (%)		
Occurrence of disease in 2011-2012						
Yes	82.5	92.5	65	72.5	10.845	0.0126
No	17.5	7.5	35	27.5		
Season of high disease prevalence						
March - April	12.5	20	25	27.5		
May - June	80	75	65	60	5.55	0.4753
July - August	7.5	5	10	12.5		
More affected age group by disease						
Chicks < 2 months age	35	40	27.5	32.5		
Lying and incubating hen	45	37.5	30	37.5	6.27	0.3938
All age group	20	22.5	42.5	30		
More affected chicken breeds						
Local breed	0	0	0	0		
Exotic breeds	80	87.5	67.5	75		0.1714
All breeds	20	12.5	32.5	25	5.00	

MHH = male headed households; FHH = female headed households; n = number.

Swatson et al. (2001) also reported that about 75% of respondents in South Africa indicated that Newcastle is the major disease that wipes out 85% of their flocks. Most farmers living in lowland areas of the study area do not give any name for the disease affecting their chickens, they simply called ‘Disease’ (locally called *Himam*) but they can easily identify the symptoms of the disease. In midland agro-ecology, however, farmers locally called *kudm* for Newcastle disease and expressed the symptoms in different ways. For example gastrointestinal disorders like diarrhea with greenish, yellowish and blood stained excreta, nasal discharges, twisted neck, dropping of wings, inability to drink and eat properly (*jine malet*) and sudden death were some of the symptoms mentioned by the farmers. They have also indicated that, the disease mostly occurs at the end of dry season and beginning of rainy seasons particularly from March to June.

According to the animal health experts in both agroecologies these symptoms are referring to Newcastle disease. The interviewed chicken owners revealed that the disease affected all chicken breeds and age groups. About 37.5% of the households in lowland and 30% in midland indicated chicks (< 2 months) as more affected age groups, 41.2% of the households in lowland and 33.75% in midland reported matured lying and incubating hens as more sensitive chickens to disease while the rest 21.2% of the households in lowland and 36.2% in midland mentioned all age group as equally affected chickens by disease (Table 8).

Regarding to breed groups, 83.75 and 71.2% of the households in lowland and midland agroecology, respectively indicated that exotic chicken breeds were more sensitive and easily affected by disease.

According to the veterinarian in Adwa and Mereb-leke districts, except for exotic breeds that were immunized against Newcastle disease before distribution, there was no regular schedule of vaccination service for local chickens but at time of wide spread disease outbreak, ring vaccination method could be practiced to control the outbreak.

Conclusion

Despite the management problems involved in rearing poultry, relatively promising performance of the local chickens in midland agro-ecology was observed which is explained in terms of high hatchability, survival and resistance to disease and feed shortage. Egg production, hatchability, survival of chicks and mortality of chickens vary with agro-ecology. The exotic breed chickens are appreciated for their more egg production but sensitive to disease, predators and feed shortage. High hatchability percentage and low survival rate of chicks were two antagonistic features of poultry production in the area. Average body weight of matured and grower chickens in both agro-ecological zones of the study area was small compared to the weight of chickens kept in intensive production system.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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Full Length Research Paper

Improving the fertilizer use efficiency and profitability of small farms by intercropping with transgenic Bt cotton

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Bt transgenic hybrid cotton productivity and profitability could not be sustained after 2007 in India which necessitated to investigate into the nutrient needs of transgenic Bt hybrid cotton and their intercropping systems. Seven Bt transgenic hybrid cotton intercropping systems with four fertilizer management practices and two plant spacings were evaluated in split plot design during 2010-12 in *Vertisols* at Central institute for Cotton Research, Nagpur, India. Present recommendation of Pigeon pea *refugea* as stripcropping (2:8) with transgenic Bt hybrid cotton reduced seed cotton yield by 69%, therefore, not accepted by 82% farmers. *Tagetus sp*. or *Dolichus lab lab* were advocated as better *refugea* with economic advantage of US $ 600 ha-1 with C: B ratio of 1:2.8. However, a net a profitability of US $768 and 662 ha-1 with a C:B ratio of 1:3.7 and 1:3 were achieved in intercropping with roselle or soybean compared to US $ 348 C:B ratio of 1:2.5 only with transgenic Bt hybrid cotton alone. This study also breaks the myth of 30% extra fertilizer dose for transgenic Bt hybrid cotton, which requires 90:20:37 N:P:K kgha-1 only and soybean intercropping requires additional 14:13:0 N:P:K kg ha-1. Any deficiencies arising can be economically corrected with two prophylactic foliar sprays of NMgB after square initiation. NPK uptake, nutrient and fertilizer use efficiencies were significantly improved in transgenic Bt hybrid cotton and intercropping with soybean and roselle (except PUE). Hand weeding cost was minimum in soybean and maximum in roselle with Pendimethalin PPI rotated with Pyrithiobac Na EPE.

Key words: *Transgenic* Bt hybrid cotton, fertilizer use efficiency, intercropping, small farms, soybean.

INTRODUCTION

Small and marginal (1.6 ha) rainfed cotton farmers (58%) invested US $ 80 ha^1 on transgenic cotton seeds with double fertilizer dose and improved the productivity, which could not be sustained after 2008 necessitated this study to investigate into the nutrient needs of transgenic Bt hybrid cotton and intercropping systems for better

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profitability. Seedling droughts followed by excess rains during 2008 to 2014 reduced transgenic Bt cotton production, productivity and total factor productivity and profitability despite of increased fertilizer consumption (Anonymous, 2013; CCI, 2013; FAI, 2008, 2012; Raju and Thakare, 2013, Reddy et al., 2012). Transgenic Bt hybrid cotton cultivation is the livelihood for 4.0 million small and marginal farmers who grow it on 6.5 million ha area, with a cash input costs of US $ 235 ha^{-1} gives a net profitability of US $ 612 ha^{-1} with a C:B ratio 1:3.6. Transgenic Bt hybrid cotton is annually rotated with soybean-chickpea, gives a profitability of US $ 893 ha^{-1} with C:B ratio of 1:4.8. Heavy and extended monsoon rains causes severe weed infestation in the absence of pre and post emergence herbicide use causing serious crop losses. Although, sequential chickpea crop could recover some crop losses with this excess rains, abuse of tractors for seed bed preparation and planting is compacting the soils with restricted root growth in rotational transgenic Bt hybrid cotton (Venugopalan, 2009). Soybean-chickpea production and productivities are not as per national demand and there is limited scope for increasing area except intercropping. The income generated by the present rainfed transgenic Bt cotton based sequential cropping systems is insufficient to get a fairly good standard of living for Indian rainfed farmers who requires atleast US $ 1000 ha^{-1} which is possible only through integrated farming systems (IFS) mode. Diversified intercropping systems (Asewar et al., 2008; Seran and Brintha, 2011; Raju and Thakare, 2013) along with boundary cultivation with arid horticulture/ forest tree species such as teak/red sandal, silvi-pastoral systems dairy and small ruminants could avert this risk to some extent, improve profitability and sustainability of transgenic Bt hybrid cotton farming by preventing leaching, runoff, soil erosion losses and better interception of rainfall. Transgenic Bt hybrid cotton based inter cropping systems were identified across the country during 2007, 2008, 2009 seasons under ICAR funded Technology Mission on Cotton (TMC) Mini Mission-I programme (Raju and Thakare, 2013). Transgenic Bt hybrid cotton + marigold intercropping system produced 0.4 t ha^{-1} more seed cotton yield than pigeon pea /non transgenic Bt cotton as *refugea* (Yenagi et al., 2011). Transgenic Bt hybrid cotton requires 100% RDF only with 29% improvement in cotton equivalent yield (CEY) in *Vertisols* (Rekha and Dhurua, 2010). Transgenic Bt hybrid cotton + soybean had maximum predators (Mote et al., 2001). Intercropped maize reduced pink boll worm damage in cotton (Kavitha et al., 2003; Seran and Brintha, 2010). Indian cotton consumes 3.5% of the total fertilizers with Fertilizer Use Efficiency (FUE) of 9.6 kg^{-1} seed cotton kg^{-1} fertilizers applied. Transgenic Bt hybrid cotton in central India is advocated to fertilize with 90:24:37 NPK kg ha^{-1} and micronutrients mixtures as soil and foliar application with a productivity of 0.5 Mg lint ha^{-1} and FUE of 7.0 kg^{-1} seed cotton kg^{-1} fertilizers applied.

Transgenic Bt hybrid cotton sample surveys found fertilizer application rate was doubled with the introduction of transgenic Bt cotton, which was clearly reflected in all India fertilizer consumption pattern (FAI, 2008, 2012; Reddy et al., 2012). However, after 2008-2012 there is a decline in factor productivity despite of increased fertilizer consumption necessitated to find out any secondary and micronutrient deficiencies arising. Soybean-transgenic Bt hybrid cotton is grown on residual fertility in central India with a productivity of 1.5 and 2.0 Mg ha^{-1} without and with fertilizers respectively. Excess fertilizers to cotton fields under saturated conditions lose 18-24 kg N ha^{-1} beyond root zone, which can be trapped, if intercropped. Sub optimal soil fertilization with foliar application of urea improved 16 to 29% seed cotton yields, however, less cost-effective than carefully planned, demand driven, timely soil-applied fertilizers (Wiedenfield et al., 2009). Calcareous soils and droughts reduce the soil availability of Zn and B. Foliar applied B at 0.2 kg ha^{-1} boric acid and Na tetra Borate, increased B concentration 8 to 11 mg kg^{-l} and 16 to 22 mg kg^{-l} respectively in cotton and soybean. Looking into increased fertilizer application/ prices in rainfed transgenic Bt hybrid cotton in India without improving productivity necessitated comprehensive validation of nutrient requirements and recommendations for enhanced FUE and profitability.

MATERIALS AND METHODS

Soil and climate of the experimental site

Experimental site was mild sloppy, medium deep *Vertisol*, Nagpur, India (21°09'N 79°09'E, altitude 331 MSL). Nagpur is located at the centre of Indian peninsula, has a tropical wet and dry climate (Köppen climate classification Aw) with dry conditions prevailing for most of the year. It receives on an average 852 mm rainfall in 48 rainy days during June to October months. However, fluctuations in the onset of monsoon rains resulted in seedling droughts of 14-29 days were most common change in the climatic features of this area. Soil analysis of experimental site observed, soil depth as 1.2 m, soil textural class clay loam, pH 8.1, organic carbon 0.45%, available N:P:K 280:6.5:249 kg ha^{-1}. DTPA extractable Zn 0.7 ppm, Mn 2.54 ppm and B 0.1 ppm. Transgenic Bt hybrid cotton is planted in 45 and 52% on shallow and medium deep *Vertisols* in central India. Farmers plant 80% cotton area as dry sowing with pre monsoon showers between 25-29th June without any pre emergence herbicides. Post emergence herbicides are being accepted by 35% of the cotton farmers during excess rains, where controlling weeds by conventional systems will be very difficult in the absence of timely intercultures and hand weedings.

Seasonal rainfall during 2010, 2011 were 1032 and 832 mm both the years received excess rains in July, August September months. Seedling droughts followed by excess rains in few rainy days became common feature of climate change with no rains June 20-30th during 2010 year adversely affected germination, but during 2011 year received no effective rains upto 24th June-14th.July and 24th July to 4th, August seedling drought received only 54.3% rains at the end of the month without any rain during October month.

A field experiment was conducted in *Vertisols* with six transgenic Bt hybrid cotton based intercropping systems in main plots with four nutrient management modules in sub plots at two transgenic Bt hybrid cotton spacings 90 x 45/ 30 cm as sub sub plots in split plot design with four replications during 2010, 2011 monsoon seasons. Cropping system were: C_1: Paired row *transgenic Bt* hybrid cotton with Cry 1Ac' MRC 6301' (PR 90/135 cm); C_2: *Transgenic* Bt hybrid cotton + pigeon pea *Maruti* (90 x 45 cm) with 4:4.4:0, C_3: C_1 + soybean 'JS-335' (45 x 10 cm) with 14:13:0, C_4: C_1+ shrub field bean 'Ankur Goldy'(45 x 10 cm) with 30:11:10, C_5: C_1 + marigold 'African tall'(45 x 22.5) with 80:35:67, C_6: C_1 + maize '*Komal*'(45 x 22.5 cm) with 48:11:10, C_7: C_1+ roselle 'local' (45 x 10 cm) with 20:9:7; C_8: C_1+ castor 'TAU-9 (45 x 22.5cm) with 24:7:0 kg ha^{-1} N: P: K respectively. Transgenic Bt hybrid cotton received a fertilizer dose of F_1: 90:20:37 + 2% Urea + 0.2% Mg + 0.06% B foliar spray twice at squaring to flowering stage; F_2: 90:20:37+5 kg Mg+2 kg B ha^{-1} as soil application at the time of sowing; F_3: 113:24:47 + 2% Urea +0.2% Mg + 0.06% B foliar spray twice at squaring to flowering stage; F_4: 113:24:47+5 kg Mg + 2 kg B ha^{-1} as soil application at the time of sowing. N fertilizers were applied in 3 equal splits at 20, 45, 65 days after sowing (DAS). Foliar correction of nutrients were made 75 and 90 DAS. Rotation of herbicides Pendimethalin 1.0 kg PPI and Pyrithiobac Na 0.035 kg a.i. ha^{-1} early post emergence applications were made respectively in 2010 and 2011 years along with two intercultures at 21 and 42 DAS followed by one hand weeding to remove uncontrolled/ resistant weeds.

METHODOLOGY

Experiment was planted when a cumulative rainfall of 150 mm was received that is, 24th, June in *Vertisol* in all the years on the same site with same randomization. Fertilizer was applied to transgenic Bt hybrid cotton paired rows as per the fertilizer treatments made in addition to proportion to the intercrop population with respective intercrops recommended dose of fertilizers. In order to use the existing hoes, only two rows of intercrops were accommodated in between two paired rows. Conservation furrows were opened to conserve runoff and provide drainage at every wide spaced transgenic Bt hybrid cotton row. Popular five tyned soybean marker (*datari*) was used for planting intercrops 45 cm a part, where 1st two rows were drilled with intercrop seed and subsequent two rows of cotton were dibbled leaving centre row blank, like this 4 sets were made in each plot (9 x 4.5 m). Soil moisture was measured by oven dry method at harvest. Economic yield, fresh and dry weights, biomass, nutrient uptake were estimated and analyzed with SAS 9.3 statistical package. Marigold flowers were harvested a day before the major Indian festivals starting from birth day of Indian elephant faced god (19th September) to festival of lamps (11th November) when farmers get double the price 0.80 US $ kg^{-1} fresh flowers compared to half in normal days and remaining left for next year seed production after mid November. Price of cotton US $ 0.83 kg^{-1}, field bean US $ 0.17 kg^{-1}, soybean US $ 42 Mg^{-1}, pigeon pea and castor US $75 Mg^{-1}, maize green cob US $ 22 Mg^{-1}, field bean US $ 167 Mg^{-1}, marigold 330 Mg^{-1}, Pendimethalin US $ 15 ha^{-1}, Pyrithionbac Na US $ 28 ha^{-1}, intercultures US $ 3 ha^{-1}, weeders US $ 0.17 h^{-1}, harvesting charges US $ 8.3 Mg^{-1} fertilizer N US$ 167, 467, 167 Mg^{-1} for NPK were considered calculating cost of cultivation and profitability.

RESULTS AND DISCUSSION

Yield performance of intercropping systems

Transgenic Bt hybrid cotton + soybean intercropping system was at par with transgenic Bt hybrid cotton + marigold intercropping system with 89 and 65% higher cotton equivalent yield (CEY) and also significantly higher CEY than with field bean intercropping system which produced 44% more CEY over paired row (PR) transgenic Bt hybrid cotton (Table 1). Transgenic Bt hybrid cotton + roselle significantly improved CEY by 111% and at par with soybean intercropping system with CEY of 89% over PR transgenic Bt hybrid cotton (Figure 1).

Uptake, use efficiencies

NPK uptake and FUE were significantly improved in both PR transgenic Bt hybrid cotton and transgenic Bt hybrid cotton + soybean intercropping system. NPK uptake, NUE, KUE, NPK FUE of the Transgenic Bt hybrid cotton + roselle / marigold intercropping systems was also significantly improved over PR transgenic Bt hybrid cotton. NPK uptake was significantly improved in transgenic Bt hybrid cotton + maize intercropping system but non significantly improved Nutrient and FUE. Fertilizer dose of 90:20:37 N:P:K kg ha^{-1} 2% Urea+ 0.2% Mg+0.06% B at squaring to flowering period also produced significantly superior and similar NPK uptake in both transgenic Bt hybrid cotton and intercropping systems FUE to that of 113:24:47 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1} soil application at the time of sowing (Table 2).

Profitability

Transgenic Bt hybrid cotton intercropped with roselle and soybean produced statistically similar profitability of US $ 768 and 662 ha^{-1} with a C:B ratio of 1: 3.73 and 1: 2.99 respectively (Table 3). However, determinate transgenic Bt hybrid cotton + indeterminate type pigeon pea strip cropping put to 69% losses due to severe irrecoverable competition. Foliar correction of nutrient deficiencies in both excess and scanty rainfall conditions by 2% Urea + 0.2% Mg +0.06% B at squaring to flowering period is significantly superior over soil application at the time of sowing at 5 kg Mg+ 2 kg B ha^{-1} with only 90:20:37 N:P:K kg ha^{-1} which was statistically similar to that of higher doses presently recommended for transgenic Bt hybrid cotton 113:24:47 N:P:K kg ha^{-1}.

Root growth, soil moisture extraction and senescence

Transgenic Bt hybrid cotton MRC 6301 had produced shallow (Table 4) tap and main lateral roots (<30 cm). Selection of ever green leaf color in transgenic Bt hybrid cotton MRC 6301 masked nutrient deficiencies by shortage fertilizer nutrient supply or inter plant competition. Significantly more nutrient deficient cotton leaves/plant were observed due to intercropping with

Table 1. Mean performance of transgenic Bt hybrid cotton based intercropping systems.

Treatment	Seed cotton yield t ha^{-1}	Intercrop yield t ha^{-1}	CEY t ha^{-1}	BMP cotton t ha^{-1}	BMP inter crop t ha^{-1}	HI of Bt cotton	HI of intercrop
PR Transgenic Bt hybrid cotton	0.9		0.9	2.86		33	
Bt hybrid cotton+pigeon pea 8:2	0.4	0.18	0.6	3.27	1.66	15	13.1
Bt hybrid cotton+ soybean 2:2	1.2	0.64	1.7	3.15	1.47	38	42.8
Bt hybrid cotton+ field bean 2:2	1.2	0.32	1.3	3.49	0.43	33	52.9
Bt hybrid cotton+ marigold 2:2	1.2	1.34	1.9	3.00	1.07	39	86.1
Bt hybrid cotton+ maize 2:2	1.1		1.1	3.39		31	
Bt hybrid cotton+ roselle 2:2	1.1	0.14	1.7	2.99	2.03	37	7.6
Bt hybrid cotton+ castor 2:2	1.1	0.09	1.1	3.25	0.20	31	49.9
SE±5%				0.35			
CD±5%	0.2	0.19	0.2		0.33	7	5.6
F1: 90:20:37 N:P:K kg ha^{-1} 2% Urea+0.2% Mg+0.06% B	1.0	0.41	1.3	3.15	1.07	33	41.3
F2: 90:20:37 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	1.0	0.39	1.2	3.13	1.06	31	42.1
F3: 113:24:47 N:P:K kg ha^{-1} 2% Urea+0.2% Mg+ 0.06% B twice	1.0	0.50	1.3	3.08	1.23	33	42.1
F4: 113:24:47 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	1.1	0.50	1.3	3.34	1.20	32	42.3
SE±5%		0.05		0.12		1	2.0
CD±5%	0.1		0.1		0.12		
90 x 45cm	0.9	0.40	1.2	2.68	1.17	35	41.7
90 x 30cm	1.1	0.50	1.4	3.67	1.11	30	42.5
SE±5%	0.0				0.06		1.2
CD±5%		0.03	0.0	0.13		1	

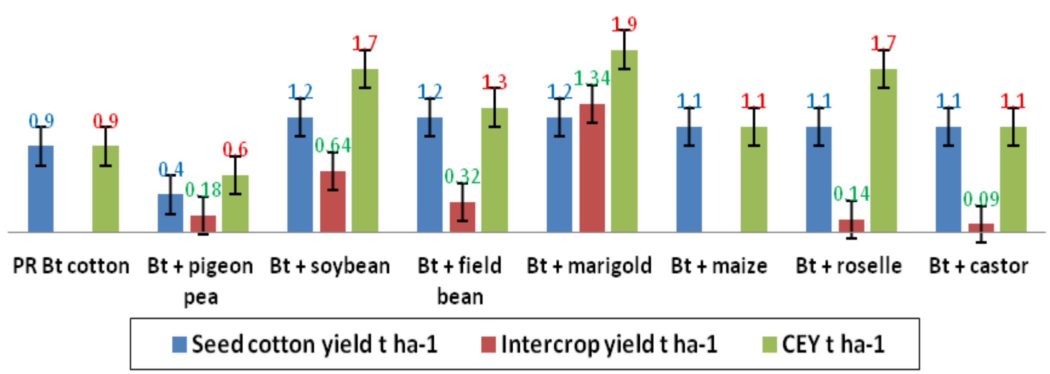

Figure 1. Cotton based intercropping systems.

Table 2. Nutrient uptake, use efficiencies as influenced by intercropping systems.

Treatment	Bt hybrid cotton									Intercrop			Bt hybrid cotton intercropping system								
	Uptake			NUE			FUE			Uptake			Uptake			NUE			FUE		
Cropping systems	**N**	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
PR Bt hybrid cotton	44	7	11	22	144	89	10	21	21				44	7	11	22	144	89	10	21	21
Bt hybrid cotton+ pigeon pea 8:2	22	3	5	20	168	93	5	9	9	26	11	17	67	25	33	24	179	101	8	18	9
Bt hybrid cotton+ soybean 2:2	59	9	15	21	150	83	13	26	26	32	10	14	89	17	26	42	228	138	22	48	26
Bt hybrid cotton+ field bean 2:2	56	8	13	22	161	91	13	27	27	43	15	19	81	15	26	35	209	119	18	32	27
Bt hybrid cotton+ marigold 2:2	62	8	12	20	152	100	13	27	27	26	10	14	129	23	34	40	243	162	20	34	34
Bt hybrid cotton+ maize 2:2	50	7	12	22	154	91	12	23	23	24	8	11	69	17	26	22	154	91	12	23	23
Bt hybrid cotton+ roselle 2:2	52	8	12	22	142	90	12	24	24	38	11	18	76	17	28	25	151	96	14	26	28
Bt hybrid cotton+ castor 2:2	52	8	13	21	141	82	12	24	24	31	10	15	60	12	17	27	158	96	12	25	24
SE±5%					18					12	3	4									
CD±5%	10	2	2	1		6	2	4	4				14	4.2	5	2	40	7	2	5	4
F1: 90:20:37 N:P:K kg ha^{-1} 2%Urea+0.2%Mg+0.06% B	51	7.4	12	21	155	88.2	12	23	23.4	31	10	15	77	17	25	29	187	110	15	29	25
F2: 90:20:37 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	47	6.8	11	21	154	92	11	22	21.6	35	13	17	73	16	24	29	185	113	14	27	23
F3: 113:24:47 N:P:K kg ha^{-1} 2%Urea+0.2%Mg+ 0.06% B twice	49	7.3	11	21	146	88.8	11	22	22.3	28	10	14	78	17	25	29	178	111	15	28	24
F4: 113:24:47 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	51	7.5	12	21	151	90.9	12	23	23.4	33	10	15	79	17	25	30	183	113	15	29	25
SE±5%	1.8	0.4		0.5	7.13	2.11				3.1	1	1	3.4	0.8	0.9	1	7	3		1	
CD±5%			0.9				1	1	1.49										1		2
90 × 45 cm	47	7	11	21	151	89.6	11	22	21.6	33	11	16	73	16	24	29	183	111	14	27	23
90 × 30 cm	52	7.5	12	21	152	90.3	12	24	23.7	30	10	15	81	17	26	29	183	112	15	30	25
SE±5%		0.3		0.3	5.22	1.37				2.3	1	1		0.8	1	0	6	2			
CD±5%	1.7		0.4				0	1	0.69				2.4						0	1	1

soybean, marigold, maize and castor, which were although not at economic threshold level compared to NCS 145 Bunny transgenic Bt hybrid cotton (Table 4). These intercroppings except maize also registered significantly lowest sucking insect and foliar diseases damage compared to sole cotton.

Fibre quality

Fibre quality was almost unaffected by droughts during seedling or termination and excess rains received during 2010 and 2011 respectively. Weaker fibres were produced by intercropping with field bean during 2011 drought year and fibre strength was improved by closer plant spacings due to non uniform fibres under excess rains of 2010 (Table 5).

Weed incidence

Lowest hand weeding cost was registered with castor /maize followed by close grown legume intercropping with soybean and field bean to maximum in sole cotton / pigeon pea strip cropping and roselle/marigold intercropping (Table 6). Castor and maize intercrops were very sensitive at young seedling stage to Pyrithiobac Na needs delayed application or directed sprays.

Herbicide tolerant weeds

Pyrithiobac Na at 0.035 kg a.i.ha^{-1} could not effectively controlled grasses(6.1 M^2) such as *Cynodon doctylon, Cyperus rotundus, Sorghum halepense, Eriochloa Polystachia, Commelina benghalensis* and broad leaved weeds (13.8 M^2) such as *Tridax procumbence, Phylanthus niruri, Parthenium hysterophorus, Merremia emarginta,*

Table 3. Profitability of transgenic Bt hybrid cotton based intercropping systems.

Treatment	Bt hybrid cotton economics US $				Intercrop economics US $			Cropping system economics US $			
Cropping systems	Gross returns	Cost of cultivation	Net returns	BCR	Gross returns	Cost of cultivation	Net returns	Gross returns	Cost of cultivation	Net returns	BCR
PR Bt hybrid cotton	600	233	367	2.50				600	233	358	2.49
Bt hybrid cotton+ pigeon pea 8:2	267	233	33	1.15	98	21	77	367	250	110	1.42
Bt hybrid cotton+ soybean 2:2	750	250	500	3.04	241	85	156	1000	333	662	2.99
Bt hybrid cotton+ field bean 2:2	767	250	517	3.03	54	34	20	817	283	532	2.87
Bt hybrid cotton+ marigold 2:2	767	250	517	3.08	156	83	74	917	333	592	2.79
Bt hybrid cotton+ maize 2:2	667	233	433	2.77	0	0	0	683	250	427	2.67
Bt hybrid cotton+ roselle 2:2	683	250	450	2.80	359	37	322	1050	283	768	3.73
Bt hybrid cotton+ castor 2:2	667	233	433	2.77	54	18	37	733	267	467	2.79
CD±5%	117	8	117	0.42	30	10	22	127	12	118	0.39
F1: 90:20:37 N:P:K kg ha^{-1} 2%Urea+0.2%Mg+0.06% B	650	250	417	2.69	152	44	108	767	283	496	2.76
F2: 90:20:37 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	600	233	367	2.49	159	43	116	717	267	451	2.60
F3: 113:24:47 N:P:K kg ha^{-1} 2%Urea+0.2%Mg+ 0.06% B twice	650	250	400	2.64	170	49	121	767	283	494	2.72
F4: 113:24:47 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	683	250	433	2.75	161	48	113	800	283	518	2.79
SE±5%		2			10	3	9				
CD±5%	33		38	0.15				46	7	43	0.14
90×45	600	233	367	2.61	152	44	109	717	267	451	2.69
90×30	700	250	433	2.68	169	49	120	817	300	529	2.74
SE±5%			17	0.06			7				0.05
CD±5%	17	2			8	2		18	2	17	

Digera arvensis, Acalypha india and Desmodium sp. Similarly, Pendimethalin at 1.0 kg a.i.ha^{-1} PPI continuous use could not controlled *Echinocloa crussgalli*. This resulted for requirement for hand weeding or graminicides besides interculture operations (Table 6).

DISCUSSION

Scope for commercial intercropping

Farmer's acceptance of intercropping is limited to poor awareness difficulty in interculture operations and weed management within row except by tribes for food security in India. Yield stagnation in cotton, soybean crops, risk associated with changing climate, price fluctuations and benefit from residual fertility can be dealt with better profitability and solutions for difficulties in intercropping adoption. Transgenic Bt hybrid cotton and soybean is cultivated in 6.87 and 9.0 million ha respectively with 1.0 t ha^{-1} productivity in Madhya Pradesh, Maharashtra and Andhra Pradesh states of central India (CCI, 2013), which can be brought under transgenic Bt hybrid cotton +soybean intercropping with improvement of 89% in CEY. This requires targeted efforts with wide scale demonstrations from public sector and soya processors extension. These results were in agreement with those observed by Raju and Thakare (2013).

Risk prone areas

Roselle and marigold intercropping will be suitable for low and high rainfall districts with sloppy terrains respectively. Both were suitable in IPM as

Table 4. Root growth and soil moisture extraction during drought year 2011-2012.

Treatment	**Lateral root spread cm**		**Soil moisture content and use**			**Red color leaves%**		
Cropping systems	**Cotton**	**Intercrop**	**20 cm**	**40 cm**	**cm**	**Disease**	**Insect**	**Nutritional**
PR Bt hybrid cotton	23.6		9.5	10.5	54.3	1.19	1.4	1.23
Bt hybrid cotton+ pigeon pea 8:2	20.4	50.9				1.58	1.7	1.49
Bt hybrid cotton+ soybean 2:2	24.3	17.0	6.2	6.8	63.3	0.71	0.7	2.27
Bt hybrid cotton+ field bean 2:2	17.8	35.4	9.0	10.7	54.8	1.15	1.4	1.27
Bt hybrid cotton+ marigold 2:2	24.4	22.3	7.6	9.4	58.5	0.59	0.9	2.21
Transgenic Bt hybrid cotton+ maize 2:2	17.3	18.5	4.2	7.3	66.0	1.01	0.9	2.53
Bt hybrid cotton+ roselle 2:2	24.1	74.5	9.0	11.0	54.7	1.14	1.5	1.39
Bt hybrid cotton+ castor 2:2	24.0	33.7	9.8	11.0	54.0	0.78	0.8	2.61
SE±5%	2.6							
CD±5%		7.4	0.9	1.87	2.04	0.51	0.6	0.84
F1: 90:20:37 N:P:K kg ha^{-1} 2% Urea+0.2%Mg+0.06% B	23.5	29.2	7.9	9.3	58.0	1.05	1.0	1.92
F2: 90:20:37 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	20.8	31.3	8.0	9.6	57.9	1.02	1.2	1.84
F3: 113:24:47 N:P:K kg ha^{-1} 2% Urea+0.2%Mg+ 0.06% B twice	22.2	28.3	7.7	9.6	58.1	0.94	1.2	2.14
F4: 113:24:47 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	21.5	28.9	7.9	9.5	57.7	1.05	1.2	1.60
SE±5%		1.8	0.5	0.59	1.28	0.15	0.1	0.33
CD±5%	1.7							
90×45 cm	21.9	31.5	8.0	9.3	58.0	0.99	1.1	1.90
90×30 cm	22.1	27.3	7.8	9.7	57.8	1.05	1.2	1.85
SE±5%	0.5		0.3	0.47	0.82	0.10	0.1	0.13

former reduces damage of sucking pests and later for bollworms acts as *refugea* at least in around largely populated *peri* urban areas for the supply of Iron rich fresh green leafy vegetable and fresh flowers in premium price early season. These results were in agreement with those observed by Raju and Thakare (2012).

Intercrops with suitable modifications

Hybrid maize for green cobs / fodder and castor TAU-9 variety both were able to improve CEY by 22%, which needs suitable genotype selection at optimum spacing under the umbrella of growth regulators. Hybrid maize in transgenic Bt hybrid cotton intercropping system is excellent soil binding intercrop after harvest winter pulses can also be drilled with residual soil moisture for *refugea* requirement for both *Helicoverpa armigera* and *Pectinophora gossypiella*. Transgenic Bt hybrid cotton with castor hybrid as a relay intercrop in high rainfall areas and stripcropping in low rainfall areas can be exploited as it can be planted after receding rains in mid September in order to avoid competition with early transgenic Bt hybrid cotton vegetative growth on residual moisture (Raju et al., 2012).

Fertilizer for intercropping

A fertilizer dose of 90:20:37 N:P:K kg ha^{-1} with foliar correction of nutrient deficiencies by 2%Urea for N, 0.2%Mg for Mg, 0.06% B for Boron deficiency is sufficient as those observed by Raju and Thakare (2012). There is no need of higher nutrients for determinate type transgenic Bt hybrid cottons with shallow root system of synchronous flowering with early retention of fruiting bodies. Separate fertilizer application to drill sown intercrops proportion to their population are to be drilled as the pit method of NPK fertilizer placement cannot be shared by 45 cm away

Table 5. Fibre quality during experimentation.

Treatment	2.5% span length		Uniformity ratio%		Micro naire		Strength g/tex	
Cropping systems	2010	2011	2010	2011	2010	2011	2010	2011
PR Bt hybrid cotton	29.3	29.1	48.3	46.6	3.7	3.13	21.0	23.0
Bt hybrid cotton+ pigeon pea 8:2	30.1	28.6	48.6	46.6	4.0	3.13	21.2	22.2
Bt hybrid cotton+ soybean 2:2	30.2	28.4	49.7	46.6	4.0	3.30	20.6	22.3
Bt hybrid cotton+ field bean 2:2	29.6	27.5	48.9	46.8	3.9	3.18	20.7	20.8
Bt hybrid cotton+ marigold 2:2	30.0	28.7	48.5	47.1	3.9	3.30	21.1	22.2
Bt hybrid cotton+ maize 2:2	29.9	28.7	49.0	47.1	4.1	3.30	20.3	22.5
Bt hybrid cotton+ roselle 2:2	29.7	29.6	48.5	45.8	3.9	3.33	21.2	23.5
Bt hybrid cotton+ castor 2:2	30.1	29.2	48.8	46.8	4.1	3.50	21.0	22.4
SE±5%	0.62	0.6	0.68	0.4	0.2	0.12	0.37	
CD±5%								1.03
F1: 90:20:37 N:P:K kg ha^{-1} 2% Urea+0.2%Mg+0.06% B	29.9	28.7	48.8	46.8	3.9	3.26	20.8	22.4
F2: 90:20:37 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	29.6	28.7	48.5	46.6	4.0	3.28	20.9	22.3
F3: 113:24:47 N:P:K kg ha^{-1} 2% Urea+0.2%Mg+ 0.06% B twice	30.0	28.6	48.9	46.7	3.9	3.25	20.8	22.4
F4: 113:24:47 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	30.0	28.8	48.9	46.6	3.9	3.26	21.1	22.5
SE±5%	0.1	0.1	0.3	0.20	0.04	0.05	0.2	0.21
90 × 45 cm	30.0	28.7	49.0	46.6	4.0	3.25	20.7	22.5
90 × 30 cm	29.8	28.7	48.6	46.8	3.9	3.27	21.1	22.4
SE±5%	0.1	0.1		0.1	0.03	0.02		0.08
CD±5%			0.3				0.3	

Table 6. Hand weeding cost incurred with Pyrithiobac Na used during 2011.

Treatment	Hand weeding costs after Pyrithiobac Na application US $\$\ m^{2}$ 22/9/2011		
Cropping systems	cotton	cotton +intercrop	Inter copping system
PR Bt hybrid cotton	95	190	284
Bt hybrid cotton+ pigeon pea 8:2	76	203	279
Bt hybrid cotton+ soybean 2:2	84	171	254
Bt hybrid cotton+ field bean 2:2	88	162	250
Bt hybrid cotton+ marigold 2:2	90	185	275
Bt hybrid cotton+ maize 2:2	119	107	225
Bt hybrid cotton+ roselle 2:2	96	193	290
Bt hybrid cotton+ castor 2:2	90	82	172
CD±5%	15	20	24
F1: 90:20:37 N:P:K kg ha^{-1} 2% Urea+0.2%Mg+0.06% B	94	161	253
F2: 90:20:37 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	90	161	251

Table 6. Contd.

F3: 113:24:47 N:P:K kg ha^{-1} 2% Urea+0.2%Mg+ 0.06% B twice	91	164	254
F4: 113:24:47 N:P:K kg ha^{-1} alongwith 5 kg Mg + 2 kg B ha^{-1}	95	161	255
SE$\pm$5%	8	4	6
90 x 45 cm	93	161	254
90 x 30 cm	92	102	253
SE$\pm$5%	3	4	5

drilled legumes during seedling droughts.

Conclusion

Transgenic Bt hybrid cotton +soybean intercropping can improve 89% in CEY, a profitability of US $ 662 ha^{-1} with a C:B ratio of 1: 2.99. A fertilizer dose of 90:20:37 for transgenic Bt hybrid cotton and 14:13:0 N:P:K kg ha^{-1} for soybean is sufficient with foliar correction of nutrient deficiencies by 2%Urea for N, 0.2%Mg for Mg, 0.06% B for Boron deficiency.

Conflict of Interest

The authors have not declared any conflict of interest.

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Review

Analysis of seed quality: A nonstop evolving activity

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The enhancement of the production processes and increasing demand for high-quality seeds, has led the seed quality control companies to technically improve their activities. Due to this demand, we searched to improve tests related to physiological seed quality, aiming to obtain analysis results that are more consistent with the findings related to field conditions, providing information about the possibilities of the seeds in producing vigorous and representative seedlings. The studies related to the tests for seed vigor evaluation are noteworthy, as they are auxiliaries to the germination test, which has its limitations, especially when regarded to the differentiation of the lots and a relative delaying in obtaining reliable results in short time periods. Therefore, the aim of this review was to describe the available information about seed quality analysis, reporting the possible incorporation of a new method for this analysis.

Key words: Rapid tests, viability, vigor.

INTRODUCTION

Evaluating the physiological potential of seeds is the main component in a program of quality control, considering that it supplies information that identifies and fixes problems during the production process, besides estimating seed performances in the field. When talking about tests of physiological seed quality for sowing and marketing purposes, we can focus on the germination test, while being conducted under ideal and artificial conditions, allows for obtaining the maximum germination percentage. However, this test has limitations, mainly, with respect to the differentiation of lots and the relative delay in obtaining results, which, over the years has stimulated the development of reliable and fast vigor tests speeding up decision making (Bertolin et al., 2011). Thus, the identification of vigor tests that supply a safe margin in regards to seed behavior in the field, have been a tireless search and a necessity, since the adverse environmental conditions impose uniformity between the germination test and the field results, thus establishing the need to identify tests that provide equivalent conditions to germination in the field, combined with all the adversities that may affect the performance of a

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cultivar. Therefore, during the last decades, the interest to develop appropriate techniques for better information about crops has been a central topic of research (Dell'aquila, 2009).

The use of seeds with high physiological potential is an important aspect that should be considered for increased productivity and, therefore, the quality control of seeds tends to be increasingly efficient, including tests that assess this aspect quickly, allowing accurate differentiation between seed lots (Fessel et al., 2010). Thus, this review aimed to describe the main information existing to date on the analysis of the physiological quality of seeds, as well as to report the possible incorporation of a new method for analysis of seed vigor.

QUALITY AND PHYSIOLOGICAL POTENTIAL OF SEEDS

The history of agriculture demonstrates that the first contacts between man and seed physiology were established from the moment the possibility of its use was discovered for propagating plants in the century LXXX a.C. (Krzyzanowski and Vieira, 1999). In this situation, besides causing profound positive changes in the habits of life, the beginning of the use of seeds for the establishment of cultures aimed at producing foods also became a source of concern (Peres, 2010). With the domestication of plant species, new challenges have emerged with the need to determine the most favorable seasons for sowing (Marcos Filho, 2005). For being an indispensable ingredient, due to its role in the agricultural chain and in human history, the seed both socially and economically, contributes to the quantitative and qualitative increase in productivity, noting that the use of high quality seeds is an important factor for the success of any culture (Gaspar and Nakagawa, 2002). The procedures adopted in the control of seed quality programs are based both on previous recommendations from research or practical experience, just like the survey data which allows the detection of problems, and the proposition of adequate solutions (Krzyzanowski and Vieira, 1999). Seed quality is basically determined by their physiological potential, which gathers information on viability and vigor of a seed lot, with the potential term translated as virtuality or set of skills to perform tasks and produce results (Marcos Filho, 2005). The test commonly used to determine the viability of the seed is germination, whose main aim is to obtain information about the value of seeds for sowing, as well as the comparison of quality of different lots (Lima et al., 2006).

Its conduction occurs under optimal conditions in order to provide maximum germination of the sample analyzed. These conditions refer to the availability of water, aeration and temperature (Marcos Filho et al., 1987; Brasil, 2009). The germination test is aimed towards at least two aspects: providing information on the potential of a lot to germinate under favorable environmental conditions and to provide high degree of standardization, with ample opportunity for repetition of the results with reasonable levels of tolerance, as long as the instructions established are followed (Marcos Filho, 1999; Brasil, 2009).

However, the germination test might overestimate the physiological potential of the seeds for not evaluating the physiological, biochemical, physical and cytological changes related to the process of deterioration, not allowing to differentiate in the field and in storing seed lots in regards to vigor (Abrantes et al., 2010). For this reason, research has performed studies to develop methods that allow the evaluation of vigor of the seed (Kikuti et al., 1999; Ávila et al., 2007; Ohlson et al. 2010). The vigor of a seed comprehends the set of properties, which determines the ability of emergence and the fast development of normal seedlings under a wide range of environmental conditions (Baalbaki et al., 2009). Thus, its basic aim is to identify properly which lots have greater potential to survive and generate good productivity under field conditions (Marcos Filho, 2005).

In the U.S.A. and in Canada, from 1976 to 1990, there was a significant increase in the use of vigor tests in laboratories of seed analysis. During this time the test of electrical conductivity was not mentioned as a vigor test although it began being mentioned as such in 1982 (Tekrony, 1983; Ferguson, 1993). This test, along with the accelerated aging and cold tests, were the aim of study by the committee of vigor of the Association of Official Seed Analysts, Inc (AOSA), which from 1983 to 1991 were considered the three most promising vigor tests (McDonald, 1993).

Searching the use of existing laboratory tests between members of ISTA (International Seed Testing Association), Hampton et al. (1992), recommended the test of electrical conductivity to evaluate the effect of pea seeds in Europe and New Zealand. With this the electrical conductivity and the accelerated aging tests became the only two recommended by the committee of vigor by ISTA (Hampton and Tekrony, 1995).

In Brazil, Krzyzanowski et al. (1991), assessing the situation of the use of vigor tests as routine among laboratory of seed analysis, concluded that, although fundamental, these tests have yet to evolve in a way that they can participate effectively in quality control programs in seed industries. In the particular case of the electrical conductivity test, its use is still very restricted to certain situations, especially to those directly related to research (Peres, 2010).

The opening of new agricultural frontiers and increased seed production in Brazil in recent years, has led seed companies to seek technical improvement of its activities primarily aimed at increasing productivity associated with an increase in product quality. Thus, both the ISTA and AOSA adopt procedures to evaluate the most adequate methodology for the inclusion in the Rules for Seed

Analysis, through benchmarking tests performed in different laboratories, under the coordination of specific committees, who evaluate purity, germination, and moisture content among other features.

To this end, a number of samples are sent to participating laboratories, accompanied by instructions that must be followed by analysts. Once in possession of the results, the Coordination Committee interprets them, verifying compatibility among the laboratories, detecting problems, diagnosing the situation and programing new test steps, until the level of standardization is satisfactory and allows the recommendation of the methodology. Therefore, it is possible to notice the occurrence of a careful process of standardization and quality control, which have the function of equating and determining the best method for monitoring vital seed (Krzyzanowski and Vieira, 1999).

The main purpose of seed analysis is to determine the quality of a seed lot and, consequently, its value for sowing (Brasil, 2009). The analysis is characterized by a detailed and critical test of a sample, with the aim of analyzing its quality in order for it to be used in research works, as well as in identifying causes and problems of quality.

In the United States (USA), various seed producing companies of big cultures have used vigor tests for the identification of lots that do not reach internal standards of quality, classification (ranking) of lots in different levels of physiological quality, evaluation of potential for the formation of regulator stocks (carry over), decision making in regards to commercialization, looking to commercialize, firstly, lots which attend to germination standards, but at the same time show lower vigor, and moreover, supply of information about the physiological quality of the lots to consumers (Frigeri, 2007).

Thus, the seed analysis is an important tool in quality control, particularly from the end of the maturation period, when the seeds reach physiological maturity. Also, there is an international consensus among researchers, technologists and producers of seeds about the importance of the determination of seed vigor and the necessity for evaluation. Information about vigor is still important for seeds of a higher commercial value, such as vegetables, which may have been pelleted (covered by films) and pre-conditioned physiologically, like in other countries. Moreover, for presenting a lesser quantity of stored reserves, they have a higher propensity to reduced vigor after physiological maturity (Kikuti et al., 2012). The cultivation of these species is done intensively and must be established using seeds that germinate rapidly and uniformly, and therefore with higher physiological quality. The demand for methods that are satisfactory in predicting seed quality, through the force of vegetable seeds serves as methodological updates from other cultures such as, hybrid corn seeds generating technological progress in search of quality in the determination of vigor tests (Peres, 2010).

Within this context, vigor tests are useful in the programs of seed production for the evaluation of physiological potential of lots with similar germination, allowing to differentiate lots based on potential of seedling emergence in the field, evaluation of storage potential, degree of deterioration, quality control post-maturity, and physiological quality, serving as a tool to aid in methods of selection during the improvement of plants, as well as to allow the effects of mechanical and thermal injuries, treatment with fungicides and other adverse factors pre and post harvest (Marcos Filho, 1999).

Often, seed lots with similar germination percentage can show different responses in the field and/or storage (Frigeri, 2007), being that the loss of germinating potential is an important indicator in loss of quality; however, it is the ultimate event of this process. Thus, the use of vigor tests is of extreme importance for monitoring the quality of seeds from maturity, because the fall of vigor precedes loss of viability (Dias and Marcos Filho, 1995).

VIGOR TESTS OF SEEDS

Although vigor tests possess different technologies, they are intended to detect significant distinctions in the physiological potential of seed lots with similar germination among themselves (Lima et al., 2006; Dutra and Medeiros Filho, 2008), classifying them into different levels of vigor, especially proportional to the response of emergence of seedlings in the field (Marcos Filho, 1999). Thus, the selection of vigor tests must meet specific goals, making it important to identify the characteristics evaluated by tests and its relation to the responses of seeds by specific situations such as performance after drying, storage potential, response to mechanical damage and weather conditions.

According to McDonald (1993), vigor tests can be classified as physical, physiological, biochemical and of stress resistance. The physical tests assess morphological or physical characteristics of the seed, which may be associated with force, such as size, density, and color of seeds X-ray tests. Tests characterized as physiological are based on specific physiological activities which have their manifestation based on vigor, such as first germination count, index of speed of germination or emergence of seedlings. However, biochemical tests evaluate changes in metabolism related to the vigor of seeds; among these are the tests of tetrazolium and of electrical conductivity. Finally, stress resistance tests, which analyze the behavior of seeds when exposed to unfavorable environmental conditions, with emphasis for the accelerated aging tests, controlled deterioration, cold, germination at low temperature and submersion in water (Marcos Filho, 2005)

Seed vigor, according to what was defined by the

International Seed Testing Association (Ista, 1995), is the degree index of physiological deterioration and/or mechanical integrity of a seed lot of high germination, representing a broad ability to establish in the environment. This vigor definition is similar to the one formulated by the Association of Official Seed Analysts (Aosa, 1983).

Vigor tests contribute in detecting these information and, consequently, are useful for decision making on the destination of a seed lot. Among these tests of vigor available, it is worth to emphasize the test of electrical conductivity, which is a fast and objective test of vigor, which can be conducted easily by various laboratories of seed analysis with minimal spending on equipment and training of employees (Marcos Filho et al., 2009).

The results of the vigor tests are comparative, since it is not possible to quantify them since all the characteristics are not measurable. In fact, the results of 60% of normal seedlings in the accelerated aging tests, of cold, first count of germination, among others, mean nothing if not compared with what was obtained for another sample of the same species and cultivar. Thus, expressions like 70% vigor are incorrect and should not be used (Ista, 1999). The impossibility of quantifying vigor generates difficulty both for the understanding of its meaning, and for comparing information obtained in different tests. With this, research has sought to translate and establish indexes that contribute to interpretation and utilization of results obtained regarding the seed vigor (Peres, 2010).

The evaluation of the physiological potential of seeds is fundamental as a base for the processes of production, distribution and commercialization of seed lots. Thus, production companies and laboratories of seed analysis should use tests that provide reproducible results that are also reliable and that indicate with certainty the quality of a seed lot, mainly in respect to vigor (Frigeri, 2007). It is noteworthy that a seed lot is made of the same species from a defined, identified and homogenous quantity of seeds with similar physical and physiological attributes. However, lots, even from the same production area, may have imperfect homogeneity in terms of germination, which is related to variations in topography and soil fertility, or even, can be influenced by crop interspersed with rainy days (Peske et al., 2006).

Vigor analyses allow racking of lots, thus allowing commercialization according to the local conditions of cultivation. Thus, lots of higher vigor may be destined to regions with bigger environmental limitations during sowing season (Peske et al., 2006). Various vigor tests are available and differ regarding to technology, time and ease of execution, being that the most studied ones are those related to initial events of the deterioration sequence (Marcos Filho, 1999), such as degradation of cellular membranes and the reduction of respiratory activity, which allow separating seed lots in regards to vigor (Abrantes et al., 2010).

The test of first count of germination is based on the principle that the samples that present higher percentages of normal seedlings during the first count, established by the Rules of Seed Analysis (Brasil, 2009), for each culture, will be the most vigorous which correlates with the index of germination speed. However, there can be a better answer than the latter reinforcing so, the affirmation that this test is of great interest to evaluate seed vigor, taking into account its practicality and execution time.

To use the germination test itself for running both tests mentioned above, all what we need is follow the norms of rules of seed analysis (Brasil, 2009), where the uniformity and speed of seedling emergence are the most important components within the current concept of seed vigor, considering the evaluation of seedling growth a logical and specific vigor test, as well as to evaluate the length of normal seedlings (Aosa,1983).

The evaluation of dry matter and length of seedlings are related to germination speed, taking into account that lots that show more vigorous seeds will originate seedlings with higher rates of development and gain of biomass due to having greater adaptability, since they use their reserves of the tissues of storage for the differentiation of the tissues, and consequently, promoting the growth and development of the embryonic stem of the seedling (Dan et al., 1987).

The methods for evaluating vigor can be classified directly when performed in the field or even under laboratory conditions, which simulate adverse factors of the field; or indirect when performed in laboratory evaluating the physical, physiological and biochemical characteristics that express the quality of seeds (Ferreira and Borghetti, 2004). In general, the low vigor of seeds is associated to reductions in speed and lack of uniformity of emergence, as well as in the reduction of initial size of the seedlings, in the accumulation of dry matter, in the leaf area and, consequently, in the rate of cultivation growth (Schuch et al., 2000; Machado, 2002; Höfs et al., 2004; Kolchinski et al., 2005). The cause of failure or reduction in speed of emergence is frequently attributed to low vigor associated to the deterioration process of the seeds (Rossetto et al., 1997).

As previously reported, various vigor tests are available and differ in methodology, time and ease of implementation, and the most studied are those related to early events of sequence deterioration (Delouche and Baskin, 1973),. since the vigor and deterioration of the seed are physiologically linked, being reciprocal aspects of quality, where deterioration has a negative connotation, while vigor has a positive connotation, as they are inversely proportional (Delouche, 2002). The seed does not start the deterioration process before reaching physiological maturity, since before this period it is not an independent unit of the parent plant yet. However, unfavorable environment conditions during maturation may determine the formation of seeds with poor physiological potential (Marcos Filho, 2005).

The tests that evaluate the initial events of deterioration the test of electrical conductivity, as previously reported, was proposed by Matthews and Bradnock (1967) in order to estimate vigor of pea seeds. This test measures the amount of electrolytes released by the seeds during soaking, which is directly related to the integrity of cell membranes (Matthews and Powell, 1981). Among the various procedures used in the determination of seed vigor, one of the alternatives that have shown promising results is the measuring of respiratory activity under laboratory conditions (Mendes et al., 2009; Aumonde et al., 2012; Marini et al., 2012), since breathing is the first metabolic activity that goes along with rehydration of the seed and the increase in this metabolic process varies from negligible values to high levels shortly after the start of imbibition (Popinigis, 1977; Ferreira and Borghetti, 2004). In this process, the oxidation of organic substances in a cellular system with gradual energy release occurs through a series of reactions, with molecular oxygen as a final electron acceptor. The respiratory substrates may be carbohydrates like starch, sucrose, fructose, glucose and other sugars or even lipids, especially triglycerides, organic acids and proteins (Taiz and Zeiger 2009; Marenco and Lopes, 2007).,

The increase in respiratory activity of the seed can be evaluated by the amount of carbon dioxide (CO_2) eliminated, by the amount of oxygen (O_2) absorbed or by the respiratory quotient (QR). The respiratory rate of the seed is influenced by its moisture content, temperature, and membrane permeability by oxygen and light strain (Popinigis, 1977). Respiration implicates in the loss of dry matter and in gas exchange, being methods that are used based on the determination of these characteristics. However, the measuring of dry matter variation requires large amount of material, besides being considered an analysis that is somewhat time-consuming to obtain the result, given that the plant material must be completely dried in an oven (Marenco and Lopes, 2007).

The methods based on gas exchange are more sensitive, require less material and are not destructive, and can be the gage measuring O_2 consumption, for example, Warburg respirometer and Clark electrode (potentiometry), in measuring liberated CO_2, using physical methods as the analyzer of infrared gas (IRGA), or physiochemical which are based on CO_2 retention on a base and quantification by titration calorimetry or conductivimetry (Maestri et al., 1998). Among the different forms of verification of seed physiological quality in the breathing process special attention is due to the high ratio between this phenomenon and seed quality (Mendes et al., 2009; Aumonde et al., 2012). Given that the greatest current interest, when evaluating the physiological quality of seeds, is to obtain reliable results in a relatively short period of time, it is expected that this review agility allows fast decision making during different stages of seed production, especially between maturation phase and future seeding (Dias and Marcos Filho, 1996).

Tests for the rapid assessment of viability or vigor represent important components in the control of seed quality programs and allow to speed up the obtaining of information by discarding lots of inferior quality during the reception at the processing unit and rationed management (Marcos Filho, 2005).

Within this context and having in mind that the analysis of physiological seed quality should be seen as a dynamic activity, that shows constant evolution, both by improving the resources available for its evaluation and the incorporation of new methods (Novembre, 2001), it becomes of extreme importance to prove the efficiency of new analysis, such as the application of the breathing test in order to obtain results with the aim of separating seed lots in regards to vigor.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

First report of *Leptocybe invasa* Fisher and La'Salle (Hymenoptera:Eulophidae) in Mozambique

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The existence of gall wasp, *Leptocybe invasa,* in Mozambique was first recorded and reported in July, 2011 in the forestry nursery from the Forestry Investigation Center (CIF) and the forestry plantations of the districts of Marracuene and Namaacha (in the province of Maputo), respectively. The formation of galls on the eucalyptus seedlings was observed through the samples collected and sent to the Agricultural Research Council Landbounavorsingsraad – Plant Protection Research Institute in South Africa for identification. *L. invasa* is the insect that causes galls on eucalyptus (causes leaves malformation); its occurrence on the central nervure and petiole defoliates and dries the plant.

Key words: *Eucalyptus*, gall wasp, *Leptocybe invasa*, quarantine pest.

INTRODUCTION

The trees from the *Eucalyptus* genus (Myrtaceae) have enormous economic importance in tropical countries (like Mozambique) due to its fast growth, low costs of establishment of its plantations and fewer demands for climatic conditions, such as the precipitation quantity (Lamprecht, 1989). Kassab (2011) reported the fact that the species of *Eucalyptus* genus have fast growth, productive capacity, ability to adapt to several different environments and their varieties can be grown in large scale (monoculture) makes it a great demand in the segment of forestry products. However, Oliveira et al. (2001) reported that monoculture (cultivation of eucalyptus) can favor pest presence; therefore, it is necessary to develop appropriate techniques to reduce the damage caused by these pests.

The implementation of monoculture as a homogeneous massive plantation favors the presence of many plague-insects populations due to food availability which may affect the forest enterprise (Ohrmart and Edwards, 1991). Despite the facts that *Eucalyptus* spp. forests have their main issues with the native pests, in Brazil, such as the leaf-cutting ants, the termites and

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Figure 1. *Eucalyptus camaldulensis* seedling attacked on the forestry nursery from the Forestry Research Centre (CIF) with typical symptoms of attack of L. invasa in the District of Marracuene, Mozambique, 2011. Photo: Cacilda J. Chirinzane.

the defoliating caterpillars; in the last few years, many quarantined pests were also reported across the globe, for examples, the red gum lerp psyllid *glycaspis brimblecombei* (Hemiptera: Psyllidae), the bronze bug *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) and *Leptocybe invasa,* gall wasp (Hymenoptera: Eulophidae), which was reported in many countries (Wilcken et al., 2011).

The gall wasp, *L. invasa,* is originally from Australia; it is dark-colored, very shiny and cannot be bigger than 1 to 5 mm; its females are very common, but only a male has been reported in Turkey (Doganlar, 2005). According to Mendel et al. (2004), it is an oviparous insect that reproduces by parthenogenesis (which means females are able to reproduce without the need of a male); the adult female lays eggs in vegetal organs (preferentially, on the central vein, on the leaves petiole, in the stem and on the apical buds of branches), which swell to form galls when the larva hatches. According to Wilcken and Berti-Filho (2008), these galls cause leaf deformation on the central vein and the petiole and defoliates and dries the pointing of the leaf on the thinner branches. These galls are caused by some substances injected by the egg-layer of the female, which block the sap flux and lead to the falling of the leaves. The authors are yet to affirm that these damages can affect the growth of the seedlings and the trees, the productivity of clones or susceptible species.

This insect has already been found in many continents, such as Asia (India, China, Thailand, Lebanon, Iraq, Israel, Jordan, Syria, Turkey, Vietnam and Iran); Europe (Portugal, Spain, France, Greece and Italy); South America (Brazil) and Africa (South Africa, Morocco, Uganda, Tanzania, Ethiopia, Algeria and Kenya) (Mendel et al., 2004; Doganlar, 2005; Mendel et al., 2007; Wilcken and Berti-Filho, 2008; FAO 2009; Hassan, 2012). This study aimed to register the first occurrence of the gall wasp in Eucalyptus species planted on the province of Maputo in Mozambique.

MATERIALS AND METHODS

In November 2009, the formation of galls could be observed in seedlings and forest plantings of *Eucalyptus saligna* in the district of Namaacha, province of Maputo in Mozambique; in February 2011, it was also reported on *Eucalyptus camaldulensis* seedlings in the forestry nursery from the Forestry Investigation Centre (CIF) in the District of Marracuene, province of Maputo, with 100% of attacked seedlings (Figure 1).

In order to capture the insect responsible for causing the galls, all parts of the plant that had holes and galls were collected and conserved into bowls (30 cm in length x 25 cm wide x 10 depth cm) and covered with a very fine mesh to facilitate ventilation. On a daily basis, the outbreak of the adult insect could be observed; the insect was collected and put into a flask containing 70% alcohol using an entomological brush. Ten insects were collected from each of the two districts in the Maputo province; a total of 20 insects were put in a 70% alcohol flask and later sent to the Agricultural Research Council Landbounavorsingsraad – Plant Protection Research Institute in South Africa for identification.

RESULTS AND DISCUSSION

According to the Agricultural Research Council Landbounavorsingsraad – Plant Protection Research Institute, the eucalyptus plants from the Maputo province were attacked by an exotic pest, *L. invasa,* commonly known as eucalyptus gall wasp (Figure 2). This insect was first reported and identified on July, 8th 2011 in the country. On severe infestations of the pest, it reduces the development of the plants, blocks the normal flux of the sap, causes deformation (twisting) and drying of the leaves and affects the production and quality of the wood (Mendel et al., 2004). The same symptoms were observed and verified from the samples collected in Mozambique.

In young plants (trees), the insect preferentially attacks the central vein of the leaves; consequently, causing their curving (Figure 3C and D).

The insect has severely attacked *Eucalyptus saligna* and *Eucalyptus camaldulensis* plants; caused the death of 2% of the plantation due to the formation of many galls on the plant (Figure 4A and B).

Visually, the *E. camaldulensis* showed higher amount of seedlings attacked in the nursery than the *E. saligna*; according to Wilcken et al. (2011), *E. camaldulensis* and hybrid clones are very susceptible to the attack of the gall wasp, which endangers its initial

Figure 2. Adult of *Leptocybe invasa* on *Eucalyptus camaldulensis* leaf collected in the district of Marracuene. Forestry Research Centre (CIF), Mozambique, 2011.Photo: Cacilda J. Chirinzane.

Figure 3. Attack symphoms of *Leptocybe invasa* in *Eucalyptus camaldulensis*, at different stages of development (A) Sowings attacks in CIF; (B) Seedlings in CIF; (C) Clear cutting showing attacks on the Seeds Production Area (Marracuene); (D) Leaf attacked on the forestry planting. CIF, Mozambique, 2011. Photos: Cacilda J. Chirinzane.

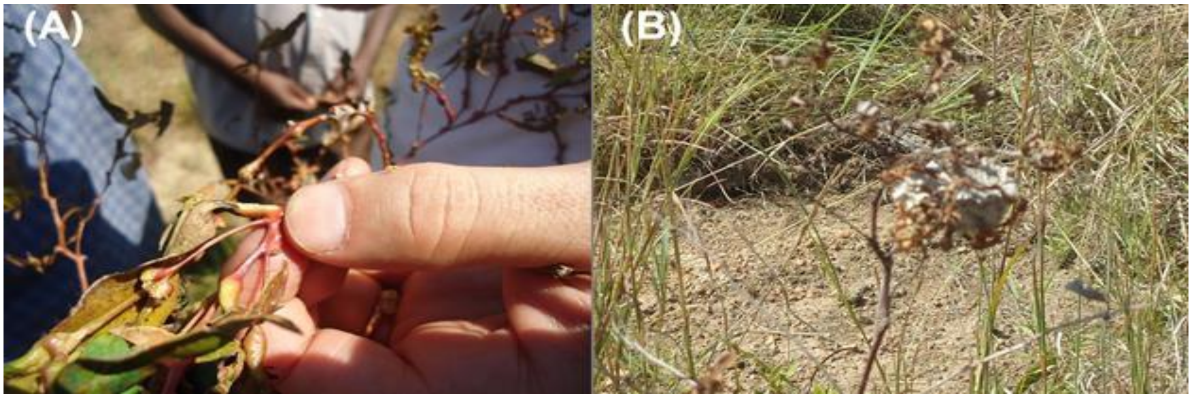

Figure 4. *Eucalyptus saligna* plants attacked by *Leptocybe invasa.* (A) Initial symptoms of vegetal dry and yellowish leaves; (B) Dead plant completely dry. Namaacha, Mozambique, 2011. Photos: Cacilda J. Chirinzane.

development (from planting to 2 years old) and causes growth reduction in height with loss of apical dominance. Thus, the use of this genetic material must be conducted in a very selective way. Wilcken and Berti-Filho (2008), also reported the susceptibility of *E. camaldulensis* to the gall wasp attack.

Conclusion

The climatic condition of Mozambique is favorable for the exotic pest, *L. invasa,* therefore, the monitoring and management of this pest is not only important but also urgent, especially in *E. camaldulensis* plantings on the region, more than 100 000 ha, and related places, the infestation levels and losses caused by the pest are required to be studied as ways to understand and elaborate the control measures and reduce the damage caused by this insect in the region.

Conflict of Interest

The authors have not declared any conflict of interest.

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Growth, yield, quality and nutrient content of pigeonpea (*Cajanus cajan*) as influenced by sunflower stover and nutrients management under pigeonpea-sunflower (*Helianthus annuus*) cropping system

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A fixed plot field experiment was carried out during 2008-2009 and 2009-2010 at New Delhi, to evaluate the ''effect of sunflower stover and nutrient management on pigeonpea under pigeonpea-sunflower cropping system''. The results indicates that sunflower stover incorporation reduced the plant growth parameters *viz.,* plant height, leaf area index (LAI), dry matter accumulation (DMA), crop growth rate (CGR), grain and stover yields, protein and nutrient contents of pigeonpea. Reduction in these parameters was more pronounced in the second year than the first. Application of P levels significantly increases growth attributes, grain and stover yields, protein and nutrient contents of pigeonpea over the control. Maximum plant height, LAI, DMA, CGR, grain yield (1.63 and 1.32 Mg/ha), protein content and nutrient status were recorded with the application of 30 kg P/ha followed by 15 kg P/ha + phosphate solubilizing bacteria (PSB). Significantly higher grain yield (1.17 Mg/ha) of pigeonpea was recorded due to residual effect of recommended dose (RD) of nitrogen and phosphorus (NP) given to sunflower, which was 10.4 and 14.7% higher over 50% RD of NP and control, respectively.

Key words: Growth, nutrient content, sunflower stover incorporation, quality.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a non traditional oilseed crop widely adopted under different agro-climatic regions owing to its thermo-photo-insensitivity, it has potential to yield of 4 to 6 t/ha crop residue and 2 to 2.5 t/ha seed yield. Its seed is used as a source of vegetable oil but its crop residue is neither use as feed for livestock nor suitable for fuel due to low energy value per unit mass. However, it contains major plant nutrients in the range of 0.45 to 0.60% N, 0.15 to 0.22% P and 1.80 to 1.94% K along with secondary and micronutrients (Babu et al., 2014), so recycling of its residue in the soil may be one of the best alternative practices for replenishing the depleted soil fertility and improving the physical, chemical and biological properties of the soil.

However, some researchers have reported allelopathic effects of sunflower residue on different crops, which put

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a question on choice of crop after sunflower and itsresidue incorporation (Velu, 1989). Allelopathy of sunflower residue affects the crop growth in various ways. Schon and Einhelling (1980) demonstrated that, incorporation of dried sunflower leaf material into the soil inhibited germination and growth of succeeding grain sorghum. Water soluble toxic substances could leak from the plant and from decomposing residue causing allelopathic interference. Leachates from the plants have been shown to suppress seed germination and vegetative propagules, and early seedling growth of *Cyprus rotundus* (Babu and Kandasanmy, 1997). Pulses are important food-use crop. They are able to fix nitrogen to meet their own requirement and are also beneficial to the succeeding crops by providing nitrogen sustaining soil health. Among the pulses, pigeonpea is the second most important crop of India next only to chickpea. In India, it is cultivated over an area of 3.6 Mha, with production of 2.6 Mt. However, its productivity is very low that is, 709 kg/ha. Among the various reasons for low productivity, one finds the role of phosphorus in plant growth of paramount importance. In pigeonpea, phosphorus has been thought to play an indirect role in nodule initiation as it increases the symbiotic relationship between nitrogen fixing bacteria such as *Rhizobium* sp (Jacobsen, 1985). The phosphorus status of several leguminous plants have been reported to increase the nitrogen (N) content in plant tissues and the growth of the host plant (Israel, 1987). However, cost of phosphatic fertilizers is continuously increasing due to limited supply. Hence, there is need to explore the possibilities of saving phosphatic fertilizers, without sacrificing economic yields. In this regard, biofertilizers could play a crucial role by increasing the availability of phosphorus and other nutrients to the crops (Selvakumar et al., 2012). Among the biofertilizers, phosphorus solubilizing micro-organism play a significant role for improving growth, yield attributes and yield of pigeonpea by enhancing the phosphorus availability (Singh and Yadav, 2008). Phosphate solubilizing bacteria (PSB) increase the availability of P in the soil by solubilizing the residual or fixed soil-P (Singh et al, 2008). In view of the limited information available on the comparative assessment of sunflower stover and nutrient management on pigeonpea, a fixed plot field experiment was conducted over two consecutive seasons to study the effect of sunflower stover and phosphorus management on growth, yield, quality and nutrient content of pigeonpea (*Cajanus cajan*) under pigeonpea-sunflower (*H. annuus*) cropping system.

MATERIALS AND METHODS

A fixed plot field experiment was carried out during 2008-2009 and 2009-2010 to evaluate the effect of sunflower stover and nutrient management on growth, yield, quality and nutrient content of pigeonpea under pigeonpea-sunflower cropping system at New Delhi, India. It is situated at a latitude of 28°40' N, longitude of 77°12' E and altitude of 228.6 m above the mean sea level (Arabian Sea). The soil of experimental field was sandy clay loam belonging to the order Inceptisol and having 145.0 kg/ha alkaline permanganate oxidizable N, 17.5 kg/ha available P, 226.0 kg/ha 1 N ammonium acetate exchangeable K and 0.40% organic carbon. The pH of soil was 7.5 (1:2.5 soil and water ratio). Field capacity, permanent wilting point and bulk density recorded were 17.0% (w/w), 6.30% (w/w) and 1.46 Mg/m^3, respectively in 0 to 15 cm soil depth. *Kharif* season experiment in the first year was laid out in split-plot design, assigning sunflower stover incorporation (8 t/ha) and no stover incorporation (control) to main plots and combination of P levels and biofertilizers (Control, 15 kg P/ha, 15 kg P/ha + PSB and 30 kg P/ha) to sub-plots. The *spring* season experiment was laid out in split-split plot design in which three treatments of nitrogen and phosphorus (NP) rates to sunflower crop {Control, 50% recommended dose (RD) of NP and RD of NP (80 kg N + 15 kg P/ha} were applied in sub-sub plots. Data for *Kharif* season experiment in second year was laid, recorded and analyzed in split-split plot design to investigate the residual effect of NP doses applied to *spring* season crop in sub-sub plots. All the treatments were replicated thrice during both the years. The plot size was 17.4 × 15.0 m for main plots and 2.40 × 15.0 m, 2.40 × 4.0 m for sub-plots and sub-sub plots, respectively. Main field was irrigated, ploughed with tractor-drawn disc plough followed by harrowing after the soil reached to tilth conditions and levelling was done with land leveler. Sunflower stover of the general sunflower grown during the *spring* season of 2008 and experimental crop of the *spring* season 2009 was chopped with the help of chopper and incorporated in the soil as per treatments (8 t/ha) before the preparation of field for sowing of pigeonpea. The recommended starter dose (25 kg/ha) of N for pigeonpea was supplied through urea [after subtracting the N supplied from diammonium phosphate (DAP)]. DAP was used to supply phosphorus as per treatment. Phosphorus was placed 3 to 5 cm below the seed with the help of mechanical applicator. Seeds of pigeonpea were inoculated with the PSB culture 'Microphos' containing inoculum of *Pseudomonas striata*. The Microphos was thoroughly mixed with 10% jaggery solution (It is an amorphous form of unrefined and non-distilled sugar prepared from the juice of plants (sugarcane) that contains a considerable amount of sucrose or sugar).

The seed to be inoculated with Microphos as per treatment was heaped on a clean polythene sheet. The inoculants slurry was poured on it and was mixed with seed uniformly. The inoculated seed was air-dried in shade and used for sowing. Pigeonpea ''Pusa 992'' was sown at the seed rate of 15 kg/ha by 'pora' method (Special type metallic tube attached with desi plough) on 16^{th} June, 2008 and 18^{th} June, 2009, as per treatments in rows 60 cm apart, plant to plant spacing was maintained 15 to 20 cm apart by adopting gap filling and thinning at appropriate time. For weed control, pre-emergence spray of stomp (pendimethalin) at a rate of 1.0 kg/ha was done. Beside, herbicide application one hand weeding was done at 30 days after sowing (DAS). Irrigation to crop was provided only to supplement the rainfall. For control of blister beetle and pod borer in pigeonpea, two spraying of monocrotophos at a rate of 0.04% were given. Pigeonpea was grown as per recommended practices and was harvested on 9 and 14^{th} of November during both the years of experimentation (2008 and 2009), respectively. Calendar of cultural operations are given in Table 1.

Plant height of five randomly selected and tagged plants in each plot was measured from the base to the tip of the plant at 30, 60, 90 and 120 DAS. Leaf area was measured by separating leaves of five randomly selected plants from the stem and cleaned with de-ionized water and then dried with tissue paper. The area of fresh green leaves for each treatment was measured by using leaf area meter (Model LICOR 3000, USA) and was expressed in cm^2/plant. Leaf area index (LAI) was calculated at 30, 60, 90 and 120 DAS using the formula as suggested by Evans (1972).

Table 1. Calendar of cultural operations in pigeonpea.

S/N	Operation	Date of operation	
		2008-2009	2009-2010
1	Collection of soil samples for physico-chemical analysis	16.06.08	18.06.09
2	Field preparation	16.06.08	18.06.09
3	Lay out and bed preparation	18.06.08	20.06.09
4	Sunflower stover incorporation	19.06.08	22.06.09
5	Fertilizer application	20.06.08	24.06.09
6	Sowing of crop	21.06.08	24.06.09
7	Pre -plant incorporation of pendimethalin at 1.0 l/ha	20.06.08	23.06.09
8	Thinning and gap filling	14.07.08	14.07.09
9	Intercultural operations	22.07.08	24.07.09
10	Irrigation		
i	First irrigation	10.09.08	08.07.09
ii	Second irrigation	03.10.08	22.10.09
11	Plant protection measures		
i	First spraying of insecticide.	08.10.08	06.10.09
ii	Second spraying of insecticide.	29.10.08	02.11.09
12	Harvesting	08.12.08	14.12.09
13	Threshing	22.12.08	28.12.09

LAI = Total leaf area per plant (cm^2) / Land area under per plant (cm^2)

These plants were sun-dried for 2 to 3 days and oven-dried at 60-65 ± 2°C for 48 h and dry weight was recorded with the help of an electronic pan balance (Mettler, Type K7T, Swiss made) and expressed as g/plant. Crop growth rate (CGR) were calculated by using the fallowing formula and expressed as g/m^{-2}/day.

$$CGR = W_2 - W_1/T_2 - T_1 x A$$

Where = W_2 and W_1 are final and previous dry weight, T_2 and T_1 is the time of final and previous observation and A is the area per plant.

At the time of maturity, the net plots (2.40 × 15.0 m) were harvested and grain and stover yields were recorded after sun drying and expressed in Mg/ha. For the calculation of harvest index, economic yield (grain) was divided by grain+stover yield and multiplied by 100 and expressed in percentage. For quality determination, protein content and protein yield was calculated by the following formula.

Protein content = Nitrogen content × 6.25

Grain protein yield (kg/ha) = 6.25 × nitrogen uptake in grain (kg/ha)

The plant samples collected for dry matter accumulation (DMA) estimation were ground into fine powder and pass through a 40-mm mesh sieve and used for chemical analysis to find out the nitrogen and phosphorus content in plants at 60, and 120 DAS and at harvest. Nitrogen and phosphorus content was estimated by Kjeldahl's method and Vanado-molybdophosphoric yellow colour method, respectively. The nutrient content was expressed in percentage. All the data obtained from pigeonpea for 2 consecutive years of study were statistically analyzed using the *F*-test the procedure given by Gomez and Gomez (1984). Critical difference (CD) values at p = 0.05 were used to determine the significance of differences between means.

RESULTS

Growth

In general, plant height (Table 1), LAI (Figures 1 and 2), DMA (Figures 3 and 4) and CGR (Table 3) increased as the crop advanced in age and reached to the maximum at maturity except LAI and CGR, which recorded increase only up to 90 and 120 DAS, respectively. Initially, plant growth in terms of plant height, LAI, DMA and CGR was slow up to 30 days of sowing and thereafter, the rate of increase took place at a faster rate and reached at peak between 60 and 90 DAS with respect to plant height (Table 2) and LAI and between 90 to 120 DAS with respect to DMA and CGR in both years and it declined towards maturity. The values of growth parameters recorded during 2008 were considerably higher at all the growth stages than 2009. Perusal of data indicates that sunflower stover incorporation had adverse effect on the growth parameters, as a result lower plant height, LAI, DMA and CGR were recorded at all the growth stages of pigeonpea during both years of experimentation over the control. The effect of sunflower stover incorporation on plant height and CGR was significant during both the season except at 30 DAS with respect to plant height and 30 and 120 DAS with respect to CGR. Sunflower stover incorporation reduces the LAI and DMA significantly only

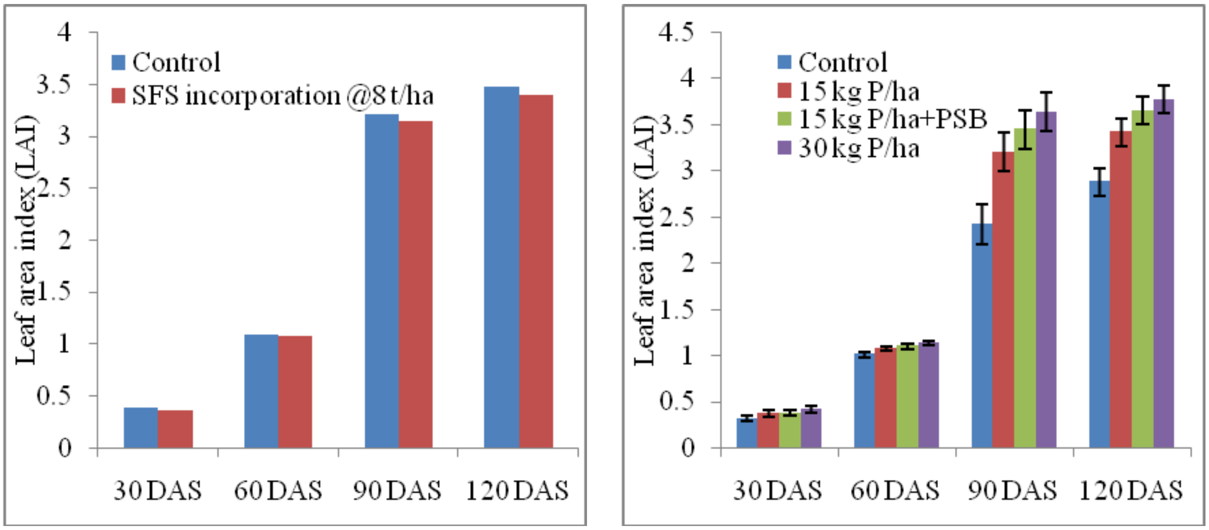

Figure 1. Effect of sunflower stover and nutrient management on the leaf area index (LAI) of pigeonpea-2008. The *vertical bars* indicate C.D. at p = 0.05

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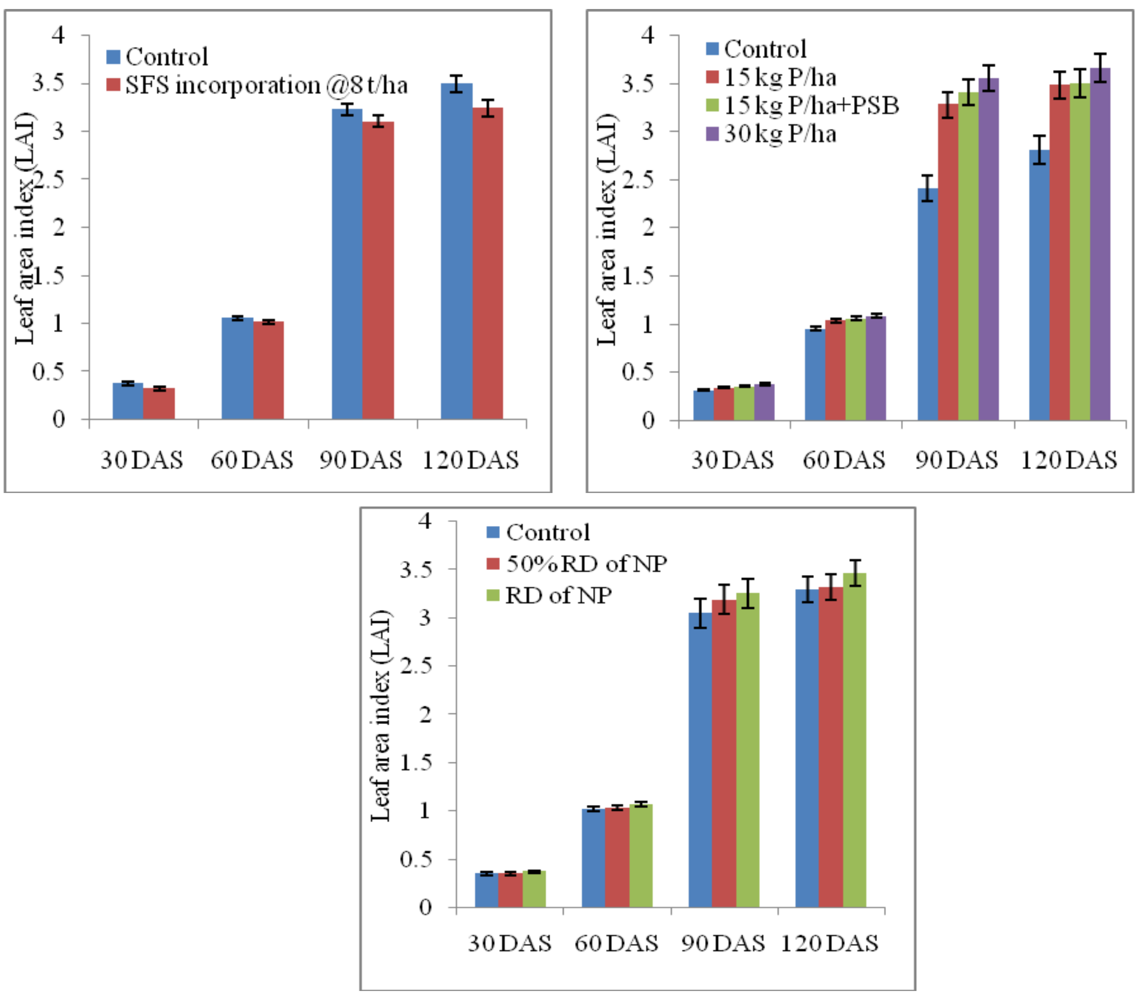

Figure 2. Effect of sunflower stover and nutrient management on leaf area index (LAI) of pigeonpea-2009. The *vertical bars* indicate C.D. at p = 0.05.

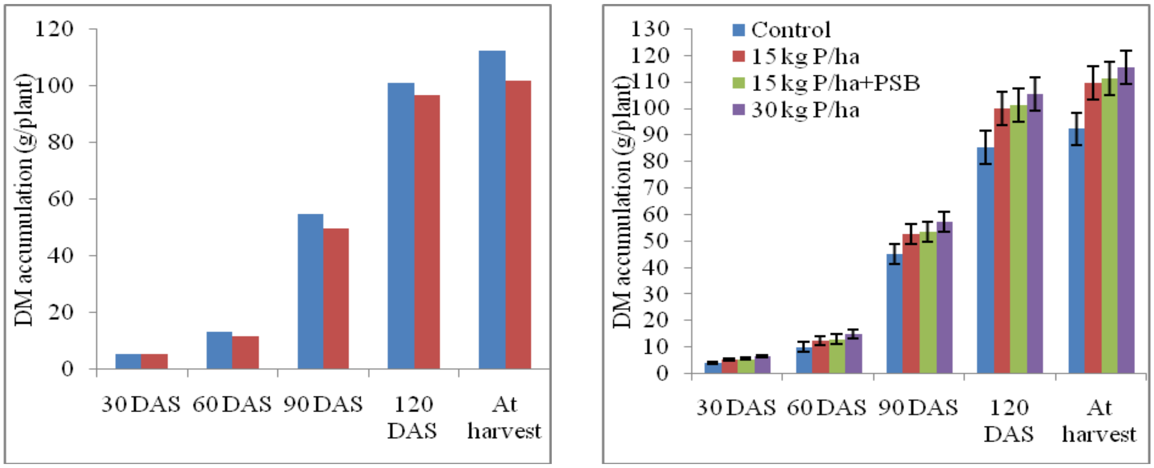

Figure 3. Effect of sunflower stover and nutrient management on dry matter accumulation (DMA) (g/plant) of pigeonpea-2008. The vertical bars indicate C.D. at p = 0.05.

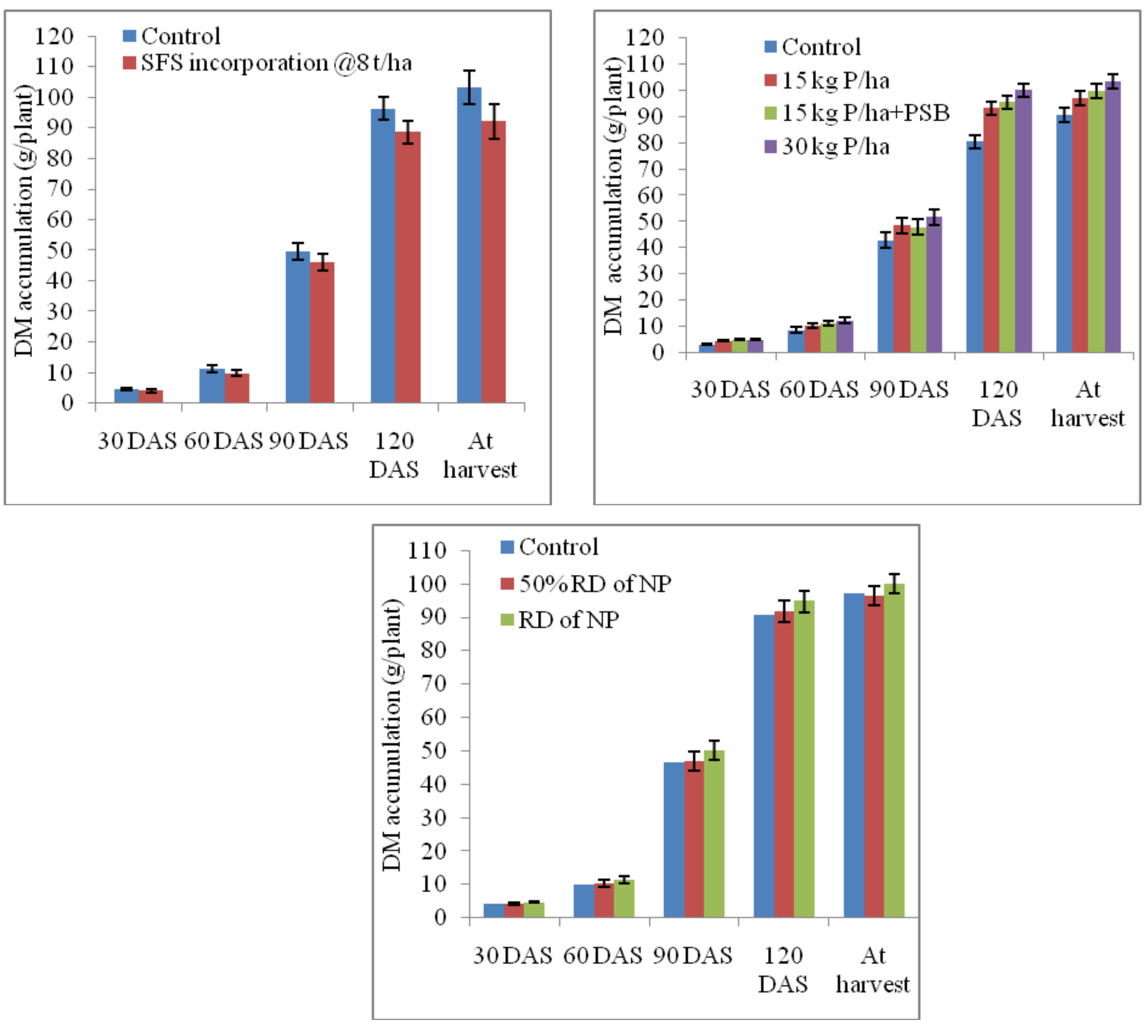

Figure 4. Effect of sunflower stover and nutrient management on dry matter accumulation (DMA) (g/plant) of pigeonpea-2009. The *vertical bars* indicate C.D. at p = 0.05.

Table 2. Effect of sunflower stover and nutrients management on plant height of pigeonpea.

Treatment	Plant height (cm)									
	30 DAS		60 DAS		90 DAS		120 DAS		At harvest	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Direct effect of SFS management										
Control	39.3	36.8	92.5	83.0	159.1	145.3	197.0	191.2	210.0	196.4
SFSI at 8 t/ha	32.1	28.7	86.6	72.6	151.1	139.2	187.9	180.7	187.4	184.2
SEm±	1.2	0.5	0.74	1.1	1.17	0.9	1.4	1.1	2.6	1.7
CD (p = 0.05)	NS	2.9	4.49	6.8	7.09	5.3	8.6	6.6	15.8	10.4
Direct effect of P levels										
Control	29.2	28.0	80.0	71.3	145.3	138.5	175.5	173.2	179.0	178.8
15 kg P/ha	35.5	33.1	90.5	78.2	156.3	141.6	193.0	187.5	197.7	188.3
15 kg P/ha + PSB	36.8	33.3	92.6	79.5	156.8	142.3	195.5	189.0	205.8	194.2
30 kg P/ha	41.3	36.7	95.2	82.1	161.9	146.4	205.8	194.1	212.4	199.9
SEm±	0.8	0.6	1.68	1.0	1.5	0.6	1.6	1.0	2.2	1.4
CD (p = 0.05)	2.4	1.9	5.18	3.2	4.5	2.0	5.1	3.0	6.8	4.2
Residual effect of NP doses applied to sunflower										
Control	-	31.9	-	76.8	-	140.9	-	183.8	-	187.2
50% RD of NP	-	32.3	-	76.9	-	141.3	-	185.0	-	189.9
RD of NP	-	34.2	-	79.6	-	144.5	-	189.0	-	193.9
SEm±	-	0.6	-	0.6	-	0.7	-	1.0	-	1.2
CD (p = 0.05)	-	1.8	-	1.7	-	2.0	-	3.0	-	3.5

SFSI: Sunflower Stover Incorporation, RD of NP: 80 kg N + 15 kg P/ha

during 2009. P-levels significantly improve the plant height, LAI, DMA and CGR over the control at all growth stage of pigeonpea during both years. Except CGR at 30-60 DAS, at this stage control was on par with 15 kg P/ha. Among the P levels, application of 30 kg P/kg recorded the highest plant height, LAI, DMA and CGR over rest of the treatments at all the growth stages. Plant height with 30 kg P/ha was significantly higher at all the growth stages except 60 DAS, where it remained on par with 15 kg P/ha + PSB during the both years of study. The application of phosphorus at 30 kg P/ha recorded significantly higher LAI at 30 and 60 DAS during the both years and at 90 and120 DAS during 2009. Application of 30 kg P/ha remained statistically on par with 15 kg P/ha + PSB at 90 and 120 DAS during first year (2008). The application of 30 kg P/ha being on par with 15 kg P/ha + PSB at 30 and 90 DAS in 2008 and at 60 and 120 DAS, and at harvest in 2009, produced the highest dry matter at all stages of crop growth. NP doses applied to sunflower had significant residual effect on plant height, LAI, DMA and CGR of pigeonpea under pigeonpea-sunflower cropping system. Residual effect of RD of NP registered significantly higher plant height, LAI, DMA and CGR over ½ RD of NP and control except LAI at 120 DAS, where RD and ½ RD remained at par.

Yield and harvest index

Results of treatment effects on grain, stover, yields and harvest index of pigeonpea are presented in Table 4. In general, grain and stover yields of pigeonpea were higher during first year as compared to second year irrespective of treatments. During first year, approximately 29% higher grain yield was recorded as compared to second year. Similar trend was observed with respect to stover yield. Sunflower stover incorporation recorded lower grain and stover yields during both years over the control, but the margin was significant only during second year. In second year, grain, and stover yields reduction due to sunflower stover incorporation was around 25.8% over the control (no stover incorporation). Application of different levels of P caused marked increase in grain and stover yields over the control. Among the different levels of P, application of 30 kg P/ha recorded significantly higher grain yield (1.63 and 1.32 Mg/ha) over rest of the P levels during the both years of study.

Application of 15 kg P/ha induced 41.4 and 50% increase in grain yield over control in respective season. Percent increase due to 15 kg P/ha + PSB was 52.5 and 57% and due to 30 kg P/ha, was 65 and 78% over control during 2008 and 2009, respectively. Residual effect of

Table 3. Effect of sunflower stover and nutrient management on crop growth rate (CGR) of pigeonpea.

Treatment	CGR ($g/m^2/day$)									
	0 - 30 DAS		30 - 60DAS		60 - 90DAS		90 - 120 DAS		120 DAS - At harvest	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
SFS management										
Control	1.51	1.32	2.20	1.84	11.47	10.61	12.70	13.34	1.99	1.32
SFSI at 8 t/ha	1.49	1.14	1.82	1.60	10.45	9.98	12.53	12.34	1.24	1.14
SEm±	0.04	0.02	0.03	0.02	0.23	0.08	0.52	0.11	0.05	0.02
CD (p = 0.05)	NS	0.15	0.20	0.10	NS	0.51	NS	0.70	0.28	0.13
P levels										
Control	1.16	0.90	1.66	1.52	9.70	9.47	10.85	11.93	1.19	0.90
15 kg P/ha	1.49	1.25	1.96	1.61	11.16	10.61	13.10	12.58	1.69	1.25
15 kg P/ha + PSB	1.56	1.36	2.07	1.76	11.23	10.19	13.23	13.30	1.74	1.36
30 kg P/ha	1.80	1.42	2.36	2.01	11.75	10.90	13.30	13.56	1.84	1.42
SEm±	0.05	0.03	0.09	0.04	0.32	0.18	0.50	0.17	0.09	0.03
CD (p = 0.05)	0.14	0.10	0.28	0.11	1.00	0.56	1.55	0.52	0.27	0.10
NP doses applied to preceding sunflower										
Control	-	1.17	-	1.64	-	10.13	-	12.58	-	1.17
50% RD of NP	-	1.20	-	1.65	-	10.14	-	12.78	-	1.20
RD of NP	-	1.32	-	1.88	-	10.60	-	13.17	-	1.32
SEm±	-	0.02	-	0.05	-	0.14	-	0.16	-	0.02
CD (p = 0.05)	-	0.07	-	0.14	-	0.39	-	0.46	-	0.06

SFSI: Sunflower stover incorporation, RD of NP: 80 kg N+15 kg P/ha.

50% RD of NP on the grain and stover yields of pigeonpea was not observed over the control, while that of RD of NP was significant over 50% RD of NP and control.

Significantly, higher grain (1.17 Mg/ha) and stover (4.7 Mg/ha) yields were recorded with residual effect of RD of NP, which was 10.37% higher over residual effect of 50% RD of NP and 14.70% over control with respect to grain yield. Sunflower stover incorporation failed to affect the HI statistically during the both years. HI was not influenced due to P levels during first year (Table 4). Application of 30 kg P/ha recorded maximum HI (21.5 and 20.5%) during both years. NP doses applied to preceding sunflower caused significant variation in harvest index of succeeding pigeonpea under pigeonpea-sunflower cropping system. Significantly higher harvest index (19.60%) was recorded due to residual effect of RD of NP over control.

Quality

Data pertaining to protein content in grain and its yield in pigeonpea is given in Table 4. Effect of sunflower stover management was not observed on protein content and yield in grains of pigeonpea during first year. In the second season, significantly lower protein content (2.5%) and protein yield (27.84%) was recorded due to sunflower stover incorporation. Application of 30 kg P/ha recorded the maximum protein content in grain. However, it remains statistically on par with other 15 kg P/ha and 15 kg/ha + PSB. Significantly higher protein yield (331.3 and 271.4 kg/ha) was recorded with the application of 30 kg P/ha over rest of the treatment during the both years of study, which lead to 78.8 and 50.9% increase in the protein yield over control. Residual effect of NP applied to sunflower was not observed on protein content in grains of pigeonpea. Significantly higher protein yield (237.6 kg/ha) was recorded due to residual effect of RD of NP compared to the residual effect of 50% RD of NP and control. This increase in protein yield was 13.2 and 18.4% over 50% RD of NP and control, respectively.

Nutrients content

N and P content in plant (at 60 and 120 DAS) and in grain and stover at harvest are given in Tables 5 and 6, respectively. Data showed that N and P content declined from 60 DAS to harvest stage. N and P content was more in grain. At 60 and 120 DAS, sunflower stover incorporation reduced N and P content in plants significantly over no stover incorporation only during 2009. During 2008, effect of P-levels (15 kg P/ha,

Table 4. Effect of sunflower stover and nutrient management on grain, stover yields, harvest index and protein content and yield of pigeonpea.

Treatment	Grain yield (Mg/ha)		Stover yield (Mg/ha)		Harvest index (%)		Protein content (%)		Protein yield (kg/ha)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
SFS management										
Control	1.42	1.24	5.7	5.2	20.1	19.2	19.8	20.0	282.3	251.0
SFSI at 8 t/ha	1.35	0.92	5.0	3.9	21.3	19.1	19.6	19.5	267.2	181.1
SEm±	0.02	0.02	0.17	0.04	0.34	0.13	0.12	0.07	5.0	4.3
CD (*p* = 0.05)	NS	0.11	NS	0.23	NS	NS	NS	0.42	NS	26.0
P levels										
Control	0.99	0.74	3.9	3.4	20.3	17.7	18.6	17.9	185.2	131.8
15 kg P/ha	1.40	1.11	5.6	4.7	20.2	19.1	19.8	20.1	277.4	223.0
15 kg P/ha + PSB	1.51	1.16	5.8	4.9	20.9	19.3	20.2	20.4	305.1	238.0
30 kg P/ha	1.63	1.32	6.0	5.1	21.5	20.5	20.3	20.5	331.3	271.4
SEm±	0.03	0.02	0.18	0.07	0.67	0.31	0.19	0.28	6.3	6.4
CD (*p* = 0.05)	0.09	0.07	0.54	0.22	NS	0.97	0.59	0.87	19.4	19.8
NP doses applied to preceding sunflower										
Control	-	1.02	-	4.4	-	18.7	-	19.5	-	200.7
50% RD of NP	-	1.06	-	4.4	-	19.1	-	19.6	-	209.9
RD of NP	-	1.17	-	4.7	-	19.6	-	20.1	-	237.6
SEm±	-	0.02	-	0.06	-	0.23	-	0.2	-	4.8
CD (*p* = 0.05)	-	0.07	-	0.16	-	0.66	-	NS	-	13.8

SFSI: Sunflower stover Incorporation, RD of NP: 80 kg N+15 kg P/ha.

Table 5. Effect of sunflower stover and nutrient management on N content of pigeonpea.

Treatment	N content (%)							
	60 - 120 DAS				At harvest			
	Shoot				Grain		Stover	
	2008	2009	2008	2009	2008	2009	2008	2009
SFS management								
Control	2.22	2.04	1.53	1.45	3.17	3.19	1.27	1.29
SFSI at 8 t/ha	2.20	1.99	1.50	1.41	3.14	3.12	1.25	1.25
SEm±	0.043	0.008	0.025	0.006	0.013	0.011	0.005	0.005
CD (*p = 0.05*)	NS	0.049	NS	0.037	NS	0.067	NS	0.028
P levels								
Control	2.12	1.90	1.42	1.33	2.98	2.87	1.2	1.17
15 kg P/ha	2.21	2.01	1.50	1.44	3.17	3.21	1.25	1.28
15 kg P/ha + PSB	2.24	2.06	1.57	1.46	3.23	3.26	1.29	1.3
30 kg P/ha	2.26	2.08	1.57	1.49	3.25	3.28	1.32	1.34
SEm±	0.028	0.009	0.025	0.008	0.033	0.045	0.026	0.008
CD (*p = 0.05*)	0.087	0.027	0.077	0.023	0.1	0.139	0.081	0.023
NP doses applied to preceding sunflower								
Control	-	2.00	-	1.41	-	3.11	-	1.26
50% RD of NP	-	2.01	-	1.43	-	3.13	-	1.27
RD of NP	-	2.03	-	1.44	-	3.22	-	1.28
SEm±	-	0.009	-	0.007	-	0.032	-	0.007
CD (*p = 0.05*)	-	NS	-	NS	-	NS	-	NS

SFSI: Sunflower stover incorporation, RD of NP: 80 kg N + 15 kg P/ha.

Table 6. Effect of sunflower stover and nutrient management on P content of pigeonpea.

Treatment	P content (%)							
	60 - 120 DAS				At harvest			
	Shoot				Grain		Stover	
	2008	2009	2008	2009	2008	2009	2008	2009
SFS management								
Control	0.34	0.29	0.13	0.15	0.25	0.27	0.11	0.13
SFSI at 8 t/ha	0.27	0.23	0.12	0.12	0.23	0.24	0.10	0.10
SEm±	0.017	0.003	0.005	0.005	0.005	0.003	0.003	0.001
CD (p = *0.05*)	NS	0.019	NS	0.030	NS	0.021	NS	0.008
P levels								
Control	0.19	0.13	0.09	0.09	0.22	0.23	0.09	0.09
15 kg P/ha	0.31	0.27	0.12	0.13	0.23	0.25	0.10	0.11
15 kg P/ha + PSB	0.34	0.31	0.14	0.16	0.25	0.26	0.10	0.13
30 kg P/ha	0.37	0.33	0.16	0.17	0.26	0.29	0.11	0.13
SEm±	0.016	0.009	0.006	0.005	0.007	0.006	0.004	0.003
CD (p = *0.05*)	0.048	0.029	0.019	0.017	0.021	0.017	0.012	0.009
NP doses applied to preceding sunflower								
Control	-	0.24	-	0.12	-	0.24	-	0.11
50% RD of NP	-	0.26	-	0.14	-	0.26	-	0.11
RD of NP	-	0.28	-	0.15	-	0.28	-	0.13
SEm±	-	0.010	-	0.005	-	0.005	-	0.003
CD (p = *0.05*)	-	0.030	-	0.013	-	0.015	-	0.008

SFSI: Sunflower stover incorporation, RD of NP: 80 kg N + 15 kg P/ha.

15 kg P/ha + PSB/ha and 30 kg P/ha) on N content in plant was found to be statistically at par with each other but significantly superior over the control, while in 2009, N content in plant at 30 kg P/ha being on par with 15 kg P/ha + PSB and significantly higher over 15 kg P/ha and control (Table 5). With respect to P content, application of 30 kg P/ha was slightly better than 15 kg P/ha + PSB but statistically at par with each other. Application of 30 kg P/ha recorded maximum P content at 60 DAS (0.37 and 0.33%) and at 120 DAS (0.16 and 0.17%). This led to 94.7 and 153.8% increment in P content at 60 DAS and 77.7 and 88.9% at 120 DAS over the control (Table 6). At harvest stage, negative effect of sunflower stover incorporation on N and P content in grain and stover was not observed during 2008, while in 2009 sunflower stover incorporation reduced N and P content both in grain and stover significantly. At harvest stage, P application resulted in higher N and P content in seed and stover over control.

Among the P levels, differences were not significant during the both years with respect to N except in second year where application of 30 kg P/ha recorded significantly higher N content over the 15 kg P/ha. P application was effective to enhance P content during the both years, the increment was continued till the next dose but the rate was reduced. Above all, various P treatments were far better than absolute control (no P). Among the various levels of P, application of 30 kg P/ha recorded maximum P content in grain (0.26 and 0.29%) and in stover (0.11 and 0.13%), but it was statistically at par with 15 kg P/ha + PSB during the both years of study. The residual effect of NP doses applied to sunflower was not found significant on N content in succeeding pigeonpea. The residual effect of NP applied to sunflower had significant effect on the P content in grain and stover of succeeding pigeonpea crop under pigeonpea-sunflower cropping system in 2009. Residual effect of RD of NP recorded significantly higher concentration of P in grain (0.28%) and stover (0.13%) over the control.

DISCUSSION

Growth

Sunflower stover incorporation had an adverse effect on the growth parameters, as a result lower plant height were recorded at all the growth stages of pigeonpea during the both years of experimentation over the control.

The effect of sunflower stover incorporation on plant height and CGR was significant during both season except at 30 DAS with respect to plant height (Table 2) and 30 and 120 DAS with respect to CGR (Table 3). However, significantly lower values of LAI and DMA only during 2009. This might be due infestation of wilt disease and also due to the buildup of allelo-chemicals in soil due to continuous cultivation of sunflower crop and incorporation of sunflower stover in the same plot and also during second year of experiment initial phase of crop experienced with heavy rainfall. Heavy rainfall besides water logging may have induced distribution of allelochemicals into the soil profile. Leachates of allelochemicals reduced the seed germination, root growth, enzymatic activity, cell division, hormonal activity mainly GA and auxins, photosynthesis and respiration (Batlang and Shushu, 2007). These results were supported by the findings of Anjum and Bajwa (2010) and Nawab et al. (2011). P-levels significantly improve the plant height, LAI, DMA and CGR over the control at all growth stage of pigeonpea during the both years. Except CGR at 30 to 60 DAS, at this stage the control was similar to the 15 kg P/ha treatment (Table 3). Plant height with 30 kg P/ha was significantly higher at all the growth stages except 60 DAS, where it remained similar to the 15 kg P + PSB/ha treatment during the both years of study. With regards to LAI, application of phosphorus at a rate of 30 kg P/ha recorded significantly higher LAI at 30 and 60 DAS during both years and at 90 and120 DAS during 2009 (Figures 1 and 2). Similarly in case of DMA, application of 30 kg P/ha being similar to 15 kg P + PSB/ha at 30 and 90 DAS in 2008 and at 60 and 120 DAS and at harvest in 2009, produced the highest dry matter at all stages of crop growth (Figures 3 and 4). However, both the treatments remained statistically at par in case of CGR at most of the crop period. Results indicate that PSB enhanced P solubilization and increased available P, which may have facilitated better nitrogen utilization and thus better crop growth. The overall improvement in crop growth due to phosphorus application seems to be on account of its pivotal role in early formation of roots and aerial parts through cell division, their proliferation and increased microbial activities in root nodules and energy transfer and oxidation reduction reactions. This might have improved effective utilization of soil nutrients by crop and grater biological nitrogen fixation through enhancement in nitrogenase activity (Tisdale et al., 1995), retaining more leaf area (Pandey and Sinha, 2002). These results are corroborated with the findings of Singh and Yadav (2008) and Chaudhari et al. (2010). Residual effect of RD of NP registered significantly higher plant height, LAI, DMA and CGR over ½ RD of NP and control except LAI at 120 DAS, where RD and ½ RD remained at par. This was probably due to the carry over effect of nutrients applied to preceding crop. Corroborative findings were also reported by Shivran et al. (2000).

Yield and harvest index

In general, grain and stover yields of pigeonpea were higher during first year as compared to second year irrespective of treatments (Table 4). This might be due to higher infestation of wilt disease and accumulation of allelochemicals due to growing of sunflower continuously in same plot during the second year of experimentation. Sunflower stover incorporation recorded lower grain and stover yields during the both years over the control, but the margin was significant only during the second year. With regards to HI, sunflower stover incorporation failed to affect the HI statistically during both the years (Table 4). It might be due to higher concentration of secondary and primary metabolites of sunflower due to continuous growing of sunflower and decomposition of sunflower stover, causing more inhibitory effect on the plant growth and yield of pigeonpea. Narwal et al. (2003) detected chlorogenic acid, caffeoylquinic acid and neochlorogenic acid through chromatographic studies in sunflower stems at varying concentrations, indicating that these phenolic compounds might be responsible for growth and yield reductions in succeeding crops. Rana et al. (2004) also reported that sunflower as preceding crop caused 51% reduction in the yield of succeeding legume crops compared to maize as a preceding crop. The results of the present investigations are in accordance with the findings of Narwal et al. (1999) and Ashrafi et al. (2008). Application of different levels of P caused marked increase in grain and stover yields over the control. Application of 30 kg P/ha recorded maximum HI (21.5 and 20.5%) during the both years. These results are supported by the findings of Singh and Yadav (2008). Residual effect of 50% RD of NP on the grain and stover yields of pigeonpea was not observed over the control, while that of RD of NP was significant over 50% RD of NP and control. Residual effect of RD of NP, which was 10.37% higher over residual effect of 50% RD of NP and 14.70% over control with respect to grain yield. With respect to HI, NP doses applied to preceding sunflower caused significant variation in harvest index of succeeding pigeonpea under pigeonpea-sunflower cropping system. Significantly higher harvest index (19.60%) was recorded due to residual effect of RD of NP over control.

Quality

Quality of harvested product is as important as the volume of the produce. A healthy crop raised under favourable climatic condition, in general give quality produce and here the role of nutrient management becomes significant. Effect of sunflower stover management was not observed on protein content and yield of pigeonpea during first year. In the second season, significantly lower protein content and protein yield was recorded due to sunflower stover incorporation. This might be due to secretion and accumulation of allelochemicals by continuous growing of sunflower and decomposition of sunflower stover. Allelochemical retards the protein synthesis in plants (Einhelling, 1986). Reduction in protein content due to the higher concentration of sunflower allelochemicals was also reported by Stodder et al. (1993) in pea and Mehboob et al. (2000) in linseed. Protein yield is a product of protein content and grain yield. Sunflower stover incorporation reduces the grain yield of pigeonpea and ultimately protein yield. Reduction in grain yield of pigeonpea due to sunflower allelopathy was reported by Pal and Sand (2006). Protein content is a qualitative trait and is significantly influenced by P application. Among the P levels, application of 30 kg P/ha resulted in the greatest protein content in grain, but it was not statistically different than the 15 kg P/ha and 15 kg + PSB/ha treatments. Significantly higher protein yield was recorded with the application of 30 kg P/ha over rest of the treatment during the both years of study. P being responsible for synthesis of nucleic acid and an ingredient of phospho-proteins, play a central role in synthesis of protein. Improvement in protein content and grain protein yield due to phosphorus application has also been reported by Jat and Ahlawat (2001) and Singh and Yadav (2008). Residual effect of NP applied to sunflower was not observed on protein content. Although, significantly higher protein yield was recorded due to residual effect of RD of NP compared to the residual effect of 50% RD of NP and control.

Nutrient content

N and P content in plant at 60 and 120 DAS and in grain and stover at harvest are given in Tables 5 and 6, respectively. N and P content in shoots declined between 60 and 120 DAS. N and P content in shoot and in seed and stover at harvest were reduced by sunflower stover incorporation during both year, but it was only significant during 2009. This might be due to negative allelopathic effect of sunflower. With regard to P levels, during 2008, effect of P-levels (15 kg P/ha, 15 kg P + PSB/ha and 30 kg P/ha) on N content in plant was found to be similar but significantly greater than the control, while in 2009, N content in plant at 30 kg P/ha was significantly higher over 15 kg P/ha and control treatments. With respect to P content, P application was effective in enhancing P content during both years (Table 6). Moreover, pigeonpea growth and yield generally responded better to P application than no P. Application of 30 kg P/ha resulted in maximum P content in shoot at 60 DAS, 120 DAS and at harvest in grain and stover but it was not significantly different than the 15 kg P + PSB/ha treatment during both years of study. This could simply be attributed to phosphorus providing more balanced nutrition for plants resulting in higher photosynthetic efficiency that favour growth and yield. When P availability is enhanced in a symbiotically N-fixing system; N content in plants is normally increased (Buresh and Smithson, 1997). Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and improving biological nitrogen fixation (Kucey et al., 1989). PSB increased the P content in plant because some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Hilda and Fraga, 2000). The increases in nutrient content with phosphorus fertilization are in line those of Jat and Ahlawat (2001) and Chaudhari et al. (2010). The residual effect of NP doses applied to sunflower was not found significant on N content of succeeding pigeonpea. However, the residual effect of NP applied to sunflower had significant effect on the P content in grain and stover of succeeding pigeonpea crop under pigeonpea-sunflower cropping system. Residual effect of RD of NP recorded significantly higher concentration of P in grain and stover over the control. This was due to the fact that higher grain yield recorded with the residual effect of RD of NP.

Conclusion

Based on above study, it is concluded that continuous incorporation of sunflower stover in the fixed plot may cause the negative effect on growth, productivity, protein content and nutrient status of pigeonpea. In India, pigeonpea responses well up to 30 kg P/ha, hence for obtaining higher yield of pigeonpea use of 30 kg P/ha may be recommended.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Preliminary genetic diversity Analysis of introduced and local Zimbabwe cowpea landraces

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Cowpea, *Vigna Unguiculata* plays an important role in human diets because of its good protein quality and high nutritional value. On hand are two cowpea lines sourced from a local Zimbabwean humanitarian organization, with an aim to diversify the local germplasm base of cowpea. Activities involved characterizing and evaluating the foreign sourced lines alongside local ones for morphological and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) diversity. Five seed characteristics were evaluated, namely seed size, color, uniformity, presence or absence of seed spots and hilum color using specified scales. For RAPD-PCR analysis, a set of five oligonucleotides was used. DNA extraction, quantification, amplification, electrophoresis and band analysis of presence or absence of bands was carried out. Data was collected and an ANOVA was carried out using genstat discovery software. After an ANOVA, principal components analysis (PCA) and hierarchical cluster analysis were used to examine distribution patterns in the germplasm. Significant differences were observed for the five variables (p<0.001). Both morphological and molecular data for the cowpea lines show limited diversity from results of multivariate analysis. The dendrogram for morphological data shows an overall similarity index of 78%, whereas for molecular data shows a similarity index of 75%. Of the two foreign lines, the white seeded foreign line, NGO1, seems to be consistently diverse from the rest. It will be a valuable addition to the collection and will be useful for possible introgression in future. The genetic diversity for the collection is generally low and may require continued enrichment with introductions in future.

Key words: Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) characterization, variables, primers, electrophoresis, gel scoring.

INTRODUCTION

Cowpea, *Vigna unguiculata* is a tropical grain legume which plays an important nutritional role in developing countries of the tropics and subtropics, namely sub-Saharan Africa, Asia, Central and South America (Badiane et al., 2012; Fang et al., 2007; Singh et al., 1997). The African origin of cowpea was suggested as early as 1847 and since then no one is contesting the idea because wild cowpea plants are found only in tropical Africa and Madagascar. However, where the crop was first domesticated is still uncertain (Pasquet, 1999).

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The genus *Vigna* currently includes around 80 species of which *unguiculata* is one (Ba et al., 2004). The crop is grown for consumption of its young leaves green pods and dry seeds (Zannou et al., 2004). The residues are important as stock feed (Sharawy and El-Fiky, 2002). Cowpea is often termed the “poor man’s meat” because the seed protein contents range from 23 to 32% of seed weight and are rich in lysine and tryptophan, and a substantial amount of mineral and vitamins, folic acid and vitamin B (Huaqiang et al., 2012). Cowpea is reported as having superior nutritional qualities to dry beans (*Phaseolus vulgaris* L.) (Spiaggia et al., 2008). The Food and Agriculture Organization, 2009, acknowledges that of the twelve and a half metric tons of cowpea produced globally, more than sixty four percent are from resource-poor, subsistent production. Cowpea is reported as the most cultivated and the most consumed among grain legumes especially in Asia and in tropical Africa (Diouf, 2011).

Cowpea can grow under low fertility conditions due to its ability to fix nitrogen and withstand the low pH (Prasanthi et al., 2012). The plant is generally drought tolerant and is effectively used in rotation with cereals for soil fertility restoration. However, the yields produced which average 1 ton/ha in sub-Saharan regions, fail to meet the needs of consumers (FAO, 2009). In drought prone areas like Southern Africa, in particular Zimbabwe, cowpea usually survives dry spells and is one of the few crop species that may escape moisture stress due to its short life span (Nhamo et al., 2003). Its importance as a rescue species during humanitarian crisis cannot be overemphasized as the grain is often donated to human communities struggling for nutrition.

However, the genetic diversity in the species is reportedly narrow, in spite of substantial variation in seed color, seed proteins, plant type, pod type and seed size among cultivated cowpeas (Sharawy and El-Fiky, 2002). Morphological characterization is the first step in the description and classification of germplasm collections (Sarutayophat et al., 2007).

Information regarding genetic diversity is a key component for the development of novel and desirable traits (Kholghi et al., 2011). Molecular characterization is also increasingly becoming an important source of genetic information. Molecular markers have been observed to detect more genetic diversity than morphological and protein-based markers in cowpea as they can identify functional as well as neutral genetic variation (Zannou et al., 2008). Sometimes morphological attributes do not necessarily reflect real genetic relationships as the environment has significant effect on morphological traits.

Estimating genetic diversity and determining the relationships among germplasm collections enhances efficient germplasm, management and genetic improvement (Iqbal et al., 2014). The study was carried out as a preliminary evaluation of two NGO lines meant for drought relief, although some farmers in Zimbabwe now seem interested in them alongside local landraces. This may be important to enrich the genetic base of cowpea which is important because germplasm with wider genetic base provides buffer and resilience against climatic and other environmental changes and ensures sustainable food security (Adewale et al., 2011). It is hoped they can be adopted in the national breeding program for a more widespread evaluation and eventual distribution.

MATERIALS AND METHODS

Germplasm

Twelve lines of cowpea *Vigna Anguiculata* were used for morphological characterisation and Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis. Two of the lines were obtained from a local Zimbabwean humanitarian organisation working in Mashonaland Central. These lines were named NGO 1 and NGO 2 in the study. They were meant for drought relief but however farmers now seem keen to perpetuate them along local landraces. Ten other lines were obtained from the national gene bank, six Zimbabwean landraces, Nhepetera, Chikoromo, Nyadahwa 1381, Zai Redahwa, Tsumbe and Nyadahwa 1444 and an additional four foreign lines also kept at the gene bank namely Red Teak, Tinyawa Kalula, Tinyawa Kongonzi and Indumba. For RAPD-PCR analysis, common bean was used as control. A complete list of the lines is shown in Table 1. A picture of seed taken for each accession in the same order as in the table from left to right is shown in Figure 1.

The experiment was carried out in the Biosciences Department, Bindura University of Science Education, Bindura, Zimbabwe. Seed characteristics were scored after which RAPD-PCR characterization was carried out in the laboratory at African institute of Biomedical Research.

Morphological characterisation

The lines show diversity in terms of seed characteristics e.g. colour, shape, size etc. Five seed variables namely seed size, seed colour, seed uniformity, hilum colour, presence and absence of seed spots were evaluated using scales specified in Table 2. Five seeds were scored for each entry in order to carry out ANOVA and also calculate means.

Genstat Discovery Version 4.0 software was used to analyse all the measured traits. The same software was used to carry out the hierarchical cluster analysis and also to establish the level of relatedness among the lines with respect to seed characteristics.

RAPD-PCR characterisation

The following stages were followed towards RAPD-PCR analysis, DNA extraction, quantification, PCR

optimization, amplification, electrophoresis and band analysis. For extraction, the cetyl trimethylammonium bromide (CTAB) protocol was used for the extraction of DNA from the plant tissues (Harisha, 2007; Hoelzel, 1998).

DNA extraction

Fresh leaf tissue was obtained separately from the seedlings of the twelve cowpea lines. They were placed in a mortar, liquid N_2 added to lyophilize the plant tissue before grinding to a fine powder with a

Table 1. Names and accession numbers of the lines used in the study.

S/No	Name of line	Accession number
1	Red Teak	1529
2	Tinyawa Kalula	1778
3	Nhepetera	1358
4	Chikoromo	1491
5	Tinyawa Kongonzi	1777
6	NGO1	-
7	Nyadahwa	1381
8	Zai Redahwa	1349
9	NGO2	-
10	Tsumbe	1366
11	Nyadahwa	1444
12	Indumba	1538

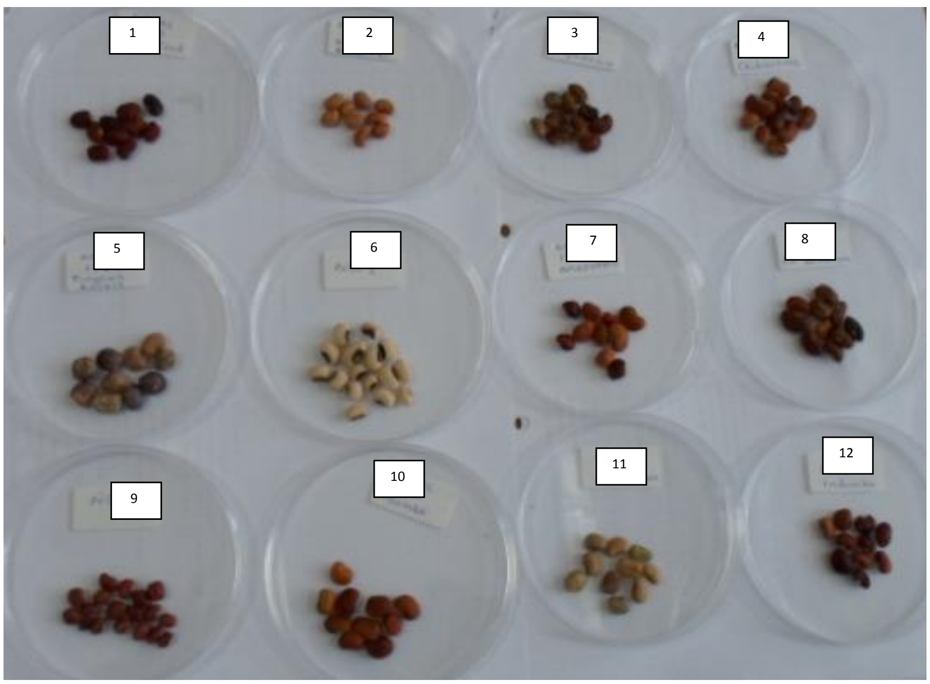

Figure 1. The seed for the twelve lines represented in pictures following their order in the table.

pestle. Two milligram of the fine powder was weighed and put into 2 ml Eppendorf tubes. One milliliter of CTAB buffer previously heated at 65°C for approximately 20 min was added. The contents were mixed gently and incubated in the water bath for 2 h at 65°C. The tubes were removed from the water bath cooled down for five minutes at room temperature five hundred microlitres ofchloroform/iso-amyl alcohol (24:1) were added. It was then mixed by gentle inversion or rocking approximately 100 times. The mixture was spinned in a table-top centrifuge for 10 min at 3600 round per minute (rpm). Six hundred microlitres of clear liquid was pipetted and incubated at room temperature for 15 min after which 400 ul of ice cold isopropanol was added.

The mixture was mixed by gentle inversion for 15 min till a precipitation was formed and then spinned in a centrifuge at 12 000 rpm for 10 min. The isopropanol was decanted carefully and 1 ml of 70% ethanol added, then mixed by inversion. The mixture was centrifuged at 12000 rpm for 10 min. The supernatant was poured off carefully by inverting the tube to obtain the pellet of DNA. The pellet was dried by leaving the tubes open in the laminar air flow. Hundred microliters of TE buffer of pH 8.0 was added and rocked gently overnight in the cold room to dissolve the DNA.

DNA quantification and quality check

DNA quantification was carried out using the UV spectrophotometer. For each sample, 5 ul of the DNA were pipetted and mixed with 995 ul of TE buffer in 1 ml quartz microcuvette. The mixture was read at 260 nm against a TE-buffer blank. The actual quantity of the DNA was calculated by converting the optical density

Table 2. Variable list for morphological characterization.

Variable	Measurement
Seed size	Seed size was rated on a scale of 1 to 3 where 1 is small 2 is medium and 3 is large
Seed colour	Seed colour was rated on a scale of 1 to 5 where 1 is cream, 2 is green, 3 is brown, 4 is red and 5 is black
Seed uniformity	Seed uniformity was rated on a scale of 0 to 1 where 0 is non uniform and 1 is uniformity
Seed spots	Seed spots where rated on a scale of 0 to 1 where 0 is no spots and 1 is spots
Hilum colour	Hilum colour was rated on a scale of 1 to 5 where 1 is cream, 2 is green, 3 is brown, 4 is red and 5 is black

Table 3. List of primers used in the amplification of the twelve lines.

Name of primer	Sequence	References
A03	5'-AGTCAGCCAC-3'	
A07	5'-GAAACGGGTG-3'	
AP1	5'-GGTGCGGGAA-3'	
AP5	5'-AACGCGCAAC-3'	Sharawy and El-Fiky, 2002
OPC-05	5'-GATGACCGCC-3'	Zannou et al. (2008)
OPB-10	5'-CTGCTGGGAC-3'	Prasanthi et al. (2012)

(OD) readings to ug/ml (a reading of 1.0 at OD260 is equivalent to 50ug of DNA/ml) (Weising et al., 1995). The conversion also took into account the dilution factor. Finally all the quantified samples were aliquoted into dilutions of 4 ng/ul as required by the PCR conditions used.

The Random Amplified Polymorphic DNA analysis was carried out using primers that have been successfully used in cowpea by other researchers in various studies predominantly molecular characterisation studies (Sharawy and El-Fiky, 2002; Zannou et al., 2008; Prasanthi et al., 2012). A gel run of the twelve lines plus a common bean control was carried out to determine the success of the extraction process.

List of primers

The list of primers in Table 3 were tried for use in the study. The primers were sourced from University of Cape Town South Africa.

The RAPD-PCR procedure

The primers were used for both amplification and detection of polymorphisms among the cowpea lines. Temperature cycling was carried out on the Gene Amp 2400 thermocycler. A number of reaction contents and protocols were tried by performing permutations between 3 sets of different reaction contents against 3 sets of different reaction conditions (Nienhuis et al., 1995). Each set of reaction was performed in 10 ul volumes containing, 1X RAPD buffer, 1.1 mM deoxynucleotide triphosphates (dNTPs), 4 mM primer, 1.25 units *Taq* DNA polymerase and 20 ng of genomic DNA. The only differences among the 3 sets were on the magnesium concentrations.

Amplification was performed in 0.2 ml tubes using a thermocycler (Eppendorf, Germany) programmed for initial denaturation at 94°C for 3 min followed by 45 cycles of denaturation at 94°C for 1 min at 35°C for 1 min and 72°C for 2 min. The amplification was completed at 72°C for 7 min and holding temperature of 4°C (Zannou et al., 2008).

RAPD-PCR products were separated run on a 1.5% agarose gel (Weising et al., 1995). The molten gel was stained with a 10 mg/ml ethidium bromide stock to a final concentration of 0.5 ug/ml before it had set (Henry, 1997). To each well, 10 ml of the sample in 1X loading dye, were loaded. The samples were electrophoresed in 0.5X TBE buffer at 80 V (1-5 V/cm) for 2 h.

RESULTS AND DISCUSSION

Morphological characterization

The lines show diversity in terms of various seed characteristics that were scored namely seed colour, hilum colour, seed size, presence and absence of seed spots and seed uniformity. ANOVA results were generated using two-way analysis of variance (ANOVA) using Genstat Discovery Edition 4 software. The lines showed significant differences with respect to all of the variables namely seed size, seed spots, seed uniformity, hilum colour and seed colour, with $p<0.001$. Table 4 shows the means, standard error and the p values.

The means for the 12 lines are presented in Table 4 for the purposes of multivariate analysis. Principal component analysis (PCA) and hierarchical cluster analysis were used for the construction of a scatter plot and a dendrogram respectively.

Principal components analysis

PCA first converted the five variables to two vectors which can be plotted on a 2 dimensional scatter plot (Figure 2). The scatter plot produced after principal components analysis showed that most of the lines are clustered together in a large group, x-coordinates -2 to 1 and y coordinates -1.7 to 1.2 on the two dimentional plane. This group which is on the left hand plane includes

Table 4. Means, standard error and p valuesfor the five variables recorded.

Variable entry	Seed size	Seed colour	Seed spots	Uniformity	Hilum colour
Chikoromo	3.00	4.00	0.00	1.00	3.00
Tinyawa kongonzi	1.00	3.00	0.00	1.00	3.00
Nyadahwa 1444	3.00	5.00	1.00	1.00	3.00
Tsumbe	2.00	3.00	0.00	1.00	3.00
Zai redahwa	1.00	4.00	0.00	1.00	3.00
Indumba	2.00	3.50	0.00	0.00	5.00
Nhepetera	2.00	2.80	0.00	0.00	1.00
Tinyawa Kalula	3.00	5.00	1.00	1.00	2.00
Red teak	2.00	4.00	0.00	0.00	3.00
Nyadahwa 1381	2.00	5.00	1.00	0.00	3.00
NGO 1	1.75	1.00	0.00	1.00	5.00
NGO 2	0.80	4.00	0.00	1.00	3.00
Standard error	0.207	0.167	0.00	0.00	0.00
P value	<0.001	<0.001	0.00	0.00	0.00

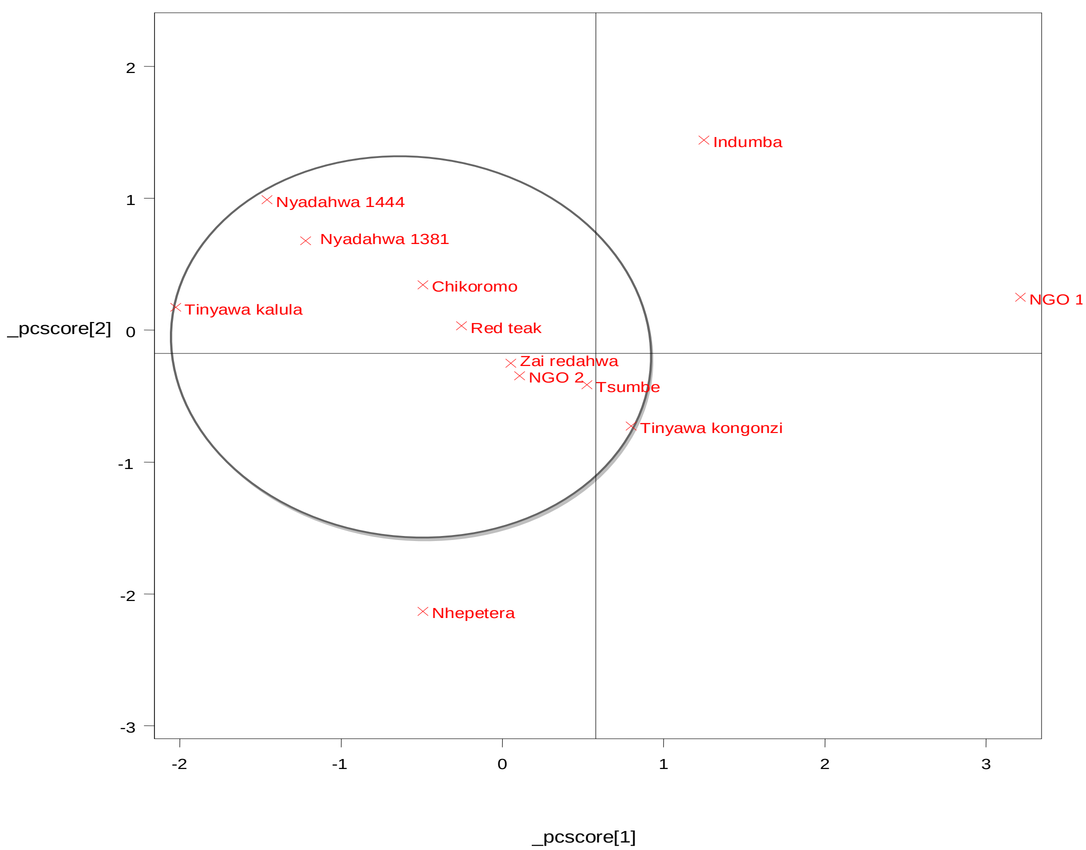

Figure 2. The scatter plot produced after principal components analysis was carried out on the twelve lines in genestat software. Cluster analysis shows a spread of the lines maily bebtween x= -2.1 to 1 and y=-1.7 to 1.3. Three outliers, Nhepetera, NGO1 and Indumba are observed.

entries, Nyadahwa 1444, Nyadahwa 1381, Tinyawa Kalula, Chikoromo, Red Teak, Zai Redahwa, NGO2, Tsumbe, Tinyawa Kongonzi and NGO2 with three outliers, Indumba, NGO1 and Nhepetera. This is an

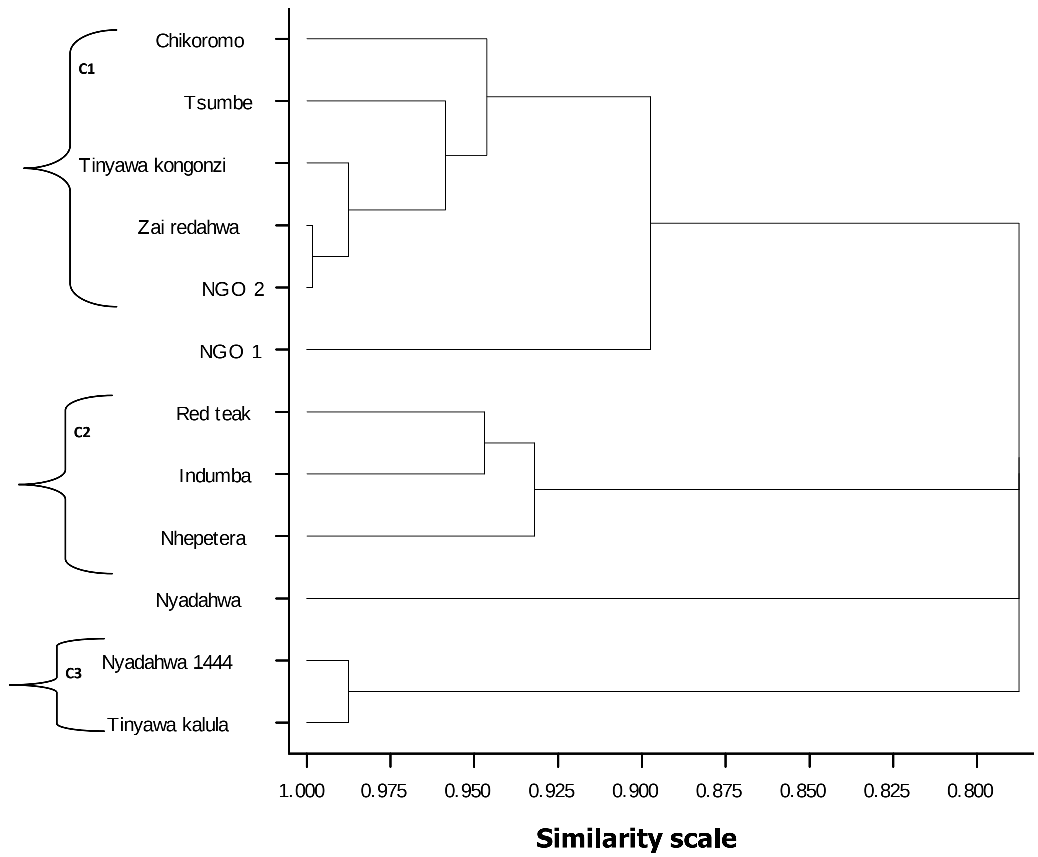

Figure 3. A dendrogram for the lines generated using nearest neighbour cluster analysis using genstat software.

indication of similarity or uniformity within the group and the outliers may be significantly diverse from the rest.

Cluster analysis

After hierarchical cluster analysis a dendrogram was constructed and presented in Figure 3. On a similarity scale of 0 to 1, total similarity index for the collection is 0.78. Zai redahwa and NGO2 are the most similar whereas Nyadahwa is the most dissimilar from the rest. Three main clusters are observed labelled C1, containing lines, Chikoromo, Tsumbe, Tinyawa Kongonzi, Zai redahwa and NGO2, C2 containing lines, Red Teak, indumba and Nhepetera and C3 containing lines, Nyadahwa 1444 and tinyawa Kalula. Each cluster has lines which are most related amongst themselves. Nyadahwa and NGO1 do not belong to any particular clusters.

Molecular characterisation

When the DNA samples were run on the gel to ascertain success of the extraction process, the following gel picture was captured (Figure 4). The DNA shows very faint bands showing some presence of the genetic material.

PCR analysis results

The banding patterns for primers AP5 and A07 are shown in Figures 5 and 6 respectively. Figure 5 shows DNA polymorphisms detected in the 12 accessions screened using primer AP5. While primer A07 was unable to clearly distinguish these accessions (Figure 6). Primer AP5 managed to detect polymorphic bands showing important variations among these accessions.

Principal components analysis

PCA converts the 8 alleles into two vectors which were again plotted on a 2 dimensional scatter plot. The scatter plot produced after principal components analysis showed the lines are distributed all over the plane some above y=0 and the other group below y=0. Figure 7

Table 5. Results of scoring of gel from primer AP5.

Germplasm	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8
Tinyawa Kalula	0	0	0	0	0	0	1	1
Tinyawa kongonzi	0	0	0	1	0	0	1	1
Tsumbe	0	0	1	1	0	0	1	1
NGO1	0	0	0	0	0	0	0	1
Nyadahwa 1444	0	0	1	1	0	0	0	1
NGO2	0	0	0	0	0	0	1	1
Indumba	0	0	0	0	0	0	0	0
Nyadahwa 1381	0	0	0	1	0	0	0	1
Chikoromo	0	1	1	0	0	0	0	1
Red Teak	0	0	0	0	0	1	0	0
Zai redahwa	0	1	1	0	0	1	0	0
Nhepetera	0	0	0	0	0	1	0	1

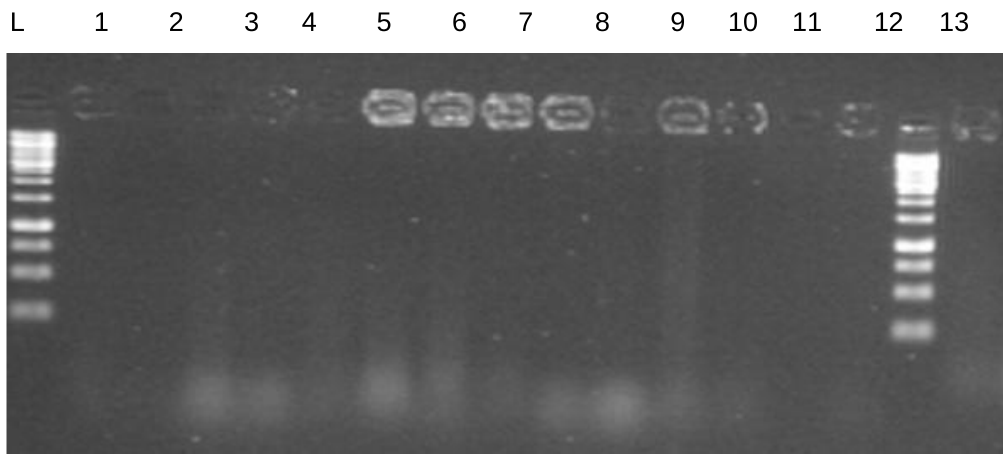

Figure 4. A gel showing presence or absence of genomic DNA.

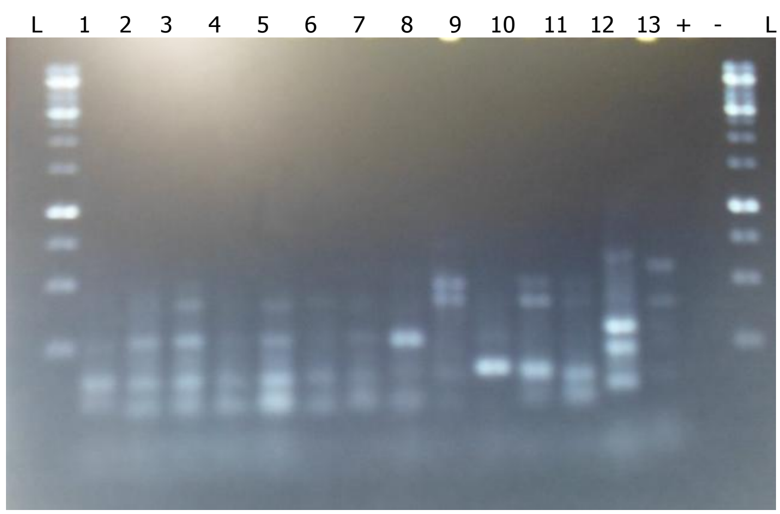

Figure 5. A gel generated with primer AP5 of sequence 5'-AACGCGCAAC-3'. L is 1 kb molecular marker, 1 to 12 are cowpea samples, 13 is a bean samples and – and + are positive and negative controls.

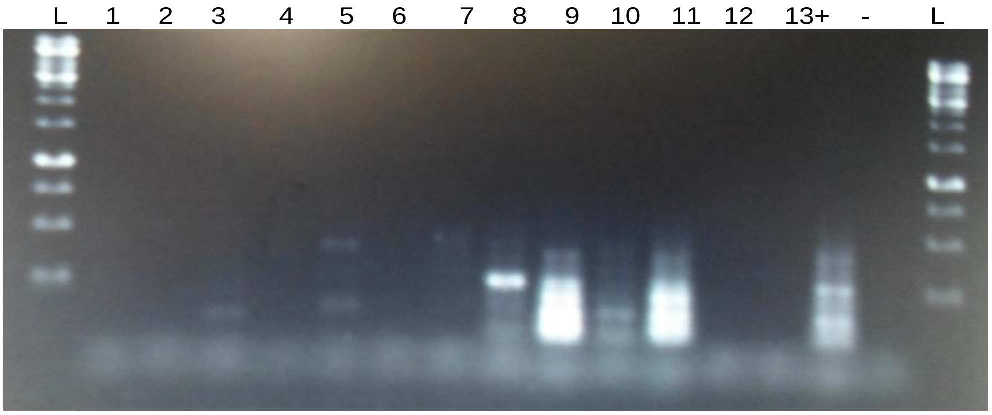

Figure 6. A gel picture generated with primer A07 of sequence 5'-GAAACGGGTG-3'. L is 1 kb molecular marker, 1 to 12 are cowpea samples, 13 is a bean samples and – and + are controls. The polymorphic bands are not clear with this primer. Primers AP1 and OPB 10 showed monomorphic bands when gels were run. The gel in Figure 5 for primer AP5 was used for scoring and the results were recorded in Table 5, where 0 is absence and 1 is presence of a band.

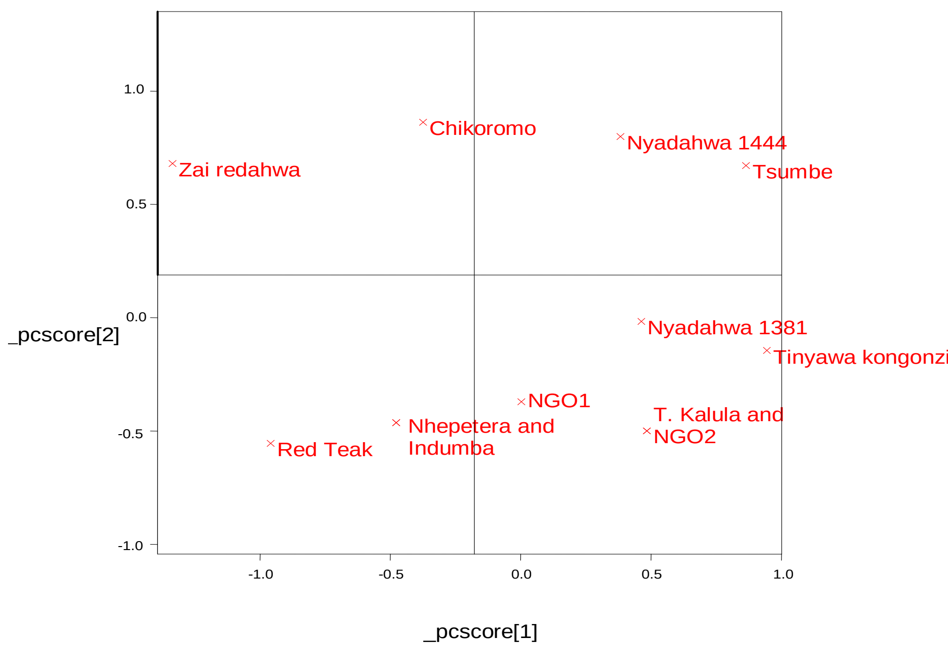

Figure 7. Scatter plot generated for principal component analysis in GenStat on the twelve line. Four of the lines have duplicated positions on two places in the scatter plot.

shows the scatter plot produced after principal component analysis was carried out.

The distribution is highly clustered together when the coordinates are examined which have small intervals although visually they seem widely distributed.

Cluster analysis

The dendrogram for RAPD-PCR data shows an overall similarity index of 75% or dissimilarity of 25%. It presents one main cluster containing eight lines (Tsumbe, NGO2, Nyadahwa, Tinyawa Kalula, Nhepetera, Zai Redahwa, Nyadahwa and Tinyawa Kongonzi. Red Teak and NGO 1 are the most dissimilar from the rest while Chikoromo and Indumba are the most similar (Figure 8).

The distribution pattern for morphological data are observed as different. Despite efforts to enrich a sample of cowpea lines from the gene bank, the genetic diversity is consistently low. It is comparable to literature reports that say genetic diversity in cowpea is generally low in spite of substantial variation seed color, seed proteins,

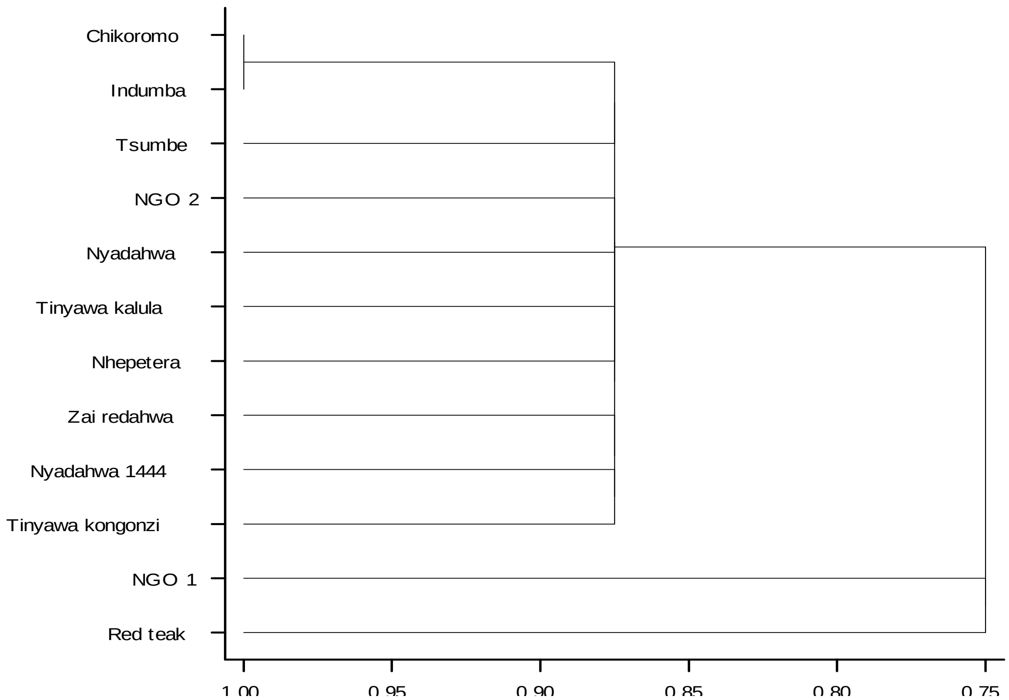

Figure 8. A dendrogram generated after nearest neighbour cluster analysis of the lines for RAPD-PCR scores. Multivariate analysis for the molecular data shows limited variation among the lines.

plant type, pod type and seed size among cultivated cowpeas (Sharawy and El-Fiky, 2002). According to Huaqiang et al. (2012) the genetic diversity among cowpea cultivars they studied ranged from 0.1742 to 0.4054 on a scale of 0 to 1. Malviya et al. (2012) reported genetic diversity levels ranging 0.2 to 0.4 on diversity analysis of ten Indian cultivars.

Conclusion

Both morphological and molecular data for the cowpea lines show limited diversity after multivariate analysis was carried out. The germplasm collection may require continuous enrichment. The white seeded line NGO1, consistently seem to be diverse from the multivariate analysis carried out. It will certainly be a valuable addition and will be useful for possible introgression in future.

Conflict of Interest

There are no conflicts of interests regarding this publication.

ACKOWLEDGMENTS

This work was partially funded by the Postgraduate Research Center, Bindura University of Science Education - the purchase of primers and laboratory fees at African Institute of Biomedical Research (AiBST). The National Gene Bank of Zimbabwe was the source of some of the germplasm used in the study. Laboratory technical support from AiBST staff is also greatly appreciated.

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Full Length Research Paper

Response of *Jatropha curcas* plants to changes in the availability of nitrogen and phosphorus in Oxissol

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The present study aimed at identifying the correct nitrogen and phosphate fertilization for the maximum productivity of *Jatropha curcas* plants. The study was carried out at the experimental field of the Ipameri Campus of the State University of Goiás. Three-year-old physic nut plants under full production were used. After analysis of the soil classified as Oxissol, pH correction was performed in accordance with technical recommendations for the species. The experiment followed a randomized block design with a 4 × 2 factorial arrangement [four doses of N (0, 100, 200 and 300 kg/ha) and two levels of phosphate fertilization (0 and 50 kg/ha). Fertilization was made in semicircle following the crown projection, 20 cm away from the stem. The lower leaf concentration of phosphorus in plants treated with 50 kg ha^{-1} in relation to plants fertilized with phosphorus shows there was no deficiency in the plants that did not get phosphorus and possibly the 50 kg ha^{-1} represented the excess of phosphorus due to the high content of organic matter. However, the high organic matter was not sufficient to meet the demand for nitrogen due to the high demand for *J. curcas* for this nutrient. Seed productivity presented a significant increase in relation to nitrogen fertilization. For 3-year-old plants cultivated at a spacing of 4 × 2 m, a fertilization of 81 kg ha^{-1} is thus recommended.

Key words: Biofuel, mineral nutrition, growth, nitrogen, phosphorus.

INTRODUCTION

The increase in greenhouse gases in the earth's atmosphere has compromised natural resources and intensified the search for renewable energies. The search for an energy alternative to fossil fuels requires the evaluation of renewable sources with low impact on the environment like biofuels (Matos et al., 2014).

The choice of the alternative source of energy must be made carefully with an impact as low as possible on the environment. In this context, biofuels are an option which may partially substitute fuels derived from oil and natural gas in internal combustion engines and in other types of energy generation (Freitas et al., 2011; Pan and Xu, 2011). Biofuels pollute less for emitting less compounds in the combustion process, mainly CO_2 and the

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Production process is cleaner than with fossil fuels.Brazil is the country with the greatest potential for biofuel production because it has vast expanses of arable land, plenty of water, and soil and climate diversity that enables the exploitation of various species adapted to different climates and biomes (Freitas et al., 2011).

Biodiesel production in Brazil is based only on one raw material, namely soybean, which represents 73.92%. Beef suet, cotton and other fat materials contribute 21.21, 2.45 and 2.42% of the production, respectively (Matos et al., 2014). It is thus necessary to diversify the source of raw material used in biodiesel production through introduction of potential species like *Jatropha curcas* L.

J. curcas is an oil-producing plant of the Euphorbiaceae family, popularly known as physic nut. It is a fast-growing shrub, able to initiate production from the seventh month of planting, remaining productive for approximately 40 years (Dias et al., 2007). *J. curcas* has various favorable attributes enabling its commercial exploitation, like fast growth, easy propagation, perennial plant, high oil content (between 33 and 38%) and quality, evidencing its high economic potential (Sujatha et al., 2008).

J. curcas is considered as a rustic, drought-resistant plant, suitable for the most diverse soil and climate conditions, surviving in marginal lands of low natural fertility (Arruda et al., 2004). The plant grows in various soil types, including sandy, stony, saline, alkaline and rocky soils, which, nutritionally and physically speaking, limit the full development of the roots (Carvalho et al., 2011).

Despite high adaptability and rusticity, one may presume that the highest genetic potential is exploited in adequate fertilization conditions. Well-performed fertilization makes significant productivity gains possible in most cultivated plants. Although it is a rustic plant, higher seed productivity is obtained with use of fertilizers and soil pH correction (Carvalho et al., 2011; Freitas et al., 2012; Mohapatra et al., 2011; Laviola and Dias, 2008).

Among essential nutrients for the growth and production of *J. curcas*, we may highlight phosphorus and nitrogen for affecting the initial growth and the establishment of the plant, the vegetative development and the seed productivity. According to Freiberger et al. (2014), phosphate fertilization is as limiting to the growth and establishment of *J. curcas* as fertilization with all other nutrients, in the seedling phase. Nitrogen is the nutrient the plant requires in the greatest quantity (Laviola and Dias, 2008). It is considered as the main nutrient for the development of the plant, as it is directly involved in growth and photosynthetic activity (Freitas et al., 2012).

In order that *J. curcas* become competitive with other oil-producing plants on the Brazilian energy stage, aspects related to the productivity of the plant need to be elucidated, since the species lacks basic agronomic information (Fernandes et al., 2013).

The present study aimed at identifying the correct nitrogen and phosphate fertilization for the maximum productivity of *J. curcas* plants.

MATERIALS AND METHODS

Experimental design

The study was carried out in the experimental field of the State University of Goiás, Ipameri Campus (latitude 170° 43' 19''S, longitude 480° 09' 35''W, Altitude 773 m), Ipameri, Goiás, Brazil. This region has basically two well defined seasons: the wet season, from October to April, and the dry season, from May to September.

Three-year-old clones of *J. curcas* plants were used at a spacing of 4 × 2 m. The clones come from plant populations naturally found in the State of Goiás. After analysis of the soil classified as Oxissol, pH correction was performed in accordance with technical recommendations for the species (Dias et al., 2007). The chemical analysis of the soil gave the following values: pH 5.55, 4.3% of O.M., 0.0 mg dm^{-3} of P, 129 mg dm^{-3} of K, 0.10 $cmol_c\ dm^{-3}$ of Al, 6.5 $cmol_c\ dm^{-3}$ of Ca, 29 $cmol_c\ dm^{-3}$ of Mg, 84.8% of V, 35.90 $cmol_c\ dm^{-3}$ of S and 42.33 $cmol_c\ dm^{-3}$ of CEC. The experiment was set up as a random block design, in a 4 × 2 factorial arrangement, with four doses of nitrogen (0, 100, 200 and 300 kg/ha), two doses of phosphorus (0 and 50 kg/ha), five replications and two-plant slot. All plants were fertilized with the same amount (50 kg/ha) of potassium chloride, following recommendations of Laviola and Dias (2008).

The nitrogen fertilization were divided in three equal applications in the days 10/30/2013, 30/11 / 2013 and 01/05/2014. The phosphate and potash fertilizers were applied in its entirety on 08/20/2013. Fertilization was made in semicircle following the crown projection, 20 cm away from the stem.

On 02/20/2014 and 02/21/2014 the following evaluations were made: number of branches, plant height, stems diameter, leaf area, crown diameter, total chlorophyll and carotenoids, phosphorus and nitrogen leaf content. Seed productivity was analyzed between 04/05/2014 and 06/10/2014.

Morpho-agronomic characteristics

Plant height, stem diameter and crown diameter were measured using graduated ruler and digital pachymeter. The evaluation of the number of branches was made through counting. To evaluate the leaf area, we used a graduated (cm) tape measure, measuring width and length of the totally expanded leaf (between the 3^{rd} and 5^{th} pair of leaves). Then we calculated the leaf area following the recommendations of Severino et al. (2007). Seed productivity was evaluated through harvesting and threshing of the fruits of each plant and weighing of the seeds.

Nitrogen, phosphorus and potassium content in the leaves were determined in the Soil Science Laboratory of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. Nitrogen was determined using the Kjeldahl method with sulfuric acid digestion. To determine phosphorus contents, dry and ground plant material was submitted to nitric-perchloric digestion (Johnson and Ulrich, 1959).

Physiological characteristics

To determine the total chlorophyll concentration (photosynthetic pigments), leaf disks of known area were removed and placed in glasses with dimethyl sulfoxide. Subsequently, extraction of the samples was performed in a bain-marie at 65°C for 1 h. Aliquots were removed for spectrophotometric reading at 490, 646 and 663 nm. Chlorophyll *a* (Cl *a*), chlorophyll *b* (Cl *b*) and total carotenoid contents were determined following the equation proposed by Wellburn (1994).

Table 1. Summary of the analysis of variance of the plant height, stem and crown diameter, number of branches and leaf area of physic nut plants cultivated under different doses of nitrogen and phosphorus.

Variation source	DOF	Mean squares				
		Height (m)	Stem diameter (mm)	Crown diameter (m)	Nr. branches	Leaf area (cm^2)
Block	4					
P	1	0.028^{ns}	74.6^{ns}	0.02^{ns}	62.3^{ns}	7.70^{ns}
N	3	0.22^{*}	299.9^{ns}	0.22^{ns}	37.0^{ns}	1834.2^{*}
N x P	3	0.033^{ns}	83.8^{ns}	0.02^{ns}	45.1^{ns}	537.4^{ns}
Error	28	0.87	1942.5	0.09	61.6	331.2
CV (%)		7.9	7.8	10.9	16.7	10.3
Treatments		**Averages**				
- N - P		2.50	120.4	2.89	47.4	179.7
- N + P		2.64	129.1	2.78	46.8	167.8
100 N - P		2.88	136.8	2.74	46.0	174.6
100 N + P		2.85	129.5	2.67	52.4	154.1
200 N - P		2.56	132.9	2.91	41.5	164.2
200 N + P		2.77	136.4	2.81	46.8	174.4
300 N - P		3.01	138.2	3.02	49.0	194.6
300 N + P		2.95	143.1	3.10	46.2	205.8

*Significant at 5% probability; ns = not significant by F test.

Statistical procedures

Analyses of variance (ANOVA) were performed following the random block design with a factorial arrangement of 4 × 2 (four doses of nitrogen: 0, 100, 200 and 300 kg/ha and two doses of phosphorus: 0 and 50 kg/ha) and five blocks. Regression analysis for the quantitative variables was made using software SISVAR 5.3 (Ferreira, 2011).

RESULTS AND DISCUSSION

Statistical analysis of the data showed that there was no significant interaction between nitrogen and phosphorus doses (Tables 1 and 2). Thus, the nitrogen doses interfere with the analyzed variables regardless of the phosphorus doses. The factors are thus independent and, in this case, were studied separately. The plant height and leaf area showed significant differences in function of the nitrogen doses (Table 1).

Leaf concentrations of carotenoids (Car), total chlorophylls (total Cl) and nitrogen (N) in the leaves showed no significant variation in relation to the nitrogen and phosphorus doses applied to the soil (Table 2). However, phosphorus leaf concentration showed significant variation in relation to phosphorus doses applied to the soil. Seed productivity showed significant variation in function of the nitrogen doses.

The maximum points were 81.6 kg nitrogen to get 1483 kg ha^{-1} of *J. curcas* seeds. We observed that as nitrogen doses were incremented, there was an increase in plant height (Figure 1), and that phosphorus leaf concentration was lower in the treatments with applications of more phosphorus to the soil. Productivity and leaf area showed quadratic variations in relation to the nitrogen doses.

Under natural conditions, nutritional stress can cause irreversible damage in *J. curcas*. High adaptability to soil and climate variations of *J. curcas*, besides maintenance of the growth under nutrition stress condition can represent expansion of the agricultural frontier by getting economic return in areas until then inappropriate, as later discussed.

The lack of significant interaction between doses of nitrogen and phosphorus shows that these factors acted separately on the analyzed variables. Regardless of supplied nitrogen and phosphorus doses, the plants behaved similarly as for plant growth (stem and crown diameters and number of branches). However, plant height increased linearly with the nitrogen doses, showing that heavy fertilizations with this nutrient can intensify plant growth, more specifically plant height. The increment in the vegetative growth of *J. curcas* is a common effect of treatments with increasing nitrogen doses (Oliveira and Beltrão, 2010; Freitas et al., 2012). Competition for assimilates between the growth and the filling of seeds may have contributed to a lower productivity of plants submitted to high doses of nitrogen. Under such circumstances, the larger leaf area of plants under high doses of nitrogen may be related to higher photosynthetic activity in function of the higher demand for assimilates. According to Freitas et al. (2012), the photosynthesis of *J. curcas* plants increases linearly with the doses of nitrogen. These authors used doses from 50 to 350 kg ha^{-1} of urea.

Seed productivity did not increase proportionally to the

Table 2. Summary of the analysis of variance of carotenoids (Car), total chlorophylls (total Cl), nitrogen (N), phosphorus (P) leaf concentrations and productivity of physic nut plants cultivated under different doses of nitrogen and phosphorus.

Variation source	DOF	Mean squares				
		Car (g kg^{-1})	Total Cl (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	Productivity (kg ha^{-1})
Block	4					
P	1	0.002^{ns}	0.38^{ns}	0.42^{ns}	0.73*	109341^{ns}
N	3	0.001^{ns}	0.09^{ns}	4.31^{ns}	0.17^{ns}	158013*
N × P	3	0.006^{ns}	0.19^{ns}	11.30^{ns}	0.07^{ns}	32942^{ns}
Error	28	0.007	0.15	7.04	0.17	45412
CV (%)		20.7	7.8	8.8	16.8	14.6
Treatments		**Averages**				
- N - P		0.36	2.09	29.3	2.5	1414.6
- N + P		0.33	2.38	31.0	2.1	1544.2
100 N - P		0.35	2.08	28.6	2.6	1655.4
100 N + P		0.34	2.58	31.8	2.6	1737.3
200 N - P		0.32	2.32	31.1	2.7	1226.6
200 N + P		0.32	2.13	28.4	2.3	1562.5
300 N - P		0.30	2.33	30.1	2.5	1313.1
300 N + P		0.39	2.53	28.7	2.2	1344.2

*Significant at 5% probability; ns = not significant by F-test.

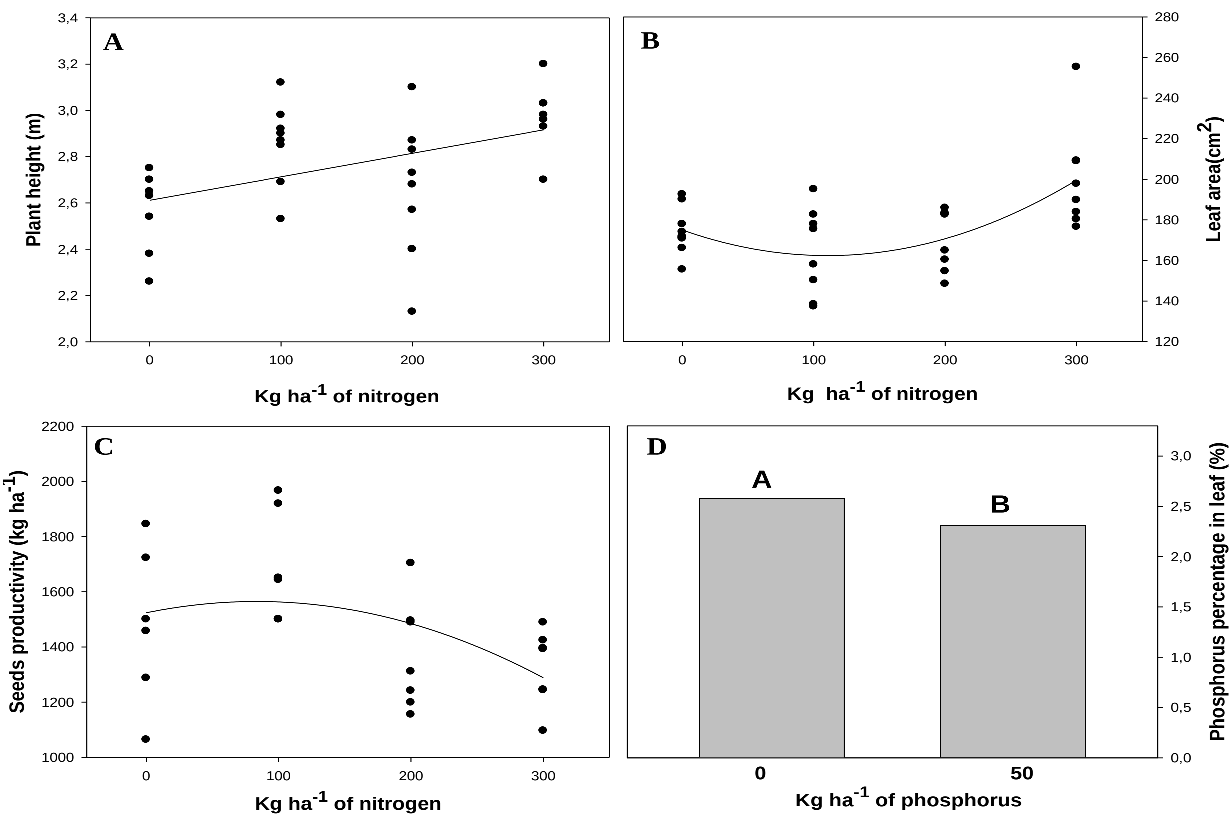

Figure 1. Equations of regression for plant height "A" (Y = 2.61 + 0.0010x, R^2 = 0.81*), leaf area "B"(Y = 174.99 Y - 0.23x + 0.001x^2, R^2 = 0.64*) seed productivity "C" (Y = 1523.76 + 0.98x - 0.006x^2, R^2 = 0.80*) and phosphorus percentage in leaf "D" of *Jatropha curcas* L. plants, fertilized with different doses of nitrogen and phosphorus. Averages followed by the same capital letter do not differ, 5% of probability by F test.

increase of nitrogen fertilization. The maximum seed productivity (1483 kg ha^{-1}) occurred with 81.6 kg ha^{-1} of nitrogen, that is, 65 g of nitrogen per plant. This result corroborates that of Laviola and Dias (2008), who recommended 60 g of nitrogen per plant. Thus, for a 3-year-old plant, cultivated at a spacing of 4 × 2 m, fertilization with 65 g of N is recommended. Heavy fertilizations with amounts of nitrogen fertilizer higher than 65 g per plant intensify plant growth and harm seed productivity. However, subsequent studies with several crop cycles after stabilization of productivity are necessary to get a consistent fertilization recommendation. Studies with plants younger than 5 years show a divergent variation of the vegetative growth and seed productivity (Laviola and Dias, 2008; Freitas et al., 2012; Carvalho et al., 2013). The fertilization of a crop depends on the demands for vegetative and reproductive growth, nutrient supply by the soil and losses in fertilizer (Laviola and Dias, 2008).

Phosphate fertilization incremented productivity and/or growth of *J. curcas* (Freire et al., 2011). *J. curcas* is sufficiently responsive to phosphate fertilization in the initial growth period (Laviola and Dias, 2008; Freiberger et al., 2014), but application of doses from 135 to 200 g of phosphorus per plant shows no significant variation of the vegetative growth (Sousa et al., 2011). In the present study, the application of 40 g per plant did not modify vegetative growth and seed productivity. The liberation of phosphorus by the organic matter of the soil may possibly have contributed to the lack of significance of the analyzed variables. The lower leaf concentration of phosphorus in plants treated with 50 kg ha^{-1} in relation to plants fertilized with phosphorus shows there was no deficiency in the plants that did not get phosphorus and possibly the 50 kg ha^{-1} represented the excess of phosphorus due to the high content of organic matter. However, the high organic matter was not sufficient to meet the demand for nitrogen due to the high demand for *J. curcas* for this nutrient.

Conclusions

We recommend fertilization of 81 kg ha^{-1} (65 g/plant) of nitrogen in 3-year-old *J. curcas* plants cultivated at a spacing of 4 × 2 m. In 3-year-old plantations, soils with organic matter content higher than 4%, phosphate fertilization is not recommended for not increasing seed productivity.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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