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Full Length Research Paper

Investigating viability of the premium influenced land agro-usage structure for production of African leafy vegetables in Vihiga and Jinja

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Land subdivision has reduced land for agricultural production resulting in its intensive cultivation. This has lowered soil fertility which has contributed to reduction in the diversity of African Leafy Vegetables thus restricting the otherwise traditional dietary diversity that was once beneficial to smallholder farmers. As land continues to decline, there needs to be some impetus in place that can retain the diversity of African Leafy Vegetables. This study therefore recognized the need to niche the African Leafy Vegetables to a none-competing, specially constructed raised cropping bed referred to us the Premium Influenced Land Agro-usage structure (PILA). A study to investigate the viability of the PILA structure for production of vegetable crops was undertaken in Vihiga and Jinja. The objective of the study was to evaluate the benefits of the PILA structures. PILA structures were constructed on 20 smallholder farms in Vihiga and Jinja. Vegetable crops *Solanum scabrum, Cleome gynandra, Amaranthus hybridus*) and exotic vegetables (*Daucas carota*) were grown on these structures. The same procedure was done on farmers' conventional plots (Flat beds). Analysis to compare the performance of vegetable crops between the PILA and Flat beds was done using Genstat. The net present value was used to assess the viability of the structure for long term use. Results indicated high significant differences (p≤0.001) in yield and height of vegetables crops grown on PILA and flat beds, (PILA yield (kg/ha) was 42254 versus 27772 for flat beds, PILA height in (cm) was 14.8 versus 10.8 for flat beds). Comparisons in vegetable performance between seasons showed better performance of vegetable crops in the Long Rains than the Short Rains seasons for both sites with significant difference (p=0.001) in yield (kg/ha) for the Long Rain (LR) was 36064 against 33962 for the Short Rain (SR), mean height (cm) for LR was 13 against 12.5 for SR, mean branching (score out of 3) for LR was 2.5 against 2.4 for SR. Also significant differences in vegetable performance were detected between Vihiga and Jinja in height and yield; mean yield (kg/ha) for Vihiga was 34962 and 36064 for Jinja, mean height (cm) for Vihiga was 12.8 and 16.6 for Jinja. The PILAs had a high net present value (KSH191390) compared to flats beds (KSH122087). Vegetable crops on PILA structure performed better than on Flat beds, the PILA structure can be promoted for production of vegetables in areas with small land sizes like the urban and peri-urban. However, there is need to increase the acceptability and adoption of the structure through awareness.

Key words: Raised bed planting, land size, premium land agro-usage structure, african leafy vegetables, dietary diversity.

INTRODUCTION

Land subdivision as a result of population pressure has resulted in reduced land for agricultural production which has had an effect on soil fertility. Traditionally, farmers would restore soil fertility by leaving part of their land uncultivated for many years while new and more fertile land was cultivated for food production. The small land sizes have otherwise destabilized this traditional system of maintaining soil fertility (Amadalo et al., 2003). For instance, the current land holdings on smallholder farms are approximately 0.4 ha which is usually considered to be below the FAO recommendation for subsistence food purposes of 1.4 ha / household (FAO, 2008). Consequently, long-duration natural fallows are no longer possible. The apparent implication of the low soil fertility status and reduced land holding is the decline in the abundance and distribution of phyto-diversity found on smallholder farms (Tittonell et al., 2005).

The declining quantity, distribution and consumption of edible phyto-diversity has led to reduction in the diversity of African leafy vegetables (ALV) grown on the smallholder farms thus restricting the otherwise traditional dietary diversity that was once beneficial to the locals (Vorster et al., 2008; Abukutsa-Onyango, 2008; Mitra and Pathak, 2008). Recent studies have shown that ALV's such as *Curcubita maxima, Amaranth* spp., *Cleome gynandra* and *Solanum nigram* are mineral micro-nutrient (MiMi) richer than cereal crops such as maize and sorghum (Akundabweni et al., 2010). In fact, almost all the leafy vegetables are good sources of micronutrients including iron and calcium as well as vitamins A, B complex, C and E. For example, *Amaranth* contains a multiple of these nutrients compared to *Brassicca oleracea* (IPGRI, 2003; Abukutsa-Onyango, 2007). Some of the African leafy vegetables even contain micro-nutrients content higher than those found in their exotic counterparts (Steyn et al., 2001; Odhav et al., 2007; Nangula et al., 2010). These indicates that the consumption of these leafy vegetables has both nutritional, health and a potential role to play in the mitigation of 'hidden hunger' [Hidden hunger is a condition manifested in increased malnourished children and adults because of lack dietary diversity (Hughes, 2008)].

Unfortunately, because of intense cultivation of the small land holdings, these ALVs can easily be marginalized in favour of the major agronomic crops. For instance there is increased production of some staple crops like maize at the expense of vegetable crops resulting in low dietary diversity. Diets poor in leafy vegetables may lead to xerophthalmia (a form of blindness) associated with vitamin A deficiency. It is also recognised that a diet rich in energy but lacking other essential components can lead to a heart disease, diabetes, cancer, and obesity (Frison et al., 2004). These conditions are no longer associated with affluence; they are on the increase among poor people from urban and rural areas in developing countries. A diverse diet offers nutritional buffers and there should be a key policy reform to combat this unhealthy trend (Johns and Sthapit, 2004). Since no approaches are possible in expanding the land resource, sustainable utilization of the limited land parcels for increased yield and dietary diversity is paramount (Mutiga et al., 2011).

Raised beds have been widely used in the production of commercial crops like rice, wheat and maize than vegetable crops. (Aquino, 1998; Hobbs and Gupta., 2003; Limon-Ortega et al.,2003,2006). Raised beds concentrate a large percentage of crops on a small piece of land thus increasing yield. Raised bed planting has also been shown to offer better weed control, water and fertilizer management, thus leading to the lower inputs of water and fertilizers and higher stress-resistance (Tripathi et al., 2005; Kong et al., 2010). Additionally, raised beds create a micro-climate (Microclimate- In this context, micro-climate refers to creation of an internal warm climate by plants that makes plant mature fast) in the field of the growing crop that reduces crop lodging and disease incidences (Fahong et al., 2004).

Other studies have shown that raised-bed planting reduces seed mortality rates, increases water- and nitrogen (N)-use efficiency, and improves soil quality. In addition, less labour is required for irrigation and fertilizer is better managed relative to conventional flat planting (Limon-Ortega et al., 2000). This therefore represent the social-economic benefits likely to be derived from using raised beds for production of crops. Can raised beds be improvised to enable production of vegetable crops in areas with land as a scarce resource?

This study sought to investigate the viability of the premium influenced land agro-usage structure (PILA), a land use innovation for production of ALV. The PILA is an improvised raised bed to enable production of vegetable crop.

MATERIALS AND METHODS

The study sites

The study sites were Jinja-Uganda and Vihiga-Kenya, as shown in Figure 1.

The study period

The study was done in the long and short rain of year 2011. The long rain season covered the months of April, May , June and July

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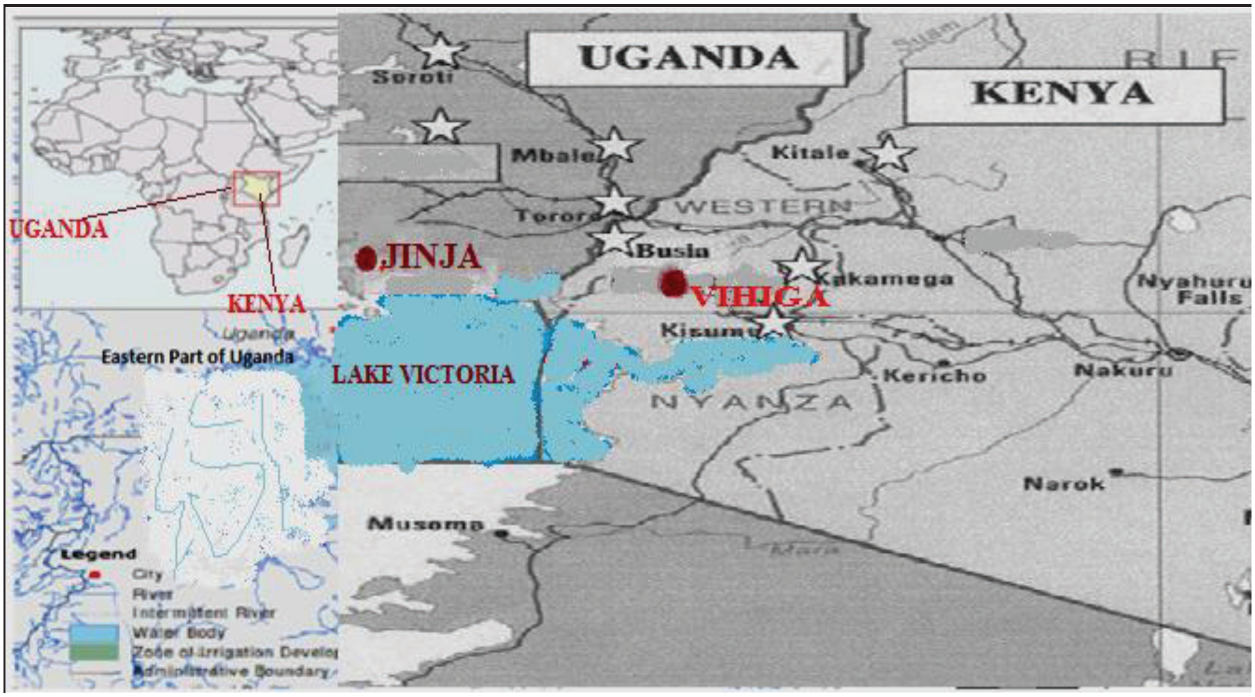

Figure 1. A map showing the study sites; Jinja (Lat. 1° 1.5' S; 29° 30.9' E) and Vihiga (Lat. 0° 15'N; Long. 34° 30'E).

Figure 2. Premium influenced land agro-usage structures (1) compared to flat bed (2).

while season 2 was the short rain season covered the months of September, October November and December.

The experimental design

The treatment was the PILA structure. PILA[1] Structures were established on 10 smallholder farms in Vihiga and the same number in Jinja (Figure 2 diagram 1). Each PILA was designed in three layer stair-case raised bed with each succeeding layer smaller than the preceding one. African Leafy Vegetables (*Solanum scabrum, Cleome gynandra, Amaranthus hybridus*) and exotic vegetables (*Daucas carota*) were planted on these beds. Weekly monitoring of the plots was done to determine their performance. The following agronomic appeal attributes were taken; vigour and robustness, plant height, branching and leaf density. Yield was also determined. A similar procedure was done on the flat beds as shown in Figure 2 diagram 2. The Flat beds[2] were the farmers' conventional way of planting vegetables. The measurements of PILA and flat beds were kept the same (21.3 m^2). The plant spacing was also similar. However, the size of the Flat bed could not contain the seed density as had been applied on the PILA structures. The seed density used on PILA structure was 300 gm compared to 250 gm that was appliedo n the flat bed.

[1]PILA structure was the treatment factor. This is an improvised raised beds constructed in a special way. See explanation of how the structures were constructed in description 1.2.4.

[2]The Flat beds were the control plots. These were the farmers conventional planting beds where farmers ploughed long rows and planted crops.

Construction of premium influenced land agro-usage structures

The beds were prepared using old sacks, posts and manure. Each bed measured 21.3 m^2. Land preparation by clearing to remove unwanted trash was done on the specific site where the beds were to be situated. The initial procedure involved taking measurements of the bed using a tape measure and a rope. This was done by making a central spot for the bed. A diameter measuring 240 cm from the central spot was then marked. The bed was then divided into three micro-beds measuring 60 cm in diameter. Vertical posts of 40 cm long were put all round the first stair from the ground. Filling materials (a mixture of stones and plant material) were then put up to the 20 cm mark from the ground. The purpose of putting stones was to help in strengthening and prevent sinking of the soil in case of rain. The remaining 20 cm up was filled with a mixture of soil and manure. The second stair case was constructed by erecting posts up to the 60cm length from the ground. Filling materials were put to 40 cm mark, a mixture of soil and manure was then put in the remaining 20cm length. The same procedure was repeated for the third and fourth stair cases. Posts were used to provide support. Sheeting of harvesting sacks was then put round to help in retaining the soil and control soil erosion in the case of rainfall.

Determination of costs and benefits of the PILA structures and flat bed

The costs for production and the corresponding revenue of vegetable crops contained in the PILA structures and flat beds were determined. The annual crop net benefits were computed by taking the total revenue less total variable costs as in the formula:

GMy=TRy-TCy

Where GM was the gross margin, TRy was the total revenue, TC total costs and y a selected vegetable crop.

The net present values of vegetable crops were then calculated for a period of 30 years at the rate of 12%. The 12% was the average rate of inflation for the past 10 year according to the World Bank Data (Appendix 3). Assumption made included; the rate of inflation of 12% would remain constant for the next 30 years, the 30 years period was the time that a person could be actively involved in farming, the cost of constructing the premium influenced land agro-usage structures would be incurred in the first year and after every five years, the costs of the flat beds would be the same throughout the farming period.

To compute the NPVs of the PILA structures, the NPVs of vegetable crops growing on the premium influenced land agro-usage structures were summed as in the following formula;

NVPpl=NPVi+NPVj+…….NPVz

Where NPVpl was the net present value of the PILA Structures, while NPVi, NPVj and NPVz were the net present values of various vegetable crops grown on the PPILA structures. The same procedure was repeated with the Flat cropping beds. A comparison of the NPVs of the PILA structures and flat cropping beds was done to determine the most viable cropping bed.

The NPV or discounted cash flow method was used as it is a preferred method for evaluating the economic worth of an investment, because it considers the time value of the entire stream of net cash flows over the life of the investment (Casler et al., 1993).

Data analysis

Data analysis was done using Genstat version 14 and excel. Results were presented in table and graphs.

RESULTS

Seasonal variations in the means of the agronomic appeal attributes of selected vegetable crops produced on the PILA Structures

There was a high significant difference (P≤0.001) in vegetable performance between the long rain and short rain seasons in the means of the following agronomic appeal attributes; Yield and height, as shown in Tables 1 and 2. A significant difference of (P=0.001) was observed in the following agronomic indicators; branching and disease incidences in both Vihiga and Jinja. In Jinja, vegetable crops had higher yields, longer height, better leaf density, low disease prevalence than in Vihiga. Generally vegetable crops performed better in the long rain season as compared to the short rain season in both Vihiga and Jinja (Appendix 2).

Differences in the means of the agronomic indicators of selected vegetables grown on PILA structures and flat beds

There was a high significant difference (P≤0.001) in vegetable crops grown on PILA structures and flat beds in the following agronomic appeal attributes; yield and height. A low significant differene was oberved for leaf density, branching and disease prevalence as shown in Tables 3 and 4.

Generally vegetable crops grown on PILA structures performed better than the ones that were grown on flat beds (Figure 3). Notice the effective use of space in the PILA structure compared to flat beds.

Variations in agronomic appeal attributes of selected vegetable crops grown on PILA Structures in Jinja and Vihiga

There was a high significant difference in yield and height (P≤0.001) of vegetables crops grown in Jinja compared to the ones that were grown in Vihiga as shown in Table 2. The difference in the following crop indicators was however significantly lower; leaf density (P=0.004), branching (P=0.004) and disease prevalence (P=0.070) as shown in Table 5. Generally, vegetable crops grown in Jinja showed a better performance compared to ones that were grown in Vihiga. More analysis is as shown in Appendix 2.

The germination percent of vegetable crops on PILA and flat beds

The germination percent of vegetable crops growing on PILA was higher than on Flats beds except for *S. Scabru* (Figure 4).

Table 1. Seasonal variations in the means of the agronomic indicators of vegetable crops grown on PILA Structures in Vihiga.

Season	Yield in (kg/ha)	Height in (cm)	Leaf density (score out of 3)	Branching (score out of 3)	Disease prevalence (score out of 3)
Long rain	36064	13	2.4	2.5	2.5
Short rain	33962	12.5	2.4	2.4	2.4
cv%	24.7	68.8	17.1	18.6	16.3
P-value	≤0.001	≤0.001	0.075	0.001	0.001
Least significance difference	273.4	0.543	0.02	0.03	0.01524
Standard error	197.1	0.201	0.00976	0.01498	0.00927

CV, Coefficient of variation.

Table 2. Seasonal variations in the means of the agronomic indicators of vegetable crops grown on PILA Structures in Jinja.

Season	Yield in (kg/ha)	Height in (cm)	Leaf density (score out of 3)	Branching (score out of 3)	Disease prevalence (score out of 3)
Long rain	40064	18	2.8	2.5	2.5
Short rain	36962	15.5	2.3	2.3	2.2
cv%	24.7	68.8	17.1	18.6	16.3
P-value	≤0.001	≤0.001	0.001	0.001	0.001
Least significance difference	400.4	0.743	0.012	0.05	0.01624
Standard error	234.1	0.3601	0.00876	0.01898	0.01127

CV, Coefficient of variation.

Table 3. Differences in the means of the agronomic appeal attributes of selected vegetables on PILA Structures and Flat cropping beds in Vihiga.

Vegetable	Treatment	Yield in (kg/ha)	Height (cm)	Leaf density	Branching	Disease prevalence
Amaranthus hybridus	PILA	47440	15.8	2.6	2.7	2.8
	Flat bed	21360	11.9	2.2	2.3	2.1
Solanum scabrum	PILA	44600	16.2	2.5	2.6	2.8
	Flat bed	27160	10.5	2.1	2.2	2.3
Cleome gynandra	PILA	47440	14.2	2.7	2.7	2.8
	Flat bed	21360	12.5	2.2	2.3	2.4
Daucus carota	PILA	24672	11.7	2.4	2.4	2.7
	Flat bed	20081	9.3	2.3	2.1	2.0
P-value		≤0.001	≤0.001	0.191	0.01	0.061
Least significance difference		273.4	0.543	0.0234	0.0246	0.543
Standard error		139.4	0.277	0.0119	0.0125	0.0078
cv%		12.3	67.1	15.3	15.5	9.6

CV, Coefficient of variation.

Analysis of the viability of PILA Structures versus flat beds using NPV method

There was a high significant difference (P≤0.001) in the mean Net NPV of the PILA structures and flat beds as shown in Table 6. More analysis is given on Appendix 3 and 4.

DISCUSSION

Seasonal effect on vegetable crop performance grown on PILA structures

There was a difference in crop performance between the short and long rain seasons across all the two sites of

Table 4. Differences in the means of the agronomic indicators of selected vegetables on PILA structures and flat cropping beds for Jinja.

Vegetable	Treatment	Yield in (kg/ha)	Height (cm)	Leaf density	Branching	Disease prevalence
Amaranthus hybridus	PILA	49302	18.8	2.6	2.7	2.8
	Flat bed	35981	13.9	2.2	2.3	2.1
Solanum scabrum	PILA	43720	14.2	2.5	2.6	2.8
	Flat bed	20465	9.5	2.1	2.2	2.3
Cleome gynandra	PILA	55813	15.2	2.7	2.7	2.8
	Flat bed	36279	11.5	2.2	2.3	2.4
Daucus carota	PILA	30046	11.3	2.4	2.4	2.7
	Flat bed	18604	8.3	2.3	2.1	2.0
P-value		≤0.001	≤0.001	0.187	0.02	0.071
Least significance difference		273.4	0.543	0.0234	0.0246	0.543
Standard error		139.4	0.277	0.0119	0.0125	0.0078
cv%		12.3	67.1	15.3	15.5	9.6

Figure 3. A caption of vegetable crops growing on 1 PILA and 2 Flat beds.

studies (Vihiga and Jinja). The long rain season indicated better crop performance compared to the short rain season mostly in the yield. The difference in yield were likely caused by a variation in the amount of rainfall. The long rain season normally receive high amounts of rainfall compared to the short rain season (Okoola et al., 2008). High amount of rainfall positively interacts with soil nutrients to give a high crop yield. Differences in seasonal vegetable production have also been reported in cowpea (*V. unguiculata*) as in a study by Chesney et al. (2010) and Kimithi et al. (2009) also found that the yield of chick pea was high in the long rain period as compared to the short rain period.

Difference in the performance of selected vegetables crops grown on premium influenced land agro-usage structures between Jinja and Vihiga

There was a high significant difference in the performance of vegetables grown on PILA structures in both Vihiga and Jinja in yield and height (Table 5). There were however small significant differences in the leaf density, branching and disease prevalence in the two study sites. This would have been as a result of differences in soil properties and climatic conditions across the two study sites. Even though the two study sites are found in the Lake Victoria Basin, differences in

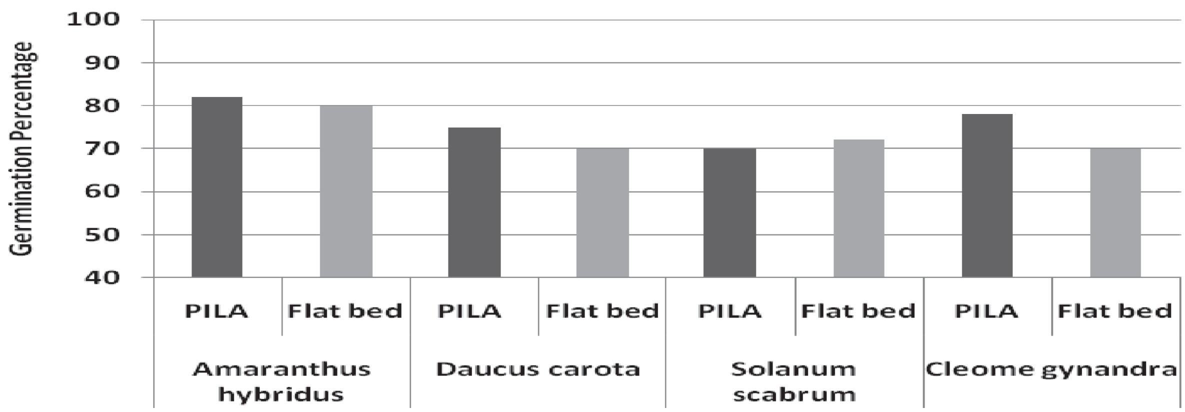

Figure 4. Germination percentage of vegetable crops growing on the PILA and Flat beds.

Table 5. Differences in the agronomic appeal attributes of selected vegetable crops grown on PILA Structures in Jinja and Vihiga.

Variety		Yield in (kg/ha)	Height in (cm)	Leaf density (score out of 3)	Branching (score out of 3)	Disease prevalence (score out of 3)
Amaranthus hybridus	Vihiga	42174	18.1	2.5	2.7	2.9
	Jinja	47907	19	2.5	2.7	2.8
Solanum scabrum	Vihiga	48230	10.8	2.7	2.7	2.8
	Jinja	40465	17.7	2.5	2.8	2.7
Cleome gynandra	Vihiga	51301	13.2	2.6	2.8	2.9
	Jinja	51163	17.2	2.6	2.7	2.7
Daucus carota	Vihiga	25488	9.4	2.5	2.6	2.7
	Jinja	31302	13.3	2.6	2.7	2.8
cv%		11.2	64.9	15.9	13.4	10.1
P-value		≤0.001	≤0.001	0.004	0.004	0.070
Least significance difference		553.8	1.717	0.075	0.066	0.051
Standard error		199.7	0.619	0.027	0.024	0.019

CV, Coefficient of variation.

climatic and soil properties are noticeable. Similar results on differences in crop performance as a result of variations in soil conditions in the Lake Victoria Basin, have been documented by Fungo et al. (2011).

Performance of vegetable crops grown on PILA structures compared to flat bed

There was a high significant difference in vegetable crop performance between the PILA structures and flat beds. Vegetable crops grown on PILA Structures performed better in the following agronomic appeal attributes; yield, height, leaf density, branching and disease prevalence compared to the ones that were grown on flat beds. The performance of vegetable crops on PILA Structures could have been attributed to better utilization of space, solar energy, water and nutrients.

Vegetable crops grown on PILA Structures had a higher germination percentage, were densely packed compared to the ones on Flat beds. Although the plot sizes and spacing was kept the same for PILA and flat beds, the seed densities varied. The design of the PILA structures permitted a special arrangement of the rows resulting in a higher seed density than on the flat bed. This arrangement could not be replicated on the Flat beds. This would have caused the vegetable crops grown on the PILA structures to have more yield than on the flat beds. Notice the effective utilization of space on the PILA beds as shown in Figure 3. The vegetable crops on PILA grew taller than on the Flat beds. But whether this was as a result of competition or sunlight need be investigated.

Table 6. Mean NPV of PILA Structures versus NPV of Flat beds for Vihiga.

	Mean NPV	Standard deviation	Standard error
PILA	191390	25007	4566
Flat bed	122087	25508	4657

NPV is the net present value; PILA is the premium influenced land agro-usage structure; N=60, test statistic t=10.63 on 58 degrees of freedom, P≤0.001.

Creation of an internal micro-climate also helped in reducing disease incidences and promoting growth as well as ensuring better nutrient use. Similar findings on better performance of crops grown on raised beds have been recorded by Wang et al. (2011) in a study on morphological and yield responses of winter wheat (*Triticum aestivum)* to raised bed planting. Other studies by Singh et al. (2010) and Fahong' et al. (2004) have recorded similar findings.

Comparison of the cost and benefits of the PILA Structures and flat beds

The NPV of the PILA structures were more than for the flats bed. This could be attributed to better crop performance. The total revenue that was obtained from vegetable crops contained on PILA Structures was higher than on flat beds in year 1 as shown in Appendix 1. This is because costs used for production of vegetable crops grown on flat beds were low compared to PILA Structures. Costs of production for vegetable crops contained on PILA structures included costs of construction (purchase of sheeting materials and rope). These costs were not incurred in making flat beds. As the years progressed as shown in Appendix 1, the revenue obtained from vegetable crops grown on PILA structures became higher and continuously increased than the revenue that was obtained from vegetable crops that were grown on flat beds. This made the net present value that was obtained from vegetables crops grown on PILAStructures to be higher compared to flat beds.

Conclusion

Vegetable crops grown on the PILA structures performed better compared to the ones that were grown on the flat beds. This was shown in the high yield, reduced disease incidences and the high net present value of the vegetables crops that were produced on PILA Structures in comparison to the Flat beds. PILA structures as an innovation or technology could be suitable for home vegetable growing preferably under high family land population pressure and/or less tillable land. Because of its micro-climate, a PILA Structure planting is known for uniform special plant arrangement and therefore good seedling growth and plant produce of an attractive marketable appearance, that is, (premium sale value). Its relevance is thus as follows: (a) Convenient to fit the Premium PILA structures into a main household compound setting; (b) None-competitive in space to an already overcrowded arable piece of land; (c) Within reach for constant care and protection of a high premium value crop.

Conflict of Interest

The authors have not declared any conflict of interest.

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Appendix 1: PILA versus flat bed analyisis.

Analysis of variance table					
Variate: Height					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Replications stratum	2	44.58	22.29	0.30	
Plot treatment (PILA versus flat)	1	15174.56	15174.56	205.91	<.001
Vegetable type	3	20722.24	6907.41	93.73	<.001
Plot treatment (PILA versus flat) *Vegetable type	3	438.79	146.26	1.98	0.114
Residual	3830	282254.12	73.70		
Total	3839	318634.30			
Variate: Yield in ha					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replications stratum	2	0.000E+00	0.000E+00	0.00	
Plot treatment (PILA versus flat)	1	2.013E+11	2.013E+11	10788.30	<.001
Vegetable type	3	1.724E+11	5.747E+10	3079.39	<.001
Plot treatment (PILA versus flat) *vegetable type	3	1.892E+10	6.308E+09	338.01	<.001
Residual	3830	7.148E+10	1.866E+07		
Total	3839	4.641E+11			
Site variations					
Variate: Yield in ha					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Site treatment (Vihiga and Jinja)	1	8.528E+09	8.528E+09	98.99	<.001
Residual	958	8.253E+10	8.615E+07		
Total	959	9.106E+10			
Variate: Height					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Site treatment (Vihiga and Jinja)	1	7891.4	7891.4	67.17	<.001
Residual	958	112556.8	117.5		
Total	959	120448.2			

Appendix 2: Analysis of the costs and benefits of constructing PILA:

Costs and revenue analysis of selected vegetable crops grown on PILA.	
Cost	**Figure in Ksh**
Amaranthus hybridus	
Land	***
Labour	1000
Pegs	***
Manure	***
Filler materials	***
Purchase of 50 empty sacks at 50	2500
Purchase of ropes	300
Fertilizer	200
Total costs (TC)	**4000**
Revenue	
total sales for season 1 (82 kg at Ksh 60)	4920
total sales for season 2 (80 kg at Ksh 60)	4800

Appendix 2. Contd.

Total Revenue (TR)	**9720**
total benefit (TV-TC)	**7240**
Solanum scabrum	
Land	***
Labour	1000
Pegs	***
Manure	***
Filler materials	***
Purchase of 50 empty sacks at 50	2500
Purchase of ropes	300
Fertilizer	200
Total costs (TC)	**4000**
Revenue	
total sales for season 1 (90 kg at Ksh 65)	5850
total sales for season 2 (81 kg at Ksh 65)	5265
Total Revenue (TR)	**11,115**
total benefit (TV-TC)	**7115**
Cleome gynandra	
Land	***
Labour	1000
Pegs	***
Manure	***
Filler materials	***
Purchase of 50 empty sacks at 50	2500
Purchase of ropes	300
Fertilizer	200
Total costs (TC)	**4000**
Revenue	
total sales for season 1 (85 kg at Ksh 65)	5525
total sales for season 2 (77 kg at Ksh 65)	5005
Total Revenue (TR)	**10530**
total benefit (TV-TC)	**6530**
Daucas carota	
Land	***
Labour	1000
Pegs	***
Manure	***
Filler materials	***
Purchase of 50 empty sacks at 50	2500
Purchase of ropes	300
Fertilizer	200
Total costs (TC)	**4000**
Revenue	
total sales for season 1 (60kg at Ksh 30)	1800
total sales for season 2 (70 kg at Ksh 30)	2100
Total revenue (TR)	**3900**
total benefit (TV-TC)	**-100**

*** Provided locally. Prices of vegetables provided by Kisumu Uchumi Supermarket; total revenue, 35265; total costs, 16000; total vegetable crop benefits, 19265.

Appendix 2. Contd.

Analysis of the costs and revenues of constructing flat beds	
Cost	**Figure in Ksh**
A. hybridus	
Land	***
Labour	1000
Manure	***
Fertilizer	200
Total costs (TC)	**1200**
Revenue	
Total sales for season 1 (60 kg at Ksh 60)	3600
Total sales for season 2 (67kg at Ksh 60)	4020
Total revenue (TR)	7620
Total benefit (TV-TC)	**6420**
S. scabrum	
Land	***
Labour	1000
Manure	***
Fertilizer	200
Total costs (TC)	**1200**
Revenue	
Total sales for season 1 (63 kg at Ksh 65)	4095
Total sales for season 2 (55 kg at Ksh 65)	3575
Total revenue (TR)	7670
Total benefit (TV-TC)	**6470**
C. gynandra	
Land	***
Labour	1000
Manure	***
Fertilizer	200
Total costs (TC)	**1200**
Revenue	
Total sales for season 1 (53kg at Ksh 65)	5525
Total sales for season 2 (62 kg at Ksh 65)	5005
Total revenue (TR)	7475
Total benefit (TV-TC)	**6275**
D. carota	
Land	***
Labour	1000
Manure	***
Fertilizer	200
Total costs (TC)	**1200**
Revenue	
Total sales for season 1 (45kg at Ksh 30)	1350
Total sales for season 2 (53 kg at Ksh 30)	1590
Total revenue (TR)	2940

Appendix 2. Contd.

Total benefit (TV-TC)	**1740**

*** Provided locally; Prices of vegetables provided by Kisumu Uchumi Supermarket;Total revenue, 25705; total costs, 4800; total crop benefits, 20905.

Appendix 3. Analysis of Inflation rate.

Year	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**Average**
Inflation rate (%)	10.95	14.5	9.8	26.8	10.1	10.7	14	9.65	5.72	6.88	12

Appendix 4. Analysis of the net present value of PILA and Flat bed.

Years	1	2	3	4	5	6	7	8	9	10	11	12	13	14
PILA (*A. hybridus*)														
Total benefits	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720
Total costs	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	1200	4000	1200	1200
Net benefits	5720	8520	8520	8520	8520	5720	8520	8520	8520	8520	8520	5720	8520	8520
NPV	**Ksh 63129.9**													
Flat (*A. hybridus*)														
Total benefits	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420
NPV	**Ksh 51714.3**													
PILA (*S. scabrum*)														
Total benefits	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115
Total costs	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	1200	4000	1200	1200
Net benefits	7115	9915	9915	9915	9915	7115	9915	9915	9915	9915	9915	7115	9915	9915
NPV	**Ksh74366.92**													
Flat (*S. scabrum*)														
Total benefits	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470
NPV	**Ksh 52117.04**													
PILA (*C. gynandra*)														
Total benefits	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530
Total costs	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	1200	4000	1200	1200
Net benefits	6530	9330	9330	9330	9330	6530	9330	9330	9330	9330	9330	6530	9330	9330
NPV	**Ksh 69654.5**													
Flat (*C. gynandra*)														
Total benefits	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275
NPV	**Ksh 50546.28**													
PILA (*D. carota*)														
Total benefits	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900

Appendix 4. Contd.

Total costs	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	1200	4000	1200	1200
Net benefits	-100	2700	2700	2700	2700	-100	2700	2700	2700	2700	2700	-100	2700	2700
NPV	**Ksh 16248.77**													
Flat (*D. carota*)														
Total benefits	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740

Years	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
PILA (*A. hybridus*)																
Total benefits	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720	9720
Total costs	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200
Net benefits	8520	5720	8520	8520	8520	8520	5720	8520	8520	8520	8520	5720	8520	8520	8520	8520
NPV																
Flat (*A. hybridus*)																
Total benefits	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620	7620
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420	6420
NPV																
PILA (*S. scabrum*)																
Total benefits	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115	11115
Total costs	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200
Net benefits	9915	7115	9915	9915	9915	9915	7115	9915	9915	9915	9915	7115	9915	9915	9915	9915
NPV																
Flat (*S. scabrum*)																
Total benefits	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670	7670
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470	6470
NPV																
PILA (*C. gynandra*)																
Total benefits	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530
Total costs	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200
Net benefits	9330	6530	9330	9330	9330	9330	6530	9330	9330	9330	9330	6530	9330	9330	9330	9330
NPV																

Appendix 4. Contd.

Flat (*C. gynandra*)																
Total benefits	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475	7475
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275	6275
PILA (*D. carota*)																
Total benefits	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900	3900
Total costs	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200	4000	1200	1200	1200	1200
Net benefits	2700	-100	2700	2700	2700	2700	-100	2700	2700	2700	2700	-100	2700	2700	2700	2700
Flat (*D. carota*)																
Total benefits	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940	2940
Total costs	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Net benefits	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740	1740

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Full Length Research Paper

Allelopathic effect of *Casuarina equisetifolia* L. on wheat, associated weeds and nutrient content in the soil

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Allelopathy is an ecological phenomenon that recently attracted researchers' attention as a new approach in weed control. Pot and field experiments were conducted to study the allelopathic effect of *Casuarina equisetifolia* L. leaf litter, one of three most common Casuarina species in Egypt. The influence was studied during two successive seasons (2012-2014) on wheat and associated weeds through foliar application of the aqueous extract and incorporating Casuarina leaf litter in the soil. In pot experiment, the results showed remarkable improvement in the growth of wheat under screen house conditions at the different rates of incorporation (15, 30 and 45 g dry L/kg soil). On the contrary, associated weed (that is, canary grass) was adversely affected and recorded up to 62% inhibition over the control. Germination was less affected both for crop and weed. Slight increases in macro (N, P, K, Ca and Mg) and micro (Fe, Mn, Zn and Cu) elements were recorded in the shoots of wheat, 21 days after sowing, compared to the control. Good response otherwise was noted in increasing soil content of the different nutrients. Under field conditions, the incorporation of dry leaf litter at a rate of 1 ton/fed (fed = 0.42 ha) caused increases in wheat biomass (65 days after sowing) by up to 27.5% in comparison with the untreated control. On the other hand, weed biomass was significantly reduced by up to 27%. Foliar application (twice) of water extract was most effective. Wheat biomass increased by up to 42.9% versus 51.2% reduction in weed biomass compared to the untreated control. Hand pulling treatment for weeds was the best regarding the increase of wheat yield (up to 66.9%) and its components (from 24.4% to >100%) followed by foliar application of the extract and then incorporation of leaf litter in the soil. From these results it can be concluded that spraying water extract of Casuarina leaf litter (comparing to incorporation the litter in the soil) has a high degree of effectiveness which ensures safe use in controlling weeds in wheat fields.

Key words: Allelopathy, cannary grass, casuarina leaf litter, nutrients, weed, wheat.

INTRODUCTION

Chemical pesticides cost the world a lot. Water, air and soil contaminations and natural ecological balance upsetting are of the most harmful aspects of pesticides. Herbicides are the main class of pesticides worldwide.

The world consumption of herbicide is about 48% of the total manufactured pesticides in the world (Gupta, 2011). Herbicides cause a great damage to the environment if compared with the other groups. Searching for the alternatives is then necessary. Allelopathy is the harmful or beneficial effect of an organism upon another, including plants and microorganisms, through the release of one or more bioactive agents (e.g., allelochemicals) into the environment. As the world's attention tends to search for new alternatives to herbicides, the natural products from plants and microbes received the greatest attention. Plants produce a wide array of secondary metabolites that can be used directly in controlling weeds or as a skeleton for new synthetic herbicides (Duke and Lydon, 1987; Duke et al., 2002). Aside from being safer and economically sounder than commercial herbicides, they might open the door for new discoveries related to new sites of action (Duke, 1990).

Various aspects of the allelopathy phenomenon have been studied. Crops affect crops, crops affect weeds, weeds affect weeds, weeds affect crops and microorganisms affect crops have been studied extensively (Mandava, 1985). *Casuarina equisetifolia* L. is one of the important trees in Egypt. It is a fast growing, evergreen, nitrogen fixing multipurpose tree. Casuarina wood is widely used as a source of energy. It is also grown along rivers for the purpose of fixation and reduction of evaporation. In the new reclaimed lands it is grown in rows along field boundaries as windbreaks. It is also being grown in the neglected wasteland/poor lands with the aim of reclaim soils (Pahwa, 1988). The roots of *C. equisetifolia* harbor nitrogen-fixing microbial assemblages that allow the host tree to colonize and thrive in low nutrient soil conditions that many other species cannot tolerate (Swearingen, 1997). Casuarina species are strongly suspected to have allelopathic properties, as evidenced by the total or near absence of the understory species which are otherwise present around it (Batish and Singh, 1998; Batish et al., 2001). The leaf leachates collected under canopy of Casuarina trees were found to have a deleterious effect on species like *Medicago sativa* L., and *Ageratum conyzoides* L. (Batish et al., 2001). Extracts from fresh and brown (dead when collected) Casuarina needles were also effective against duckweed plants (Sutton and Portier, 1989). Phenolics, terpenoids and organic cyamides were identified as allelopathic agents in the different parts of the tree including fresh needles, female cones and even leaf litters (June, 1976; Batish and Singh, 1998; Buehler, 2010). Quercetin-3-α-araboside and quercetin-3-β-glucoside were recognized as biologically active allelochmicals in *C. equisetifolia* that are responsible for the autotoxicity in Casuarina plantations (Jian et al., 2013). Kaempferol-3-α-rhamnoside and luteolin-3,4-dimethoxy-7-β-rhamnoside were also isolated and identified as autotoxins in *C. equisetifolia* (Deng et al., 1996). Thus, the aim of the present work is to study the allelopathic potential of Casuarina leaf litter on the growth and development of wheat and associated weeds as well as on the nutrient content both in soil and in wheat plants.

MATERIALS AND METHODS

Pot experiment

The allelopathic effect of dry leaf litter of *C. equisetifolia* was studied under screen house conditions on wheat (*Triticum aestivum* L., cv. Sakha-93) and one of the most common weeds in wheat fields in Egypt namely canary grass (*Phalaris minor* L.). The effect on soil properties and nutrient content in wheat shoots was also studied. Casuarina content of the different macro- and micronutrients was also investigated using methods of Cottenie et al. (1982).

Material preparation

The dry leaf litter of Casuarina was collected under the tree in the Experimental Research and Production Station of the National Research Centre, Al-Emam Malek Village, Nubaria District, Al-Behaira Governorate, Egypt. The dry materials was chopped into 1 to 5 cm pieces with an electric fodder cutter, then grounded to pass a two-mesh (4.48 mm) screen in a laboratory grinder. The ground material was mixed with loamy sand soil (88.2% sand, 4% silt and 7.8% clay according to the methods of Black et al. (1981) at rate of 15, 30 and 45 g/kg soil (equivalent to 0.5, 1.0 and 1.5 ton/fed, resp.; fed=feddan= 0.42 ha). Plastic pots (12 cm in diameter x 8 cm depth) were filled with 1 kg of soil/Casuarina ground leaves, while control pots were filled only with loamy sand soil.

Cultivation methods

Twenty grains of wheat and thirty five seeds of canary grass were sown per pot. Water was added as required to avoid water stress. Recommended NPK fertilizers were applied throughout the period of experiment (21 days after sowing). The experiment was applied in completely randomized design with three replications for each treatment.

Data collection

Germination took place 5 days after sowing; the number of emerged seedlings (wheat and weeds) was recorded until emergence ceases. At 21 days after sowing (DAS), data on vegetative growth including shoot and root lengths (cm), fresh and dry weights (g/plant) as well as dry root/shoot ratios were estimated for both wheat and weeds. Soil chemical analysis (that is, organic matter, OM; Electric conductivity, EC; pH; $CaCo_3$) was realized according to the procedure described by Jackson (1973). Macro- (that is, K, Mg, Ca and Na) and micro-elements (that is, Fe, Mn and Zn) concentrations were determined in wheat shoots 21 DAS as mentioned by Cottenie et al. (1982).

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Table 1. Physical and chemical properties of experimental soil at Numbaria District.

	Physical properties			
Particle size distribution	**Sand**	**Silt**	**Clay**	**Texture**
	68.70	24.50	6.80	Silty loam
	Chemical properties			
	pH*	**EC** dS/m**	**Ca CO_3 (%)**	**OM (%)**
	7.80	0.18	7.07	0.16
Soluble cations (meq/L)	**Ca^{++}**	**Mg^{++}**	**K^{+}**	**Na^{+}**
	3.00	2.00	0.32	2.09
Soluble anions (meq/L)	$CO^{=}_3$	HCO^{-}_3	Cl^{-}	$SO^{=}_4$
	-	1.41	0.70	5.30
Available macronutrients (mg/100 g soil)	**Total N**	**K**	**P**	**..**
	15.00	9.40	16.00	..
Available micronutrients (ppm)	Fe	Mn	Zn	Cu
	7.80	3.30	1.86	4.00

*Soil pH was measured in 1.25 L soil - water suspension. ** EC was measured as dS/m in soil paste extract.

Field experiments

In this trial we studied the allelopathic effects of the aqueous extract or dry leaf litter of *C. equisetifolia* leaves on wheat growth and yield as well as on associated weeds under field conditions. The study was conducted during two successive seasons (2012/2013 and 2013/2014) at Experimental Research and Production Station of the National Research Centre, Al-Emam Malek Village, Al-Nubaria District, Al-Behaira Governorate, Egypt. The physical and chemical soil properties of the experimental site are presented in Table 1.

The aqueous extract of Casuarina leaf litter were prepared by soaking 1 kg of the ground tissues in 5 L of distilled water under lab conditions (20 to 25°C) for 24 h in darkness. The extract was filtered and used immediately under field conditions. The Casuarina extract was applied as post-emergence (30 and 45 day DAS). Spraying was done by Knapsak hand sprayer. The treatments were then: hand pulling of weeds (twice at 30 and 45 DAS); foliar application (twice at 30 and 45 DAS) of aqueous extract of Casuarina leaf litter at a rate of 200 L/fed (20% based on dry leaf matter); incorporation of dry ground tissues of Casuarina leaf litter (1 ton/fed) into the soil at sowing time and control (unweeded).

The treatments were arranged in a randomized complete block design with three replicates for each treatment. The experimental unit consisted of 20 rows of wheat, each 15 cm apart by 3.50 meters long (10.5 m^2). Soil was prepared for cultivation by removing the residue of the previous crop, plowing twice and dividing into rows. Wheat grains cv. Sakha-93 were sown by drilling seeds manually in the rows at a rate of 60 kg/fed. Sowing date was in mid November in both seasons. Potassium sulphate fertilizer (50 K kg/fed) in the form of K_2O (48%) was added 30 days after wheat sowing. Nitrogen fertilizer (120 kg N/fed) was added as ammonium sulphate (20.6% N) in six equal supplies after complete germination and over a two weeks period till spike emergence stage. Sprinkler irrigation was applied as needed by plants. All others standard agronomic practices for wheat cultivation were done.

Data collection

Wheat and weeds dry weight

During both seasons the dry weight of weed and wheat plants (g) was recorded in 1 m^2 from each plot at 65 days after sowing. The weight was determined after drying in a forced oven at 70°C until constant weight.

Yield and its components

Wheat plants were manually harvested in the first week of May in both seasons. The tillers in 0.25 m^2 from each plot were cut and counted to determine number of spikes/0.25 m^2. The plant height (cm), No. of spikelets/spike, No. of grains/spike, length of spike (cm), weight of spike (g) and grains weight of spike (g) were also determined from 20 randomly selected tillers. Wheat was threshed manually to determine grain, straw and biological yields per plot (3 x 3.5 m) and then per fed. Harvest and crop indexes were calculated by dividing seed yield by biological yield and straw yield, respectively.

Statistical analysis

All data were subjected to analysis using M-STAT-C statistical analysis program (MSTAT, 1988). LSD test were used to compare between means.

RESULTS

Pot experiment

Germination and seedling growth

The results showed an appreciable effect of Casuarina leaf litter in improving growth of wheat under screen house conditions with a marked decline in the growth of associated canary grass weed (Tables 2, 3 and Figures 1 to 3). Generally, the data recorded an increase of 16.8% to >100% and 18% to >100% for the fresh and dry weights of wheat respectively unlike the effect on weed growth which inhibited by up to 62% in the two

Table 2. Effects of *C. equisetifolia* leaf litter incorporated in soil on wheat growth 21 days after sowing under screen house conditions.

Treatment		Casuarina leaf litter incorporation rate (g/kg soil)				LSD at 5%
Character		**0.0**	**15**	**30**	**45**	
			Season 2012/2013			
Germination (%)		58.33	63.33	95.83	65.00	13.92
Shoot weight (g/pot)	Fresh	1.31	2.86	4.50	1.53	0.25
	Dry	0.22	0.38	0.49	0.31	0.03
			Season 2013/2014			
Germination (%)		63.33	66.73	76.703	73.33	8.30
Length (cm)	Shoot	11.80	13.25	15.35	13.75	1.59
	Root	3.70	5.75	6.40	6.00	0.40
	Total	15.50	19.00	21.75	19.75	1.45
Fresh weight (g/pot)	Shoot	2.05	3.00	3.08	2.57	0.22
	Root	1.59	1.92	3.65	2.79	0.28
	Total	3.64	4.92	6.73	5.36	0.45
Dry weight (g/pot)	Shoot	0.61	0.72	0.86	0.74	0.08
	Root	0.68	0.83	2.00	1.20	0.07
	Total	1.29	1.55	2.86	1.94	0.12
Root/Shoot dry ratio		1.13	1.15	2.34	1.63	0.32

Table 3. Effects of *C. equisetifolia* leaf litter incorporated in soil on growth of canary grass weed 21 days after sowing under screen house conditions (Combined analysis of two seasons).

Treatment		Casuarina leaf litter incorporation rate (g/kg soil)				LSD at 5%
Character		**0.0**	**15**	**30**	**45**	
Germination (%)		42.90	62.90	35.20	32.40	8.78
Length (cm)	Shoot	16.67	17.00	14.00	13.00	1.60
	Root	20.33	21.00	16.00	15.33	1.54
	Total	37.00	38.00	30.00	28.33	2.88
Fresh weight (g/pot)	Shoot	2.29	2.60	1.78	1.69	0.16
	Root	7.87	7.25	3.05	2.99	0.25
	Total	10.16	9.85	4.83	4.68	0.20
Dry weight (g/pot)	Shoot	0.89	1.01	0.69	0.66	0.06
	Root	3.54	3.26	1.37	1.35	0.11
	Total	4.43	4.28	2.07	2.00	0.09
Root/Shoot dry ratio		3.97	3.22	1.98	2.05	0.30

successive seasons. The germination responded to a lesser extent. It recorded an increase of between 8.5 to 64.1% for wheat (Table 2 and Figures 1, 2) and up to 24.5% reductions for canary grass weed (Table 3 and Figure 3). It should be noted that the effect on wheat decreased in general with higher doses of incorporated leaf litter compared to low and moderate doses (Figures 1 and 2). Good response was also recorded for shoot and root lengths either for canary grass or wheat.

Macro- and microelements in Casuarina leaf litter, wheat shoots and soil

Analyzing Casuarina leaf litter revealed good composition of macro- and micronutrients including N,P,K, Ca, Mg and Fe, Mn, Zn, Cu respectively (Table 4). The chemical analysis of the macro- and microelements in treated plants of wheat showed a moderate impact of Casuarina leaf litter incorporation on increasing N, P, K, Ca, Mg, Fe,

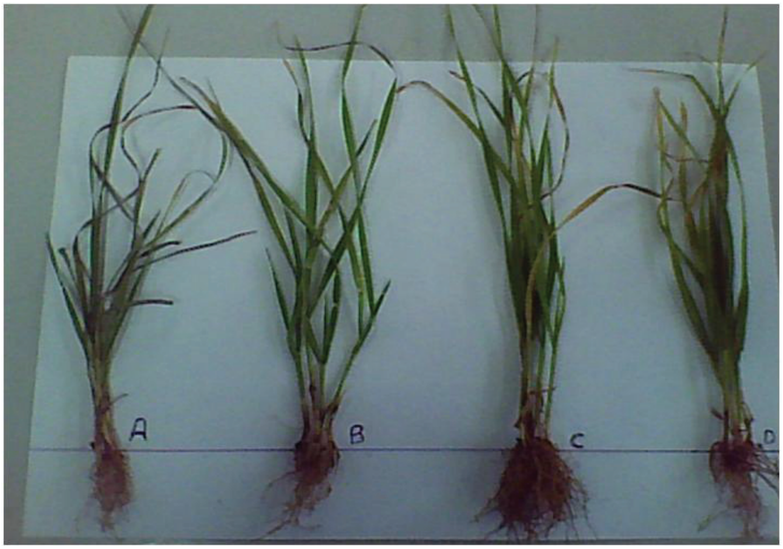

Figure 1. Effect of *C. equisetifolia* leaf litter incorporated in soil at different rates on wheat growth 21 days after sowing under screen house conditions. A, B, C, D= 0, 15, 30, and 45 g/kg soil, respectively.

Control 15 g/kg soil 30 g/kg soil 45 g/kg soil

Figure 2. Effect of *C. equisetifolia* leaf litter incorporated in soil at different rates (15, 30 and 45 g/kg soil) on wheat aerial growth.

Control 15 g/kg soil 30 g/kg soil 45 g/kg soil

Figure 3. Effect of *C. equisetifolia* leaf litter incorporated in soil at different rates (15, 30 and 45 g/kg soil) on aerial growth of canary grass weed.

Table 4. Casuarina content of macro- and micronutrients.

Character	Macronutrients (%)					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Casuarina chemical composition	1.40	0.14	0.68	1.55	0.24	126.00	96.00	14.90	6.00

Table 5. Wheat content of macro- and micronutrients at 21 days after sowing of wheat and incorporation of Casuarina leaf litter in soil under screen house conditions.

Character		Macronutrients (%)					Micronutrients (ppm)			
Treatment		**N**	**P**	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Zn**	**Cu**
Casuarina leaf litter incorporation rate (g/kg soil)	0.0	3.02	0.09	1.12	0.33	0.15	135.50	8.80	2.21	1.10
	15	3.25	0.12	1.13	0.42	0.15	178.50	9.00	2.47	1.38
	30	3.44	0.16	1.17	0.48	0.15	209.00	12.30	2.72	1.38
	45	3.88	0.17	1.20	0.51	0.15	220.00	13.20	6.46	1.93
LSD at 5%		**0.10**	**0.04**	**0.01**	**0.02**	**ns**	**3.40**	**1.00**	**0.67**	**0.53**

Table 6. Soil content of macro- and micronutrients at 21 days after sowing of wheat and incorporation of Casuarina leaf litter in soil under screen house conditions.

Character		Macronutrients (mg/100 g soil)					Micronutrients (ppm)			
Treatment		**P**	**K**	**Mg**	**Na**	**Ca**	**Fe**	**Mn**	**Zn**	**Cu**
Casuarina leaf litter incorporation rate (g/kg soil)	0.0	1.10	4.30	3.96	4.10	174.00	1.10	2.21	0.56	0.18
	15	2.01	4.80	4.66	6.10	187.50	1.50	3.09	0.89	0.09
	30	2.10	7.40	5.28	9.20	185.00	2.50	3.96	1.03	0.10
	45	2.30	7.10	5.04	9.80	208.00	3.00	4.80	1.88	0.14
LSD at 5%		**0.01**	**0.62**	**0.36**	**0.71**	**9.55**	**0.55**	**0.20**	**0.21**	**ns**

Table 7. Chemical analysis of soil 21 days after wheat sowing and incorporation of Casuarina leaf litter in soil.

Character		Chemical proprieties (%)			
Treatment		**$CaCo_3$**	**OM**	**EC(dS/m)**	**pH**
Casuarina leaf litter incorporation rate (g/kg)	0.0	2.61	0.20	0.33	8.87
	15	1.73	0.23	0.21	8.76
	30	1.71	0.19	0.33	8.80
	45	1.52	0.19	0.42	8.81
LSD at 5%		**0.12**	**ns**	**0.07**	**0.06**

Mn, Zn and Cu nutrients (Table 5). The chemical analysis of soil regarding its content of macro- and micro-nutrients 21 DAS otherwise showed high efficiency in increasing P, K, Mg, Na, Ca, Fe, Mn and Zn components under treatments supplied with Casuarina leaf litter compared to untreated treatment (Table 6). Some variations regarding soil pH, E.C, OMand calcium carbonate content were recorded (Table 7).

Field experiment

Wheat and weed growth

The results of using dry litter and aqueous extract of Casuarina leaf litter under field conditions were promising either in increasing wheat growth or suppressing associated weeds (Table 8). Foliar application of the

Table 8. Effect of *C. equisetifolia* leaf litter incorporated in soil in comparison with foliar application of the aqueous extract of the litter on growth of wheat and associated weeds 65 days after sowing under field conditions.

Character	Wheat dry weight (g/m^2)		Weeds dry weight (g/m^2)	
Treatment	**2012/2013**	**2013/2014**	**2012/2013**	**2013/2014**
T1	164.00	104.00	29.13	18.20
T2	116.00	88.00	34.20	27.44
T3	94.00	78.67	46.00	41.08
T4	81.13	68.99	58.16	56.33
LSD at 5%	5.51	9.62	6.09	9.39

T1: Hand pulling (twice at 30 and 45 DAS), T2: Post-emergence application of aqueous extract of Casuarina leaf litter (20%) at a rate of 200L/fed (sprayed twice 30 and 45 days after sowing), T3: Incroporation of 1 ton of ground leaf litter of Casuarina per fed at wheat sowing time,T4: Unweeded (control).

Table 9. Effect of *C. equisetifolia* leaf litter incorporated in soil in comparison with foliar application of the aqueous extract of the litter on yield components of wheat under filed conditions.

Treatment		**T1**	**T2**	**T3**	**T4**	**LSD at 5%**
Character		**Season 2012/2013**				
Plant height (cm)		85.00	77.33	71.33	65.67	8.03
Spike characteristics	Number/0.25 m^2	100.00	81.33	76.00	61.33	7.05
	Length (cm)	12.50	11.33	10.33	9.33	1.15
	Weight (g)	1.00	0.89	0.70	0.51	0.15
	Grains weight (g)	0.80	0.58	0.54	0.47	0.11
Spikelet's no/spike		17.33	16.33	15.00	13.67	1.37
		Season 2013/2014				
Plant height (cm)		79.33	73.00	65.00	58.00	6.82
Spike characteristics	number/0.25 m^2	120.00	116.00	104.00	86.33	11.79
	Length (cm)	12.33	11.33	10.67	9.50	1.07
	Weight (g)	1.10	0.83	0.61	0.50	0.12
	grains weight (g)	0.70	0.63	0.51	0.40	0.07
Spikelet's no/spike		17.00	15.67	15.00	13.67	1.45

T1: Hand pulling (twice at 30 and 45 DAS), T2: Post-emergence application of aqueous extract of Casuarina leaf litter (20%) at a rate of 200L/fed (sprayed twice 30 and 45 days after sowing), T3: Incroporation of 1 ton of ground leaf litter of Casuarina per fed at wheat sowing time,T4: Unweeded (control).

aqueous extract at a rate of 200 L/fed was more effective than soil incorporation treatment (1 ton/fed). Incorporation of dry leaf litter increased wheat growth (plant biomass) by up to 27.5% and reduced weed growth by up to 27% in comparison with the untreated control. Foliar treatment caused wheat growth to increase by 27.55 and 42.98% and weed growth to decrease by 41.19 and 51.28% during the two successive seasons. As expected hand pulling of weeds was the best technique as far as wheat and weed growth is concerned; an increment of >100% was estimated in the first season versus 50.7% in the second seasons for wheat growth, meanwhile a reduction in weed growth was estimated in between 49.9% in the first season and 67.7% in the second season on this treatment (Table 8).

Wheat yield and its components

Wheat yield and different other parameters including spike attributes, plant height, straw yield and biological yield were also significantly affected by Casuarina leaf litter used (Tables 9 and 10). Post-emergence application treatment was still in the lead compared to adding leaf litter to the soil. The foliar treatment achieved up to 47.79% increases in grain yield, compared to soil incorporation treatment which recorded only 33% increases as compared with untreated control. Hand

Table 10. Effect of *C. equisetifolia* leaf litter incorporated in soil in comparison with foliar application of the aqueous extract of the litter on wheat yield under filed conditions.

Treatment		T1	T2	T3	T4	LSD at 5%
Character			**Season 2012/2013**			
Yield (ton/fed)	Grain	2.27	2.01	1.81	1.36	0.04
	Straw	7.31	6.49	5.32	4.49	0.31
	Biological	9.58	8.50	7.14	5.85	0.31
Harvest index (%)		23.7	23.7	25.4	23.3	0.82
Crop index (%)		31.1	31.0	34.1	30.3	1.41
			Season 2013/2014			
Yield (ton/fed)	Grain	1.97	1.59	1.51	1.33	0.03
	Straw	6.18	4.79	4.49	3.99	0.59
	Biological	8.15	6.38	6.00	5.32	0.61
Harvest index (%)		24.23	24.89	25.19	24.93	ns
Crop index (%)		32.03	33.15	33.67	33.21	ns

T^1: Hand pulling (twice at 30 and 45 DAS), T^2: Post-emergence application of aqueous extract of Casuarina leaf litter (20%) at a rate of 200L/fed (sprayed twice 30 and 45 days after sowing), T^3: Incroporation of 1 ton of ground leaf litter of Casuarina per fed at wheat sowing time, T^4: Unweeded (control).

pulling treatment was most effective. It caused an increase in wheat grain yield by up to 66.9% and different other characteristics in a range of 24.4% to >100%.

DISCUSSION

Casuarina is a widespread tree. Its allelopathic effect is well established (Singh, 1993; Jadhav and Gaynar, 1995; Patil and Hunshal, 2004; Wu-xing, 2010). Both fresh parts and litters are involved. In our current study wheat growth was well improved in contrast to weeds (e.g., canary grass) which were negatively affected in this regard. Of the many experiments comparable with our current results is the work by Leela et al. (2014) who referred to the good allelopathic effect of *C. equisetifolia* leaf extracts. Researchers believe that Casuarina trees employ allelopathy to eliminate competing plant species, as evidenced by the absence of the nearby species around and under the tree (Batish and Singh, 1998; Batish et al., 2001). Self-inhibitory effect is also commonly observed in Casuarina colonies as many autotoxins are released suppressing root growth of its own seedlings (Liu et al., 2007). Phenolics, terpenoids and organic cyamides were reported as active allelochemicals in Casuarina trees (June, 1976; Batish and Singh, 1998; Buehler, 2010). These researches provide evidences on supporting our findings in many places regarding the allelopathic effect of Casuarina leaf litters on wheat (positively) and associated weeds (negatively).

Regarding the effect of Casuarina leaf litter on the macro- and micronutrients, the results were not consistent between the plants and soil. The treatment with Casuarina had a good impact in increasing the soil content of the different micro- and macronutrients while a poor effect was noted on wheat plants. *C. equisetifolia* litter is rich in the different nutrients as we reported earlier and this may explain in a positive way what has been obtained from results on increasing the soil content of the different element. *C. equisetifolia* leaf litter may works in enriching the soil of the different elements that could benefit wheat plants. However, some researchers believe that *Casuarina* debris is able to decrease soil pH and that has a dramatic effect on the capacity of the soil to retain nutrients (Ussiri, 2006). Barritt and Facelli (2001) demonstrated emphatically that Casuarina spp. litter has both physical and chemical effects.

Increasing yield and its components is a normal situation due to suppressing weed growth and increasing chemical soil fertility. The good effects of post-emergence application of the extract compared to adding leaf litter into the soil could be due to the absence of many of the biotic and abiotic factors that interfere with the phytotoxic action of Casuarina leaf litter in the soil. From the above results we can note the beneficial effect of Casuarina leaf litter on both wheat and weed growth, positively and negatively respectively. However, the economic side remains the crucial point in judging the reliability of using these natural products in the effective weed control in various crops. May be the advances in biotechnology and chemistry could solve many of our problems related to develop natural products as herbicides.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Inheritance and production of multiple small fruits per node, in *Abelmoschus* species, to meet consumer's demand, in the West African region

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Inheritance and fruit production studies were carried out with an *Abelmoschus esculentus* cultivar, Okpa mkpe (P_1), which expresses a mutant trait that produces multiple small fruits per node (msfpn) and two elite *A. esculentus* cultivars, Awgu early (P_2) and Mpi ele (P_3), as well as a high yielding *A. callei* cultivar, Ogolo (P_4); all of which produce the conventional solitary fruit per node (sofpn). The aim was to meet consumer's need for small sized fresh marketable okra fruits in the region. The inheritance studies showed that the mutant trait was controlled by a pair of dominant genes. The fruit morphometric studies showed that the *A. esculentus* cultivars and their hybrids differed significantly in length of fruit (LOF), diameter of fruit (DOF), circumference of fruit (COF), area of fruit (AOF) and volume of fruit (VOF). The direct cross of the mutant parent to the two elite *A. esculentus* cultivars showed that all the F_1 hybrids produced small sized fruits. On the average, multiple small sized marketable fruits were produced on 61% of fruit-bearing nodes of P_1, 58% of P_1 x P_2 hybrid and 52% of P_1 x P_3 hybrid fruit-bearing nodes, respectively. The percentage reduction in fruit parameters of the msfpn fruits in comparison with the sofpn fruits ranged from 14.28 to 23% for LOF, 10.47 to 15.12% for DOF and 11.18 to 18.89% for COF fruit parameters. Attempts to cross the msfpn parent (P_1) with an *A. callei* (late okra) elite cultivar, P_4, proved inconclusive. The discovery of the msfpn trait on an *A. callei* cultivar, Ojo ogwu, creates a possible opportunity for transferring the trait among *A. callei* cultivars. It is concluded that exploitation of the msfpn mutant trait could result to meeting okra consumers' need for small sized fruits in the region and pave the way for exporting small sized fruits; which are in great demand by okra canning industries overseas.

Key words: *Abelmoschus* species, mutant trait inheritance, multiple fruits per node, small sized fruits.

INTRODUCTION

The two edible okra, *A. esculentus* (early okra) and *A. callei* (late okra), which are characterized by the production of solitary flowers at the nodes, are short day plants with critical day lengths (CDL) of 12½ and 12¼ h

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respectively. The implication of their different CDL is that while early okra can flower at any time of the year in Nigeria, late okra only starts flowering around September when day length shortens considerably (Njoku, 1958; Oyolu, 1977; Nwoke, 1980; Siemonsma, 1982).

The fact that Africa is held as the centre of origin of okra (Purseglove, 1968; Karakoltsidis and Constantinides, 1975; Martin and Ruberte, 1978; Vickery and Vickery, 1979), notwithstanding, not much has been done to improve okra cultivars, especially in the West African region, to meet consumers demand. High yielding exotic okra introduced into the region fail to meet local consumer's needs; hence *in situ* improvement efforts becomes a research imperative (Okonkwo, 1981). Similar challenges have been documented in some okra producing regions of the world. Abdelmageed et al. (2012), reported that despite the increase in okra and other vegetable production in Turkey, yet the production does not meet the demand of the population. Consequently, any attempt to increase productivity like seeking better cultivars than those presently grown would certainly be of value.

Okra consumers in the region prefer small sized fruits necessitating research effort in this direction. Fatokun and Oken'ova (1979) observed that fruit size is an important yield component because an average Nigerian consumer prefers small-sized fruits. Also the processing and packaging industries in the developed countries, where okra is consumed, prefer small podded varieties (Sistrunk et al., 1960). Incidentally, in the region, emphasis had been placed on increasing yield by breeding for larger fruits rather than breeding for higher number of small sized fruits per plant.

A mutant early okra type that bore msfpn was isolated from our pool of local okra germplasm during screening. The (msfpn) mutant isolated looked quite different from the supernumerary inflorescence mutant described by Fatokun et al. (1979), because it produces normal shaped small sized fruits directly from the axil of the node and not as an extension of the petiole. Additionally the report of one-year old *A. callei* cultivars, Ojo Ogwu, which produces msfpn on sympodial-like branches on its trunk, is new to the author's best of knowledge. Since Fatokun et al. (1979) reported the existence of supernumerary inflorescence in *A. esculentus*, scarcely any follow-up research work is known to have been directed at exploring the potentialities of exploiting this mutation for the improvement of fresh okra fruit production in the region. Reawakening research interest in this neglected but important mutant trait (which is suspected to have more undiscovered variants) which can boost the production of small sized okra fruit in the region is a long overdue step in the right direction.

This present study reports on the inheritance pattern of this mutant trait and the production of small sized hybrid fruits, through crosses, between the mutant parent and the two elite *A. esculentus* and one *A. callei* cultivars. It equally reports for the first time, to the author's best knowledge, the occurrence of this mutant trait in *A. callei*.

MATERIALS AND METHODS

Inheritance of msfpn mutant trait

Three local *A. esculentus* cultivars P_1, P_2, P_3 (Table 1 and Figures 1 to 3) and one local *A. callei* cultivar, P_4 (Table 1 and Figure 4), were used in the separate crosses. Three pre-germinated seeds of each of the three parents were sown in medium sized black polythene bags measuring 12cm in diameter and 25 cm deep, filled with a mixture of top garden soil, poultry manure and river sand in the ratio of (3:1:1). The bags were placed on wooden benches in the screen house in the Botanic garden, University of Nigeria, Nsukka. Ten days after the emergence of the two juvenile leaves, the seedlings were thinned down to one plant per bag. Watering was done twice daily, morning and evening. Mature flowers were emasculated very early each morning when they were due to open, adopting nondestructive emasculation technique (NDET), as described by Udengwu (2007). Table 2 gives the details of the direct and reciprocal crosses.

When the hybrid seeds were dry they were harvested and stored in desiccators, using anhydrous Calcium chloride pellets as dehydrant. Three pre-germinated seeds, from the stored F_1 hybrid seeds, as well as the parental seeds, were planted per stand, in holes about 1 cm deep in the Botanic garden, on flat beds measuring 3 x 3 m with 15 cm as the within row spacing and 30 cm as the between row spacing, following standard cultural practices. The hybrids were completely randomized and there were three replicates with the three parents serving as guard rows. Chicken manure was applied at the rate of 10.75 kg per plot. Ten days after the emergence of the two juvenile leaves, the seedlings were thinned down to one per stand. The plants were rain fed. When the plants were in bloom, the hybrid plants were selfed to obtain the F_2 seeds. The F_1 hybrid plants were also backcrossed to the recessive parents to study the segregation in the backcross generations (Table 3). The selfed and backcross flowers were tagged.

The beds for the backcross were similar to that of the F_1 while that for the F_2 measured 10 x 2 m with plant spacing similar to that of the F_1. There were three replicates in a randomized complete block design. When the first fruits produced attained the age of 9 days, counts were made with respect to expression of the msfpn mutant trait, treating the replicates as a single population. Chi-square test was used in the analyses of the data. However as a result of the small population size of the experimental plants, Yates correction formula for small numbers and continuity, according to Steel and Torrie (1960) was used. The formula used was:

$$\chi^2 = \sum (\left| 0 - E \right| - 0.5)^2 / E$$

Expression of msfpn mutant trait in hybrid plants and fruit size studies

One hundred and twenty seeds of each of P_1, P_2, P_3, F_1 of P_1 x P_2 and F_1 of P_1 x P_3, from the inheritance studies, were soaked in tap water overnight and all the seeds that floated were discarded. All the plantings were done on flat beds measuring 4 x 3.0 m. The cultural practices were similar to the one used for the inheritance of msfpn mutant trait. There were 10 experimental plants per row giving rise to 50 plants per block in a randomized complete block design. The plants were sprayed weekly with Vetox 85 for the control of both the leaf and fruit borers. The plants were rain-fed throughout the period of the studies. Harvesting of fresh marketable

Table 1. Fruit bearing characteristics of the 4 okra cultivars.

Ogba mkpe	Awgu Early	Mpi ele	Ogolo
A very late-early flowering okra, *A. esculentus*, which produces long clusters of fruits per node (supernumerary inflorescence) on some nodes but can also produce solitary fruits on some nodes. High yielding but not very mucilaginous.	A very early flowering okra type *A. esculentus*, which produces short but stout solitary fruits per node, Very mucilaginous and high yielding.	A moderately late-early flowering okra *A. esculentus,* which produces solitary very long fruits per node. High yielding but not very mucilaginous.	An elite late flowering okra *A. callei* which produces long highly mucilaginous solitary fruits per node. Fruits are devoid of stiff hairs and remain fibreless at the age of 12 days.

Table 2. Details of direct and reciprocal parental crosses for the inheritance of msfpn in okra.

S/No	Parental Crosses	Nature of Cross
a)	P_1 (**msfpn**) x P_2 (**sofpn**)	Direct Cross
	P_2 (**sofpn**) x P_1 (**msfpn**)	Reciprocal Cross
b)	P_1 (**msfpn**) x P_3 (**sofpn**)	Direct Cross
	P_3 (**sofpn**) x P_1 (**msfpn**)	Reciprocal Cross
c)	P_1 (**msfpn**) x P_4 (Late **sofpn**)	Direct Cross
	P_4 (Late **sofpn**) x P_1 (**msfpn**)	Reciprocal Cross

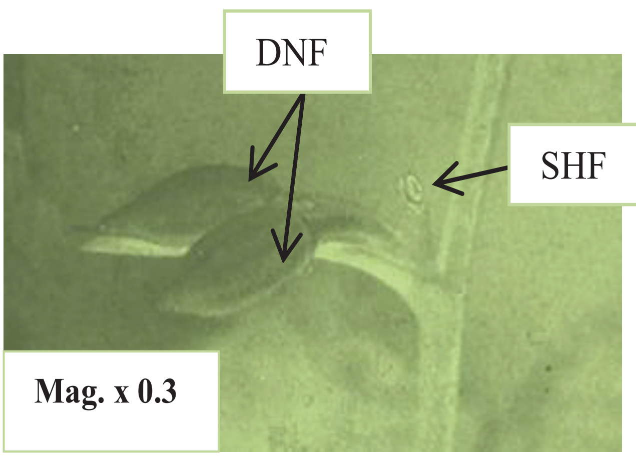

Figure 1. *A. esculentus* (Ogba mkpe) with three fruits on one axil. One fruit already harvested (SHF), two still developing (DNF)(msfpn).

fruits from the parents and the two hybrids was carried out every 5 days. As the flowers opened and formed fruits they were tagged to distinguish the expression of msfpn fruits from the sofpn fruits. Number of nodes that expressed the msfpn mutant trait was counted from 30 randomly and evenly selected plants from all the blocks and the mean expression of the trait was determined for each of the germplasm. Harvesting of fresh fruits for morphometric measurements were restricted to 6 day old fruits. Thirty fruits randomly and evenly selected from the three blocks were used for each of the measurements for all the germplasm. ANOVA was used for the analysis of collected data, while LSD (5%) was used to separate the means.

RESULTS

Inheritance pattern of the msfpn trait and number of genes governing the trait.

The results of the various crosses and their tests for significance, using the modified Chi-square statistic, have been summarized (Table 6). In the cross between P_1 and P_2, the F_1 showed that all the plants produced msfpn fruits for both the direct and reciprocal crosses. The segregation of the F_2 plants showed that for the direct cross out of the 349 plants raised, 250 plants produced msfpn flowers while 98 produced sofpn inflorescence. For the reciprocal cross, out of a total of 385 plants raised, 272 produced msfpn flowers while 113 produced sofpn inflorescence. This is in agreement with a ratio of 3 msfpn flowers: 1 sofpn inflorescence. The backcross of the msfpn flower F_1 parents to the sofpn inflorescence parent produced 256 msfpn flower producing offspring and 225 sofpn with sofpn inflorescence; for the direct cross. The backcross involving the reciprocal cross produced 228 plants with msfpn flowers and 198 plants with sofpn inflorescence. Both backcrosses produced a ratio of 1 msfpn flower: 1 sofpn inflorescence.

For the second cross P_1 x P_3, the F_1 showed that all the hybrid plants produced exhibited msfpn flowering pattern. The segregation of the F_2 plants showed that for the direct cross, out of the 341 plants raised, 243 were of the msfpn flower type while 98 were of the sofpn inflorescence type. For the reciprocal cross out of the 303 plants raised in the F_2, 215 produced msfpn flowers while

Table 3. Details of F1 and backcrosses for the inheritance of msfpn inflorescence trait in okra.

S/No	F1 and Backcross Crosses	Nature of Cross
a)	P_1 (**msfpn**) x P_2 (**sofpn**),	F_1 Selfed
	P_2 (**sofpn**) x P_1 (**msfpn**)	F_1 Selfed
	P_2 x (P_1 x P_2)	F_1 Backcrossed
	P_2 x (P_2 x P_1)	F_1 Backcrossed
b)	P_1 (**msfpn**) x P_3 (**sofpn**),	F_1 Selfed
	P_3 (**sofpn**) x P_1 (**msfpn**)	F_1 Selfed
	P_3 x (P_1 x P_3)	F_1 Backcrossed
	P_3 x (P_3 x P_1)	F_1 Backcrossed
c)	P_1 (**msfpn**) x P_4 (**sofpn**),	F_1 Selfed
	P_4 (**sofpn**) x P_1 (**msfpn**)	F_1 Selfed
	P_4 x (P_1 x P_4)	---
	P_4 x (P_4 x P_1)	---

Table 4. Phenotypic expression of msfpn in Parents, F_1, F_2 and backcross generations in okra.

Parents and crosses	Plants with msfpn inflorescence	Plants with sofpn fruiting habit	Total population	Expected phenotypic ratio	χ2	Probability
P_1	37	--	37			
P_2	--	36	36			
P_3	--	32	32			
P_4	--	40	40			
i) P_1 x P_2, F_1	52	--	52			
P_2 x P_1 , F_1	64	--	64			
P_1 x P_2, F_2	250	98	349	3:1	1.6876	0.40 - 0.30
P_2 x P_1 F_2	272	113	385	3:1	3.658	0.20 - 0.10
P_2 x (P_1 x P_2) BC_1	256	225	481	1:1	1.8711	0.40 - 0.30
P_2 x (P_2 x P_1) BC_1	228	198	426	1:1	2.1126	0 .30 - 0.20
ii) P_1 x P_3, F_1	43	--	43			
P_3 x P_1 , F_1	47	--	47			
P_1 x P_3, F_2	243	98	341	3:1	2.3469	0 .30 - 0.20
P_3 x P_1 F_2	215	88	303	3:1	2.1763	0 .30 - 0.20
P_3 x (P_1 x P_3) BC_1	255	214	469	1:1	3.4115	0 .20 - 0.10
P_3 x (P_3 x P_1) BC_1	259	212	471	1:1	4.4925	0.20 - 0.10
iii) P_1 x P_4, F_1	12	--	12			
P_4 x P_1 , F_1	19	--	19			
P_1 x P_4, F_2	. --	--	--			
P_4 x P_1, F_2	. --	--	--			

88 plants were of the sofpn inflorescence type.

The proportion of plants produced for both the direct and reciprocal crosses agreed with a ratio of 3 msfpn flower type: 1 sofpn inflorescence type. The back cross of the F_2 plant from the direct cross with the sofpn inflorescence parent gave rise to 214 msfpn flower producing plants and 255 sofpn inflorescence plants. For the reciprocal cross, 259 plants were of the msfpn flower

Table 5. Means of five fruit morphometrics for the four okra cultivars.

Cultvar	Characteristics				
	LOF	DOF	COF	AOF	VOF
P_1	5.49±.09[a]	2.75±.07[a]	6.61±.10[a]	11.58±.13[a]	36.02±.23[a]
P_2	8.43±.13[b]	4.49±.08[b]	12.62±.18[b]	32.67±.32[b]	103.27±.43[b]
P_3	12.75±.19[c]	3.09±.05[c]	8.54±.12[c]	33.46±.56[b]	107.41±.37[c]
P_4	10.39±.18[d]	3.82±.07[d]	11.09±.18[d]	31.74±.32[b]	97.59±.44[d]
LSD	0.39	0.21	0.38	2.18	1.94

Means on each column bearing the same letters do not differ significantly at LSD 0.05, **LOF**= Length of Fruit, **DOF**=Diameter of Fruit, **COF**=Circumference of Fruit, **AOF**= Area of Fruit, **VOF**= Volume of Fruit.

Table 6. Summary of Analysis of variance for five fruit morphometrics.

			ANOVA FOR LOF		
	SS	**df**	**MS**	**F**	**p**
Between:	850.761	3	283.587	411.776	0.000
Within:	79.888	116	0.689		
Total:	930.649	119			
			ANOVA FOR DOF		
	SS	df	MS	F	p
Between:	53.921	3	17.974	118.329	0.000
Within:	17.620	116	0.152		
Total:	71.542	119			
			ANOVA FOR AOF		
	SS	df	MS	F	p
Between:	9,984.440	3	3,328.147	821.347	0.000
Within:	470.039	116	4.052		
Total:	10,454.479	119			
			ANOVA FOR VOF		
	SS	df	MS	F	p
Between:	101,670.528	3	33,890.176	9,825.709	0.000
Within:	400.099	116	3.449		
Total:	102,070.627	119			

LOF= Length of Fruit, **DOF**=Diameter of Fruit, **COF**=Circumference of Fruit, **AOF**= Area of Fruit, **VOF**= Volume of Fruit.

type while 212 were of the sofpn inflorescence type. These two results also conformed to a ratio of 1 msfpn flower type: 1 sofpn inflorescence type.

In the third cross P_1 x P_4 and its reciprocal P_4 x P_1, all the F_1 produced plants that expressed the msfpn mutant trait. To produce the F_2 plants, the F_1 selfed plants gave very poor germination rates which necessitated termination of the studies.

Expression of msfpn mutant trait and fruit size studies

The results showed that the four cultivars used for the studies- P_1, P_2, P_3 and P_4 differed in their fruit bearing characteristics (Table 1; Figures 1 to 4). The five fruit morphometric characteristics, LOF, DOF, COF, AOF and VOF measured for the four parents as well as the hybrids(P_1 x P_2 and P_1 x P_3) differed significantly from each other(LSD .05)(Table 5). The ANOVA for the five fruit morphometrics (Table 6) showed that very highly significant differences were recorded for the five characteristics studied for both the parents and the hybrids.

Table 7 shows the mean expressions of the msfpn mutant trait on P_1, the mutant parent and the direct cross hybrids- P_1 x P_2 and P_1 x P_3. The percentage expression of the trait on the mutant parent was 61%, while it was 58% in the direct P_1 x P_2 hybrid and 52% in the hybrid, P_1 x P_3. The details of the comparison of the fruit size of

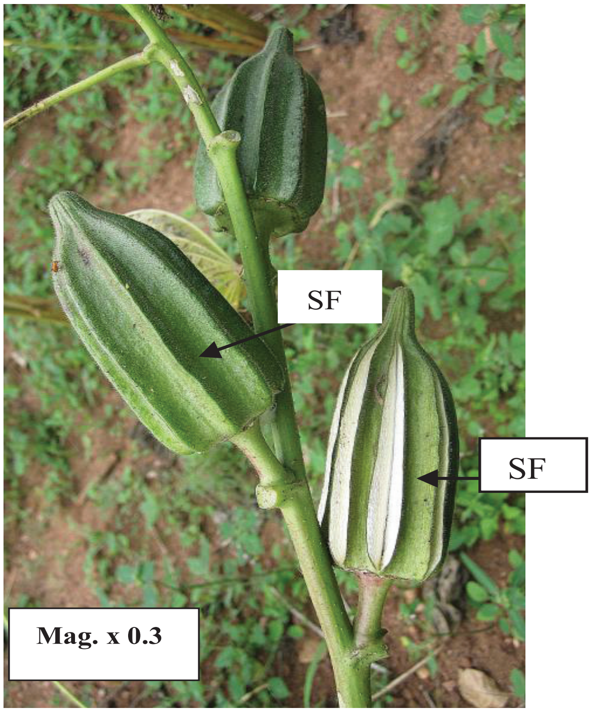

Figure 2. An early okra, *Abelmoschus esculentus* cultivar (Awgu early) showing typical sofpn fruiting habit

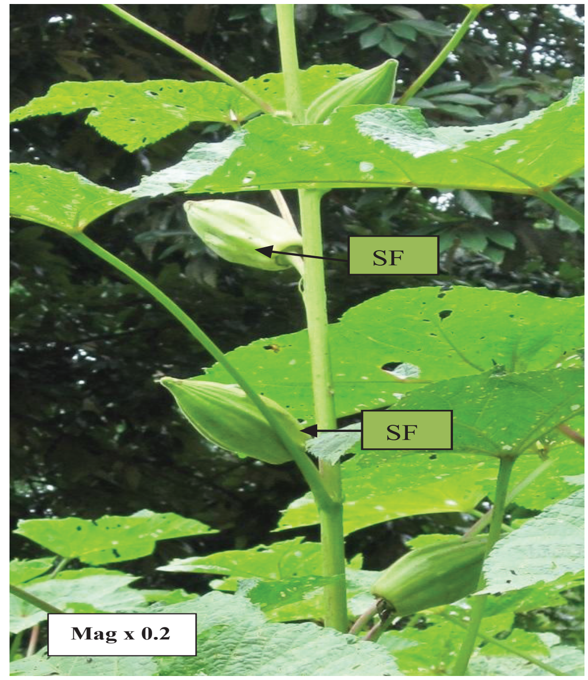

Figure 4. A late okra, *Abelmoschus callei* cultivar (**Ogolo**) showing typical sofpn fruiting habit.

Figure 3. An early okra, *Abelmoschus esculentus* cultivar (Mpi ele) showing typical sofpn fruiting habit.

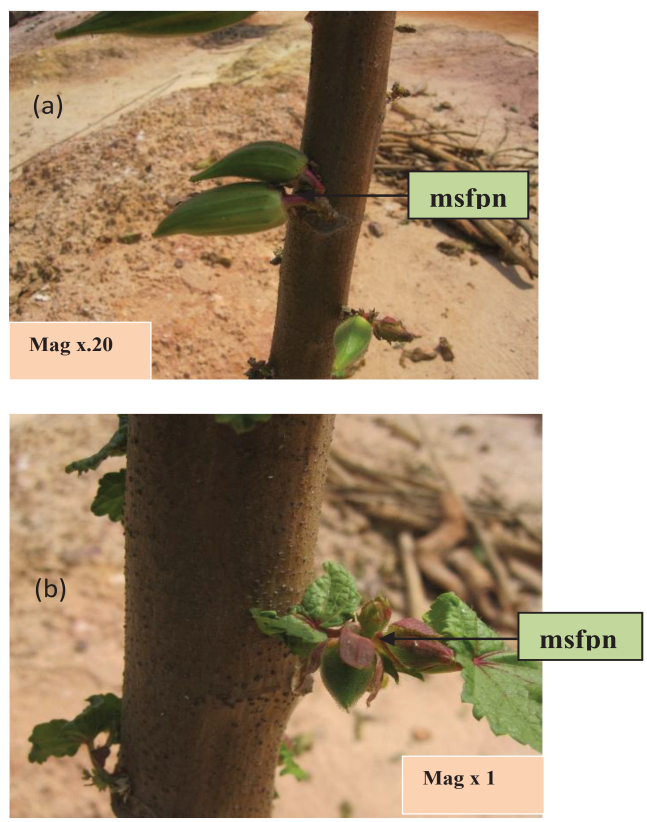

Figure 5. (a) and (b) One-year old late okra cultivar (Ojo ogwu) showing msfpn on sympodial-like branches on its trunk.

Table 7. Mean Expression of multiple small fruits per node (msfpn) on mutant parent and F_1 hybrids.

Cultivar/Hybrid	Mean No. of fruit bearing nodes	Mean No. of nodes with msfpn/plant	Mean No. of nodes with sofpn/plant	Percentage expression of mutant trait
P_1	10.10±.45	6.1±.74	3.9±.72	61
P_1 x P_2	9.30±.37	5.8±.52	3.5±.51	58
P_1 x P_3	9.00±.22	5.2±.46	3.8±.47	52

Table 8. Percentage reduction in fruit parameters based on expression of mspfn mutant gene.

Cultivar/Hybrid	Mean parameter of fruits of multiple fruit per node(mfpn)			Mean parameter of fruit from solitary node(soln)			%age reduction in fruit parameter		
	LOF	DOF	COF	LOF	DOF	COF	LOF	DOF	COF
P_1	5.49	2.75	6.61	7.20	3.24	8.15	23.70	15.12	18.89
P_1 x P_2	**6.59**	**3.31**	**9.92**	**8.00**	**3.72**	**11.20**	**17.62**	**11.02**	**11.42**
P_1 x P_3	9.00	2.65	8.26	10.5	2.96	9.30	14.28	10.47	11.18

the msfpn parent plant with those of sofpn parents are presented (Table 8). Reduction in fruit size due to the msfpn mutant fruits produced on the parent plant was 23.70% for LOF. For DOF it was 15.12% while it was 18.89% for COF. For the direct cross hybrid (P_1 x P_2), the reduction in fruit parameters were 17.62%, 11.02% and 11.42% for LOF, DOF and COF respectively. In the second direct hybrid cross (P_1 x P_3), the reductions were 14.28%, 10.47% and 11.18% for LOF, DOF and COF, respectively. For the F_1 hybrid of the third direct cross, P_1 x P_4, only 20% of the seeds germinated while for the reciprocal cross, P_4 x P_1, the germination rate was merely 5%. The very few F_1 plants that were raised, all expressed the msfpn mutant flowering pattern, though the few seeds they produced were generally small and without embryos. For the F_2 generation, reduced size of the seeds coupled with very poor seed germination necessitated discontinuation of the studies.

Towards the end of this study, the msfpn mutant trait was identified on the fat trunk of a one year old *A. callei* cultivar, Ojo Ogwu (Figs. 5a and b), which had lost all its leaves, with signs of the branches drying up; while the trunk still appeared fresh, with new small leaves forming around. The plant was found growing wild and alone in a relatively moist soil in December, when relative humidity is known to be at its lowest level in the area. This is probably the first report of this mutant trait on late or West African, okra. The germplasm of this isolated *A. callei* cultivar has been preserved in our seed bank. Its full study and characterization is currently going on and will form part of a separate report.

DISCUSSION

The results of the inheritance studies suggests that a pair of genes dominant to the normal genes governing solitary flower production governs the inheritance of this msfpn mutant trait in early okra. Fatokun et al. (1979) had studied the inheritance of supernumerary inflorescence in *A. esculentus* and reported that it was governed by a pair of genes (SiSi) which were dominant over the genes governing the conventional solitary fruiting habit (s_is_i). Incidentally the mutant type described by Fatokun et al. (1979) produced more than one fruit as an extension of the petiole. Some of the fruits produced showed distorted features which may compromise their ready acceptance by okra consumers. On the other hand the msfpn mutant reported in this study consistently formed normal shaped fruits from the axils of the leaves at each of the multiple fruit bearing nodes. As the older initiated fruits were harvested, the younger ones developed. With regular harvesting of developed fruits at the age of six days, all the initiated fruits could be harvested, resulting to the production of increased number of smaller sized fruits. However, without regular harvesting, most of the other initiated flower buds fail to form new fruits and abscission follows.

The genes controlling the msfpn mutant trait may be identical to the ones already described by Fatokun et al. (1979) or they could be allelic to it. One is more inclined to suggest that a multiple allelic set of genes may be involved in the inheritance of this trait. This will become obvious when many more mutant types are isolated and multiple crosses are carried out among them. It is interesting that this mutant trait is governed by a pair of Mendelian genes which is characteristic of many other genes controlling other traits in okra which have been reported to be simply inherited. Martin et al. (1981) noted that a surprisingly large number of characteristics in okra are inherited in a simple fashion with high heritabilities;

which suggested that they are controlled by relatively few genes. In their opinion the large chromosome number of okra provides an excellent opportunity for very wide recombination. Obviously hybrid plants expressing this mutant trait could be grown by okra farmers. With the production of small sized fruits, consumer's demand can easily be met and the door opened for export since canning industries overseas equally prefer small sized fruits (Sistrunk et al., 1960). Reduction in the size of fruits produced on the nodes expressing the mutant trait on both the mutant parents as well as the hybrids could be attributed to competition for assimilates by many fruits on a node. Kress (1981) and Stephenson (1981) observed that resource limitations as well as sibling competition are responsible for the reduced fruit size in Angiosperms. The reasons for the sterility observed in the F_1 hybrids and F_2 plants from the direct and reciprocal crosses (P_1 x P_4 and P_4 x P1) are not unconnected with the barrier to gene flow known to exist between *A. esculentus* and *A. callei* (Martin, 1982; Siemonsma, 1982). This is attributed to the wide variation in chromosome number among the two species. Whereas *A. esculentus* has a genomic chromosome number of 2n=130, *A. callei* has 2n= 194 (Singh and Bhatnagar, 1976). Hamon and Hamon (1991) observed that interspecific hybrids between *A. esculentus* and *A. caillei* can be obtained artificially, but at experimental stations and in the field very low rates of cross fertilization are observed. In addition, the sterility of the F_1 hybrids makes their genetic participation in subsequent generations unlikely.

The very highly significant results of the fruit morphometric studies are indicative of the genetic diversity that exists among the cultivars and the hybrids. This can be exploited for the further advancement of okra with respect to fruit size to meet consumers' demand in the region. Many reports equally indicate that a good understanding of the genetic variability that exists among cultivated species of the genus *Abelmoschus,* in the different characters, is a useful tool in the genetic improvement of the crop (Ariyo, 1990; Bisht et al., 1995; Kiran-Patro and Ravisankar, 2004; Omohinmin and Osawaru, 2005). For the hybrid small fruit production studies, observations in the field showed that the selfing of P_1 over 4 generations always produced plants with msfpn, but only on 61% of the flower bearing nodes. For the direct hybrids, P_1 x P_2, it was produced on 58% and for P_1 x P_3 on 52%, of the flowering nodes. Though the reasons for the observed diverse floral behaviour of P_1 are not known, they may not be unconnected with some of the complexities involved in floral initiation and development in Angiosperms which are still poorly understood (Prusinkiewicz et al., 2007; Hake, 2008; Lippman et al., 2008; Vollbrecht and Schmidt, 2009; McKim and Hay, 2010; Feng et al., 2011; MacAlister et al., 2012; Park et al., 2012). The role of cadastral genes may not be ruled out. For P_2, P_3 and P_4 the flowers and fruits produced over the 4 generations were always of the solitary types. From the evolutionary point of view, the sofpn habit may be considered primitive in comparison to the msfpn type; whose occurrence in okra is thought to be a recent development based on the knowledge that members of the Malvaceae family produce more of solitary flowers, unlike members of the Solanaceae family, that commonly produce multiple fruits per node.

According to Dimech (2011) the advantages of the inflorescence mode is all about reproduction compared to a single primitive flower. There may be dozens or even hundreds of flowers in an inflorescence, with many seeds or fruits for each flowering. Increased pollination is an important bonus. Massing flowers together makes them more visible to pollinating insects and birds. In their report, Fatokun et al. (1979) noted that the flowering pattern of okra whereby only one flower and eventually one fruit is produced per node places a limit on the number of fruits that can be produced per plant. The existence of supernumerary inflorescence, wherein more than one flower is borne per node, provides a means of increasing total yield.

The expression of this mutant trait in *A. callei*, the edible okra which is indigenous to West Africa is reported for the first time (to the author's best of knowledge). This trait was found only on the trunk of very robust one-year old plants. Simpson (2006) explained that plants with sympodial growth have a specialized lateral growth pattern in which the apical meristem is terminated. The apical meristem can either be consumed to make an inflorescence or other determinate structure, or it can be aborted. Growth is continued by a lateral meristem, which repeats the process. While it is still pre-mature to draw conclusions about the expression of this trait in this cultivar, the observation further points to some of the yet to be studied aspects of flower and fruit development in the genus *Abelmoschus;* which are germane to increasing fresh fruit production in edible okra.

Obviously, when the reported occurrence of this mutant trait on a cultivar of *A. callei* is fully studied, it could facilitate ready transfer of the trait among late okra cultivars, resulting to possibly overcoming the barrier to gene flow known to exist between *A. esculentus* and *A. callei* hybrids (Martin, 1982; Siemonsma, 1982; Hamon and Hamon, 1991). This could equally result to production of small sized *A. callei* fruits, which will facilitate meeting okra consumer's demand for small sized fruits during the dry season; when *A. callei* (dry season okra) becomes readily available as *A. esculentus* (rainy season okra) is winding up fruit production, under natural growing conditions, in the region. The production of small sized fruits through successive planting of small fruited, *A. esculentus* and *A. callei* cultivars, could result to the availability of small sized okra fruits for the greater part of the year, under rain-fed conditions in the region

Conclusion

The current study confirms the monogenic pattern of inheritance of the Mendelian genes controlling the msfpn mutant trait. Variants of the trait could exist in the population, and these could be identified when more extensive screening are carried out, for incorporation into okra improvement program in the region. The results indicate that the dominant msfpn inflorescence mutant trait in *A. esculentus* can be easily transferred to other *A. esculentus* cultivars that produce large fruits which consumers discriminate against. With the production of small sized fruits, consumer's demand can easily be met and opportunity created even for export, since canning industries overseas equally prefer small sized fruits. The identification of the mutant trait on an *A. callei* cultivar is reported for the first time (to author's best knowledge) and when fully studied could facilitate the transfer of the mutant trait to *A. callei* cultivars that produce large fruits. The expression of the mutant trait in both *A. esculentus* and *A. callei* cultivars could result to the production of small sized okra fruits all the year round in the region.

Conflict of Interest

The authors have not declared any conflict of interest.

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Oil content variability and genetic divergence in half-sib families of *Prunus armeniaca* L. in Kashmir Valley – India

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This research work is a part of NOVOD, Board sponsored project entitled "National Network on Integrated Development of Wild apricot (*Prunus armeniaca*)". The experiments were conducted to study the genetic variability-cum-diversity for seed and seedling characters among 25 half-sib families of *Prunus armeniaca* L. collected from different coordinates/locations of Jammu and Kashmir, India. Seeds of 25 candidate plus trees were collected and graded to constitute seed lots of different candidate plus trees (CPT). After the dimensional measurements for fruit, seed and kernel characters, part of the seeds from seed lots of each family/CPT were analyzed for oil content estimation. Part of the constituted seed lots of each family/CPT were sown in open field environmental conditions in the nursery following randomized block design (RBD), with a view to assess the expression of genetic diversity using non-hierarchical Euclidean cluster analysis. Intercrossing of divergent groups would lead to greater opportunity for crossing over, which releases hidden variability by breaking linkage. Candidate plus tree progenies were grouped into six clusters under open field environment. Inter-cluster distance was found to be highest between cluster II and VI, revealing their genetic closeness from high to medium. On the basis of inter and intra cluster distance cluster no. II and VI may be considered as diverse and can be utilized for hybridization when selecting genotypes for breeding purposes. Fruit length followed by fruit breadth and seedling height contributed maximum to the total divergence and played a prominent role in creating the genetic diversity.

Key words: Oil content, variability, cluster, genetic divergence, *Prunus armeniaca.*

INTRODUCTION

During the last few years, the domestic consumption of edible oils has increased substantially and has touched the level of 18.90 million tonnes in 2011 in India. By 2017 the per capita consumption of vegetable oils is expected to increase to 16 kg/person/year, thereby the demand is likely to touch 20.4 million tonnes (NMOOP, 2014). Due to ever increasing demand for edible oils in India, it was felt as the immediate need of the hour to go for alternate options for increasing production of edible oil seeds so as to cut shorts the import bill. Attention was paid to

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increase the production through tree borne oil seeds which are non-traditional oil crops. Wild apricot (*Prunus armeniaca* L.) is one amongst the many tree borne oil seed crops of India and very important in temperate regions of Himalaya. It has assumed greater significance in the recent past because of its being a potential source of edible oils. Kernels yield up to 53% of edible oil, however, little or negligible efforts have been taken for its genetic improvement.

P. armeniaca L. belongs to family "Rosaceae" and includes many varieties of cultivated and wild apricots which grow throughout north-western Himalaya between an elevation of 1,000 to 3,000 m. It's importance is well realized especially in temperate region as fuel, fodder, feed and small timber. It is one of the important multipurpose trees in the region under existing system of agroforestry (Singh and Chaudhary, 1993). The fruit of wild apricot is unfit for table purpose due to high acids and low sugars. The seed (stones) yields 27 to 33% of kernels and the kernels yield up to 53% of edible oil. Kernels are bitter in taste due to the presence of cyanogenic glycoside amygdalin (Montgomery, 1969). Oil has 94% unsaturated fatty acids (Gandhi et al., 1997) and 75% oleic acid (Aggarwal et al., 1974). The oil is utilized for cooking, body massage and as raw material for cosmetic and pharmaceutical industry (Pamar and Sharma, 1992). Kernel weight which is directly related with oil yield is a complex character and it is dependent on a number of nut components. Having direct association and relationship among themselves, characters such as kernel length, breadth and weight are of paramount importance for making selections based on any of the three characters.

A characteristic feature of all living organisms is the immense natural variability present for various characters in most of the populations. In nature, widespread species variations are present between populations growing under different geographical conditions. Therefore the selection of appropriate plus tree/seed sources/genotype assumes the foremost importance in plantation. Selection of the superior tree is one of the major factor affecting establishment and productivity. For proper utilization of observed variation in a species, it is prerequisite to know the extent of variation and its cause, whether it is due to genetic (heritable) or due to the environmental factors (non-heritable). The proportion of total variation, which is heritable is termed as heritability in broad sense (Lush, 1937), knowledge of its magnitude gives an idea about scope of effecting/bringing genetic improvement in a species through selections.

Intercrossing of divergent groups would lead to greater opportunity for genetic material (gene) crossing over to release hidden variability by breaking linkage. Progeny derived from such diverse crosses are expected to show wide spectrum (scope) of genetic variability and provide a greater scope for isolating transgressive segregants in the advance generation. Hence, these genotypes might be used in multiple crossing programme to recover transgressive segregants (Thoday, 1960).

MATERIALS AND METHODS

The present experimental investigations were conducted in the valley of Kashmir in three districts of Kupwara, Srinagar and Budgam, enjoying both moist and dry temperate coniferous and deciduous forest types. This part of experimental work was carried out under the research project entitled "National Network on Integrated Development of Wild apricot (*P. armeniaca* L.)" sponsored by NOVOD, Board Project, Ministry of Agriculture, Govt. of India.

In the present study, candidate plus trees (genotypes) of a sample of (n = 25) of wild apricot based on tree check method were selected and marked. Table 1 gives the characteristics features of selected candidate plus trees in terms of their source of collection, approximate age in years (Information collected from the owner of the plus trees), height (m), diameter at breast height (cm), canopy (m^2), time of its flowering, fruiting and total fruit yield (kg). Selected trees were free from insect-pest incidence. From each individual plus tree (genotype) more than ten kilogram of fruits were collected soon after ripening. By using vernier caliper, dimensional morphological data was recorded on the following characters:

(1) Fruit, stone and kernel length (from base to apex)
(2) Fruit, stone and kernel breadth (edge-wise from the centre) and
(3) Thickness (from middle of the fruit, stone and kernel).

250 g of kernels in 5 replicates were used for oil percentage analysis. 300 seeds (number) from each plus tree were sown in open nursery beds at a depth of 1.0 cm in five replicates under open field environmental conditions using randomized block design (RBD) at Forest nursery, Faculty of Forestry, SKUAST-K Shalimar for further evaluation of their progenies. The nursery site is located at an altitude of 1,850 m amsl within the coordinates of 34°-05/N latitude and 74°-50/ E longitude, receiving a mean annual rainfall of about 660 mm and mean temperature of 13.3°C. Minimum temperature of the area may drop to -7°C in winter months while as maximum temperature may touch to 35°C in summer. Soil at the experimental site is neutral having available nitrogen of 100 kg/ha, phosphorus 10 kg/ha and potassium 200 kg/ha. A uniform pre-treatment was given to the seeds before sowing by soaking them in warm water, allowed to cool and kept soaked for 48 h. Regular watering was carried out as per requirements. Germination data was recorded soon after the emergence of plumule above soil for consecutive 21 days from the date of sowing. Observations on seedling height, collar diameter and number of branches per seedling were taken after one full-grown season for 20 seedlings/replication/plus tree.

To understand the significance of difference among 25 different plus trees, data was subjected to analysis of variance (ANOVA). Least significant difference (LSD) was calculated and plus trees were ranked for the variables studied using a computer software programme "SPSS". Coefficient of variation (CV %) among studied traits were calculated as described by (Panse and Sukhatme, 1967). Genotypic and phenotypic coefficient of variation, and heritability (broad sense) were calculated using the method of (Kempthorne, 1957). Genetic divergence and cluster information was assessed by non-hierarchical Euclidean Cluster Analysis (Spark, 1973).

Statistical analysis

The data was analyzed statistically for the assessment of analysis

Table 1. Passport details and morphological observations of 25 selected candidate plus trees of *P. armeniaca* L.

S/No	Accession No.	Source of collection	Approximate age (yrs)	Height (m)	Diameter (cm)	Canopy (m^2)	Total fruit yield (kg)	Time of flowering	Time of fruiting
01	CPT102	Kralpora (Kupwara)	53.33	25.33	46.0	14.06	55	Last week of April	Last week of June
02	CPT 103	Rawatpora (Kupwara)	62.66	28.00	28.0	12.25	50	-do-	-do-
03	CPT 104	Shimnagh (Kupwara)	58.00	33.33	32.0	12.25	60	-do-	1st week of July
04	CPT 105	Teetwal (Kupwara)	57.66	29.00	28.0	16.00	55	-do-	Last week of June
05	CPT 106	Teetwal (Kupwara)	68.33	46.00	48.0	36.00	65	-do-	-do-
06	CPT 107	Dildar (Kupwara)	70.00	35.66	30.0	9.00	50	-do-	-do-
07	CPT 108	Handwara (Kupwara)	65.33	31.33	42.0	25.00	60	-do-	1st week of July
08	CPT 109	Chowkibal (Kupwara)	64.00	30.00	36.0	12.25	55	-do-	-do-
09	CPT 110	Drugmulla (Kupwara)	69.66	34.33	38.0	30.25	60	-do-	-do-
10	CPT 111	Sempora (Srinagar)	59.00	26.00	27.0	9.00	50	2nd week of April	-do-
11	CPT 112	Sempora (Srinagar)	63.33	41.33	25.5	20.25	45	3rd week of April	2nd week of June
12	CPT 113	Khonmoh (Srinagar)	67.33	37.66	35.0	12.25	40	-do-	-do-
13	CPT 114	Yechhnambal (Srinagar)	70.66	30.33	24.0	6.25	50	3rd week of April	1st week of July
14	CPT 115	Zowur (Srinagar)	69.00	26.00	40.0	25.00	38	-do-	-do-
15	CPT 116	Chak (Srinagar)	64.33	38.66	35.0	16.00	40	-do-	-do-
16	CPT 117	SKUAST-K (Srinagar)	59.00	40.33	35.0	30.25	45	-do-	-do-
17	CPT 118	Shalimar (Srinagar)	64.66	38.33	28.0	9.00	35	-do-	-do-
18	CPT 119	Kurhama (Ganderbal)	57.00	34.00	35.0	16.00	30	-do-	-do-
19	CPT 120	Wakura (Ganderbal)	62.33	41.66	32.0	12.25	25	-do-	-do-
20	CPT 121	Aahan (Ganderbal)	58.00	29.00	38.0	9.00	40	-do-	-do-
21	CPT 122	Zazuna (Ganderbal)	62.66	37.33	45.0	42.25	35	-do-	-do-
22	CPT 123	Nunar (Ganderbal)	63.33	36.66	30.0	9.00	40	-do-	-do-
23	CPT 124	Dab (Ganderbal)	71.00	45.00	47.0	18.00	45	-do-	-do-
24	CPT 125	Kondbal (Ganderbal)	64.66	38.66	38.0	14.35	60	-do-	-do-
25	CPT 126	Choora (Ganderbal)	68.33	37.33	34.0	22.10	35	-do-	-do-

of variance, variance component, heritability, genetic gain, correlation and genetic divergence in completely randomized design (CRD) and randomized block design (RBD) for growth and biomass traits.

Critical difference (CD)

The critical difference (CD) was calculated as under:
CD = S.E x $t_{0.05}$ (error degree of freedom)

Where: S.E is the standard error of difference calculated as:

$$S.E. = \sqrt{\frac{2 \times MESS}{R \times T}}$$

MESS = Mean sum of square due to error, R = Number of replication, T= Number of treatments $t_{0.05}$ = Tabulated value of t at 5 per cent level of significance. Mean difference between any two families greater than calculated CD value was taken as significant difference.

Variability parameters

$$PCV(\%) = \sqrt{\frac{Vp}{\bar{X}}} \times 100 \qquad GCV(\%) = \sqrt{\frac{Vg}{\bar{X}}} \times 100$$

Where: Vp = Phenotypic variance, Vg= Genotypic variance, PCV (%)= Phenotypic coefficient of variation, GCV (%)= Genotypic coefficient of variation, $\overline{X}$ = Population mean of the character.

Coefficients of variation

Coefficients of variation were calculated as given by Pillai and Sinha (1968).

$$CV (\%) = (SD/\overline{X}) \times 100$$

Where: CV = Coefficient of variation, SD = Standard deviation, $\overline{X}$ = Population of mean.

Heritability (broad sense)

Heritability (borad sense) was calculated as suggested by Burton and Devane (1953) and Johnson et al. (1955).

$$h^2 = \frac{Vg}{Vp} \times 100$$

Where: h^2 = Broad sense heritability, Vg = Genotypic variance, Vp = Phenotypic variance.

Seedling height (cm)

Height was measured from collar region up to the apex of leading shoot at the end of growing season.

Collar diameter (mm)

Collar diameter was also measured at the end of growing season with the help of a digital Vernier caliper.

Germination percent

Germination percent was calculated as the number of seeds sown and the number of seeds germinated, expressed in percentage.

$$\text{Germination percent} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \times 100$$

RESULTS AND DISCUSSION

Fruit, stone and kernel characteristics

Table 2 presents data pertaining to 25 Candidate plus trees of wild apricot with variation in fruit, stone kernel and oil content characters. Analysis of variance indicated significant differences among 25 different candidate plus trees for all the studied characters. Oil content per cent recovered from kernels showed significant variations and ranged between 47.20% for CPT116 to 50.79% for CPT 110. Maximum value of 50.79% oil content recorded for CPT 110, was followed by CPT117 with 50.40% oil content, however both differed significantly. Six sources recorded above 50% of oil content. There is less than 4.00% oil content variation among all the CPTs, but most of the CPTs differ significantly from one another thereby implying that this character can be exploited for tree improvement programme. Morphometric characteristics of fruit, stone and kernels also recorded significant variations. Maximum fruit size was recorded in CPT 103 (length 31.76 x breadth 31.33 x thickness 29.90 mm). However, this had no relationship with stone and kernel size which was found different in different CPTs irrespective of their fruit size. Maximum seed length of 21.25 mm was recorded for CPT 119. Highest kernel length of 15.30 mm, breadth of 10.25 mm and thickness of 5.48 mm was recorded in CPT 118,103 and 104 respectively.

Germination, survival and seedling characteristics

Data presented in Table 3 revealed highly significant differences through analysis of variance among germination and all the morphological characters studied viz., germination percent, survival per cent, seedling height, seedling collar diameter and number of branches/seedling. The maximum value for germination percent (71.00), survival percent (46.00), seedling height (102.71 cm), seedling collar diameter (6.94 mm) and number of branches per seedling (4.66) were recorded in CPTs – 124, 106, 123, 106 and119 respectively. It has been demonstrated that seeds of a single species when collected from different coordinates (locations/altitudes) differ in viability, germination, growth and biomass performance, as reported by Isik (1986) in *Pinus brutia* , Todaria and Negi. (1995) in some Himalayan tree species and Chauhan et al. (1996) in *Alnus nepalensis.* Rapid genetic gain is the result of selection among CPTs which differ significantly in seed and seedling traits, similar findings were reported by Dangasuk et al. (1997) in *Faidherbia albida*.

Variations refer to observable differences in individual for a particular trait. These differences may partly be due to genetic factors and partly due to environmental effect. The observed value of a trait is the phenotypic value of that individual. The related magnitude of these components determines the genetic properties of any particular species. The extent of variation observed in germination per cent (CV-7.21%), survival per cent (CV-10.67%), seedling height (CV-5.78%), seedling collar diameter (CV-14.95%) and number of branches per seedling (CV-18.23%) was found to be moderately high (Table 3).

Table 2. Variation in fruit, seed, kernel characteristics and oil content (%) in different candidate plus trees of wild apricot (*P. armeniaca* L.)

S/No	CPT	Fruit length (mm)	Fruit breadth (mm)	Fruit thickness (mm)	Stone length (mm)	Stone breadth (mm)	Stone thickness (mm)	Kernel length (mm)	Kernel breadth (mm)	Kernel thickness (mm)	Oil content (%)
01	CPT (WA)102	20.46	18.85	13.05	16.10	14.88	9.34	12.68	8.68	4.84	48.39
02	CPT (WA)103	31.76	31.31	29.90	20.25	15.60	10.29	13.02	10.25	5.34	49.40
03	CPT (WA)104	18.72	15.58	12.65	14.61	10.55	9.68	10.97	6.16	5.48	50.30
04	CPT (WA)105	23.12	22.84	20.20	20.33	15.82	10.04	13.60	8.88	5.37	47.50
05	CPT (WA)106	21.11	21.31	18.56	19.47	16.21	10.12	12.81	8.55	4.65	48.60
06	CPT (WA)107	22.44	25.65	22.46	19.84	15.03	9.79	12.94	8.65	5.16	47.80
07	CPT (WA)108	25.88	24.50	22.88	20.15	16.13	9.86	13.58	8.15	5.26	50.20
08	CPT (WA)109	24.65	24.03	21.79	21.22	15.77	9.74	12.95	6.49	4.47	49.20
09	CPT (WA)110	24.35	22.75	20.60	19.60	15.14	9.58	13.44	7.87	4.75	50.79
10	CPT (WA)111	23.24	24.15	20.04	18.93	15.00	9.89	14.55	9.11	4.77	48.60
11	CPT (WA)112	25.49	25.36	22.40	19.31	15.57	9.88	13.47	9.24	4.66	47.80
12	CPT (WA)113	22.93	20.52	17.67	19.94	15.31	10.41	13.14	9.37	5.40	49.20
13	CPT (WA)114	26.94	27.83	24.39	20.30	16.10	9.94	13.43	9.37	5.11	47.69
14	CPT (WA)115	24.72	23.62	21.14	21.01	15.37	9.58	12.17	8.67	4.98	50.10
15	CPT (WA)116	25.01	21.65	20.22	20.02	15.37	9.77	12.96	9.45	5.25	47.20
16	CPT (WA)117	27.67	25.74	24.94	20.81	16.05	9.91	12.98	8.80	4.90	50.40
17	CPT (WA)118	26.22	24.98	23.26	19.63	15.83	9.85	15.30	10.14	4.91	49.50
18	CPT (WA)119	26.35	25.26	23.32	21.25	16.23	10.22	13.72	9.83	5.12	49.00
19	CPT (WA)120	28.94	27.86	25.74	20.91	15.59	9.63	13.02	8.84	5.32	48.50
20	CPT (WA)121	28.29	26.69	25.16	19.90	16.22	9.59	12.33	8.46	4.51	47.80
21	CPT (WA)122	27.89	26.76	23.24	20.01	16.32	9.84	14.30	9.18	4.92	48.59
22	CPT (WA)123	27.37	27.46	23.46	20.92	15.88	9.84	14.60	9.47	4.85	48.99
23	CPT (WA)124	29.24	28.07	26.76	21.24	16.07	9.76	13.84	9.46	4.94	50.10
24	CPT (WA)125	30.49	28.26	25.43	20.38	15.64	9.91	13.28	9.31	4.96	48.70
25	CPT (WA)126	20.79	20.25	18.59	15.62	16.33	10.05	10.66	8.79	4.84	47.80
Mean		25.36	24.45	21.91	19.67	15.52	9.86	13.19	8.85	4.99	48.88
C.V.		3.44	3.43	3.73	3.65	3.60	2.96	5.63	6.24	7.36	0.30
S.E.		0.49	0.48	0.47	0.42	0.32	0.17	0.44	0.32	0.21	0.02
C.D. 5%		1.41	1.37	1.34	1.21	0.93	0.48	1.25	0.93	0.60	0.15
Range	Lowest	18.72	15.58	12.65	14.61	10.55	9.34	10.97	6.16	4.47	47.20
	Highest	31.76	31.31	29.90	21.25	16.33	10.41	15.30	10.25	5.48	50.79

Table 3. Germination and seedling characters of 25 CPTs of *P. armeniaca* L.

S/No.	Character		Germination (%)	Survival (%)	Seedling height (cm)	Seedling collar diameter (mm)	Number of branches/ seedling
01	CPT (WA)102		53.33	25.33	98.99	4.28	4.33
02	CPT (WA)103		62.66	28.00	85.83	5.20	3.33
03	CPT (WA)104		58.00	33.33	101.33	4.16	4.33
04	CPT (WA)105		57.33	29.00	94.42	4.57	3.66
05	CPT (WA)106		68.33	46.00	89.21	6.94	2.33
06	CPT (WA)107		68.00	35.66	79.79	5.96	3.33
07	CPT (WA)108		65.33	31.33	93.97	6.34	4.00
08	CPT (WA)109		64.00	30.00	89.38	6.73	2.66
09	CPT (WA)110		69.66	34.33	100.34	5.30	3.66
10	CPT (WA)111		59.33	26.33	94.03	4.28	3.33
11	CPT (WA)112		63.33	41.66	86.76	4.52	3.33
12	CPT (WA)113		67.33	37.66	82.17	5.84	2.66
13	CPT (WA)114		70.66	30.66	95.39	6.53	3.33
14	CPT (WA)115		69.33	25.66	87.04	4.59	4.33
15	CPT (WA)116		64.00	37.33	78.21	3.99	4.00
16	CPT (WA)117		59.33	39.00	83.91	4.79	2.33
17	CPT (WA)118		66.00	41.00	79.21	4.22	3.33
18	CPT (WA)119		57.00	35.00	99.79	5.50	4.66
19	CPT (WA)120		62.33	41.66	85.83	6.90	3.66
20	CPT (WA)121		58.33	29.66	94.81	5.55	4.66
21	CPT (WA)122		62.33	37.66	75.03	4.19	3.66
22	CPT (WA)123		63.33	36.66	102.71	4.75	2.33
23	CPT (WA)124		71.00	45.00	76.15	6.28	2.33
24	CPT (WA)125		64.33	38.00	81.53	5.03	3.66
25	CPT (WA)126		66.00	37.66	94.52	4.77	4.33
Mean			63.62	34.94	89.21	5.25	3.50
C.V.			7.21	10.67	5.78	14.95	18.23
S.E.±			2.65	2.15	2.97	0.45	0.36
C.D. 5%			7.53	6.12	8.47	1.28	1.04
Range		Lowest	53.33	25.33	75.03	3.99	2.33
		Highest	71.00	46.00	102.71	6.94	4.66

Cluster analysis and percent contribution of characters studied to total genetic gain

In measuring genetic distance between populations and differentiating population at early stages in variability studies, seed and seedling characters can be used as a quantitative character in defining a genotype. As tree characters measured in natural population are amenable to geographical and environmental interactions, seedling characters measured in different environment are more useful in differentiating population at preliminary stage (Hedge et al., 2004).

The analysis of variance revealed the existence of significant difference among 25 plus tree progenies for all the traits, indicating the existing of huge genetic variability. The cluster pattern of 25 candidate plus tree progenies/genotypes under open field environmental conditions is given in Table 4. Under open field environment they were grouped into six clusters. Cluster I recorded the highest number of 12 families (CPT-105, 126, 119, 111, 108, 114, 110, 115, 107, 116, 113 and CPT 120) followed by Cluster III with nine (9) families (CPT-117, 123, 122, 125, 118, 112, 124, 103 and CPT 109) under open field environment. Families of Candidate plus trees occupying same cluster numbers, indicate their genotypic stability with respect to the eco-geographical coordinates. Families of candidate plus tree formed same groups in different clusters indicating that even thought the genotypes (parents) were selected from different eco-geographical areas, the genetic make-up along with breeding system, heterogeneity, and unidirectional selection pressure may be the cause of genetic diversity among different families of candidate plus tree, besides geographical variation to some extent. The cluster pattern in *Bombex ceiba* and *Eucalyptus terreticornis* proved that geographical variation need not necessarily be related to

Table 4. Distribution of 25 CPTs in different clusters in open field environmental conditions.

Cluster	I	II	III	IV	V	VI
I	**0.00**	4.92	4.77	7.43	5.29	8.20
II		**0.00**	5.08	7.80	6.04	10.20
III			**0.00**	8.325	5.34	9.24
IV				**0.00**	7.96	7.23
V					**0.00**	9.22
VI						**0.00**

Table 5. Inter and Intra cluster distances of 25 CPT progenies of *Prunus armeniaca L.*

Cluster	Total No. of CPTs in each cluster	Notation of CPTs
I	12	CPT(WA)-105, 126, 119, 111, 108, 114, 110, 115, 107, 116, 113, 120
II	1	CPT(WA)-121
III	9	CPT(WA)-117, 123, 122, 125, 118, 112, 124, 103, 109
IV	1	CPT(WA)-102
V	1	CPT(WA)-106
VI	1	CPT(WA)-104

genetic diversity (Chaturvedi and Pandey, 2001; Surendran and Chandrasekharan, 1984). Intercrossing of divergent groups would lead to greater opportunity for crossing over, which releases hidden variability by breaking linkage. Progeny derived from such diverse crosses are expected to show wide spectrum of genetic variability providing a greater scope for isolating transgressive segregants in the advance generation. Hence, these genotypes might be used in multiple crossing programme to recover transgreesive segregants (Thoday, 1960). As revealed by Table 5 inter-cluster distance was found to be highest between cluster II and VI (10.20) under open field environmental conditions followed by 9.24 between cluster III and VI. Studies in linseed and maize have revealed that the material is vulnerable to the variable environmental conditions (Murthy et al., 1973; Prasad and Singh, 1990).

Mean performance of the clusters with respect to different character (Table 6) indicated that highest mean values for oil content (50.30%) and seedling height of 101.33 mm were recorded in cluster VI , whereas maximum mean values for kernel thickness (20.24 mm) kernel length (12.35 mm), kernel breadth (8.91 mm), kernel thickness (5.11 mm) and germination percent of 64.69 was recorded in cluster I. Cluster II recorded maximum values for fruit length, thickness and seed breadth and number of branches per seedling. Cluster V recorded maximum values for seed thickness (10.12 mm), survival 46% and seedling collar diameter of 6.94 mm. Cluster IV did not record highest mean even for a single parameter studied. The present results also get support from (Gupta and Patil, 1988) in *Leucaena latisiliqua* and (Manga and Sen, 2000) in *Prosopis cineraria*. Contribution of different characters to total divergence is illustrated in Figure 1. Fruit length contributed maximum (39.33%) followed by fruit breadth (11.00%) and seedling height (9.67%) under open field environmental conditions. Knowledge of percent contribution to total divergence gives us an idea about scope of effecting genetic improvement through selection of desired traits.

Conclusion

The cluster pattern proved that geographical variation need not necessarily be related to genetic diversity (Chaturvedi and Pandey, 2001; Surendran and Chandrasekharan, 1984). Intercrossing of divergent groups would lead to greater opportunity for genetic material (gene) crossing over, to release hidden variability by breaking linkage. Progeny derived from such diverse crosses are expected to show wide spectrum of genetic variability provided a greater scope for isolating transgressive segregants in the advance generation. Hence, these genotypes might be used in multiple crossing programme to recover transgreesive segregants (Thoday, 1960).

On the basis of inter and intra cluster distance cluster no. II and VI may be considered as diverse and can be utilized for hybridization when select genotypes for breeding purposes. Therefore for getting heterosis, the genotypes from cluster I, II, V and VI with high cluster means for majority of characters can be utilized for hybridization in the further tree improvement programme of this species.

Table 6. Mean and Grand Mean values for various characters in different clusters for 25 CPT progenies of *P. armeniaca* L.

Cluster/ characters	I	II	III	IV	V	VI	Grand mean
Oil content (%)	48.70	47.80	49.18	48.39	48.60	50.30	48.83
Fruit length (mm)	24.56	28.29	27.86	20.46	21.11	18.72	23.50
Fruit breadth (mm)	23.91	26.69	26.88	18.85	21.31	15.58	22.20
Fruit thickness (mm)	21.44	25.16	24.57	13.05	18.56	12.65	19.24
Seed length (mm)	20.24	19.90	20.42	16.10	19.47	14.61	18.46
Seed breadth (mm)	15.62	16.22	15.86	14.88	16.21	10.55	14.89
Seed thickness (mm)	9.90	9.59	9.89	9.34	10.12	9.68	09.75
Kernel length (mm)	13.35	12.33	13.75	12.68	12.81	10.97	12.65
Kernel breadth (mm)	8.91	8.46	9.15	8.68	8.55	6.16	08.32
Kernel thickness (mm)	5.11	4.51	4.88	4.84	4.65	5.48	04.91
Germination (%)	64.69	58.33	64.03	53.33	68.33	58.00	61.12
Survival (%)	33.52	29.66	37.44	25.33	46.00	33.33	34.21
Seedling height (cm)	90.46	94.87	84.50	98.99	89.21	101.33	93.23
Seedling collar diameter (mm)	5.38	5.55	5.08	4.28	6.94	4.16	5.23
Number of branches/ seedling	3.75	4.66	3.00	4.33	2.33	4.33	3.73

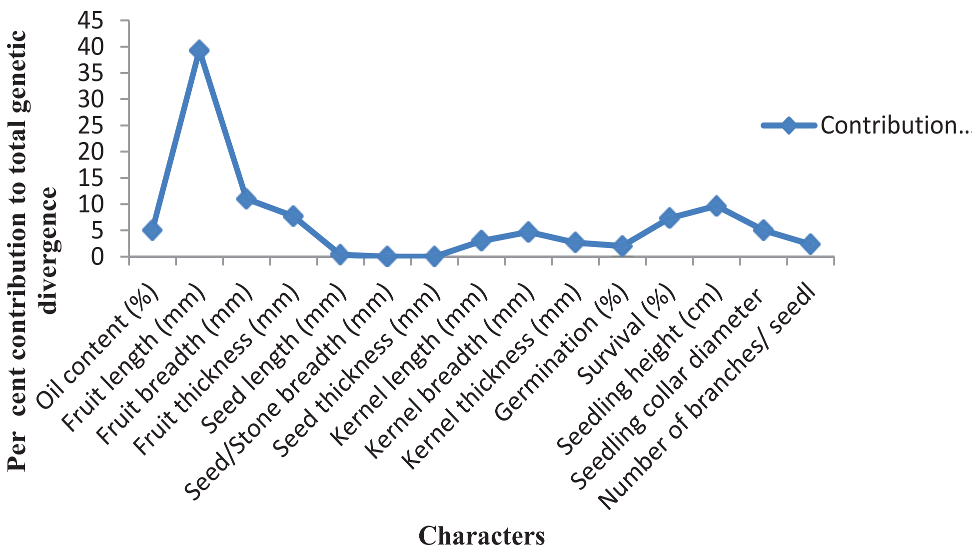

Figure 1. Percent contribution of each character to total divergence for 25 CPTs of *P. armeniaca* L.

Conflict of Interest

The authors have not declared any conflict of interest.

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Enzymatic and biological assessment of sulfonylurea herbicide impact on soil bacterial communities

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Soil microorganisms play an important role in the decomposition of organic and inorganic matter in soil, being capable of breaking down different types of xenobiotic substances in soil. Application of herbicides in crops is commonly used in agriculture to increase agricultural productivity and weed destruction. Two sulfonylurea herbicides (tribenuron methyl and nicosulfuron) were applied to the soil samples in different doses. The effect of the herbicides on soil enzymatic activities and on the main groups of microorganisms involved in the soil nitrogen cycle (ammonifying, nitrifying and denitrifying bacteria) was studied under laboratory conditions. As far as the values of enzymatic activities are concerned, it was determined that they were strongly inhibited by the application of these two herbicides. Values of the dehydrogenase and urease activities from the experimental variants, most closely reflects the inhibitory effect of herbicides. Enzymatic indicator of soil quality shows values recorded between 0.390 to 0.541 for soils treated with tribenuron-methyl, respectively 0.401 to 0.431 in those treated with nicosulfuron. The results showed that nicosulfuron had a lower inhibitory effect on the enzymatic activity of soil from tribenuron-methyl. Ammonifying, nitrifying, and denitrifying bacteria responded differently to the action of herbicides, depending on the dose that was applied. Bacterial indicator of soil quality recorded values between 1.645 to 1.706 for soils treated with tribenuron-methyl and 1.651 to 1753 in those treated with nicosulfuron. Tribenuron-methyl and nicosulfuron affect negative bacterial metabolic activity and bacterial communities.

Key words: Nicosulfuron, tribenuron-methyl, soil, enzymatic activity, bacteria nitrogen cycle.

INTRODUCTION

Most xenobiotic substances used in agriculture have more or less toxic effects on living organisms. Studies attempt to determine the impact of xenobiotic substances on soil microorganism communities, given the role these

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communities play in the biogeochemical cycle in nature. Application of xenobiotic substances in arable crops to destroy weeds and pests results in irreversible soil pollution. This process has toxic effects on soil microorganism communities, which are essential players in organic matter decomposition. Among the negative effects of xenobiotic substances applied on soil are their quantitative and qualitative reduction, biochemical activity inhibition and changes in community structure which affect its diversity and weight of microbial communities. The present study comparatively investigates the impact of two sulfonylurea herbicides, that is, tribenuron-methyl and nicosulfuron, on microorganisms that are involved in the nitrogen cycle in the soil.

Sulfonylurea herbicides are a distinctive group of herbicides that are widely used in arable crops to destroy weeds. They have become very popular, due to their low toxicity to small mammals, low application rates and effective weed control (Sarmah and Sabadie, 2002).

The application of herbicides to destroy weeds in arable crops has beneficial effects on agricultural production, but negatively influences soil microorganism growth, metabolic activity, and population dynamics (Sannino and Gianfreda, 2001; Zhou et al., 2009). Such negative effects were regularly reported for other xenobiotic substances, such as fungicides (Latijnhouwers et al., 2000), insecticides (Yao et al., 2006; Cycon et al., 2010) or heavy metals (Avidano et al., 2005; Gülser and Erdoğan, 2008), when investigating their impact on soil ecosystems.

The impact of sulfonylurea herbicides on soil microorganism communities has been studied for chlorsulfuron, thifensulfuron, amidosulfuron (Borozan et al., 2010), tribenuron-methyl, thifensulfuron-methyl (Andersen et al., 2001), bensulfuron-metil (Saeki and Toyota, 2004) and metsulfuron-methyl (Wang et al., 2010).

Cinosulfuron and bensulfuron applied in normal rates and 100 times higher had no effect on the total bacteria number and respiratory activity in the soil, but inhibited nitrification. Of the two sulfonylurea herbicides applied, bensulfuron showed more toxic effects than cinosulfuron on 17 bacteria strains and 12 fungal strains used for analysis (Gigliotti and Allievi, 2001).

A combination of two sulfonylurea herbicides (60% bromoxynil + 3% prosulfuron) used to provide a broad spectrum weed control in arable crops indicated effects on microorganism growth and activity. Their impact on bacterial communities (heterotrophic bacteria, but mostly nitrifiers), actinomycetes and fungi was demonstrated. The dehydrogenase activity proved to be an important indicator of the side effects of these herbicides (Pampulha and Oliveira, 2006).

Cinosulfuron, another sulfonylurea herbicide, causes the inhibition of microorganism community development in the soil. Aerobic bacteria, autotrophic nitrifying bacteria, *Azotobacter* bacteria and fungi showed increased sensitivity to this herbicide (Allievi and Gigliotti, 2001).

Enzymatic activities of soil biota are reliable biomarkers for assessing the effects of herbicide application on ground and below-ground ecosystems. Consequently, certain enzymes, such as oxidases (e.g., dehydrogenase, catalase) or hydrolases (e.g., protein phosphatase, urease, inverters), are routinely used for this purpose (Benitez et al., 2004). Among various bacterial enzymes, the dehydrogenase activity provides the most `natural` approach for understanding the impact of herbicides on agricultural ecosystems because it is directly related to soil respiratory processes; therefore, this effect criterion is often employed in toxicity tests with herbicides.

Xenobiotic biodegradation takes place mainly within rhisophere, wherein the populational diversity and abundance of bacterial biotas are higher than in the upper or lower soil horizons. This allows an effective decomposition of various chemical compounds, such as metsulfuronmethyl (Ghani and Wardle, 2001), dichlorophenoxyacetic acid (Shaw and Burns, 2004), and atrazine (Piutti et al., 2002). Several groups of soil bacteria are able to use herbicides as an energy source for their growth and development. For example, gram-negative and gram-positive bacteria (van Eerd et al., 2003; Gimsing et al., 2004) as well as heterotrophic bacteria (Merini et al., 2007; Zabaloy et al., 2010) were shown to use glyphosate as nitrogen, carbon, or phosphor source. As a result, assessing the structure of local soil microbiota may allow researchers to identify anthropic-induced soil disturbance.

This study involves highlighting the action of xenobiotic substances (herbicides of the sulfonylureas group), tribenuron-methyl and nicosulfuron on the community of microorganisms implicated in biogeochemical cycle of nitrogen in soil and on the main enzymatic activities that decompose organic matter.

MATERIALS AND METHODS

Uncontaminated soil (cambic chernozem) was sampled to 0 to 20 cm from the experimental fields of the Banat's University of Agricultural Sciences and Veterinary Medicine of Timisoara (Romania). The samples were spiked, under laboratory conditions, with two sulfuronureic herbicides, namely tribenuron-methyl and nicosulfuron. Tribenuron-methyl, also known as 2-methox-6 -[[[[(4-methyl-1 ,3,5-triazine-2-il) methylamino] carbon] sulfone] benzoic amino acid is also present in herbicides from local markets under the trade name „Helmstar" (Tellurium Chemical, Romania). Nicosulfuron known as 2 - [[(4,6-dimethoxypyrimidin-2-il) aminocarbon] aminosulfon]-N, N-dimethyl-3-pyridinecarboxamide is also present in herbicides from local markets under the trade name „Mistral" (ISK Biosciences Europe, Romania).

Soil treatment with herbicides

The soil was passed through a 2 mm sieve and put in polyethylene bags. Different doses of herbicides were prepared using distilled water, and then used for spiking the soil samples so that the soil humidity was 40%. The dose of herbicide applied onto the dry soil was calculated assuming a uniform distribution in the plow layer

(Atlas et al., 1978). Apart from the control group (R: 0 µg tribenuron-methyl, 0 µg nicosulfuron) the following herbicide concentrations were considered in the present study: (1) group A, A1: 0.6 µg tribenuron-methyl, A2: 0.4 µg nicosulfuron; (2) group B, B1: 1.2 µg tribenuron-methyl, B2: 0.8 µg nicosulfuron; (3) group C, C1: 1.8 µg tribenuron-methyl, C2: 1.2 µg nicosulfuron; (4) group D, D1: 3 µg tribenuron-methyl, D2: 2 µg nicosulfuron; (5) group E, E1: 4.2 µg tribenuron-methyl, E2: 2.8 µg nicosulfuron. After herbicide application, the samples were incubated for 7 days, at 28°C.

Enzymatic activity

The following enzymatic activities were assessed: catalase activity (CA), actual dehydrogenase activity (ADA), potential dehydrogenase activity (PDA), and urease activity (UA).

CA was determined by using the permanganometric method, that is, titration with a 0.05 N $KMnO_4$ solution until a weak pink coloration occurred). The enzymatic activity was expressed as milligrams of undecomposed oxygenated water (H_2O_2) per gram of soil (Dragan-Bularda, 2000).

Actual dehydrogenase activity (ADA) and potential dehydrogenase activity (PDA) were detected using the method described by Casida et al ., (1964). This technique involves the incubation of soil samples with TTC (2, 3, 5-triphenyltetrazolium chloride) for 48 h, at 37°C. ADA and PDA was shown as milligrams formazan per gram of sediment/soil. The adsorbance of reduced TTC was measured at 485 nm.

Urease activity (UA) assesses the rate of urea decomposition in ammonia (NH_3) and carbon dioxide (CO_2). The reaction mixture was incubated at 37°C, for 24 h. The ammonia was extracted with a 2 N potassium chloride (KCl) solution, and determined by spectrophotometric nesslerization at 445 nm; the corresponding calibration curve was created using an ammonium chloride (NH_4SO_4) solution (Dragan-Bularda, 2000).

Ecophysiological groups of bacteria

Serial dilutions were performed from the herbicide-spiked soil samples (viz. 10^{-1} to 10^{-6}). These solutions were incubated on elective culture media for 7 to 21 days (28°C), depending on the preferences of investigated groups of bacteria.

Samples were incubated for a week at 27°C on Ashby culture medium to determine the number of nitrogen-fixing bacteria (NFB). The presence of aerobic NFB resulted in formation of a fine velum on culture medium surface or/and in marked and adherent rings on test-tube walls. Depending on the species, the veil was either greenish yellow (*A. vinelandii*) or brown (*A. chroococum*). In contrast, anaerobic NFB (*Clostridium* sp.) induced the formation of gas bubbles in positive test tubes (Zarnea, 1994).

The culture medium for ammonifying bacteria (AMB**)** had the following chemical composition: NaCl (0.5 g), peptone (2 g), distilled water (1000 ml). The samples were incubated under anaerobic conditions for 14 days, at 22°C. AMB number was assessed based on the reaction between ammonia and Nessler reagent; the Alexander table was used as screening benchmark (Cusa, 1996).

The culture medium for nitrifying bacteria (NB) consisted of standard saline traces (50 ml), $(NH_3)SO_4$ (0.5 g), $CaCO_3$ (1 g), and distilled water (950 ml). Samples were incubated for 20 days, at 28°C. Tubes containing nitrate were identified with diphenylamine sulphuric acid. A blue colour reaction showed that nitrite and nitrate were formed, and therefore, the tube was scored positive (Dunca et al., 2007).

The denitrifying bacteria (DNB) were grown in selective culture medium, which contained: standard saline solution (50 ml), KNO_2 (20 g), glucose (10 g), KCO_3 (5 g), oligoelement solution (1 ml), and distilled water (1000 ml). The samples were incubated at 28°C, for 7 to 15 days, and diphenylamine-sulphuric acid was added in each test tube. Positive samples were colourless due to nitrate metabolization by DNB (Dunca et al., 2007).

Indicators of soil quality

The enzymatic indicator of soil quality (EISQ) and the bacterial indicator of soil quality (BISQ) were calculated by using the formulas (1) and (2), as proposed by Muntean (1995-1996, 1996):

$$EISQ = 1/n\ \Sigma\ Vr\ (s)/Vmax\ (i) \quad (1)$$

where: EISQ - enzymatic indicator of soil quality, n - number of enzymatic activities, Vr (i) – individual, real value, Vmax (i) – theoretical, maximum, individual value.

$$BISQ = 1/n \times \Sigma\ \log10\ N \quad (2)$$

where: BISQ - bacterial indicator of soil quality, n - number of ecophysiological groups, N - number of bacteria belonging to each ecophysiological group.

Statistical analysis

Statistical data interpretation was conducted with Microsoft Excel 2003 and Statistica 10 software packages.

RESULTS AND DISCUSSION

Our study showed that PDA and ADA are both inhibited by tribenuron-methyl and nicosulfuron application (Figure 1). However, ADA values were higher than PDA values, and therefore, this biomarker is more reliable when assessing the effect of these two herbicides on soil bacterial communities. A recent study showed that metsulfuron-methyl and 2,4-D (syn. 2,4 dichlorophenoxyacetic acid) greatly inhibited dehydrogenase activity (Zabaloy et al., 2008). Similarly, glyphosate application resulted in a significant decrease for the same enzymatic activity (Araújo et al., 2003; Zabaloy et al., 2008). Moreover, dehydrogenase activity proved to be an important indicator of side effects that are associated with the use of sulfuronureic herbicides (Pampulha and Oliveira, 2006). This information suggests that ADA may serve as relevant field biomarker for assessing the effect of herbicide application on soil bacterial biota.

The current experiment revealed that CA can be either stimulated or inhibited by herbicide application, depending on the herbicide type and exposure dose. UA and PDA, however, were, by far, less sensitive when applying different doses of tribenuron-methyl and nicosulfuron. Field studies found that metsulfuron-methyl reduced urease, amylase, and protease activity (Ismail et al., 1998). Similar results were reported for other herbicides, such as glyphosate or glufosinate, which displayed a dose-dependent effect on soil enzymatic activity (Accinelli et al., 2002; Accinelli et al., 2007; Lupwayi et al., 2007). In contrast, bromine application

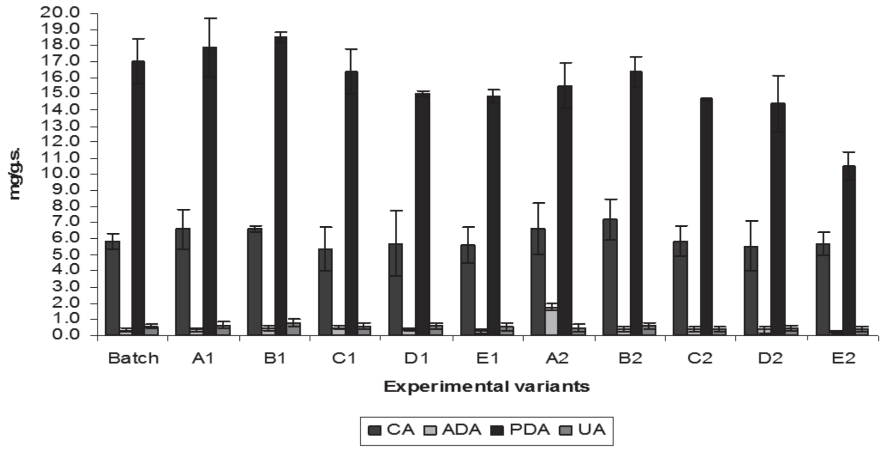

Figure 1. Variation of enzymatic activity for the investigated soil samples (A1. soil treated with tribenuron-methyl DN, B1. soil treated with tribenuron-methyl 2xDN, C1. soil treated with tribenuron-methyl 3xDN, D1. soil treated with tribenuron-methyl 5xDN, E1. soil treated with tribenuron-methyl 7XDN, A2. soil treated with nicosulfuron, B2. soil treated with nicosulfuron 2xDN, C2. soil treated with nicosulfuron 3xDN, D2. soil treated with nicosulfuron 5xDN, E2. soil treated with nicosulfuron 7xDN, Batch untreated soil, CA - catalase activity, ADA - actual dehydrogenase activity, PDA - potential dehydrogenase activity, UA – urease activity).

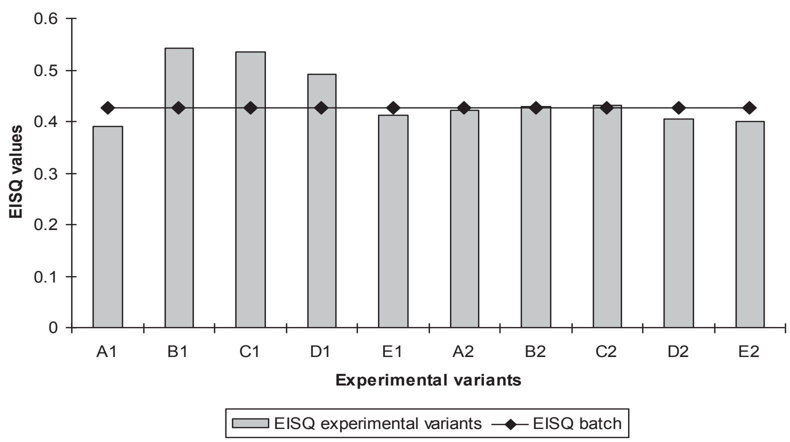

Figure 2. EISQ values in investigated soil samples (A1. soil treated with tribenuron-methyl DN, B1. soil treated with tribenuron-methyl 2xDN, C1. soil treated with tribenuron-methyl 3xDN, D1. soil treated with tribenuron-methyl 5xDN, E1. soil treated with tribenuron-methyl 7XDN, A2. soil treated with nicosulfuron, B2. soil treated with nicosulfuron 2xDN, C2. soil treated with nicosulfuron 3xDN, D2. soil treated with nicosulfuron 5xDN, E2. soil treated with nicosulfuron 7xDN, Batch untreated soil, EISQ-enzymatic indicator of soil quality).

had discrepant effects on different soil enzymes (that is, cellulase, alkaline phosphatase, acid, sulphatase); thus, this compound was found to significantly reduce cellulase and sulphatase activity, but to increase phosphatase activity, as a potential resistance mechanism to herbicide action (Omar and Abdel-Sater, 2001).

Enzymatic indicator of soil quality shows values recorded between 0.390 to 0.541 for soils treated with tribenuron-methyl, respectively 0.401 to 0.431 in those treated with nicosulfuron. The measured values of EISQ were higher for tribenuron-methyl than for nicosulfuron, thereby indicating that nicosulfuron is more toxic for soil bacterial biota than tribenuron-methyl (Figure 2).

Our result indicated that herbicide application diminished in a dose-dependent manner AMB, NB, and NFB (*Clostridium* genus) number in soil. In contrast, DNB

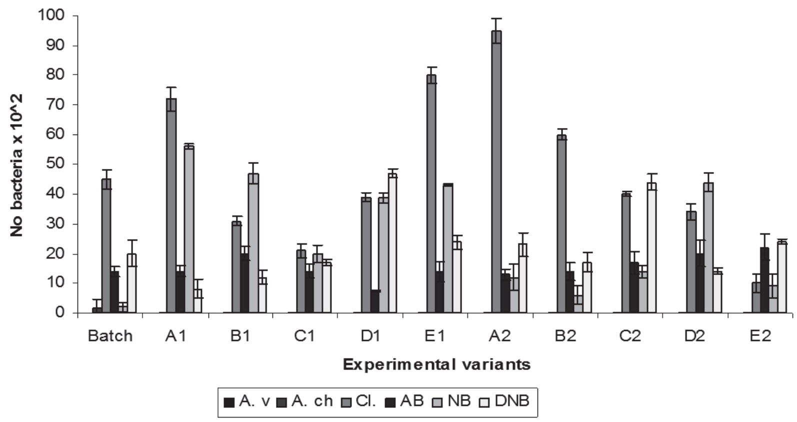

Figure 3. Quantitative population dynamics for investigated bacterial groups (A1. soil treated with tribenuron-methyl DN, B1. soil treated with tribenuron-methyl 2xDN, C1. soil treated with tribenuron-methyl 3xDN, D1. soil treated with tribenuron-methyl 5xDN, E1. soil treated with tribenuron-methyl 7XDN, A2. soil treated with nicosulfuron, B2. soil treated with nicosulfuron 2xDN, C2. soil treated with nicosulfuron 3xDN, D2. soil treated with nicosulfuron 5xDN, E2. soil treated with nicosulfuron 7xDN, Batch untreated soil, A.v-*Azothobacter vinellandi*, A.ch-*Azothobacter chroococcum*, Cl- *Clostridium* genus, AB- ammonifying bacteria, NB- nitrifying bacteria, DNB- denitrifying bacteria).

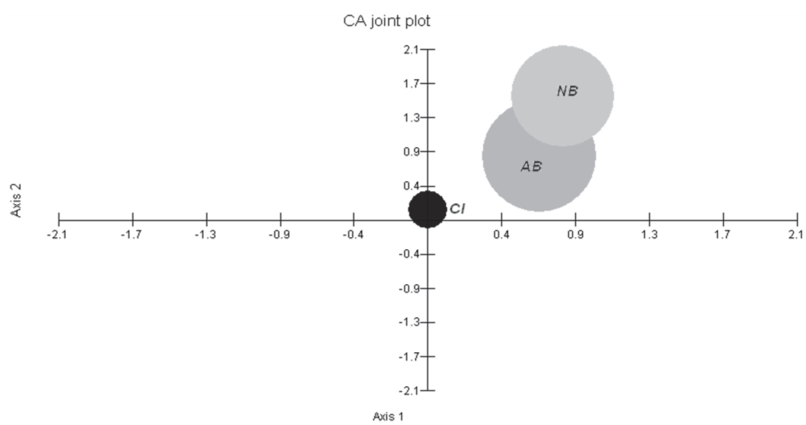

Figure 4. CA joint plot bubble representation (NB- nitrifying bacteria, AB-ammonifying bacteria, Cl – *Clostridium* genus).

displayed a more heterogeneous pattern of variation (Figure 3), and hence, the quantitative population dynamics for this bacterial group cannot serve as a pertinent biomarker of herbicide-induced soil disturbance. The bacteria of the *Clostridium* genus are the most sensitive bacterial group to tribenuron-methyl and nicosulfuron impact, with the strongest effect being reported when the exposure dose was seven-fold higher than the normal dose. In addition, quantitative population dynamics for these bacteria revealed a linear trend with exposure dose.

In this study, NFB of the *Clostridium* genus showed a distinctly different behaviour to herbicide exposure when compared to AMB, NB, and DNB. Thus, CA graphical representation revealed that highest dissimilarity between the herbicide type and exposure dose for these bacteria (0.000 to 0.109), whereas AMB and NB displayed similar responses to tribenuron-methyl and nicosulfuron exposure, irrespective of exposure dose: AMB: 0.320 to 0.646; NB: 0.290 to 0.782 (Figure 4). This observation is supported by the results of cluster analysis (Figure 5).

The bacterial indicator of soil quality (BISQ) reflects the effects of tribenuron-methyl and nicosulfuron on soil bacterial biota. Bacterial indicator of soil quality recorded values between 1.645 to 1.706 for soils treated with tribenuron-methyl and 1.651 to 1753 in those treated with

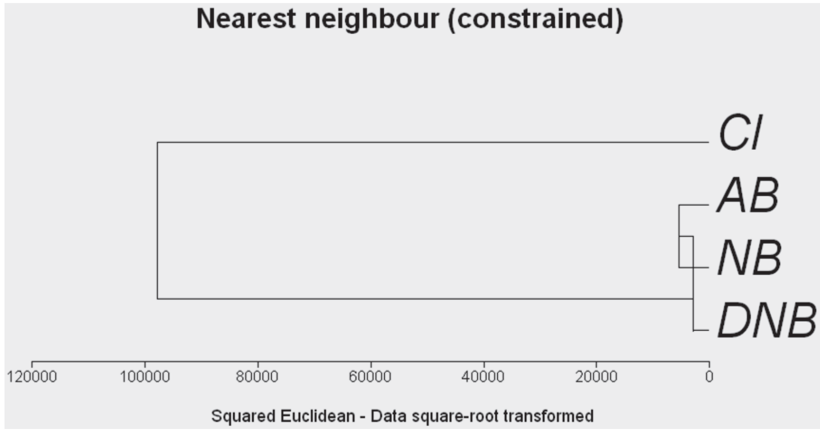

Figure 5. Cluster statistic analysis (Cl – *Clostridium* genus, AB- ammonifying bacteria, NB- nitrifying bacteria, DNB-denitrifying bacteria).

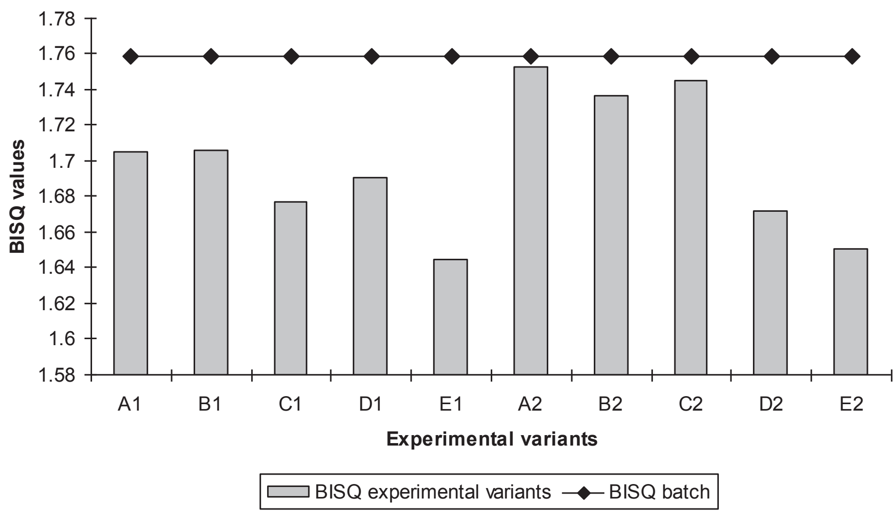

Figure 6. BISQ values in investigated soil samples (A1. soil treated with tribenuron-methyl DN, B1. soil treated with tribenuron-methyl 2xDN, C1. soil treated with tribenuron-methyl 3xDN, D1. soil treated with tribenuron-methyl 5xDN, E1. soil treated with tribenuron-methyl 7XDN, A2. soil treated with nicosulfuron, B2. soil treated with nicosulfuron 2xDN, C2. soil treated with nicosulfuron 3xDN, D2. soil treated with nicosulfuron 5xDN, E2. soil treated with nicosulfuron 7xDN, Batch untreated soil, BISQ-bacterial indicator of soil quality).

nicosulfuron. In our study, BISQ values were lower for nicosulfuron (range: 1.645 to 1.706) than those reported for tribenuron-methyl (range: 1.651 to 1.753). This suggests that tribenuron-methyl is more toxic than nicosulfuron for bacterial communities that are involved in nitrogen cycle in soil (Figure 6).

Conclusions

The effects of nicosulfuron on soil bacteria enzymatic activity are lower as compared to tribenuron-methyl. The actual and potential dehydrogenase activities, as well as urease activity are essential for assessing the effects of xenobiotics substances on metabolic activity of soil microbial biota. Tribenuron-methyl and nicosulfuron induce a dose-dependent inhibition of enzymatic activity of ammonifying, nitrifying and denitrifying bacteria in soil. Tribenuron-methyl and nicosulfuron affect negative bacterial metabolic activity and bacterial communities.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

Factors affecting feed intake, body weight, testicular size, and testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) serum concentrations in peri-pubertal male camels

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Body weight, testes development and serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were determined and compared in two groups of peri-pubertal male dromedaries. Daily maximum and minimum ambient temperatures and dry and wet-bulb temperatures were recorded. Temperature-humidity index was calculated. The camels were divided into two equal groups of nine camels each, of comparable body weight: Group (A) received a diet of 13% crude protein and 2.9 MCal metabolizable energy (ME) with added premix, while Group (B) received a non-pelleted diet of alfa-alfa and wheat straw at the ratio of 1:3 giving 12.4% CP and 2.7 MCal ME. Individual feed intake was calculated after 14 days of adaptation. Feed offered and orts were recorded daily throughout the experimental period (24 months). Animals were fed diets containing 1:3 alfa alfa:wheat straw. Blood samples were collected from 5 camels in each group at 15-day intervals during the experimental period. None significant difference in total body weight was found between groups A and B throughout the entire experimental period (p >0.05). Group averages of daily feed intake for the entire period were 5.82 kg in group A and 7.08 kg in group B, respectively (p<0.05). The latter group had significantly larger testicular size than group A (p<0.05), seasonal difference in testicular size was also significant (p<0.05); serum FSH level was significantly higher in group B than group A (p<0.05), whereas serum testosterone and LH levels were comparable in the two groups.

Key words: *Camelus dromedaries*, body weight gain, testicular size, testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH).

INTRODUCTION

In addition to nutrition and health, one of the most important factors affecting camel productivity is its low reproductive performance and long reproductive cycle (Aboul-Ela, 1991). These are major obstacles to the growth of dromedary camel populations (Tibary and Anouassi, 1997) since in camels, as in other farm animals, a high level of fertility is an important perquisite for breeding, genetic improvement and increased production.

Al-Qarawi et al. (2000) classified reproductive stages of male camels according to age as follows: pre-pubertal, (<3 years); peri-pubertal (3 to less than 5 years); mature, (5 to <15 years), and aged (≥15 years). They reported that plasma testosterone concentration in peri-pubertal male camels was 3.2±0.4 ng/ml. Others reported that male camels as young as 3 years old may be sexually active and may be used for mating (Matharu, 1966; El-Wishy and Omer, 1975; Gombe and Odour-Okele, 1977; Arthur et al., 1985).

Studies in India showed that the breeding season of camels extends from December to March that is, during the period of short day length (Matharu, 1966). Similar short day breeding seasons were reported in the Sudan (Musa and Abusineina, 1978). However, limited attempts have been made to manipulate the onset of the breeding season or to extend it in camels (Ott, 1991; Musa et al., 1993; Tompson and Johnson, 1995). Osman et al. (1979) reported that the size and weight of the testes in camels are affected by the age and season of the year. Animals on a good plane of nutrition reach puberty relatively early. The influence of body weight on puberty appears to be more marked than the influence of age; Abdel-Rahim et al. (1994) and Abdel-Rahim (1997) reported a highly negative correlation between testicular dimensions and age at the onset of spermatogenesis. Testosterone controls most of the reproductive functions in male animals (Hafez and Hafez, 2000) and its level is significantly increased in male camels during rut (Azouz et al., 1992). El-Bahrawy and El-Hassanein (2011) reported that serum testosterone concentration in camels started to rise during pre-rut, attaining maximum level during rut, then decreased towards basal level during post-rut. Rateb et al. (2011) reported that the average serum testosterone value was significantly lower in sub-fertile compared to fertile camels. Overall, immature camels had a significantly lower serum testosterone level (Al Qarawi and ElMougy, 2008).

In a previous study on the association between feed and body weight gain, testicular development and serum testosterone in pre-pubertal camels, a non-significant difference was recorded in total body weight gain between two camel groups receiving diets similar to those used in the present study (Al Saiady et al., 2013). Also no significant difference in testicular size was recorded between the two groups at the start of the experiment. On the other hand, serum testosterone level was significantly higher in group A receiving the pelleted feed as compared to group B receiving the non-pelleted feed. The aim of the following study was to evaluate the effect of pelleted versus non-pelleted, and season of the year, on daily feed intake, body weight, testes development and serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in peri-pubertal male camels.

MATERIALS AND METHODS

Animals and diet

The experiment was conducted at the Camel Breeding, Range Protection and Improvement Center in Al-Jouf area, Kingdom of Saudi Arabia during 2011 - 2012. Eighteen dromedary peri-pubertal male camels (*Camelus dromedarius*) aged around 3 years, were used in the study. The animals belonged to the *Mijahim* breed, the main dairy camel breed in Saudi Arabia, and were housed and hand fed. They were divided into two groups, A and B, of matched average body weights. Each group consisted of 9 camels. Group A camels received a commercial diet with 13% crude protein (CP) and 2.9 Mcal metabolizable energy (ME) in addition to mineral-vitamin premix, while Group B received a traditional non-pelleted diet used at the Center, with 12.43% CP and 2.7 MCal/kg ME and without premix (Table 1).

Individual feed intake was calculated after 14 days adaptation period. Feed offered and orts were weighed and recorded daily throughout the experimental period (24 months). Both diets had a roughage:concentrate ratio of 1:3. Group A diet was offered in pelleted form incorporating both roughage and concentrate components. Fresh water was available *ad lib*. For serum biochemical analyses, morning blood samples were collected by jugular venipuncture at 15-day intervals from five animals designated for blood sampling in each group. Serum was then separated from clotted blood samples by centrifugation and frozen at -20°C until analyses. Total protein, albumin, glucose and cholesterol values were determined in the samples, while total globulin was estimated as the difference between total protein and albumin and the albumin globulin ratio was calculated.

Ambient temperature

Maximum and minimum ambient temperatures and dry and wet–bulb temperatures were recorded daily, using a dry and wet bulb thermometer. Temperature-humidity index (THI) was calculated (Maust et al., 1972):

$$THI=0.72\ (Tdb + Twb) + 40.6$$

Where Tdb = dry bulb temperature; Twb = wet bulb temperature.

Measurements and laboratory analysis

The following parameters were determined: (i) body weight (in Kg) at 15 day intervals. The animals were weighed after 10 h of fasting using a platform scale Mettler Toledo©, 3000 kg capacity; (ii) total body weight gain in kg (iii) daily weight gain in kg/ day. The serum testosterone, FSH and LH concentrations were determined using specific ELISA kits (Diagnostic Automation Inc. CA. SA). Testicular volume was determined as the method described by Weibel (1989) using the following formula:

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Table 1. Diet composition and chemical constituents (Dry mater basis).

Items	Diet A	Diet B
Raw materials %		
Barley	60.22	62.23
Wheat bran	9.63	12.08
Soya Meal 48%	4.25	-
Salt	0.47	-
Limestone	2.10	-
Acid buf	1.00	-
Molasses	3.00	-
Premix	0.30	-
Alfalfa	19.03	15.23
Wheat straw	-	10.46
Nutrients %		
Dry matter (DM)	90.20	92.52
Crude Protein (CP)	13.08	12.43
Crude Fiber (CF)	10.19	15.35
Calcium	1.67	0.35
Phos.	0.42	0.27
Salt	0.78	1.38
ME Mcal/kg	2.9	2.7

Where ME= Metabolisable Energy.

$(\pi \times L \times B \times T)/6$

Where π = 3.14, L = length of the longitudinal axis of the testis, B = breadth of the testis, T = Thickness of the testis.

Statistical analysis

Data were subjected to statistical analysis using Windows SAS program (SAS, 2000). Data for changes in body weight were analyzed according to the following model:

$Y_{ij} = \mu + T_i + e_{ij}$

Where Y_{ij} is the observation of the dependent variable obtained from J^{th} animal of I^{th} treatment, μ is the overall mean, T_i is the effect of i^{th} treatment (i = A or B); and e_{ij} is the residual term.

For testicular size and hormone levels the model was:

$Y_{ijk} = \mu + T_i + S_j + e_{ijk}$

Where Y_{ijk} is the observation of the dependent variable obtained from K^{th} animal of I^{th} treatment, of J^{th} season, μ is the overall mean; T_i is the effect of i^{th} treatment (i = A or B); S_j is effect of j^{th} season (j = 1 to 4); and e_{ijk} is the residual term.

The General Linear Model (GLM) and Least Squares Means (LSMEANS) procedures were used.

RESULTS AND DISCUSSION

Meteorological conditions

Meteorological data were recorded during the experimental

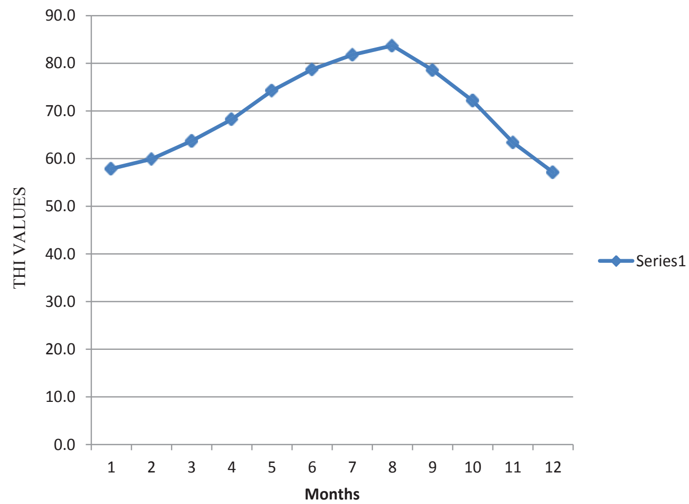

Figure 1. Average THI Rcords / two years.

period, giving an average THI of 81 (Figure 1). The hottest months of the year were July, August and September. The maximum temperature during these months ranged from 45.6 to 46.6°C while THI ranged from 78.9 to 83.1. It is documented that in the central region of Saudi Arabia, animals suffer from heat stress during summer (Al-Saiady et al., 2006). Mean initial body weight values of groups A and B camels were 381.83±36.34 and 458.00±33.64 kg, respectively. The difference was not significant (Figure 2). Total body weight gain for the entire trial period tended to be higher, but not significantly, in group A compared to group B. Average daily weight gain (DWG) in both groups was around 0.3 kg/day. This value was higher than that reported by Kadim et al. (2008), Faye et al. (2001) and Sahani et al. (1998), who stated that the daily weight gain for male camels from 18 to 24 months of age ranged from 0.111±0.015 to 0.219±0.24 kg/day. Animals in group A consumed significantly less ($P<0.05$) feed compared to group B. These results agreed with Mohamed (2006) who reported variation in camel performance when fed different types of rations. Season also significantly affected feed intake: the highest ($P<0.05$) feed intake was recorded during autumn compared to other seasons (Table 2).

Changes in testes size

The testicular size was significantly larger ($P<0.05$) in group B than group A (Table 3), while significant difference in size was found between the right and left testicles. A positive seasonal effect on the size of both right and left testicles was observed. The largest testicular size was recorded in summer and autumn, coinciding with increased levels of feed intake. These results agreed with Al-Asaad et al. (2007) who reported considerable effects on testicular dimensions due to season

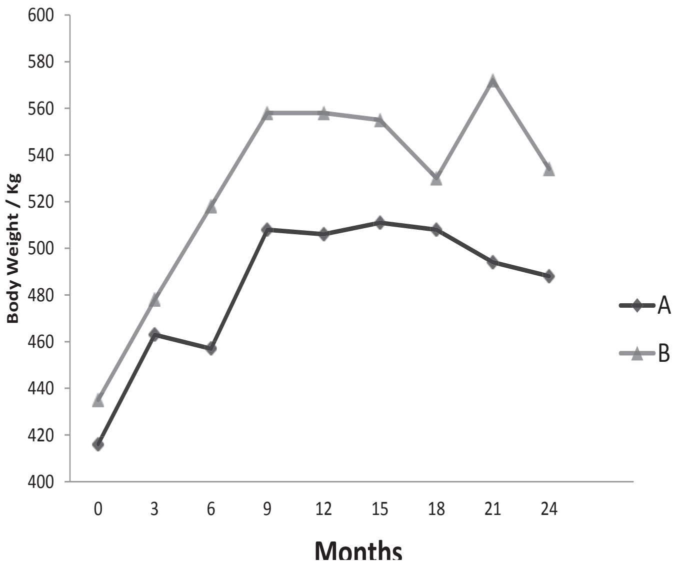

Figure 2. Changes in body weight.

Table 2. Mean ± SE for the effect of diet and seasonal effects on feed intake during the trial period.

Item	Feed Intake/kg
Diet	
A	5.82±0.03[b]
B	7.08±0.03[a]
Season	
Autumn	6.89±0.04[a]
Spring	6.15±0.04[d]
Summer	6.51±0.04[b]
Winter	6.23±0.03[c]

[a,b,c,d] Different letters within the column indicate significant difference (*P*≤ 0.05).

Table 3. Mean ± SE for the effect of diet and season on right and left testicle size.

Items	R. Testicular /cm^3	L. Testicular /cm^3
Diet		
A	456.59±18.5[b]	428.70±17.54[b]
B	565.10±16.95[a]	515.22±15.95[a]
Season		
Autumn	537.65±21.27[c]	488.99±19.98[b]
Spring	435.10±23.02[b]	422.49±21.63[a]
Summer	655.12±36.80[a]	583.02±34.56[b]
Winter	414.61±20.87[b]	393.32±19.96[a]

[a,b,c] Different letters within the column indicates significant difference (P≤ 0.05). R= right L= left.

Table 4. Effects of diet and season on serum concentrations of reproductive hormones (Mean ± SE).

Items	Testosterone ng/ml	FSH/ IU/L	LH/ IU/L
Diet			
A	2.98±0.29[ns]	0.96±0.23[b]	0.95±0.13[ns]
B	2.85±0.29[ns]	1.97±0.23[a]	1.08±0.13[ns]
Season			
Autumn	3.32±0.53[a]	0.84±0.40[b]	0.85±0.23[ns]
Spring	3.16±0.37[a]	1.83±0.29[a]	1.29±0.16[ns]
Summer	3.43±0.37[a]	1.78±0.29[a]	0.94±0.16[ns]
Winter	1.63±0.37[b]	1.43±0.29[a]	0.95±0.16[ns]

[a,b] Different litters within the column indicates significant difference (P≤ 0.05)

Table 5. Treatment effect on mean 'total protein, albumin, globulin, glucose, and cholesterol' in camel serum (Mean ± SE).

Items	Treatment (A)	Treatment (B)
Total Protein g/l	6.23±0.13	6.00±0.13
Albumin g/l	4.52±0.12	4.24±0.12
Globulin g/l	1.71±0.10	1.76±0.10
Alb/Glo	3.11±0.18	2.91±0.18
Glucose mmol/L	8.33±0.21	8.88±0.21
Cholesterol mmol/L	66.04±3.22	60.82±3.22

and age.

Serum concentrations of reproductive hormones according to diet and season are summarized in Table 4. The effect of diet on testosterone and LH concentrations was non-significant; however, Group B had higher FSH concentration compared to Group A. In the present study, the lowest levels of serum testosterone and FSH concentrations were recorded in winter and autumn, respectively. Testosterone level matched that recorded during the non-rutting season (2.89±0.26 ng/ml) by El-Bahrawy and El-Hassanein (2011) and Yagil and Etzion (1980).

Physiological status

The diets used in the present study had no significant effect on blood biochemical parameters (Table 5) and the values of the tested metabolites in both groups A and B were within normal physiological ranges (Abdel Gadir et al., 1984; Higgins and Kock, 1985; Faye and Mulato, 1991; Nyang'ao et al., 1997).

Conclusion

Nutrition affects live body weight gain, and consequently age at puberty. Peri-pubertal camels receiving a balanced diet with 13% CP, 2.9 Mcal ME and vitamin and mineral requirements, had improved body weight gain, testes size, and FSH concentration in the blood. These results support our earlier findings on pre-pubertal animals. However, more research is needed on the effect of nutrition and season of the year in decreasing age at puberty of male camels, with special emphasis on the role of vitamins or minerals.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Black-bone goat: An investigation report on new genetic resource of farm animal

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Black-bone goat is a unique local population recently discovered in Tongshan, Hubei province, China. Its geographical distribution, natural environment and ecological conditions were investigated. The Black-bone goat is mainly distributed in the mountainous area of Tongshan County. Using random sampling, its body shape and appearances, size and weight, reproductive performance and carcass characteristics were examined. The birth weight at 3, 6, 12 and 36 months of age for males were 1.83, 9.50, 14.01, 24.25 and 37.88 kg while those of females were 1.60, 8.75, 13.78, 23.53 and 34.19 kg, respectively. The live weight, dressed weight, dressing percentage, meat weight and meat percentage at 12 months was 22.32 kg, 10.95 kg, 49.02%, 8.01 kg and 35.42%, respectively. The litter size is 1.85. Based on historical materials including literature and folklore, the origin and medical applications of the Black-bone goat were analyzed. Finally, suggestions on preservation were proposed.

Key words: Black-bone goat, body weight, carcass characteristics, reproductive performance, genetic resources.

INTRODUCTION

There are 22 breeds of goats listed in Sheep and Goat Breeds in China (Zheng, 1989), but these are less than half of the total goat breeds in China. There are 31 provinces and autonomous regions in China, and each province has its own "Livestock and Poultry Breeds". There are 43 goat breeds listed in this kind of authority books (listed at URL: http://cars.hzau.edu.cn/sheep/index.php?option=com_content&task=view&id=970&Itemid=38).

A new goat breed (Dazu Black Goat) was recorded in "National Livestock and Poultry Genetic Resources" in 2009 (http://baike.baidu.com/view/1672655.htm). The majority of the goats are kept by smallholder farmers in the South of China and they are primarily used for meat production. Many indigenous goats of the middle and lower Yangtze River valley have not been officially classified into breeds (Jiang et al., 2003).

Black-bone goat is a unique local population recently identified in Tongshan County, Hubei Province. It is a very distinct native animal because of its black pigmentation, which makes many of its tissues, such as skin, bone and liver look black. According to our research, this is the only

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goat population in the world with “black bone”. Chinese people have a very long history of preference for “black food” in their culture, so this kind of goat provokes a lot of interest. Here we investigated its distribution, measured its body weight, body size, reproductive performances and carcass characteristics, and then analyzed its origin and medical applications.

METHODS OF INVESTIGATION AND RESEACH

On-the-farm investigation

According to the feedback information provided by local farmers, we investigated the new genetic resource –Black-bone goat. The investigations included distribution range, central production area, natural ecological conditions, and the local humanistic and social economic backgrounds in Tongshan County. Investigation was conducted in the form of visitation, interview and symposium in Tongshan on the history, number, and food therapy of Black-bone goat. Records concerning the history of Black-bone goat were searched in “Tongshan County Folklore Record” (Yu, 1918). Relevent meteorological data were obtained in Hubei Bureau of Meteorology.

Body weight and body size measurement

According to the information provided by farmers, there were 1129 black-bone goats in this area, and most of them were in Jiugong Mountain and Yangfanglin townships. Then 412 goats were selected randomly in these two townships to look at body appearance. The following data were measured: the birth weight of 10 males and 10 females, the weight at 3 month of age of 20 males and 25 females, the weight at 6, 12 and 36 months of age. The body length, chest girth length, withers height and cannon circumference of the goats at 6, 12 and 36 months of age were also measured. Litter size was calculated from 61 births. The reproductive performances, such as age at puberty, gestation length, length of estrous cycle, etc were recorded according to the information provided by farmers.

Carcass measurement

Four black-bone goats (two bucks and two female goats), aged one year old were selected to be slaughtered. They were held overnight without feed before slaughter. The dressed weight, dressing percentage and lean percentage were measured based on National Standard for Chinese Cattle, Sheep and Goat Industry (GB 18393, 2001). Loin-eye area and back fat thickness were measured using an Aquila Vet veterinary Ultrasound Scanner.

RESULTS

Distribution of the population

The black-bone goat is mainly distributed in the mountainous area of Tongshan County. This county is an administrative county belonging to Xianning City, about 200 km southeast of Wuhan City which is the capital of Hubei Province.

Natural environment of production area

Tongshan County is located between 114°14' and 114°58' longitude east and 29°51' latitude north which belongs to north subtropical monsoon climate zone. The total area amounts to 2680 km^2 with an average elevation of 1,400 m. It has four distinguishable seasons, consisting of muggy summers and cold winters with plenty of sunshine, abundant rainfall and long frost-free days. The annual average sunlight length is 1845 h. The average yearly temperature is 16.3°C. January is the coldest month with a minimum temperature of -20°C (February 1, 1969, Jiugongshan Tonggubao). July is the hottest month in a year with the highest temperature recorded being 40.5°C (July 9, 1978, Tongyang). There are about 226 to 248 frost free days every year. The annual precipitation amounts to 1,500 mm, over 70% of which occurs in spring and summer.

Body shape and appearances

The color of thick hair can mainly be classified into 2 types: (1) black type with black hairs and (2) black head type whose head and neck is black and the body is white (Figure 1). The black type makes up to 42% of the population and the black head type 45% (412 samples), the rest (13%) are covered with mixed coat, such as grey, black and white, brown and yellow.

In the population, 75.2% have horns and 24.8% are without horns. The shape and size of the horn vary quite differently among the population. The color of the skin is black. Other black tissues include lips, gums, teeth, tongue, nose, eye socket, eye iris, anus, vulva, tail tip, penis, and nipples (Figure 2).

Body measurements and body weight

The average birth weight of Black-bone goat is 1.83 kg for male kids and 1.60 kg for females. The weaning weight at 3 month of age is about 9.50 kg for males and and 8.75 kg for females, respectively. Body measurements and body weight of Black-bone goat are listed in Table 1.

The body weight and body size of some goat breeds in China are depicted in Table 2. The yearling weight of Black-bone goat is lower than that of famous Boer goat (36.9 kg, Schoeman et al., 1997), but is above the average of Chinese goats. So Black-bone goat is a good genetic resource for meat-type breeding in this area.

Reproductive performances

Black-bone goats have an early sexual maturation with the age at puberty being 94 to 125 days. The length of

Figure 1. Appearances of Black-bone goats a) Black type: Male, black coat, small horn (b) Black type: Female, black coat, without horn, (c) Black head type: Female, black head, white body coat, long horn and (d) Black head type: male, black head, mainly white body coat, long horn.

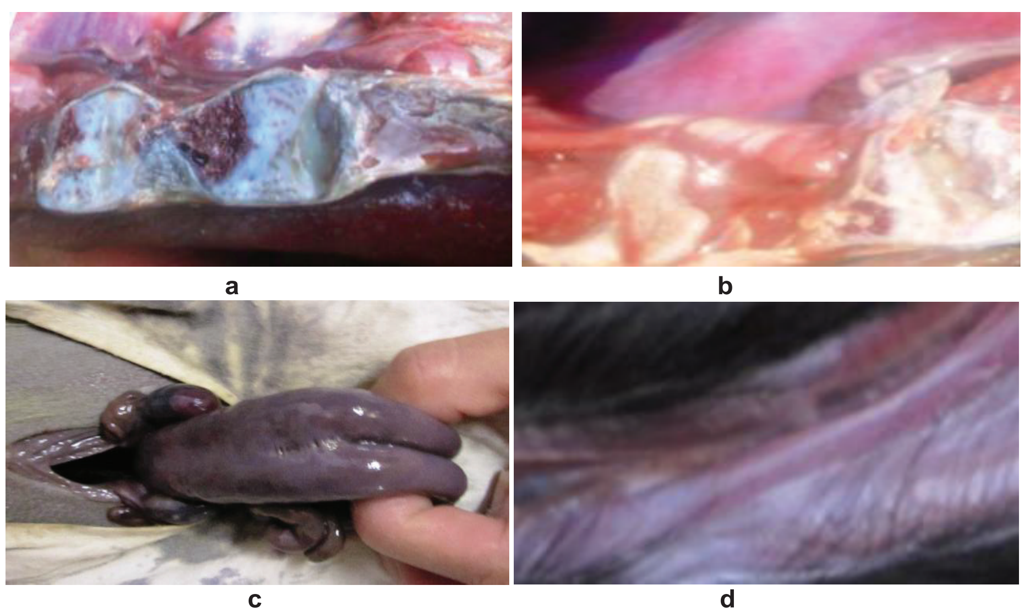

Figure 2. Black tissues of the Black-bone goats (a) Transverse section of rib, black pigmentation makes black bone, (b) Transverse section of normal native goat rib, looks white and red, (c) Uterus, ovary, oviduct and peritoneum are black and (d) Black trachea.

estrous cycle is 16 to 21 days and the duration of estrus is 42 to 59 h. The gestation length averages 147 days. The suitable age to mate for bucks is about 7 and 8 months for does. The goats can be used until they are 3 to 6 years old. Adult does are very fertile and they can come into heat in the four seasons, but breeding tends to be

Table 1. Body measurements and weight of Black-bone goat.

Month	Gender	Samples ize	Body weight	Withers height	Body length	Chest girth	Cannon circumference
6	Female	6	13.78±3.48	40.22±2.87	43.47±2.95	51.84±2.04	7.80±0.75
6	Male	5	14.01±2.14	41.88±2.06	44.85±2.43	53.14±3.42	8.10±1.21
12	Female	21	23.53±5.48	51.03±4.79	54.91±3.57	65.72±4.01	9.01±1.44
12	Male	7	24.25±5.16	54.25±5.16	56.41±3.17	66.01±2.73	9.28±0.89
36	Female	38	34.19±3.18	54.74±3.47	56.48±3.20	70.26±3.81	10.02±0.88
36	Male	4	37.88±5.15	56.71±4.02	58.14±3.08	72.34±3.19	10.47±0.76

concentrated in spring (March to May) and autumn (September to November). The average litter size is 1.85 which is normal in China (Table 2). Usually, the goats can kid twice a year or three times in two years. Most of the first litters (85.24%) are single and the remaining are twins. The litter size of multiparous goats is mainly 2 or 3 with 85% twins and 14% triplet. The litter size of Black-bone goat is similar to most of Chinese goats (Table 2).

Carcass measurement and meat quality

The live weight, dressed weight, dressing percentage, meat weight and meat percentage at 12 months was 22.32 kg, 10.95 kg, 49.02%, 8.01 kg and 35.42%, respectively.

The carcass traits of some goat breeds in China are depicted in Table 2. The carcass traits of Black-bone goats are similar to those of most Chinese indigenous goat breeds (Table 2). Most of these indigenous breeds can yield only 7 to 15 kg carcass weight, which is lower than the average carcass weight in China (13 kg) and Australia (25 kg, Wang et al., 2011).

The periosteum and marrow are black when the body is dissected (Figure 2a) and all the cartilaginous tissues are black while normal native goat bone looks white (Picture 2b). That is why local people call these goats "Black-bone" goats. The trachea, stomach, heart, liver, spleen, lung, kidney, uterus, ovary, oviduct are all black (Picture 2).

According to the results of Aquila Vet Veterinary Ultrasound Scanner, Loin eye area and back fat thickness of Black-bone bucks are 6.12 cm^2 and 0.18 cm, respectively. The values for the does are 5.82 cm^2 and 0.20 cm, respectively.

DISCUSSION

Origin of the population

Tongshan County is isolated from the outside by many high mountains. Due to the geographical constraint, the traffic condition is extremely difficult in Tongshan for a long period, so it is almost an isolated area.

According to "Tongshan County Folklore Records", there were 73 kinds of animals around Tongshan in 1910, most of them were pigs, cattle, dogs, goats and horses, and indicated that goats were one of the main livestock species during that period. In the villages, most of the goats belonged to local farmers and one family usually kept 2~5 head, sometime only one. These goats mated randomly and few new breeding stocks were brought in from the outside world, thus the goats were experiencing inbreeding. No one knows when and where and how the black-bone goats appeared in this area. We thought that the geographical isolation and continued inbreeding might be the reason.

Medicinal evaluation

The most unique feature of Black-bone goats is the black pigmentation which makes most of its tissues to be black. Fortunately, many Chinese prefer black food and it is a part of traditional food culture. In this culture, black food has many mysterious functions.

In this investigation, it was found that most of the local farmers and some civil doctors believe that Black-bone goat products (meat, bone and blood) have medicinal effects. Some people believe that Black-bone goat's blood and bone can be used as medicine to expel wind and remove damness, promote blood circulation and remove blood stasis, alleviate gastrointestinal discomfort. The medicinal value of black food is highly valued by this culture, so Black-bone goat has very high market potential. In fact, it is a good project for mountain farmers now. Farmers can sell their Black-bone goats at very high price, which may result in loss of the genetic resource as the total population size is small.

Genetic conservation

Black-bone goat has potential values for market and it is a rare and precious population, but its population size is very small. A few goats are even mixed with normal black coat goats and this may lead to hybridizing the

Table 2. Body size, body weight, carcass traits and litter size of some goat breeds in China*.

Breed	Body weight (kg)	Withers height (cm)	Body length (cm)	Chest girth (cm)	Live weight (kg)	Carcass weight (kg)	Meat weight (kg)	Dressing percentage %	Meat percentage %	Llitter size	References
Black-bone goat	23.89	52.64	55.66	65.87	22.32	10.95	8.01	49.02	35.42	1.85	This research
Matou goat	23.46	50.63	57.64	62.66	23.63	12.10	8.06	51.22	34.13	1.82	Jiang et al. (1988)
Shichuan banjiao goat	22.82	48.79	56.01	63.73	23.44	10.77	8.30	47.01	33.71	1.83	Jiang et al. (1988)
Gulin horse goat	26.14	53.06	52.74	62.71	24.27	10.02	7.49	42.07	30.02	2.14	Jiang et al. (1988)
Guizhou white goat	18.45	46.58	49.83	60.48	25.5	10.69	-	44.71	-	1.56	Jiang et al. (1988), Guo and Luo (2006)
Fuqing goat	23.32	48.44	52.83	64.82	21.22	10.97	-	51.76	-	2.36	Jiang et al. (1988)
Hui goat	18.98	50.77	54.46	65.24	21.05	9.55	7.7	44.93	36.23	2.35	Jiang et al. (1988)
Yangtse river delta white goat	-	47.25	50.61	58.55	17.79	7.35	6.76	41.32	38.00	2.70	Huang et al. (2002)
Chengdu Ma goat	27.27	56.64	60.41	66.97	26.15	11.9	9.75	50.65	42.35	1.92	Ma et al. (2007), Wang (1982)
Shannan white goat	22.5	53.20	57.60	66.20	26.7	13.1	9.7	49.1	36.3	1.62	Zhang et al. (2001), Zhu (2006)
Daiyun goat	15.83	47.00	50.44	59.12	18.13	8.13	_	45.43	_	2.31	Zhang et al. (1997), Dong et al. (2009)
Nanjiang Yellow goat	34.07	62.49	65.45	73.42	30.29	14.97	11.45	49.41	37.79	1.82	Wang (1997)

*The values of body weight, body size and carcass traits were averages for males and females at 12 months; Litter size was the average of the whole population.

population. Some methods, such as artificial insemination, embryo transfer and embryo freezing, can be used to preserve and amplify the population size. The government should start a project to protect and develop this genetic resource. As part of this project, the government may encourage farmers to raise Black-bone goat, allot areas to raise breeding stocks, to initiate nucleus breeding centers.

In short, the litter size, body weight, body size and carcass traits of Black-bone goat are similar to most Chinese indigenous goat breeds, indicating that it could be selected into meat-type goat. Further scientific works should be focused on pigmentation distribution in tissues, and genetic structure and functional genes of this unique goat population.

Conflict of Interest

The authors have not declared any conflict of interest.

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The search for productivity and pre-harvest sprouting tolerance in wheat

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The pre-harvest sprouting (PHS) in wheat *Triticum aestivum* L. is a phenomenon that reduces productivity, degrades starch and depreciates the quality of flour. The objective was to evaluate the per se performance of traits linked to grain yield and tolerance to pre-harvest sprouting in wheat genotypes from different breeding programs in Brazil. Besides, to use indirect selection based on traits showing beneficial relationships and greater heritability than the target as a selection strategy. The study was conducted in the years 2010 and 2011 using randomized complete block design with three replications. Thirty-three genotypes of different wheat breeding programs in Brazil were evaluated. The BRS 194 and Frontana genotypes are sources of tolerance to pre-harvest sprouting in wheat. The cultivar TBIO Alvorada has PHS tolerance and high grain yield, and cultivar TBIO Ivaí presenting high grain yield. The percentage of sprouted grains has negative direct relationship with the falling number in adverse cultivating environments. The falling number shows greater heritability than the percentage of sprouted grains, indicating greater reliability in selection for PHS tolerance.

Key words: *Triticum aestivum*, pre-harvest sprouting (PHS), sprouted grains, falling number.

INTRODUCTION

The pre-harvest sprouting (PHS) in wheat is the phenomenon of germination of seeds attached to the plant mother leading to degradation of starch in the conversion into sugar (Kulwal et al., 2012), a condition that makes the flour inadequate to the baking process (Emebiri et al., 2010). The higher tolerance to pre-harvest sprouting is directly linked to the rate of water absorption, level of seed dormancy and the ability to reserve mobilization during germination (Emebiri et al., 2010; Martynov and Dobrotvorskaya, 2012; Zhang et al.,

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2014a). Among the main assessment methodologies PHS highlights the counting of sprouted grains (Rasul et al., 2012) and the measurement of the enzyme alpha-amylase expressed by the falling number on whole flour (Gooding et al., 2012; Zhang et al., 2014b). It is a challenge to select plants with tolerance to PHS since the trait is quantitatively inherited and strongly affected by environmental conditions (Fofana et al., 2009; Rasul et al., 2012). The analysis of the per se performance in elite genotypes is critical to quantify the variability in grain yield traits (Luche et al., 2013) and tolerance to PHS (Ogbonnaya et al., 2008). The selection gain on these traits via correlation with the path analysis can be also decisive, highlighting the direct and indirect effects of secondary traits on the primary trait (Wright, 1921; Vesohoski et al., 2011). Furthermore, the heritability is an important property to quantify the contribution of genetic and environmental effects on the phenotype and define the selection pressure on the traits of interest (Ogbonnaya et al., 2008; Fofana et al., 2009; Krüger et al., 2011). Estimates of genetic parameters and heritability can be obtained by analysis of variance components in experiments with the same number of replications or plants in the plot (Carvalho et al., 2001; Luche et al., 2013).

The understanding of relationships and heritability of grain yield with the characters of PHS tolerance in wheat can trigger new strategies to simultaneously get more adjusted plants to meet farmer and industrial requirements. This condition reports the necessity of understanding the direct and indirect effects between traits and their stability regarding genetic and environmental effects. These effects and their interactions are important modulators of the expressed phenotype. The objective was to evaluate the per se performance of traits linked to grain yield and pre-harvest sprouting tolerance in wheat genotypes from different breeding programs in Brazil. Moreover, in the proposition of selection strategies based on indirect selection over traits of interest evidencing beneficial relationships and greater heritability.

MATERIALS AND METHODS

Experimental site and plant materials

The experiments were conducted in two successive seasons 2010 and 2011, in the field located in the city of Capão do Leão, Rio Grande do Sul, Brazil. The city is situated 31° 52' 00'' latitude south and 52°21' 24'' longitude west; at an altitude of 13.24 m, at an average annual pluviometric precipitation of 1280.2 mm. The soil is classified as Red Yellow Podzolic unit in Mapping Pelotas, which its U.S. equivalent is Typic Hapludalf (USDA, 2010). Thirty-three genotypes of wheat developed by leading Brazilian breeding programs were evaluated: TBIO Tibagi, TBIO Ivaí, TBIO Pioneiro, TBIO Itaipú, TBIO Alvorada, TBIO Sinuelo 'S', TBIO Mestre and TBIO Seleto (Biotrigo Genética Ltda); Topazio, Turquesa and Ametista (OR Melhoramento de Sementes Ltda); Quartzo, Mirante, Marfim, Valente and Supera (joint partnership of Biotrigo Genética Ltda and OR Melhoramento de Sementes Ltda); BRS Guamirim, BRS 248, BRS 194 and BRS 220 (Empresa Brasileira de Pesquisa e Agropecuária - Embrapa); Fundacep Raízes, Fundacep Horizonte, Fundacep Cristalino, Fundacep Campo Real, Fundacep Bravo, TEC Veloce, TEC Frontale, TEC Vigore, TEC Triunfo, CEP 07-136, CEP 06-167 and CEP 07-31 (Cooperativa Central Gaúcha Ltda - CCGL TEC) and Frontana (Beckmann, 1965). The cultivar Frontana released in 1940 is considered the greatest contribution of the Brazilian breeding for wheat in the world, mainly for resistance to leaf rust, the natural threshing and pre-harvest sprouting (Sousa, 2004).

Experiment design and management

The experimental design was randomized complete blocks with three replications and the sowing was performed at three different times in ten day intervals. Therefore, it enabled the harvesting of spikes at physiological maturity in the same period, even from genotypes with differences in cycle length. At harvest, only plants where the spikes had lost their green color, except the nodes of the culms, were selected. The seeding was performed in the conventional system with a density of 300 viable seeds per square meter, the experimental unit consisted of five rows of 5 m length and spaced by 0.20 m. Two applications of fungicide Folicur (tebuconazole) were performed in both years of cultivation. The fertilization and liming were carried out based on technical recommendations for the crop in the states of RS and SC (CBQFS, 2004). The control of weeds and pest plants were performed according to the recommendations of the Brazilian Commission for Research in Wheat and Triticale (RCBPTT, 2010).

Laboratory analysis

In the years 2010 and 2011 the spikes were harvested and dried at room temperature for seven days in a protected place. Subsequently, they were placed in plastic bags and stored in a freezer at -20°C. In the analysis for characterization of pre-harvest sprouting tolerance the sprouted grain (SG, in %) and falling number (FN in seconds) were evaluated. In the analysis of the SG a randomized complete block design with three replications was used, with each replicate consisting of ten spikes. The spikes were placed immersed in distilled water for eight hours and then removed and placed on paper towel to remove water excess. In the spikes the fungicide (Vitavax®) vitavax-thiram [Active Ingredient (carboxin + thiram): 200 + 200 g L^{-1}] was applied, as recommended by the manufacturer. The spikes were rolled in the vertical position in sheets of germination paper previously soaked in distilled water. By forming rollers with spikes, these were placed in plastic bags so that no moisture loss occurred. They were then placed to incubate for seven days at 20 ± 1°C in a growth chamber Biological Oxygen Demand (BOD). After that, the rolls with the spikes were removed from the growth chamber, and dried in a drying chamber at 50°C for 72 h. With the end of drying, threshing and the evaluation of the number of sprouted grains and total number of grains were performed.

In evaluating the FN, the sample of spikes was threshed and later the grains were conditioned to 15% moisture and, after 24 h, ground on Chopin mill trial (model CD1, França). After grinding of grain, 7 g were taken for the evaluation. The FN is the assessment that measures the activity of the enzyme alpha-amylase, and expresses the intensity of sprouted grains in wheat before the radicle emerges, or makes the rupture of the pericarp. The FN was determined by the falling number apparatus according to the method number 56-81B AACC (1995). The value expressed in seconds measures indirectly the enzyme activity, meaning that the shorter the fall time, the greater content and enzyme activity. In

2010, the assessments were performed in the FN handset Falling Number Perten Instruments (model Fungal 1200, France). In 2011 the assessments were performed in the FN automatic apparatus Falling Number Perten Instruments (model Fungal 1500, France). In the analysis of traits related to grain yield, the traits measured were: grain yield (GY, in kg ha^{-1}); test weight (TW, in kg hl^{-1}) and thousand grain weight (TGW, in grams). The GY, TW and TGW evaluations were performed in the laboratory after harvesting three central rows of each plot on the experimental field.

Data analysis

The data obtained were subjected to analysis of variance to estimate the components of variance in the determination of heritability according to Carvalho et al. (2001). On differentiation between genotype groups it was used the averaged plus or minus one and two standard deviations in the different traits. Analysis of Pearson correlation for the magnitude and direction of associations between traits was performed according to Falconer and Mackay (1996). The significance of correlations was assessed at p≤0.01 and was adopted, the *t* test described by Steel and Torrie (1980), with $n - 2$ degrees of freedom, according to the model $t = r/[(1 - r^2)/(n - 2)]^{0,5}$, where *r* is the correlation coefficient between X and Y traits, and the number of degrees of freedom in the levels of treatments considered, A total of $n = 198$ units of observation were obtained: two years, thirty-three genotypes with three replications. The correlations were outspread in direct and indirect effects of secondary traits on the main traits (SG and FN), the proposal described by Wright (1921). Analyses were performed with the aid of the computer software Genes (Cruz, 2006).

RESULTS AND DISCUSSION

Performance of individual genotypes

On Table 1, the SG and FN traits showed phenotypic classes of one and two standard deviations plus or minus the average of genotype standards. In 2010, the genotypes TEC Triunfo, CEP 06-137 and CEP 07-31; in 2011, FUNDACEP Bravo, FUNDACEP Raízes, TBIO Alvorada and TBIO Itaipú along with BRS 194 by joint analysis showed the lowest SG. Higher values of SG indicate seeds with lower accumulated dormancy, featuring advanced level of germination process (Gelin et al., 2007; Rasul et al., 2012). The analysis of sprouted grains in the spike was shown as an efficient trait in detecting genetic variability for tolerance to PHS (Franco et al., 2009; Rasul et al., 2012). It is noteworthy that the variability may be due to differences in the absorption of water by the grain or by differences in tolerance to germination (Chen et al., 2014). Another factor is the amount of genes of tegument color in wheat, grains of white color are more sensitive to PHS compared to red, and however, the mechanisms of action of the genes of the seed coat color have not been clearly described (Bi et al., 2014). On Table 1, the best FN values were obtained for the genotypes TBIO Mestre and BRS Guamirim (2010) and TBIO Pioneiro, FUNDACEP Cristalino and TEC Veloce (2011). Also, cultivar Frontana showed superior values in the two years of evaluation. Pre-harvest sprouting and late maturity of alpha-amylase are highlighted as key factors related to the falling number in wheat. The variability of FN may be due to genetic, environmental and genotype x environment interaction effects, that is, genotypes, genotype origin and the growing environment (Rasul et al., 2012; Zhang et al., 2014b). The balance between the hormones abscisic acid (ABA) and gibberellic acid (GA) which act on the alpha-amylase present in the aleurone layer is crucial. GA stimulates and ABA inhibits formation of alpha-amylase in dormant grains of wheat. A genetic variability for the expression of PHS was reported, identifying a strong relationship with the dormancy and balancing of hormones linked to germination (Lohwasser et al., 2013).

BRS 194 and Frontana genotypes were the most stable and PHS tolerant in per se analysis. It is emphasized that concerning the traits linked to the expression of PHS, the genotypes that showed lower SG were not those that showed higher values of FN, leading to an inconsistency between the indicator traits. Thus, analyzing the behavior of the cultivar Frontana, an internationally recognized standard for PHS tolerance (Sousa, 2004), a good fitting was observed in the FN values. Since the PHS tolerance is a quantitative trait, difficult to assess, however, the FN was shown to be the most reliable measure for PHS tolerance in wheat (Lohwasser et al., 2013; Zhang et al., 2014b). On Table 1, superior genotypes for GY were TBIO Sinuelo ‘S’, TBIO Seleto and FUNDACEP Cristalino (2010); TBIO Alvorada and Topazio (2011) and TBIO Ivaí (2010 and 2011). It is evident that the cultivars released in the Brazilian market have ceiling of grain productivity higher than the achieved in the field conditions, generally linked to absence of investment or limiting environmental conditions (Bredemeier et al., 2013; Valério et al., 2013). As reported previously, the expression of PHS is considered one of the most important factors limiting grain yield in wheat. Thus, among the PHS tolerance genotypes, TBIO Alvorada also showed higher GY, a possible source of genes for increased PHS tolerance with grain yield. Cultivar Frontana, a standard genotype for PHS tolerance was one of those that indicated reduced expression in grain yield. Although, it is important as a source of genes for PHS tolerance, Frontana does not show accumulation of favorable alleles for GY as the current cultivars, indicating the need of transferring PHS tolerance by a backcrossing strategy.

On Table 1, TBIO Sinuelo ‘S’ (2010) and CEP 06-167 and CEP 07-31 (2010 and 2011) stood out as PHS tolerant and showed significant values of TW. The cultivar BRS 194 (2010); and TBIO Tibagi, TBIO Sinuelo ‘S’, TBIO Seleto, Mirante and Marfim (2011) and Valente and Frontana in the two years of cultivation showed high TGW. It is emphasized that the TW is used in the classification and marketing of wheat in Brazil (Schmidt et al., 2009). Moreover, the TGW is important trait in wheat, showing the yield components (grain number and grain weight), closely related to the effectiveness of per se performance of the genotypes (Valério et al., 2013). In

Table 1. Performance per se of wheat genotypes for traits related to pre-harvest sprouting and grain yield.

Genotypes	Year 2010					Year 2011				
	GY (kg ha^{-1})	TW (kg hl^{-1})	TGW (g)	SG (%)	FN (s)	GY (kg ha^{-1})	TW (kg hl^{-1})	TGW (g)	SG (%)	FN (s)
TBIO Tibagi	3462	75I*	33	21	401	3699	80	41S*	24	380
TBIO Ivaí	4098S*	77	34	8	372	4929S*	80	36	19	377
TBIO Pioneiro	3234	78	34	32S*	418	3783	80	35	30	425S*
TBIO Itaipú	3160	77	34	15	372	4000	80	36	8I*	362
TBIO Alvorada	3334	77	31	8	403	4672S*	81	36	4I*	381
TBIO Sinuelo'S'	4182S*	79S*	36	10	323	4466	82	41S*	21	330
TBIO Mestre	3735	75I*	33	23	426S*	4491	80	39	15	361
TBIO Seleto	4342S*	78	36	22	362	3789	82	41S*	36S*	280I*
Quartzo	3200	77	34	15	416	4223	80	39	15	312
Mirante	3988	78	37	30S*	357	4089	81	43S*	28	348
Marfim	3190	75I*	34	25S*	439	3830	81	42S*	31	343
Valente	2690	77	46S**	23	316	3693	80	49S**	31	336
Supera	2881	75I*	36	33S**	425S*	3239	79I*	40	19	323
Frontana	2140	77	40S*	17	423S*	2068I**	78I*	42S*	16	389S*
Topazio	3500	78	31	9	198I**	5022S*	81	35	47S**	271I*
Turquesa	3564	77	34	13	337	4264	82	39	21	342
Ametista	3047	78	35	35S**	398	3772	81	37	37S*	314
Fcep Raízes	2929	78	31	11	420	3397	81	34	11I	384
Fcep Horizonte	3359	77	32	9	373	3675	81	37	14	337
Fcep Cristalino	4537S*	76	36	10	392	3894	80	37	27	386S*
Fcep Campo Real	2710	75I*	29I*	7	364	3667	81	30I*	15	332
Fcep Bravo	3328	77	28I*	21	348	3476	81	32I*	9I*	356
BRS Guamirim	1943I*	77	36	8	477S*	3969	80	39	21	378
BRS 248	2747	77	34	11	342	2840I*	79I*	34	18	342
BRS 194	2280	76	38S*	6I*	330	3369	80	40	10I*	296I*
BRS 220	2526	75I*	36	7	350	3269	79I*	40	21	364
TEC Veloce	2154	77	33	21	355	3343	81	38	33S*	390S*
TEC Frontale	2129	78	28I*	13	298I*	3516	81	31I*	28	256I**
CEP 07-136	2541	78	35	10	348	4019	80	38	20	318
TEC Vigore	3910	78	35	20	375	4461	82	38	30	358
TEC Triunfo	1995I*	77	30I*	5I*	318	3303	78I*	30I*	36S*	269I*
CEP 06-167	1710I*	79S*	30I*	6I*	377	4128	84S*	34	23	372
CEP 07-31	1647I*	79S*	32	4I*	257I*	3819	83S*	38	33S*	265I*
General Average	3036	77	34	15	367	3823	81	37	23	342
Standard Deviation	956	1,4	3,7	9,0	56,1	726,8	1,6	4,2	10,3	42,6

S^{*} and S^{**} - higher than the average of genotypes plus one and two deviation(s)-standard(s), respectively; I^{*} and II^{**} - lower than the average of genotypes plus one and two deviation(s)-standard(s), respectively; GY= grain yield; TW= test weight; TGW= thousand grain weight; SG= sprouted grains; FN= falling number.

wheat, strong interaction between genotype and environment on the phenotypic expression of GY, TW and TGW have been detected. It is necessary a continuous evaluation of performance per se in the identification of possible sources of alleles for these traits (Schmidt et al., 2009; Luche et al., 2013). Climatic variables of maximum temperature and pluviometric precipitation (Figure 1) affected the growth and development of wheat, directly influencing the productivity and quality of grain traits. In wheat the temperature rise to a limited extent in the grain filling period may increase the GY, TGW and FN, but reducing the TW. Intense pluviometric precipitation has the most drastic impact on this species, reducing the values of GY, TGW, TW, and FN (Guarienti et al., 2005).

Correlation between grain yield and PHS traits

In order to better understand the relationships between

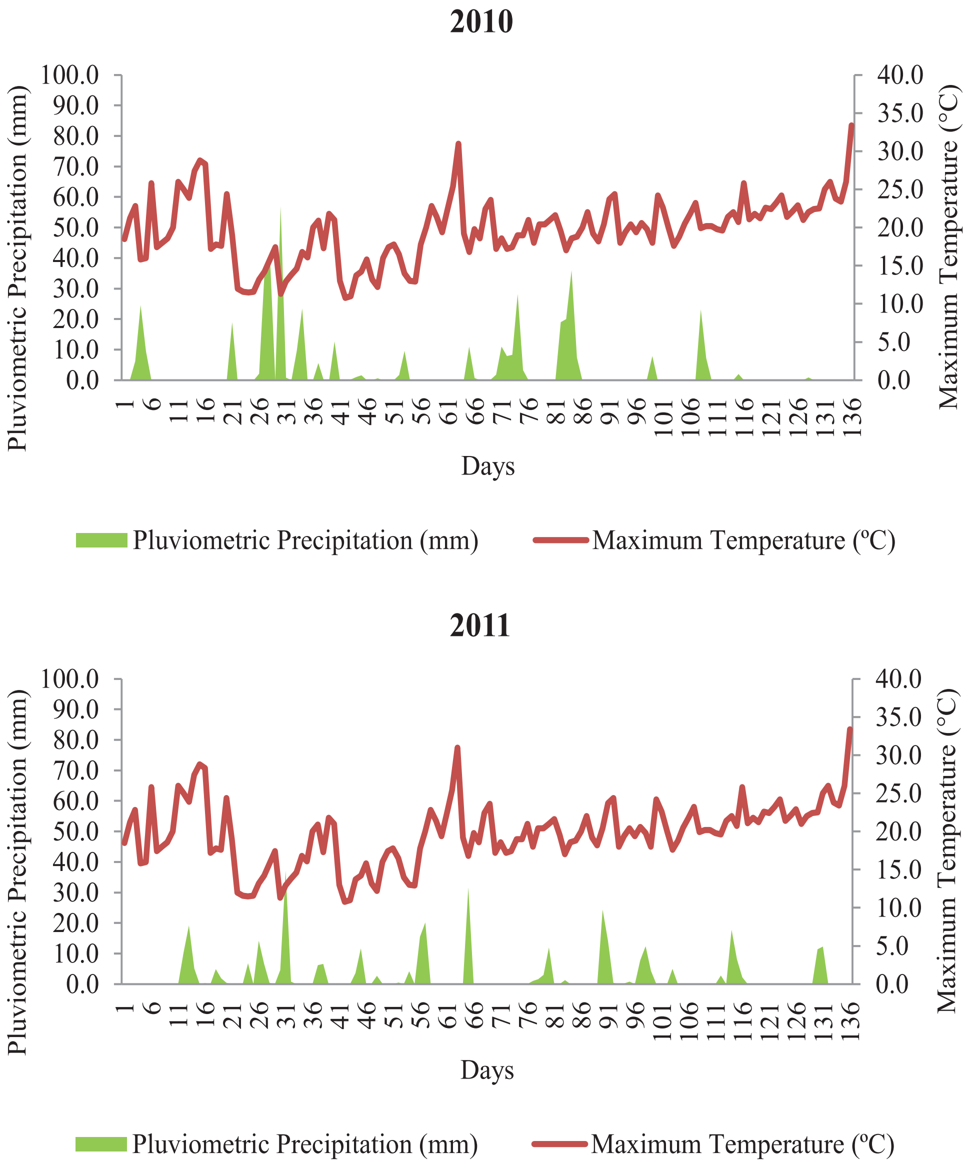

Figure 1. Pluviometric precipitation and maximum temperature during the wheat cycle cultivation in the years 2010 and 2011. Source: Agrometeorological Station of Pelotas.

traits related to grain yield and PHS tolerance, a correlation analysis was performed (Table 2). In 2010, positive phenotypic correlation was obtained for FN with TGW and SG, but with a negative association with TW. In 2011, FN showed negative association with SG and TW, indicating an inconsistency in the direct relationship between SG and FN. In the joint correlation analysis, an association of SG and FN was not observed (Table 2). The SG showed positive association with the GY, TW and TGW, while the FN showed a negative association with TGW. Also, positive correlations between GY, TW and TGW were found. TW has shown a positive association with FN, since they are important traits in the classification of wheat at the time of marketing (Schmidt et al., 2009). Moreover, it indicates efficiency for indirect selection to the increment of the grain yield. Negative correlations between TW and FN are possibly due to crop conditions, since for determining the TW the grains were obtained in the harvest maturation, the spikes staying more time exposed to high pluviometric precipitation and maximum temperatures (Figure 1). On the other hand, following standard methodology, spike sampling for determining FN was performed at physiological maturity, avoiding the most adverse environmental conditions at the end of the cycle. Also, the phenotypic correlation simultaneously includes parts attributed to genetic and environmental effects and the inclusion of sources of variation can provide more effective correlations among

Table 2. Pearson correlation among traits of pre-harvest sprouting tolerance and grain yield in wheat genotypes in the years 2010, 2011 and joint analysis.

Trait	SG	FN	GY	TW	TGW
			Year 2010		
SG	1.00	0.35**	0.24	-0.07	0.31**
FN	-	1.00	0.09	-0.28**	0.12
GY	-	-	1.00	0.11	0.11
TW	-	-	-	1.00	-0.03
TGW	-	-	-	-	1.00
Trait	SG	FN	GY	TW	TGW
			Year 2011		
SG	1.00	-0.34**	0.10	0.15	0.09
FN	-	1.00	-0.06	-0.12	0.10
GY	-	-	1.00	0.34**	-0.02
TW	-	-	-	1.00	-0.17
TGW	-	-	-	-	1.00
Trait	SG	FN	GY	TW	TGW
			Joint analysis		
SG	1.00	-0.06	0.29**	0.30**	0.30**
FN	-	1.00	-0.07	-0.31**	0.01
GY	-	-	1.00	0.44**	0.21**
TW	-	-	-	1.00	0.23**
TGW	-	-	-	-	1.00

** = Significant at 1% probability of error using the T test; GY= grain yield (kg ha^{-1}); TW= test weight (kg hl^{-1}); TGW= thousand grain weight (g); SG= sprouted grains (%); FN= falling number (s).

the traits (Krüger et al., 2011).

Path analysis between grain yield and PHS traits

In the partition of the correlations into direct and indirect effects by path analysis positive effects of FN and TGW on the percentage of SG were observed in 2010 (Table 3). Also, a positive direct effect of SG on FN was observed with reduced indirect effects of other traits. Therefore, the FN and SG show a negative relation of cause and effect, corroborating results from other experiments (Rasul et al., 2012).

The TGW and the GY in 2010 and the TGW and TW in 2011 showed positive direct effects on the SG, suggesting a direct effect of those three variables on the analysis and, therefore, a close relationship with SG. The TW showed negative direct effect on the FN (Table 3). In 2010, there was a negative direct and indirect effect via SG on FN. In 2011, there were negative direct effects of minor relevance with indirect effects on SG and TGW via TW and FN. In the joint analysis, the main contribution was the negative direct effect of TW on the FN. Therefore, it is perceived a negative association between TW and FN, contradicting other reports that showed this close positive relationship (Schmidt et al., 2009).

Heritability of traits

In the analysis of heritability, the SG and FN showed high individual heritability (Table 4). On the other hand, the heritability was reduced in the joint analysis. Because of that, one can observe that in 2011 high pluviometric precipitation occurred during the grain filling stage (Figure 1), and the method of FN proved to be adjusted in identifying genotypes more tolerant to adverse environmental conditions. Thus, it is a challenge to, identify wheat genotypes with stable expression of traits related the PHS in different growing conditions, given it is a quantitatively inherited trait (Fofana et al., 2009; Rasul et al., 2012). In the joint analysis, FN showed higher heritability than SG, indicating greater reliability in assessing PHS tolerance in wheat. However, some studies showed that the heritability for SG was considered high (Ogbonnaya et al., 2008). Studies via FN indicated high heritability in five environments evaluated, indicating a more stable and consistent trait in detection for PHS tolerance wheat (Zhang et al., 2014b). Therefore, the method of SG showed high heritability in individual, environments and reduced genetic effects on joint analysis, while the FN showed greater stability, and it can be the most suitable method for the detection of the PHS tolerant genotypes in genetic improvement of wheat.

Table 3. Estimation of direct and indirect effects on wheat by path analysis using correlations about sprouted grains and falling number as main dependent traits and the production as explanatory independent traits.

Path	Dependent trait SG			Path	Dependent trait FN		
	2010	2011	Joint		2010	2011	Joint
Falling number (FN)				**Sprouted grains (SG)**			
ED on SG	0.298	-0.338	0.004	ED on FN	0.313	-0.344	0.004
EI via GY	0.016	-0.002	-0.012	EI via GY	0.010	-0.001	0.021
EI via TW	0.001	-0.014	-0.053	EI via TW	0.019	-0.006	-0.107
EI via TGW	0.030	0.014	-0.001	EI via TGW	0.003	0.011	0.019
Overall (r)	0.35	-0.34	-0.06	Overall (r)	0.35	-0.34	-0.06
Grain yield (GY)				**Grain yield (GY)**			
ED on SG	0.182	0.042	0.172	ED on FN	0.044	-0.007	0.072
EI via FN	0.027	0.019	0.000	EI via SG	0.073	-0.035	0.001
EI via TW	0.000	0.042	0.075	EI via TW	-0.028	-0.013	-0.159
EI via TGW	0.027	-0.002	0.047	EI via TGW	0.001	-0.002	0.013
Overall (r)	0.24	0.10	0.29	Overall (r)	0.09	-0.06	-0.07
Test weight (TW)				**Test weight (TW)**			
ED on SG	-0.003	0.125	0.170	ED on FN	-0.260	-0.040	-0.360
EI via FN	-0.083	0.039	-0.001	EI via SG	-0.023	-0.053	0.001
EI via GY	0.020	0.014	0.076	EI via GY	0.005	-0.002	0.032
EI via TGW	-0.008	-0.024	0.051	EI via TGW	0.000	-0.021	0.014
Overall (r)	-0.07	0.15	0.30	Overall (r)	-0.28	-0.12	-0.31
Thousand grain weight (TGW)				**Thousand grain weight (TGW)**			
ED on SG	0.250	0.142	0.223	ED on FN	0.010	0.122	0.063
EI via FN	0.036	-0.033	0.000	EI via SG	0.095	-0.030	0.001
EI via GY	0.019	-0.001	0.036	EI via GY	0.005	0.000	0.015
EI via TW	0.000	-0.021	0.039	EI via TW	0.009	0.007	-0.083
Overall (r)	0.30	0.09	0.30	Overall (r)	0.12	0.10	0.00
R^2	0.88	0.92	0.91	R^2	0.90	0.93	0.94
Residual effect	0.22	0.15	0.17	Residual effect	0.19	0.13	0.11

R^2= Coefficient of determination; r= coefficient of phenotypic correlation; ED= direct effect; EI= indirect effect. GY= Grain yield (kg ha^{-1}); TW = Test weight (kg hl^{-1}); TGW= Thousand grain weight (g); SG= Sprouted grains (%); FN= Falling number (s).

In 2010, the heritability of GY and TW proved intermediate (Table 4). The heritability for the GY was greater than TW in 2010, while in 2011, TW was superior. It is emphasized that all the traits showed lower heritability in the combined analysis, however, heritability become more stable and reliable when estimated in more environments (Ogbonnaya et al., 2008). The expression of the GY is complex and influenced by many genes, where the genetic variance is similar to environmental variance (Hussain et al., 2013). The heritability of GY tends to be reduced when in adverse environmental conditions (Luche et al., 2013). Moreover, heritability achieves higher values in test weight and thousand grains weight (Barnard et al., 2002). Higher heritability values are indicative of a strong contribution of genetic variation on environmental expression of the phenotype and bring more effective gains on selection.

Conclusions

BRS 194 and Frontana genotypes are a source of tolerance to pre-harvest sprouting in wheat. Cultivar TBIO Alvorada has PHS tolerance and high grain yield. Cultivar TBIO Ivaí has high grain yield. The percentage of sprouted grains has negative direct association with the falling number in adverse environments of cultivation. The falling number shows greater heritability than the percentage of sprouted grains, indicating greater reliability in selection for PHS tolerance.

Conflict of Interest

The authors have not declared any conflict of interest.

Table 4. Genetic parameters estimated from the mean squares and relative contribution of the traits of pre-harvest sprouting tolerance and grain yield in wheat genotypes in the years 2010 and 2011.

Trait	Analysis of variance		Genetic parameters			
	SM_R	SM_T	V_E	V_P	V_G	h^2
		Year 2010				
Sprouted grains (%)	5.59	237.27	5.59	82.82	77.23	0.93
Falling number (s)	326.16	8972.29	326.16	3208.2	2882.0	0.90
Grain yield (kg ha^{-1})	275431	1819170	275431	790011	514579	0.65
Test weight (kg hl^{-1})	1.03	4.09	1.03	2.05	1.02	0.50
Thousand grain weight (g)	1.98	38.27	1.98	14.08	12.10	0.86
		Year 2011				
Sprouted grains (%)	16.54	289.04	16.54	107.37	90.83	0.85
Falling number (s)	152.13	5222.51	152.13	1842.2	1690.1	0.92
Grain yield (kg ha^{-1})	272275	1044913	272275	529821	257546	0.49
Test weight (kg hl^{-1})	0.91	5.17	0.91	2.33	1.42	0.61
Thousand grain weight (g)	1.92	46.17	1.92	16.67	14.75	0.88
		Joint analysis 2010/2011				
Sprouted grains (%)	47.88	331.19	47.88	95.10	47.22	0.50
Falling number (s)	727.7	11512.86	727.70	2525.2	1797.5	0.71
Grain yield (kg ha^{-1})	365368	2132654	365368	659916	294547	0.45
Test weight (kg hl^{-1})	1.2	7.12	1.20	2.19	0.99	0.45
Thousand grain weight (g)	2.74	78.53	2.74	15.37	12.63	0.82

SM_R= square means of error; SM_G= square means of treatment; V_E= environment variance; V_P= phenotypic variance; V_G= genotypic variance; h^2= heritability of trait.

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Full Length Research Paper

Reduction in growth and aflatoxin production in orange juice inoculated with *Aspergillus flavus* using *aframomum danielli*

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The survival and production of aflatoxin by *Aspergillus flavus* introduced into pasteurized (85°C;15 min) and unpasteurized orange juice treated with *Aframomum danielli* (local preservative) extracts at 0, 1, 2, 3, 4, 5 and 6% concentrations were investigated at 3 and 7 days of storage on shelf (28±2°C). There was slight increase in pH on day 3 which was more prevalent on day 7(4.77-6.85 and 6.07-7.37) for the pasteurized and unpasteurized orange juice respectively. There was no significant difference (p≥0.05) in growth and aflatoxin levels on day 3, however, luxuriant growth of *A. flavus* (0.63-1.42 g and 0.52-0.68 g) and aflatoxin production (29-156 ng/ml and 5.40-63.00 ng/ml) were recorded on day 7 respectively. The pasteurized sample (0%; control) of *A. danielli* had the highest growth and aflatoxin level followed by the sample with 6% extract with the same trend in unpasteurized juice. *A. danielli* at very low concentration showed effective inhibition of *A. flavus* growth and produced low levels of aflatoxin. 1% showed effective inhibition of *A. flavus* growth in both while 2 and 3% produced low levels of aflatoxin for pasteurized and unpasteurized products respectively.

Key words: *Aframomum danielli, Aspergillus flavus,* aflatoxin, orange juice, pasteurised.

INTRODUCTION

Sweet orange (*Citrus sinensis*) is a common fruit in warm climates and found to be rich in carbohydrates, pectin, ascorbic acid, flavonoids and essential oils. The healing properties of orange have been associated with a wide phytonutrient compounds such as flavonones, anthocyanins, hydrocinamic acids and a variety of polyphenols (Shahnawaz et al., 2013). The fruit is commonly peeled and eaten fresh or squeezed for its juice (Crowel, 1999). The peel of citrus fruit serves as natural protectant that prevents microbiological contamination of the interior flesh. Therefore, removing the peel eliminates this protective layer and subjects the edible portion to potential microbial invasion and spoilage.

Fruit juice is usually pasteurised to inactivate microbes and retard biochemical reactions which results to food spoilage. Pasteurization is mild heat treatments usually below 100°C, used to extend the shelf life of foods. The process destroys heat sensitive microorganisms such as non spore forming bacteria, yeasts, and moulds but there

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is some concern that some strains of pathogenic organisms may survive pasteurization (Aworh, 2003).

Aflatoxin is the most common mycotoxin found in foods with *Aspergillus flavus* as the major producer (Bradburn et al., 1993). Exposure to aflatoxin is wide spread in West Africa and has been implicated in carcinogenicity, mutagenicity, teratogenicity, hepatoxicity and aflatoxicosis (Bankole and Adabanjo, 2003). Bankole (2005) isolated 12 species of fungi from rotting sweet orange, 8 isolates were known to be producers of toxin while 7 of *A. flavus* obtained were aflatoxigenic producing primarily aflatoxin B1. The use of chemicals to enhance the safety of fruit juice is of great interest to the fruit industry. However, reports on the harmful effects of these synthetic preservative additives and interest of consumers in fresh foods, with reduced or no content of chemical preservations call for the research into the use of naturally derived compounds as alternative to synthetic additives.

The use of natural preservatives is a promising alternative to chemical methods in extending shelf life of products (Fasoyiro and Adegoke, 2007). Potential sources of these natural preservatives are spices, herbs, fruits, seeds, leaves, barks, and roots (Pratt and Hudson, 1996). *A. danielli* is a spice found in tropical Africa belonging to the genus Aframomum of the family Zingiberaceae. The spice has been reported to exhibit anti-oxidant and preservative properties in different oil and food systems (Adegoke et al., 2003). Antioxidant components of *A. danielli* were identified as phenolic compounds of the trihydroxy type with reducing properties (Adegoke and Gopalakrishna, 1998). Therefore, the effectiveness of *A. danielli* in reducing growth and aflatoxin production. This study was carried out to investigate the effect of local preservative (*A. danielli*) on survival of *A. flavus* in orange juice (acid food) and its ability to produce aflatoxin.

MATERIALS AND METHODS

Matured sweet orange (*C. sinensis*) were obtained at Kuto market in Abeokuta. The oranges were sorted to remove physiologically damaged and blemished ones from the bulk. Dried fruits of *A. danielli* were purchased at Bodija market in Ibadan. The innoculum, a pure culture of *Aspergillus flavus* (reference number IMI3937766) was obtained from Babcock University, Ilisan-Remo, Nigeria. The aflatoxin standard was obtained from ‘Romer Labs Inc. (Union, MO, USA)’.

Preparation of orange juice

Orange juice was prepared as described by Bruenmmar (1981). The sweet oranges were washed, peeled and the juice extracted using Kenwood juice extractor. The pulp was sieved to obtain orange juice using muslin cloth and 100 ml each was dispensed into 14 conical flasks. A part of the juice was pasteurized in a water bath at 85°C for 15 min while the other part was unpasteurized. Both samples were kept at room temperature (28±2°C) for 3 and 7 days.

A. danielli treatment and inoculation with *A. flavus*

A. danielli treatment, extraction and incorporation were carried out as described by Jatto and Adegoke (2010). Dry fruits of *A. daniell* were aspirated to release the seeds, winnowed, washed, air-dried and milled into a fine powder. This *A. danielli* powder was used in the preparation of the aqueous extract.

Cold water extraction method was used in preparation of *A. danielli* extract. A concentration of 20% was prepared; 20 g 0f *A. danielli* crude powder was weighed into 100 ml distilled water and the suspension was kept in the refrigerator for 5 days followed by filtration with Whatman no. 1. After the juice preparation, 100 ml of pasteurized and unpasteurized juice samples were transferred into sterile distilled 250 ml conical flask and treated with *A. danielli* extract (1, 2, 3, 4, 5 and 6%). The same quantity of spores suspension of *A. flavus* (6 spores/ml) was added to the samples. No spice was added to the control (0%). The treated samples were covered aseptically and stored at ambient temperature (28±2°C) for 3 and 7 days.

Determination of mycelia dry weight

The *A. flavus* growth was estimated by mycelia dry weight. 50 ml of 3 and 7 day cultures of *A. flavus* in orange juice samples were first inactivated by keeping them for 30 min in 50 ml methanol followed by 50 ml ethyl ether each on a previously weight filter paper (whatman No.1). The mycelia material on the paper was dried at 80°C for 2 h as described by Mayura et al. (1984). Each filter was cooled in desiccators and reweighed until a constant weight was obtained.

Determination of aflatoxin by ELISA method

The AgraQuant Total aflatoxin Assay, a direct competitive enzyme linked immunosorbent assay (ELISA) was used for the estimation of aflatoxin as described by Zheng et al. (2002). The test was performed as a solid phase direct competitive ELISA using horseradish peroxidase conjugate as the competing measurable entity. All reagents and kit components such as standard aflatoxin, enzyme horseradish peroxidise were manufactured by Romer Labs Inc. (Union MO USA). Eight channel pipettes was used to perform the assay which allowed 48 samples to be run at a single experiment. The representative samples were prepared by measuring 20 ml of the sample into 100 ml of 70% methanol in conical flasks, shaken and centrifuged using REML centrifuge at 1500 rpm and then filtered through Whatman no 1. The pH of the samples was adjusted to 7.0 because excessive alkaline or acidity may affect the test results.100 ml of each sample’s supernatant and standard (that is, 0, 4, 10, and 20 ppb) were dispensed into 200 ml of conjugate (horseradish peroxidase enzyme in 0.2% BSA at a dilution of 1:10000 and anti-antibody coated well and incubated at room temperature for 15 min. The plate was washed and enzyme substrate was added and allowed to incubate for additional 5 min. Stop solution was then added and the colour change from blue to yellow and read by Biotek ELISA (Elx 800-MS) at 450 nm. The intensity of the resulting yellow colour was measured optically. The results of the optical density of the standards were plotted against standard concentrations. Optical densities of the samples were compared to the optical density of the standards and the interpretation result determined on the graph.

Other measurements

The pH meter (ATC Delta 340) was used for monitoring the pH of

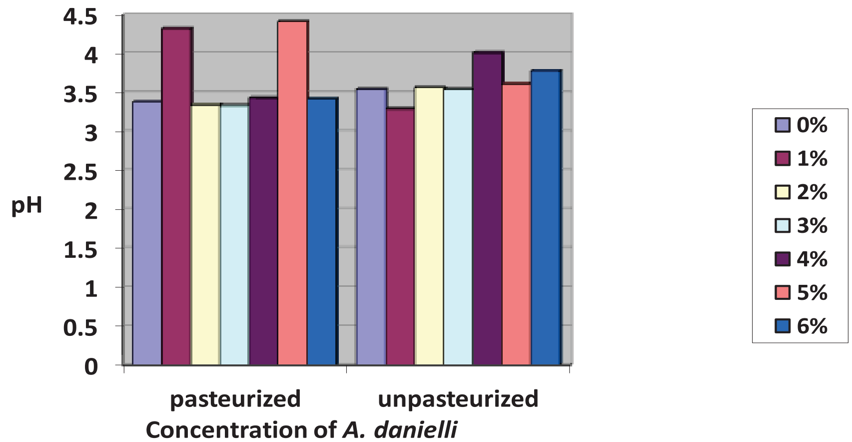

Figure 1. Effect of pH on survival of *A. flavus* and production of aflatoxin in 3 day.

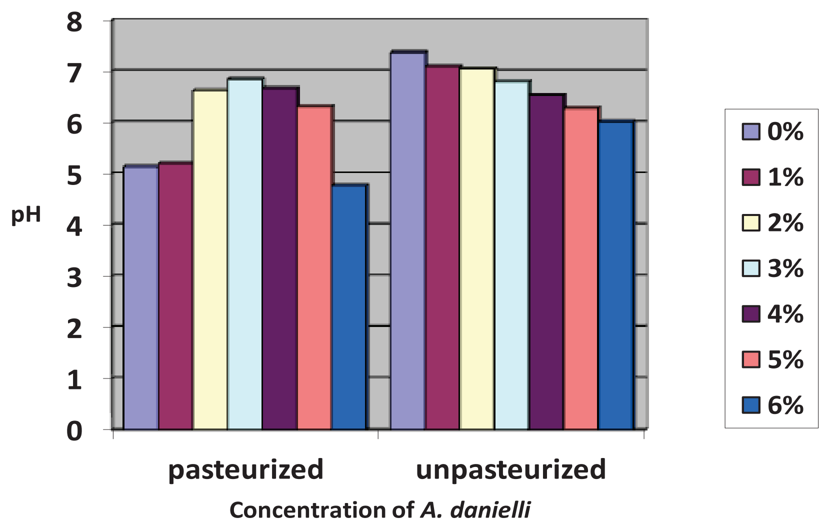

Figure 2. Effect of pH on survival of *A. flavus* and production aflatoxin in 7 day.

the samples. The soluble solid (brix content) was determined using the method described by Uma et al. (2011). The concentration of the sugar was measured directly with a brix refractomer.

Data analysis

Data obtained was analysed using Statistical Package for the Social Sciences (SPSS) version 16. The data were presented as mean and subjected to analysis of variance at 5%.

RESULTS AND DISCUSSION

A decline in pH was observed on day 3 for both pasteurized and unpasteurized samples which ranged between 4.42-3.33 and 4.01-3.29 respectively (Figure 1). This is related to the result obtained by Uma et al. (2011) in a study on determination of shelf life of cashew juice. This drop in pH was more prevalent in unpasteurized juice and may be due to greater rate of fermentation due to presence of other micro-organisms. A tremendous increase in pH was detected on day 7 (6.85-4.77) in pasteurized product and (7.37-6.02) for unpasteurized product (Figure 2).

A factor, which has aided increase in pH, was that most fungi have the capability of secreting extracellular enzymes into their environment in the process of using organic polymers present in the medium as source of carbon and energy and these enzymes secreted may increase the pH (Hussein et al., 1996). The result of mycelia dry weight showed luxuriant growth on pasteurized samples compared to unpasteurized especially at 6 and 0% perhaps due to nutrients supplied by the spice extract when in excess and the inhibition properties of pasteurization which favoured the growth of inoculated spores of *A. flavus* (Figures 3 and 4). The

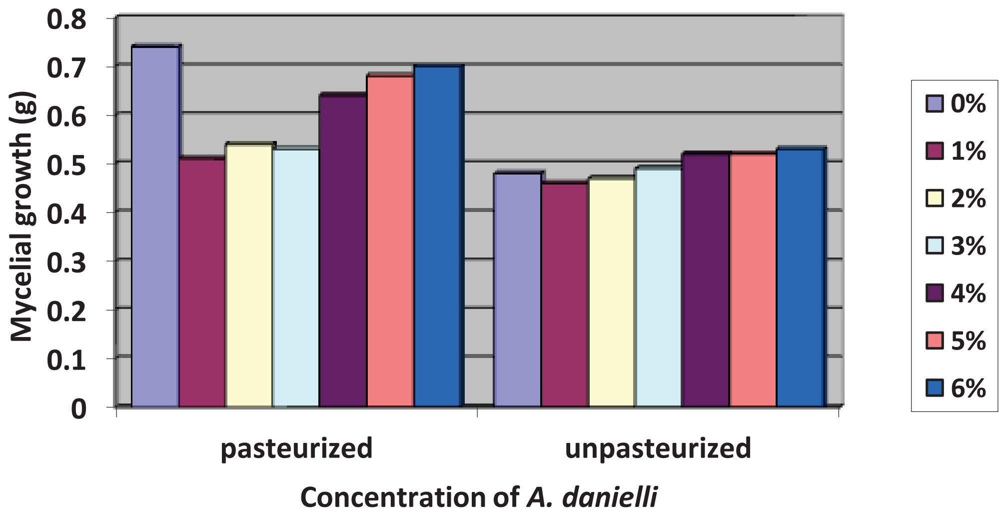

Figure 3. Effect of *A. danielli* on growth of *A. flavus* in orange juice on day 3.

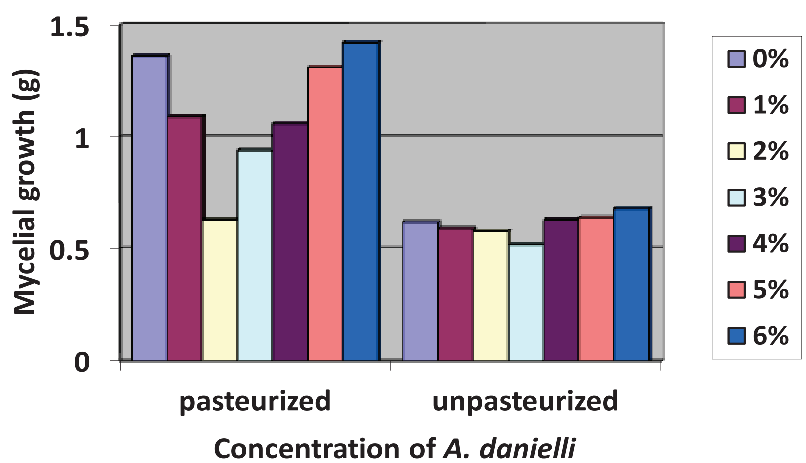

Figure 4. Effect of *A. danielli* on growth of *A. flavus* in orange juice on day 7.

increase in pH might have favoured the growth of *A. flavus* due to the fact that most fungi exhibit good growth over a wide range of pH. A very high growth was observed in 6% concentration of pasteurized sample compare with the control however aflatoxin in the later was higher. Ashaye et al. (2006) reported that some preservative constituents of *A. danielli* might have disrupted the aflatoxin biosynthetic pathway of which resulted to low production in relation to *A. flavus* growth in samples examined. There are no significant difference ($p>0.05$) in growth of *A. flavus* on day 3 for both pasteurized and unpasteurized samples with 1 and 2% *A. danielli* showed minimum growth respectively.

Aflatoxin level did not differ significantly ($p>0.05$) on day 3 for pasteurized and unpasteurized samples and differed significantly ($p<0.05$) on day 7 (Figures 5 to 8). Aflatoxin level was found to be appreciably high in pasteurized samples with the highest value of 156 and 94.50 ng/ml recorded in samples with 0 and 6% respectively. The same trend was observed in unpasteurized samples. Low levels of aflatoxin (below recommended safety limit) were found in pasteurized juice at 2 and 3% concentration on day 3 and 3% concentration on unpasteurized samples while result at 7 day were above recommended safety limits. The effectiveness of low concentration of *A. danielli* extracts is in agreement with the inhibition of the spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice A. *danielli* extract at low concentration (Adegoke et al., 2000). High levels of aflatoxin recorded with high concentration of extracts may be due to the fact that at higher treatment level, *A. danielli* extracts supplies

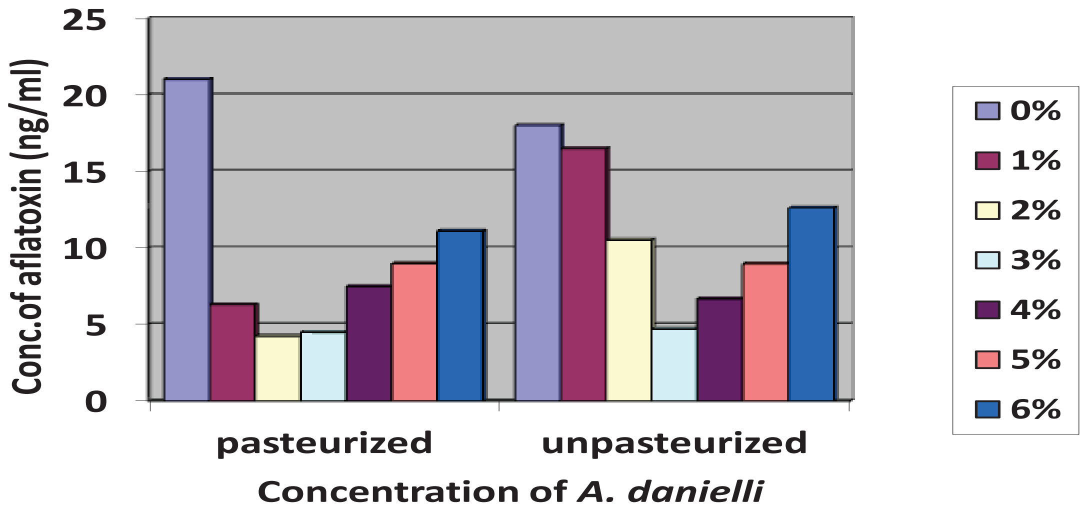

Figure 5. Effect of *A. danielli* on aflatoxin production in orange juice by *A. flavus* on day 3.

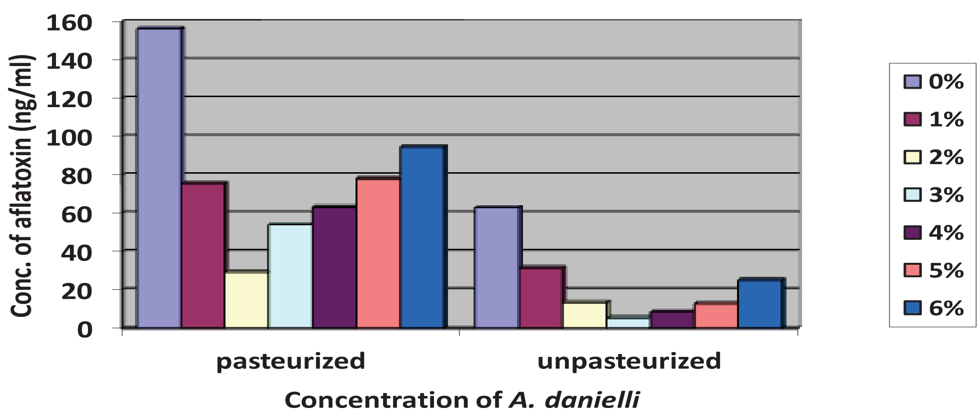

Figure 6. Effect of *A. danielli* on aflatoxin production in orange juice by *A. flavus* on 7 day.

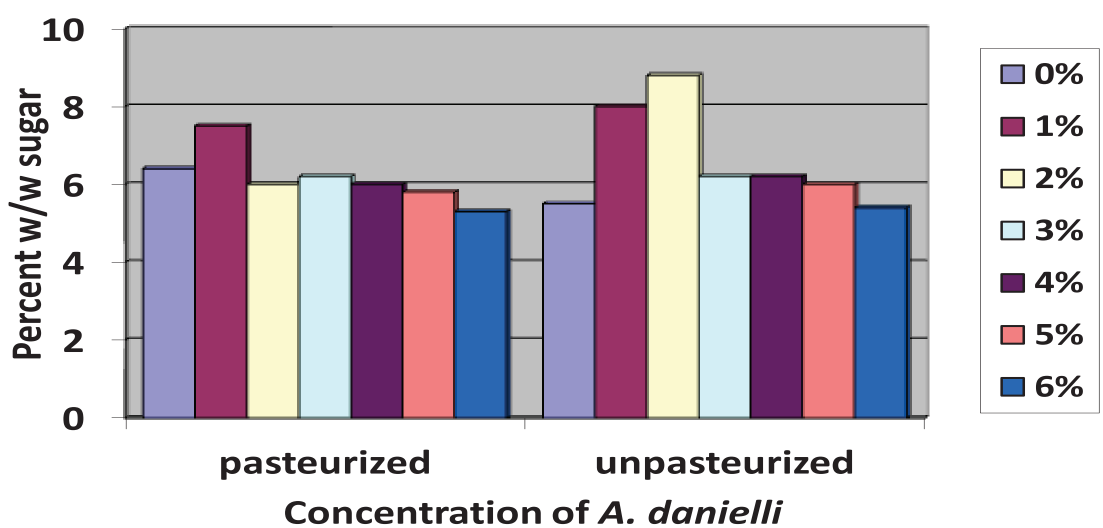

Figure 7. Effect of *A.danielli* on soluble solid of orange juice on day 3.

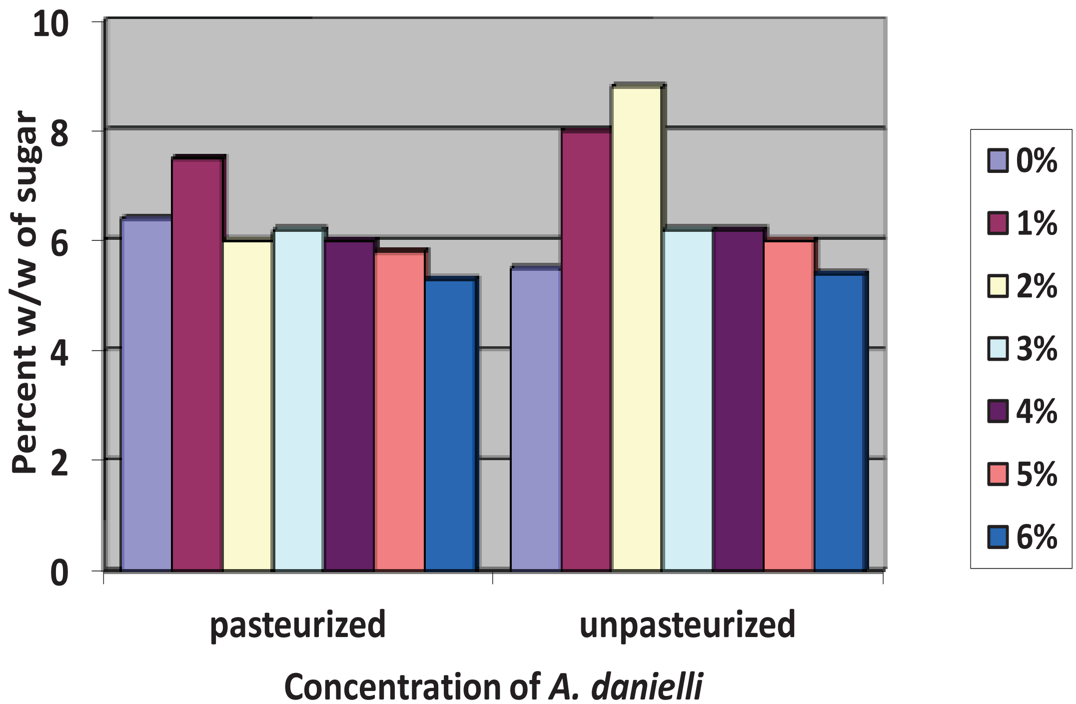

Figure 8. Effect of *A. danielli* on soluble solid of orange juice on day 7.

nutrients for mycotoxigenic mould since the organism can use a wide variety of organic compounds as sources of carbon and energy including carbohydrates, organic acids, aromatic hydrocarbons, which are present in *A. danielli*. The significant level of aflatoxin level of aflatoxin in pasteurized juice found in the study may be probably due to inactivation of other microorganisms during pasteurization process which favoured the growth of *A. flavus*.

The result obtained revealed no difference in brix levels for the treatments (11.00-7.0) and (8.90-7.0) in both pasteurized and unpasteurized orange juice on day 3 but there exist significant difference between the two products at ($p<0.05$). The same trend was recorded (7.50-5.30) and (8.8-5.4) on day 7 of storage. 1 and 2% extracts were favourable for higher retention of soluble sugar (brix) in pasteurized and unpasteurized samples respectively.

Conclusion

The results from the study showed that despite acidity of orange juice, *A. flavus* will survive and produce aflatoxin in orange. *A. danielli* has demonstrated inhibition of *A. flavus* to produce aflatoxin at very low concentration of 2 and 3% for pasteurized and unpasteurized juice respectively with average retention of soluble solid content. The *A. danielli* extract had a higher inhibitory effect in unpasteurised orange juice.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Trinexapac-ethyl in the vegetative and reproductive performance of corn

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This study aimed to measure the effects of rates of plant growth regulator trinexapac-ethyl on the development of the aerial, root parts and yield components of corn. The experiments were conducted in a greenhouse: one assay was aimed at evaluating the growth and development of the plant, in a 4 x 4 factorial arrangement, with four rates of trinexapac-ethyl (0, 125, 250 and 375 g of i.a. ha^{-1}) and four assessment periods (14, 21, 28 and 35 days after application), and the other was aimed at assessing the yield productions for corn in response to the same four rates of the regulator. The present study demonstrates that grain yield per plant was reduced with the use of trinexapac-ethyl, due to decrease in spike length, number of kernels in each row and hundred grain mass. The use of trinexapac-ethyl does not change the cycle of corn, but increases stem diameter and chlorophyll index, and reduces the plant height and spike insertion height, leaf area, dry mass of aerial part, dry mass and volume root system. The trinexapac-ethyl changes the size of the corn plants, enabling new spatial arrangements.

Key words: Leaf architecture, yield components, plant growth, plant growth regulator, *Zea mays* L.

INTRODUCTION

Corn (*Zea mays* L.) is considered one of the world's most important crops worldwide, due to several factors, particularly the nutritional characteristics of the grain that ensure large-scale consumption (Fancelli and Dourado Neto, 2004).

Modern management techniques have provided increased grain yield in corn crop, where it is possible to explore the crop production capacity by developing techniques that ensure the maximum utilization of resources (Zagonel and Ferreira, 2013). Sangoi et al. (2007) claim that corn is one of the most efficient species at converting radiant energy into biomass. Thus, taking full advantage of solar radiation is a strategy to increase the productivity of the crop.

To ensure an optimal use of solar radiation, the adoption of high densities of plants is required, as well as reduction in spacing between lines to be able to quickly capture solar radiation and retain it for a long period.

Higher plant densities are not usually used due to the tendency of the culture of lodging and self-shading of

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plants. The use of corn cultivars with lower plants, having smaller and more erect leaves may minimize the problem. However, cultivars with these characteristics are not always available or are not adapted to a given region, which requires the adoption of alternative methods such as the use of plant growth regulators.

Plant growth regulators act on plant physiology to change characteristics of leaf architecture and reduce the height of stem in order to facilitate crop treatments, reduce propensity to lodging, maximize solar radiation absorption and thus, increase yield (Zagonel and Fernandes, 2007; Zagonel and Ferreira, 2013).

In wheat crop, the use of the plant growth regulator trinexapac-ethyl, besides reducing the height and plant lodging, may change the foliar architecture, allowing greater solar radiation use and increased productivity (Matysiak, 2006; Zagonel and Fernandes, 2007; Penckowski et al., 2009). Increases in grain yield due to changes in plant height and morphology were also observed with the use of trinexapac-ethyl (TE) in upland rice (Nascimento et al., 2009; Arf et al., 2012).

Several species of grasses showed an increase in chlorophyll content, higher photosynthetic activity with the use of TE, becoming more tolerant to shading (Fan et al., 2009; Wang et al., 2009; Costa et al., 2010).

In corn crop, TE has been used experimentally, but the results may vary depending on the climate, hybrid variety, rate and time of application (Zagonel and Ferreira, 2013), as well as on the management practices.

In order to obtain more information on corn response to the use of this plant growth regulator, the present study evaluated the impact of different rates of TE on the growth and development of aerial and root parts and yield components of corn in greenhouse.

MATERIALS AND METHODS

Implementation and execution of the experiments

The study was conducted in a greenhouse with soil collected in an area under no-tillage upon straw. The soil was classified as Oxisol (EMBRAPA, 2006), where chemical analysis of 0 - 20 cm layer was performed prior to the experiment, and the following characteristics were observed: pH_{CaCl2} = 5.3, P = 12.93 mg dm^{-3}, K^+= 0.77 $cmol_c$ dm^{-3}, Ca^{2+} = 7.2 $cmol_c$ dm^{-3}, Mg^{2+} = 2.1 $cmol_c$ dm^{-3}, Al^{3+} = 0.00 $cmol_c$ dm^{-3}, H^++Al^{3+} = 4.96 $cmol_c$ dm^{-3}, $CTC_{efetiva}$ = 15.03 $cmol_c$ dm^{-3}, base saturation (V) = 67%. Based on the results of soil analysis, the seeding fertilization levels with the equivalent to 350 Kg ha^{-1} of the formulated 08-28-16 (N-P-K) was defined.

The experimental plots were formed using 25 kg capacity plastic containers, to which 20 kg of soil were added. In order to assess different characteristics of the plants two simultaneous experiments were mounted, both with completely randomized designs and random rotation of containers every seven days.

Experiment 1 was mounted to evaluate growth and development characteristics following the use of the plant growth regulator in a 4 x 4 factorial arrangement, with four replications: 4 rates of the plant growth regulator TE (0, 125, 250 and 375 g of i.a. ha^{-1}) and 4 assessment periods (14, 21, 28, 35 days after application of TE (DAA)), in a total of 64 experimental units. Experiment 2 was mounted with 4 rates of the plant growth regulator TE (0, 125, 250 and 375 g of i.a. ha^{-1}), and 4 replicates to evaluate the yield components.

Sowing was performed on October 19, 2012, with 5 seeds per container. 10 days after seedling emergence, pruning was performed and there were only two plants per container in experiment 1, and only one plant per container in experiment 2. The cultivar used was the hybrid variety Status Viptera, of early maturity, hard orange-colored grains and high yield potential. Pest and disease control was not necessary because the hybrid variety used in the experiment is resistant to major corn pests and diseases.

At V5 growth stage, nitrogen fertilization was performed with the equivalent of 153 Kg of N ha^{-1}, followed by sprinkler irrigation to prevent volatilization. At V6 growth stage, TE was applied in both experiments using a backpack motorized sprayer with 150 L ha^{-1} volume, and the containers were removed from the greenhouse and separated into groups according to the rates applied.

Assessments

In experiment 1, the following estimations were made: plant height (taking the distance between the ground and the insertion of the last fully expanded leaf), the number of fully expanded leaves and stem diameter at the second internode above the ground, with the aid of calipers, on 14, 21, 28 and 35 DAA. Also, the chlorophyll index was measured in the middle third of the last two fully expanded leaves, with an electronic meter of type ClorofiLOG model CFL 1030.

After these assessments, the aerial part of the plants was collected to calculate the leaf area of all the leaves on the plant using Li-Cor meter, model LI-3100.

The dry mass of the aerial part and of the root system was determined by placing them at 60°C forced draft oven until a constant mass was obtained. The root volume was measured using the graduated cylinder and the water displacement method. In the assessments of experiment 1 the values were derived from the average of the two plants in each container/pot.

In experiment 2, at the end of the crop cycle, the following measurements were performed: plant height and spike insertion height; and of yield components: spike length, number of rows of kernels, number of kernels in a single row, hundred grains mass and grain yield per plant. In the last two assessments, the result expressed in grams was corrected to 130 grams of water per kilogram of grains.

Statistical analysis

In experiment 1, the data were analyzed via analysis of variance (ANOVA) followed by breakdown of the sum of squares into orthogonal polynomials of degree 2. The same occurred in experiment 2.

RESULTS AND DISCUSSION

Analysis of variance in experiment 1 showed significant interaction between TE rates and assessment periods only for variables as chlorophyll index and dry mass of aerial part (Table 1).

Except for the chlorophyll index, all other variables grew linearly over time. However, in the variable, dry mass of aerial part, after breaking down the equation, the data indicate different intensities of growth with

Table 1. Summary of analysis of variance (Mean Squares) for the variables analyzed at 14, 21, 28 and 35 days after application of trinexapac-ethyl (DAA). Londrina-PR/2013.

Causes of variation	GL	Analyzed variables							
		PLH		SDI		NEL		LAR	
Rate	3	3,735.2	*	7.343	*	0.375	ns	263,598	ns
DAA	3	148,454	*	29.604	*	141.375	*	121,736	*
Rate x DAA	9	68.0	ns	0.246	ns	0.361	ns	102,395	ns
Residue	48	46.5		0.559		0.197		70,828	
CV %		4		4		4		5	
Average		166.00		19.9		12.5		5,208.13	

Cause of variation	GL	Analyzed variables							
		CPI		DMA		DMR		RSV	
Rate	3	53.76	*	2,178.3	*	461.5	*	7,414.8	*
DAA	3	334.86	*	32,831	*	4,832	*	60,947	*
Rate x DAA	9	45.30	*	258.2	*	78.4	ns	1,302.3	ns
Residue	48	6.29		24.3		57.4		844.9	
CV %		5		7		25		22	
Average		48.1		74.88		30.48		132.32	

*- Significant at 5% probability by F test, ns – not significant by F test. PLH – plant height, SDI – stem diameter, NEL – number of expanded leaves, LAR – leaf area, CPI – chlorophyll index, DMA – dry mass of the aerial part, DMR – dry mass of root system, RSV – root system volume.

increasing rates of the plant growth regulator, which has already been observed in studies with rice (Alvarez et al., 2007) and soybean (Linzmeyer et al., 2008).

As in wheat (Espindula et al., 2011), reduction in plant height was observed in the present study with increasing rates of the plant growth regulator (Figure 1A). The reduction in plant height occurs by inhibition of cell division and elongation, because TE inhibits the enzyme 3β-hydroxylase, at the end of the biosynthesis pathway of gibberellic acid, dramatically reducing the level of active gibberellic acid (GA_1), increasing its precursor (GA_{20}), with low activity (Davies, 2010). The decrease in gibberellic acid (GA_1) levels would cause the reduction of plant growth (Rademacher, 2000).

On the other hand, Zagonel and Ferreira (2013), in a field study with the same hybrid variety did not obtain reduction in plant height with the use of this plant growth regulator. The discrepancy between the results observed by Zagonel and Ferreira (2013) and those of the present study is possibly associated with the interaction between genotype and the different environments, as well as the different sowing periods. Reduction in plant height with the use of TE was also observed in wheat (Espindula et al., 2011; Contreras et al., 2012), rice (Arf et al., 2012) and soybean (Linzmeyer et al., 2008).

Although the regulator restricted the growth of corn plants, the stem diameter increased with increasing rates of TE (Figure 1B). Zagonel and Ferreira (2013), in turn, obtained quadratic response of stem diameter with the use of TE, and the diameter of the plant's stem was reduced with lower rates of TE and increased with increasing rates of this plant growth regulator.

Lozano and Leaden (2002) reported that the use of TE interferes with the internal diameter of the stem of wheat, by thickening the sclerenchyma, causing greater resistance to lodging. However, this internal thickening may or may not interfere with the external diameter of the stem of wheat. Comparison of the morphology of stems of wheat and corn showed that corn stem is completely filled, and thus, the thickening of tissues inside the stem directly affects the external diameter.

Souza et al. (2013) noticed an increase in the stem diameter of soybean plants with the use of TE, and said that it was caused by the fact that the photo assimilates were diverted from their function of plant growth regulation, which resulted in the increase in the stem diameter. This may also have occurred with the corn plants.

The number of fully expanded leaves was not affected by the use of the plant growth regulator (Figure 1C), indicating that the use of TE did not result in delay of phonological stages of corn.

The leaf area of corn plants was significantly reduced with the use of the TE plant growth regulator (Figure 1D). Zagonel and Ferreira (2013) did not observe TE effects on the leaf area of corn cultivars Status TL and Maximus TL. However, the authors observed a decrease in length and increase in width of the leaf above the spike.

The use of TE led to increases in the chlorophyll index at 14 DAA, with quadratic adjustment with the point of maximum technical efficiency (MTE) at the rate of 279.7 g i.a. ha^{-1} (Figure 2A). Nevertheless, a decrease in

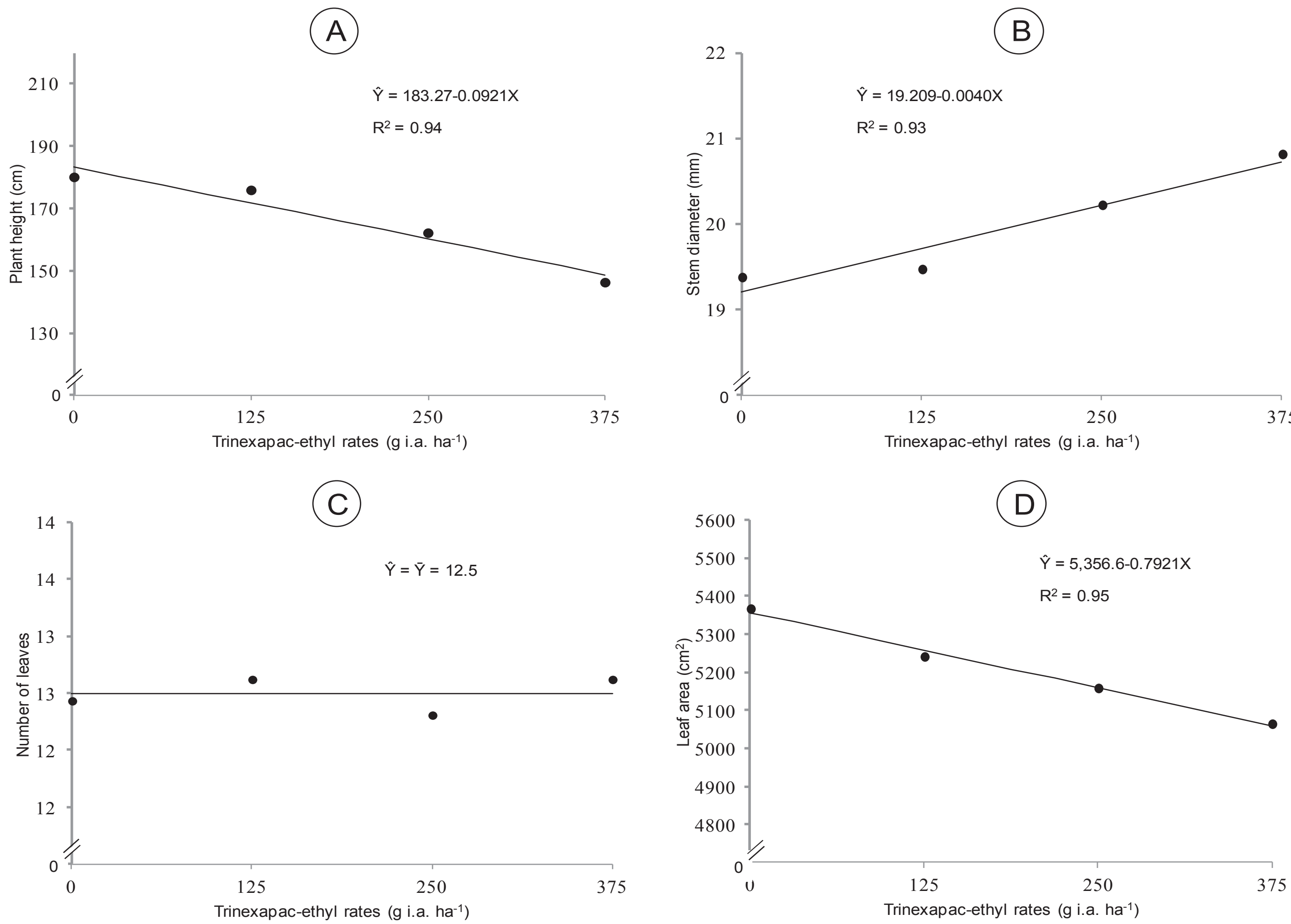

Figure 1. Plants height (A), stem diameter (B), number of leaves (C) and leaf area (D) of corn plants in response to rates of trinexapac-ethyl.

chlorophyll index was observed at 21 and 28 DAA, with increasing rates of TE. However, at 35 DAA a quadratic adjustment with slight increase at low dosage was also observed. But with rates of 108.1 g i.a. ha^{-1} (MTE) or higher, the product reduced the chlorophyll index. Espindula et al. (2009a) studied the effects of TE on chlorophyll content (SPAD index) in leaves of wheat and observed increases in chlorophyll contents with increasing rates of the plant growth regulator and affirmed that such increases are probably due to the increase in the chlorophyll content per unit area and/or volume of leaf tissue, once TE reduced the leaf area.

Analysis of the behavior of the chlorophyll index over time for each of the assessed rates (Figure 2B) shows that on day 14 DAA, all TE rates led to an average increase of 13% compared to rate 0 g i.a. ha^{-1}. However, the rate 0 g i.a. ha^{-1} continued increasing the chlorophyll index for a longer time, while the other rates led to earlier decrease of chlorophyll index at 21 DAA. However, at 35 DAA all TE rates showed chlorophyll index similar to those of the control rate, except the highest rate. A similar result was obtained by Ervin and Koski (2001) who observed an increase in the total chlorophyll content at 14 days after application, though they did not observe any effects at 28 days after application in bluegrass (*Poa pratensis* L.).

The findings demonstrate that the use of TE at the lowest rates increased the chlorophyll index earlier. The same was reported by McCann and Huang (2007), who studied the effect of TE on plants of *Agrostis stolonifera* L. and found that the chlorophyll content in the plants is significantly higher up to 10 days after application of the plant growth regulator. Also, according to the authors, these findings indicate that the application of TE improves the photosynthetic capacity of the canopy and the photosynthetic efficiency of the leaf. This effect on the chlorophyll index can be beneficial to the crop due to the increase in the photosynthetic rate of plants during the vegetative stage, which, in turn, can favor the relative growth rate and consequently, the growth rate of the crop.

The dry mass of aerial part of corn plants decreased

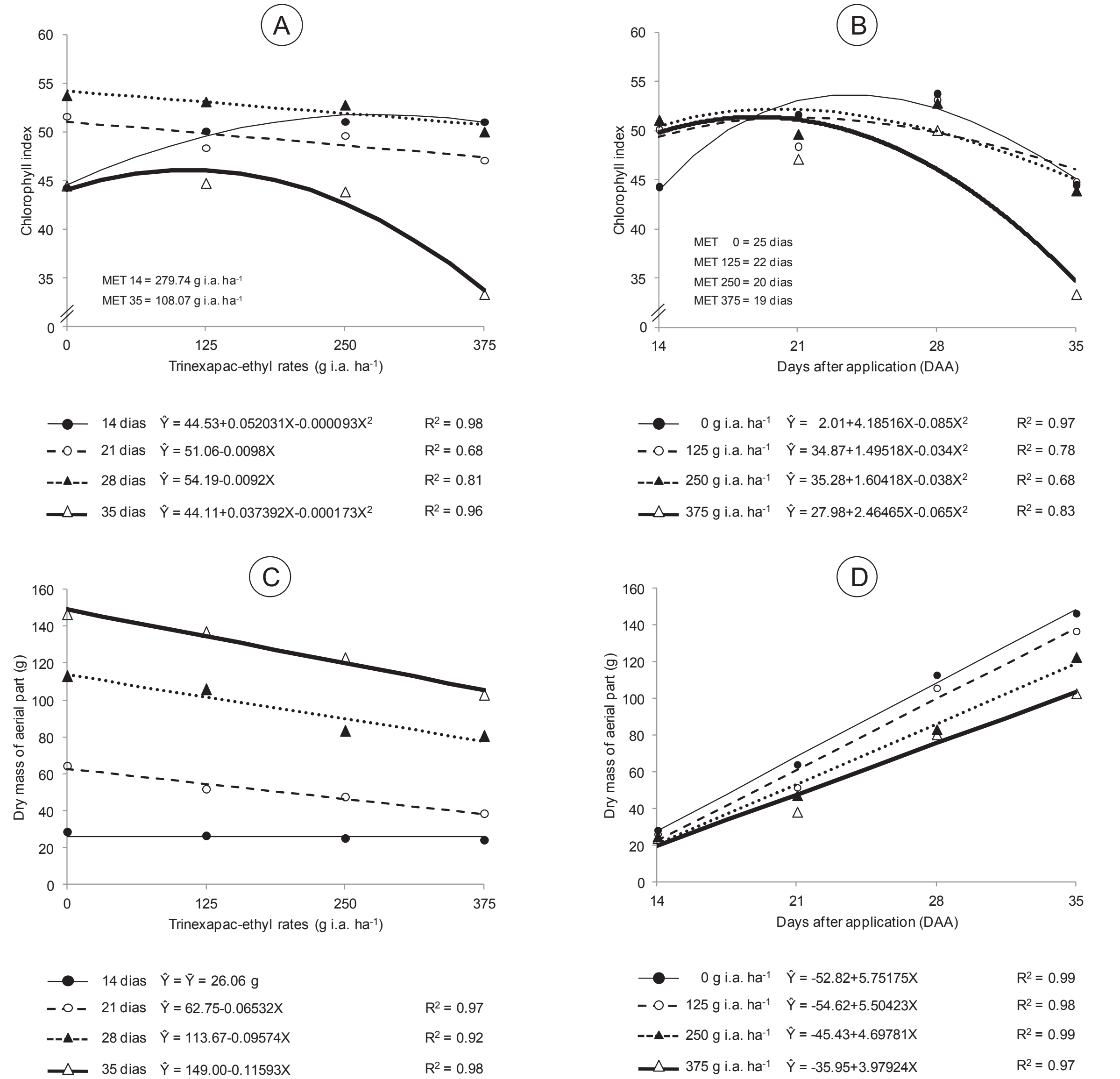

Figure 2. Chlorophyll index and dry mass of the aerial part of corn plants in response to rates of trinexapac-ethyl (A and C) and periods of assessment (B and D).

with the use of TE from at 21 DAA, and no effect was observed at 14 DAA (Figure 2C). Although it did not change the number of leaves, TE reduced the dry mass of the aerial part, which can be explained by the decrease in the plant height, which having smaller stems and leaf area showed a lower dry mass of aerial part. Similar results were described by Espindula et al. (2009b), who affirmed that this decrease is related to the lower mass of wheat plants. However, the author stresses that decrease in dry mass may be responsible for later decrease in grain yield. The highest rates led to lowest growth rate (Figure 2D) of the dry mass of the aerial part, resulting in lower values of the dry mass of aerial part at 35 DAA. The inhibition of synthesis of gibberellins by TE also reduced cell division and elongation of cells in the roots of corn plants, reducing the dry mass and the root volume (Figure 3A and B).

Measurements of TE effects are usually carried out in field studies and they concern only the aerial part of the plants. The present study highlights the effects of trinexapac- ethyl also on the reduction in the root system, which in association with the reduction in the aerial part may be responsible for reduction in individual plant yield.

In the assessments performed at the end of the crop cycle (Experiment 2), significant effects of TE were observed on the studied variables, except for the number of rows of kernels per spike.

Plant height and spike insertion height presented significantly decreased with increasing rates of the plant growth regulator (Figure 4A and B), where the highest

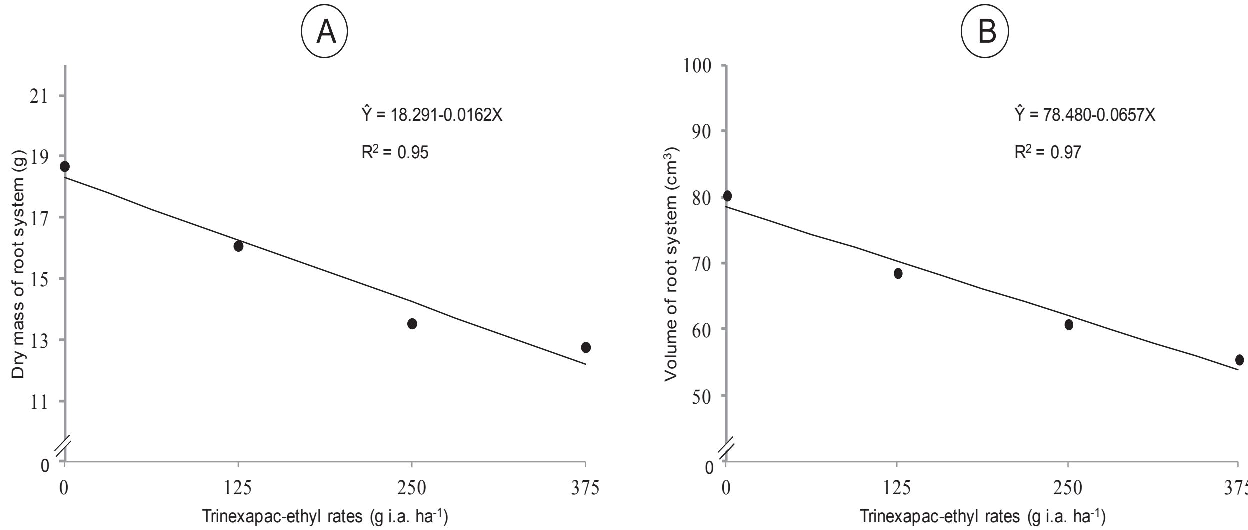

Figure 3. Dry mass (A) and volume (B) of roots system of corn plants in response to rates of trinexapac-ethyl.

rate was responsible for the lowest heights. Since TE inhibits the synthesis of gibberellins, the plants treated with rates of the plant growth regulator at V6 growth stage showed significant reduction in cell division and elongation during a period of significant stem elongation and growth (Ritchie et al., 2003), with reduction in its final height. With the achievement of a lower height of plant and spike insertion, we obtain plants with a center of gravity closer to the soil, reducing propensity to lodging (Sangoi et al., 2001). Thus, lower height of plant and spike insertion associated with higher stem diameter confirms the effects of prevention of lodging provided by the use of TE.

The number of rows of kernels per spike did not respond to the rates of TE ($\hat{Y}=\bar{Y}=$ 16.625), which was also observed by Zagonel and Ferreira (2013). The lack of response of the variable to the plant growth regulator is due to the fact that this characteristic is strongly determined by the genotype and not the environment (Nielsen, 2007).

Application of TE reduced the length of spikes and the number of kernels per row (Figure 4C and D), this reduction being linear according to the increase in the rate applied and up to 1.1 cm and 4.6 kernels per row after the application of the highest rate (375 g of i.a. ha^{-1}). Zagonel and Ferreira (2013) did not observe effects of TE rates on the number of kernels per row, regardless of the time of application, and said that this occurred because the plant growth regulator has no effects on the height of plants and other morphological characteristics.

According to Ritchie et al. (2003), the development of female inflorescences begins at V9 growth stage, and at V12 the development the number of ovules (potential kernels) and the size of spike are determined. Therefore, since TE has strong action in plant meristems, it may have impacted cell division and elongation in this period of grain determination, reducing them. Alvarez et al. (2007) reported increase in the number of shrunken grains of rice following the use of TE and relate this result to the effects of the plant growth regulation on the formation of flowers (stamens and ovaries) and in meiosis. Thesame was observed by Nascimento et al. (2009) with the use of TE in cultivation of upland rice applied during flower differentiation.

A significant reduction in the hundred-grain mass was also observed with increasing rates of TE (Figure 4E). Zagonel and Ferreira (2013) obtained quadratic responses with increases in the mass per 1000 seeds with the lowest rates and later a decrease with application of rates above 272 g de i.a. ha^{-1}. The lowest organic production of plants, as demonstrated by the lower dry mass of the aerial part, reduced the photosynthetically active area and reserves of assimilates, leading to decrease in the hundred- grain mass. The same was reported by Espindula et al. (2009a) in wheat plants. The action of the plant growth regulator in the beginning of grain formation (R1 and R2) possibly inhibited cell division and the number of cells per grain, thus restraining its potential size.

Due to the decrease in spike length, number of kernels per row and hundred grain mass, the use of TE also reduced the grain yield per plant depending on the rates, with a 22 % decrease with application of the highest rate (Figure 4F). On the other hand, Zagonel and Ferreira (2013) did not observe TE effects on the yield of corn crop. The authors affirmed that the lack of response is associated with the little effect of the product on the plants, with response being also dependent on the hybrid variety.

The decrease in grain yield in wheat is caused by

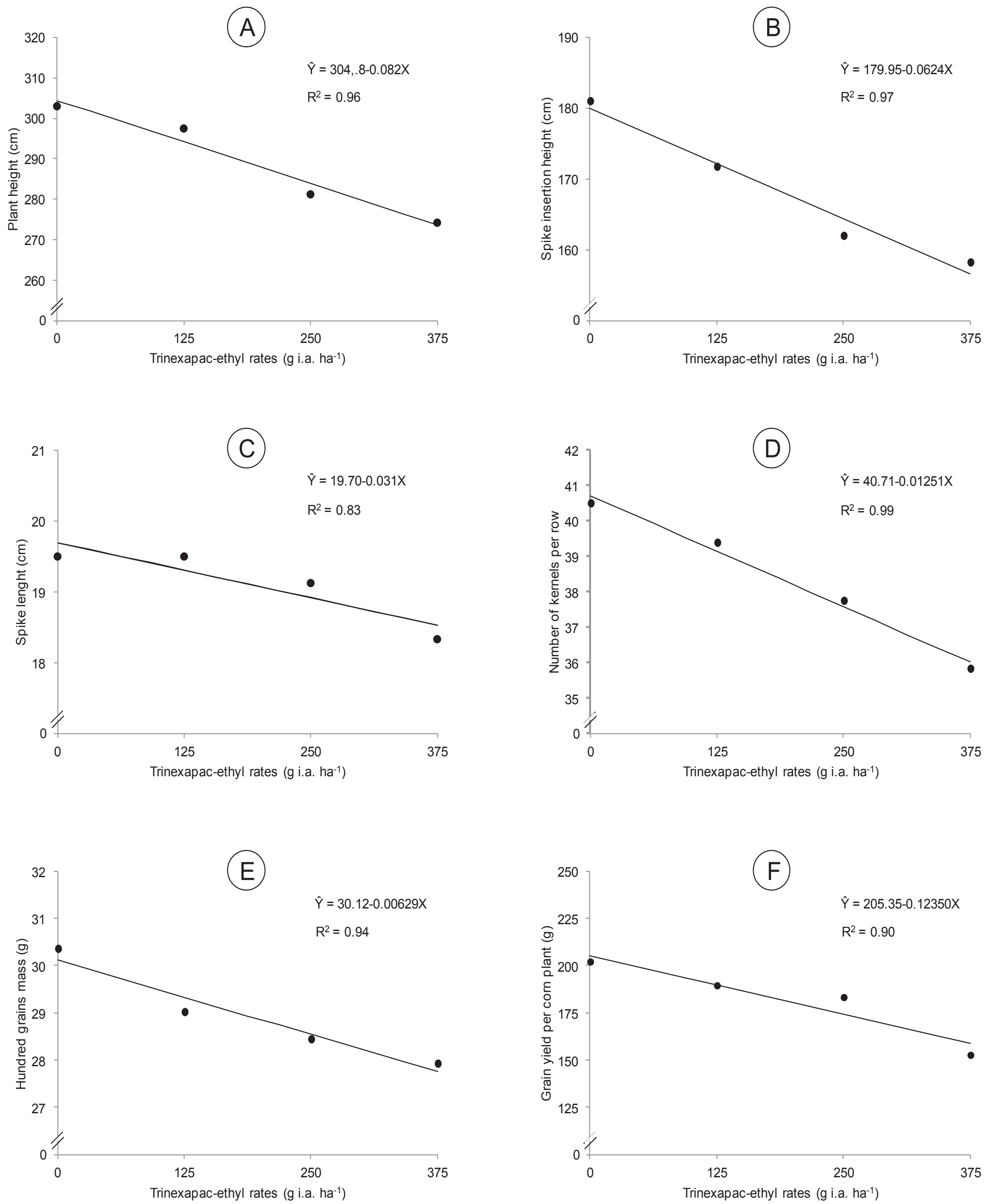

Figure 4. Plant height (A), Spike insertion height (B), spike length (C), number of kernels per row (D), hundred grain mass (E) and grain yield per corn plant (F) in response to rates of trinexapac-ethyl.

decrease in photosynthetic capacity, and reserves of assimilates in the stem was also observed by Espindula et al. (2011). Alvarez et al. (2012) affirm that there is a very close relationship between yield components and grain yield, as well as of plant height with these variables. Thus, decrease in plant height caused by TE had negative effects on yield components and on the yield of upland rice.

It is important to stress that the effects observed here concern isolated plants of a given hybrid variety cultivated in greenhouse, and further studies involving cultivars, plant arrangement and crop management are needed. The decrease in individual plant yield caused by the plant growth regulator can be balanced by a higher number of plants in the area, since the plants treated with TE showed reduced height and dry mass. At higher plant densities, with better spatial distribution, the interception of solar radiation can be optimized, with improvement of the photosynthetic capacity of the canopy and increased yield (Argenta et al., 2001). Therefore, studies on the performance of plants treated with rates of TE would help understand whether the application of the product allows the use of thickened populations, without losses in grain yield due to lower intraspecific competition.

The present study demonstrates that individual plant yield was reduced by TE, due to decrease in spike length, in the number of kernels per row and in the hundred-grain mass. The use of TE does not change the cycle of corn crop, but increases stem diameter and chlorophyll index and reduces the height of plant and spike insertion, leaf area, dry mass of the aerial part, dry mass and volume root system. The use of TE changes the size of corn plants, allowing new spatial arrangements.

Conflict of Interest

The authors have not declared any conflict of interests.

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Concentration of nutrients and chlorophyll index in pigeon pea fertilized with rock phosphate and liming in cerrado oxisol

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Soil fertilization and the management of green manure are needed to maintain and increase the soil fertility of Cerrado. Thus, the objective of this study was to evaluate the concentration of nutrients in the shoots and roots of pigeon pea that was fertilized with phosphorus sources that are associated with liming in Cerrado Oxisol. This study was conducted in a greenhouse with a completely randomized design in a 3×2 factorial arrangement with twelve repetitions. The rock phosphate, triple superphosphate and control treatments were associated with the presence and absence of liming; the sources of phosphorus were evaluated. The chlorophyll index and the concentrations of nitrogen, phosphorus, potassium, calcium, magnesium and sulfur in the shoots and roots of pigeon pea that was grown for a period of 107 days were evaluated. In general, the chlorophyll index and nutrient concentration of pigeon pea did not differ when fertilized with phosphate rock and triple superphosphate. The fertilization with rock phosphate and triple superphosphate provides a greater nutrient absorption of pigeon pea in the presence of lime.

Key words: Green manuring, *Cajanus cajan,* triple superphosphate, rock phosphate, chlorophyll index.

INTRODUCTION

The concentration of phosphorus in the soil solution of the Cerrado is generally reduced. This feature, coupled with the high adsorption capacity of phosphorus, is the main constraint on the development of any profitable farming without the use of large amounts of phosphate fertilizers to meet the crop needs in this region (Sousa and Lobato, 2003).

The sources of phosphatic fertilizers are more soluble at a high cost per unit due to their greater agronomic efficiency in the short term, but as alternatives to these sources, there are less-soluble phosphates, such as rock phosphates. However, these sources do not have nutrient availability, with a lower cost and higher residual effect on the soil, being gradually absorbed by the plants (Horowitz and Meurer, 2004).

Rock phosphates are classified per their origin and may

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belong to the group of volcanoes, where rocks are from the groups of apatite and sedimentary when derived from phosphorite rocks and phosphoric bauxites. Rock phosphates are natural phosphates of volcanic origin, due to their high degree of crystallization and low solubility, while those of sedimentary origin are more soluble (Chien 1977; Lehr and MCclellan, 1972).

Kaminski and Peruzzo (1997) observed that the sums of crop production provided by rock phosphate (Arad and Gafsa) and superphosphate were virtually the same. However, rock phosphates require soil acidity to solubilize and increase their efficiency over time. Sousa and Lobato (2003) observed a decrease in the solubility of rock phosphate with lime application, especially when applied at greater recommended amounts, to increase the base saturation to 50% dose, thereby allowing a reduction in costs in the practice of liming.

According to Pott et al. (2007), pigeon pea (*Cajanus cajan* (L.) Millsp.) is a legume with greater absorption of phosphorus via root exudates organic acids. Therefore, pigeon pea is able to potentiate the solubilization of phosphorus sources that are poorly soluble, such as rock phosphates, in addition to being able to perform symbiosis with the root nodule bacteria of the group, allowing the process of N_2 fixation.

These features cause pigeon pea to be a cover crop, able to improve the availability of soil nutrients, particularly phosphorus and nitrogen, and favoring the development of succeeding crops (Moltocaro, 2007). The positive effect of the interaction between phosphorus and nitrogen can be studied by the indirect measurement of the chlorophyll index, possibly due to the role of phosphorus in plant nutrition through the participation of adenosine triphosphate (ATP), benefiting the active process of absorption nitrogen (Malavolta et al., 1989; Prado and Vale, 2008).

Among the rock phosphates, Bayóvar is from the region of Piura in the province of Sechura, Peru, being of sedimentary origin. Bayóvar is gray, with a mash physical nature and with a guaranteed 29% of total phosphorus, 14% citric acid, and 32% calcium (Faria, 2012). In the Cerrado region, there are few studies of phosphorus fertilization using this source; therefore, this study aimed to evaluate the concentration of nutrients in the shoots and roots of pigeon pea that was fertilized with the rock phosphate Bayóvar and that is associated with liming in Cerrado Oxisol.

MATERIAL AND METHODS

This experiment was conducted in a greenhouse at the Federal University of Mato Grosso, Campus Rondonópolis, Brazil, from May to August 2011.

We used completely randomized design in a factorial 3 (triple superphosphate, rock phosphate and control treatment) × 2 (liming: presence and absence) with twelve repetitions.

The soil was from Cerrado reserve and classified as Oxisol according to EMBRAPA (2006). After collecting, the soil land passed through 4 mm mesh and placed in the experimental units, which were represented by plastic pots of 7 dm^3. The chemical and textural analyses of the soil were conducted according to the methods that were proposed by EMBRAPA (1997) and had the following characteristics in the 0 to 20 cm layer: pH in $CaCl_2$ = 4.1; Al = 1.1 cmol c dm^{-3}, Ca = 0.3 cmolc dm^{-3}, Mg = 0.2 cmol c dm^{-3}, P-Mehlich = 2.4 mg dm^{-3}, K = 28 mg dm^{-3}, S = 6.8 mg dm^{-3}, organic matter = 22.7 g dm^{-3}, V = 9.8%, clay = 367 g kg^{-1}, sand = 549 g kg^{-1} and silt = 84 g kg^{-1}.

According to the results of the chemical analysis, liming was performed only for treatments with the presence of the same, to increase the base saturation to 60%. Irrigation was performed via the gravimetric method, and throughout the experimental period, the soil moisture was maintained at 60% of the maximum capacity of water retention.

Sowing was performed using 20 seeds per pot planted two inches deep. Then, the phosphorus was applied at 200 mg dm^{-3} P_2O_5 according to the availability of the sources triple superphosphate (44% P_2O_5) and reactive rock phosphate Bayóvar (29% total P), which were incorporated into the soil.

A micronutrient fertilizer was applied using a solution containing 1 mg dm^{-3} B and Cu, 3 mg dm^{-3} Zn and Mn and 0.2 mg dm^{-3} Mo from the following sources: H_3BO_3, $CuCl_2$. $2H_2O$ $SO_4.H_2O$ $ZnCl_2$ and MoO_3, respectively. Fertilization with potassium (80 mg dm^{-3}) and sulfur (10 mg dm^{-3}) was performed 14 days after sowing using as sources KCl and $CaSO_4$, respectively. The applications were based on pilot experiments with pigeon pea grown in the same soil.

Thinning was performed on the tenth day after sowing, leaving five plants per experimental unit; and at 107 days, the chlorophyll index was determined, analyzing two leaves from the middle third of the plants, with the aid of the chlorophyll index SPAD-502 Minolta. At the end of the evaluation, the plants were cut at ground level, and the roots were separated from the soil through a 2-mm sieve using water elutriation. Then, the plant material was packaged in paper, identified and dried in an oven with forced air circulation at 65°C until obtaining a constant weight. The dried plant material was ground in a Willey mill and passed through a 2-mm sieve. Then, 5-g samples were collected to measure the nutrients (nitrogen, phosphorus, potassium, calcium, magnesium and sulfur) of the shoots and roots of pigeon pea according to the methodology of Malavolta et al. (1997).

All of the results were subjected to an analysis of variance and Tukey's test up to a 5% probability using the statistical program Sisvar (Ferreira, 2008).

RESULTS AND DISCUSSION

The significant effects were found only on the nitrogen content of the shoots and the roots of pigeon pea with fertilizing and liming, respectively. Higher nitrogen concentrations in the shoots were observed with triple superphosphate and the control treatment without fertilization and without liming and in the roots with triple superphosphate and rock phosphate in the absence of liming (Table 1).

Lima et al. (2003) observed a reduction in the leaf nitrogen concentration as a function of the evaluation time and stated that the dilution effect, which occurs when an increase in the dry mass causes a decrease in the concentration of the nutrients in the plant, occurred. This effect was observed in the control treatment and in the absence of liming (Table 1). Triple phosphate produced higher nitrogen concentrations in the shoots

Table 1. Nitrogen concentration in the shoot of pigeon pea when fertilized with phosphorus sources and liming and nitrogen concentration in the root of pigeon pea when fertilized with phosphorus sources and liming.

Phosphate fertilizer	**Triple superphosphate**	**Rock phosphate**	**Control treatment**
		Shoot Nitrogen (g kg^{-1})	
	17.27^{a}	14.83^{b}	17.34^{a}
Liming	Presence		Absence
		Shoot Nitrogen (g kg^{-1})	
	16.00^{b}		16.96^{a}
Phosphate fertilizer	Triple superphosphate	Rock phosphate	Control treatment
		Root Nitrogen (g kg^{-1})	
	16.01^{a}	15.75^{a}	14.03^{b}
Liming	Presence		Absence
		Root Nitrogen (g kg^{-1})	
	14.17^{b}		16.35^{a}

Means followed by the same letter within a first line compare the fertilization and do not differ by Tukey's test at a 5% probability. Means followed by the same letter within a second line compare the liming and do not differ by Tukey's test at a 5% probability.

Table 2. Chlorophyll index in the leaves of pigeon pea when fertilized with phosphorus sources and liming.

Phosphate fertilizer	**Triple superphosphate**	**Rock phosphate**	**Control treatment**
		Chlorophyll Index	
	43.97^{a}	44.01^{a}	40.02^{b}
Liming	**Presence**		**Absence**
		Chlorophyll Index	
	43.28^{a}		42.05^{b}

Means followed by the same letter within a first line compare the fertilization and do not differ by Tukey's test at a 5% probability. Means followed by the same letter within a second line compare the liming and do not differ by Tukey's test at a 5% probability.

and roots of pigeon pea but, however, equaled those produced by the rock phosphate Bayóvar in the roots. The roots of pigeon pea, being closer to the supply source, absorbed phosphorus from the rock phosphate Bayóvar at triple the amount from superphosphate, a source of greater solubility.

Bonfim-Silva and Monteiro (2010) evaluated the concentration of nitrogen and sulfur in the roots of Brachiaria, similar to most of the research on forage plants, emphasizing the development of the shoots, leaving the roots as a "hidden" component and not addressing the interdependence between these parts of the plant.

For green manure, the root characteristics serve as parameters for the selection of suitable species because a vigorous root system can explore and absorb nutrients from deeper soil layers, making these nutrients available to the successor cultures. Therefore, studies that address the nutritional characteristics of the root system of pigeon pea are of great importance, especially with regard to the correct practice of green manuring.

For the contents of chlorophyll in the leaves of pigeon pea, significant effects were found for fertilization and liming. The effect rock phosphate tripled that of superphosphate, promoting the chlorophyll index, as well as in the presence of lime (Table 2).

The sources of phosphate fertilizer triple superphosphate and rock phosphate were equal and allowed the highest phosphorus uptake by pigeon pea, promoting a greater concentration of nitrogen in the leaves, reflecting the results of the chlorophyll index analysis and corroborating the results in the concentrations of nitrogen in the aerial parts of pigeon pea (Table 1).

Table 3. Phosphorus concentration in the shoots of pigeon pea when fertilized with phosphorus sources and liming and phosphorus concentration in the roots of pigeon pea when fertilized with phosphorus sources and liming.

Phosphate fertilizer		Triple superphosphate	Rock phosphate	Control treatment
		Shoot phosphorus (g kg^{-1})		
Liming	Presence	0.049^{bB}	0.058^{bA}	0.640^{aA}
	Absence	0.439^{bA}	0.048^{cA}	0.649^{aA}

Phosphate fertilizer		Triple superphosphate	Rock phosphate	Control treatment
		Root phosphorus (g kg^{-1})		
Liming	Presence	0.36^{bB}	0.54^{bA}	1.36^{aA}
	Absence	0.83^{bA}	0.41^{cA}	1.44^{aA}

Means followed by the same letter within a line compare the fertilization within the liming interaction and do not differ by Tukey's test at a 5% probability. Means followed by the same uppercase letter within a column compare the liming and fertilization interaction and do not differ by Tukey's test at a 5% probability.

Bonfim-Silva et al. (2012), in a study with doses of Bayóvar rock phosphate in Cerrado Oxisol, observed a chlorophyll index of 48.4 in *Crotalaria juncea* plants, higher than that in this study.

There was an interaction between phosphorus fertilization and liming for the phosphorus concentrations of the shoots and roots of pigeon pea (Table 3). Due to the dilution effect, lower concentrations of phosphorus in the shoots and roots were observed when plants were subjected to treatments with triple superphosphate and rock phosphate, regardless of setting.

Therefore, there was no significant difference in liming when rock phosphate was used, but the response of the crop to accumulate phosphorus in the tissues was positively influenced by liming when triple superphosphate was used as a source. It is most likely that in these treatments the plants were able to absorb and metabolize necessary phosphorus loads faster due to the higher solubility of the source and the possibility of the non-existence of a root system hampered by the presence of aluminum.

Due to the concentration effect, higher concentrations of phosphorus in the shoots and roots of pigeon pea were observed when submitted to the control treatment, regardless of setting. This result, which was also observed by Fernandes et al. (2007), in which the plants retained a greater amount of phosphorus in the roots under conditions of low nutrient supply, maintained the root growth at the expense of shoots. According to these authors, one explanation for this result is that the roots, being closer to the supply source, use the limited influence of this nutrient for maintenance and growth.

Fernandes et al. (2007) studied the velvet bean, *Mucuna cochinchinensis* and jack bean observed that the phosphorus content in plants increased with increasing doses of phosphorus and lime, with the most significant effect being the interaction between the factors, suggesting that the good performance of these species as accumulators and the later recycling of nutrients depend on a minimum supply of phosphorus and liming.

The results for the concentration of potassium in the shoots and roots of pigeon pea indicate that the interaction was highly significant among the factors of fertilization and liming (Table 4).

For the shoots, triple superphosphate in the presence of lime resulted in higher concentrations of potassium, along with the treatment without phosphorus fertilization in the absence of liming (Table 4). Because the results of control treatment occurred due to the effect of concentration, as confirmed by the reduced plant growth, the results were inversely proportional to those with triple superphosphate.

In the control treatment, liming decreased the potassium content by approximately 7 g kg^{-1} when compared with the absence of liming. According Vilela et al. (2004), the influence of liming on increasing the availability of potassium is related to the increased soil cation exchange capacity, providing more exchange sites for retention, reducing leaching and promoting a greater uptake of potassium by the plants. The results of this study demonstrate the importance of the practice of liming on soils with low phosphorus availability.

Ernani et al. (2000) found that the response to lime decreased with the increase of phosphorus fertilization. Thus, the observed results are close to those of these authors; in the control treatment, there was an increased response to liming, demonstrating the importance of a proper relationship, which may result in an economy of limestone in the soils with a high content of available phosphorus and phosphorus in soils having high pH, being particularly important for the plant species in which nutrition is a high percentage of the total production cost.

For triple superphosphate, there was no significant difference for liming, but for rock phosphate in the absence of liming, there were high concentrations of potassium in the shoots of pigeon pea.

Rock phosphates are derived simply by grinding phosphate rock, may or may not pass physical processes

Table 4. Potassium concentration in the shoots of pigeon pea when fertilized with phosphorus sources and liming and potassium concentrations in roots of pigeon pea when fertilized with phosphorus sources and liming.

Phosphate fertilizer		Triple superphosphate	Rock phosphate	Control treatment
		Shoot potassium (g kg^{-1})		
Liming	Presence	6.47^{aA}	6.89^{aB}	5.54^{bB}
	Absence	6.96^{cA}	8.06^{bA}	12.15^{aA}

Phosphate fertilizer		Triple superphosphate	Rock phosphate	Control treatment
		Root potassium (g kg^{-1})		
Liming	Presence	3.89^{aA}	4.40^{aA}	4.26^{aB}
	Absence	4.29^{bA}	4.13^{bA}	7.27^{aA}

Means followed by the same letter within a line compare the fertilization within the liming interaction and do not differ by Tukey's test at a 5% probability. Means followed by the same uppercase letter within a column compare the liming and fertilization interaction and do not differ by Tukey's test at a 5% probability.

Table 5. Calcium concentration in the shoots of pigeon pea when fertilized with phosphorus sources and liming and calcium concentration in the roots of pigeon pea when fertilized with phosphorus sources and liming.

Calagem	Presence	Absence
	Shoot calcium (g kg^{-1})	
	9.66^{a}	7.57^{b}

Phosphate fertilizer	Triple superphosphate	Rock phosphate	Control treatment
		Root calcium (g kg^{-1})	
	5.44^{b}	6.20^{a}	4.42^{c}

Liming	Presence	Absence
	Root calcium (g kg^{-1})	
	6.22^{a}	4.49^{b}

Means followed by the same letter within a first line compare the liming and do not differ by Tukey's test at a 5% probability. Means followed by the same letter within a first line compare the fertilization and do not differ by Tukey's test at a 5% probability. Means followed by the same letter within a second line compare the liming and do not differ by Tukey's test at a 5% probability.

of concentration (Kaminski and Peruzzo, 1997) and do not suffer any chemical treatment (sulfuric acid). Soil acidity favors the solubilization of phosphate, making it available for use by the plants. Thus, the increased absorption of potassium by pigeon pea may provide a greater availability of this nutrient in the soil by the decomposition of biomass, allowing the recycling of this nutrient.

Richart et al. (2006) observed an increase in the foliar phosphorus concentration as a function of the dose of elemental sulfur and sulfur attributed to the influence of the reduction of the soil pH, favoring the solubility of rock phosphate.

The concentration of potassium in the roots of pigeon pea (Table 4), with higher concentrations of triple superphosphate and rock phosphate in the presence of lime and in control treatment in the absence of liming, was measured. The sources also equaled the absence of liming. According to Bedin et al. (2003), a greater reactivity of phosphates, being more readily available, would favor the absorption and utilization of nutrients, mainly for short cycle crops.

Sorato and Crusciol (2007) observed that liming increased the concentrations of potassium in the shoots and that, among the cations available to the soil after application, potassium is the most soluble in the extracts of plant residues.

For the calcium concentrations, there was a significant effect of isolated liming on the shoot, isolated to the fertilization and liming of the roots of pigeon pea (Table 5). There were higher concentrations of calcium in the shoots in the presence of lime and in the roots with rock phosphate in the presence of lime. In addition to the benefit of phosphorus, the available phosphate rock Bayóvar provides calcium as a companion nutrient, directly influencing the development of the roots and

Table 6. Magnesium concentrations in the shoots of pigeon pea when fertilized with phosphorus sources and liming and magnesium concentrations in the roots of pigeon pea when fertilized with phosphorus sources and liming.

Phosphate fertilizer	**Triple superphosphate**	**Rock phosphate**	**Control treatment**
		Shoot magnesium (g kg^{-1})	
	1.81[b]	1.63[b]	2.08[a]
Liming	Presence		Absence
		Shoot magnesium (g kg^{-1})	
	2.18[a]		1.50[b]
Phosphate fertilizer	**Triple superphosphate**	**Rock phosphate**	**Control treatment**
		Root magnesium (g kg^{-1})	
	2.30[a]	2.23[a]	1.84[b]
Liming	**Presence**		**Absence**
		Root magnesium (g kg^{-1})	
	2.79[a]		1.45[b]

Means followed by the same letter within a first line compare the fertilization and do not differ by Tukey's test at a 5% probability. Means followed by the same letter within a second line compare the liming and do not differ by Tukey's test at a 5% probability.

stimulating microbial activity and the absorption of other nutrients, in addition to being required in large quantities by N_2-fixing bacteria.

Teixeira et al. (2005) evaluated the concentrations of nutrients in millet, jack bean and pigeon pea and observed that legumes had higher calcium concentrations. This fact is of great importance as it evidences the efficiency of legumes, such as pigeon pea, as recyclers of nutrients.

More efficient species in the uptake, translocation and utilization of nutrients may be more interesting for use in the management of soils with low fertility, which present a higher adaptability and better performance (Caldeira et al., 2002).

Significant effects were isolated to fertilization and liming for magnesium concentrations in the shoots and roots of pigeon pea, respectively (Table 6).

High concentrations of magnesium occurred in the shoots in the control treatment due to the effect of concentration and the presence of lime (Table 6). In the roots, the effects of triple superphosphate and rock phosphate were equal, providing higher magnesium concentrations in the roots, along with the treatment in the presence of lime (Table 6).

Teixeira and Malta (2012) observed magnesium concentrations of 2.80 and 1.60 g kg^{-1} for jack bean and *Crotalaria juncea*, respectively.

Oliveira (2004) reported that the adequate magnesium concentration ranges from 2.0 to 5.0 g kg^{-1} for pigeon pea and that these values were observed in treatments with lime, demonstrating the importance of the practice of liming, being the form most recommended and efficient in providing the soil magnesium for plant uptake, although the authors used dwarf cultivars. However, the rate of release of nutrients from the crop residues during the process of decomposition depends on the characteristics of the species, in particular the carbon/nitrogen ratio and the location and manner in which these nutrients are found in plant tissue (Giacomini et al., 2003).

Magnesium exerts important functions in the aerial part of the structure of the chlorophyll molecule, in addition also being a cofactor of ATP hydrolysis, providing energy for nitrogen fixation (Malavolta et al., 1997).

The treatments affected the sulfur concentrations of pigeon pea. A significant interaction was detected in the fertilization and liming of the shoots ($p<0.0001$) and roots of pigeon pea (Table 7).

In bean shoots, high concentrations of sulfur with rock phosphate in the presence of lime and in the treatment without phosphorus fertilization in the absence of liming (Table 7) were observed. In the roots, triple superphosphate in the presence of lime and rock phosphate and the control treatment in the absence of liming were statistically equal and promoted higher concentrations of sulfur (Table 7), providing the roots of a higher sulfur concentration a high concentration of sulfur in the aerial parts of pigeon pea.

However, the appropriate concentration of sulfur for pigeon pea is 1.5 to 3.0 g kg^{-1} (Oliveira, 2004). Therefore, the results of this study are less than adequate for culture.

Conclusions

In general, the concentrations of nutrients and pigeon

Table 7. Sulfur concentrations in the shoots of pigeon pea when fertilized with phosphorus sources and liming and sulfur concentrations in the roots of pigeon pea when fertilized with phosphorus sources and liming.

Phosphate fertilizer		Triple superphosphate	Rock phosphate	Control treatment
			Shoot sulfur (g kg^{-1})	
Liming	Presence	0.48^{bA}	0.81^{aA}	0.60^{bB}
	Absence	0.59^{bA}	0.62^{bB}	1.02^{aA}

Phosphate fertilizer		Triple superphosphate	Rock phosphate	Control treatment
			Root sulfur (g kg^{-1})	
Liming	Presence	4.79^{aA}	1.93^{bA}	1.76^{bA}
	Absence	1.88^{Ab}	2.08^{aA}	1.45^{aA}

Means followed by the same letter within a line compare the fertilization within the liming interaction and do not differ by Tukey's test at a 5% probability. Means followed by the same uppercase letter within a column compare the liming and fertilization interaction and do not differ by Tukey's test at a 5% probability.

pea chlorophyll index did not differ by fertilization with rock phosphate and triple superphosphate.

For the nitrogen concentration in the shoot pigeon pea bean triple superphosphate promotes greater concentration of nutrients in the plant in relation to the rock phosphate. Higher nutrient concentration is observed in the absence of phosphate and lime due to the dilution effect.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

Conventional sucker with tissue culture banana production in Central India- A case study

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An economic analysis of tissue-cultured banana and sucker-propagated banana has been presented through studying their costs and returns in production. The problems in cultivation of tissue-cultured banana have been highlighted. The study has been conducted in the Jalgaon district of Maharashtra using personal interview method. The tabular analysis has been employed to find out the socio-economic characteristics, cost and returns in banana crop production through two methods. The study has revealed that tissue-cultured banana is more profitable to farmers than sucker-propagated banana. Higher price of Plantlets, lack of government subsidy on plantlets and lack of plantlets selling government outlets are the major problems in tissue culture banana production. Also, the lack in reliable market facilities, lack of expertise about identifying the pests, diseases and knowledge of plant protection and non availability of genuine planting material are problems in tissue culture banana. The study has suggested that farmers should be encouraged to adopt tissue culture banana (TCB) to get higher yield and profits.

Key words: Constraints productions, tissue culture banana, sucker.

INTRODUCTION

Banana is one of the oldest tropical fruit which is cultivated by man from prehistoric times in India with great socio-economic significance, interwoven in the cultural heritage of the country. Owing to its multifaceted uses and high economic returns it is referred to as "Kalpatharu" (a plant of virtues). It is also fourth important food crop in terms of gross value after paddy, wheat and milk products and constitutes a large proportion of the total fruit production in India. India ranks first in area and production of banana in the world. It supports livelihood of million people, with total annual production of 24870 thousand metric tonnes from 722 thousand hectare with national average of 34.5 metric tonnes per hectare during 2012-13 (Annonymous, 2012). Tamil Nadu is the leading

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producer of banana followed by Maharashtra, Gujarat, Karnataka, Andhra Pradesh and Madhya Pradesh. These six states contribute more than 78% of total banana production in the country. In banana, the difficulty to obtain large number of uniform disease free plants with high yield potential by the conventional propagation of techniques is one of the major limiting factors in increasing productivity. Conventionally, one banana plant produces only five to ten suckers in a year depending on the variety. Through tissue culture large quantities of clean banana plantlets are produced within a short period. Other advantages associated with tissue-cultured banana include optimal yield, uniformity, viral disease free planting material and true to type plants (Robinson et al., 1993).

The Indian Government has identified micro-propagation of plants through tissue culture as an industrial activity under (D&R) Act, of 1951 made effective in 1991. During 1994 there were 14 units engaged in micro-propagation of fruit crops, 12 are producing banana, 5 are aseptically multiplying strawberry, while 3 each are engaged in papaya and pineapple production. Only 6 units are producing 7 forest tree species leaving enough scope for expansion (Govil and Gupta, 1997). The micropropagation industry is particularly well suited for India as it is environment friendly and labour-intensive.

In recent years growing of tissue culture banana becoming popular in this area. In spite of the viable alternative for banana cultivation, there are problems in tissue culture banana viz., high initial investment, somoclonal variation and disease spreading through tissue culture, etc. Under these circumstances, it is essential to focus on economics of tissue culture banana and comparing of tissue culture banana cultivation with traditional (sucker propagated) method of cultivation, so as to facilitate the farmers and others concerned in appropriate decision making for the cultivation of banana. Keeping in view the importance of this technology in the agricultural economy of the Maharashtra state, the present study was formulated to assess the impact of tissue culture technology in Jalgaon district of Maharashtra with specific objectives of to estimate the costs and returns of tissue culture banana over sucker propagated banana and to identify the problems encountered in production of tissue culture banana.

MATERIALS AND METHODS

For in-depth investigations, a sample of 120 farmers was chosen using the multi-stage random sampling method. In the first stage, the districts with highest area under banana crop and availability of required primary data in Maharashtra and Madhya Pradesh during 2010-2011, namely, Jalgaon and Burhanpur districts were selected for the study. Secondly, Raver, Sawda, talukas were purposively selected for the study as the cultivation of tissue culture banana and sucker's banana, respectively, was mostly concentrated in these talukas. At the third stage , four villages from among the predominantly cotton-growing villages in each of these talukas were randomly selected. Finally, a sample of 60 farmers from selected villages was randomly chosen. For evaluating the specific objectives of the study, requisite primary data pertaining to the agricultural year 2010-2011 were collected from the sampled farmers by personal interview method with the help of pre-tested and well-structured schedule.

Analytical tools and techniques employed

The impact indicators like changes in crop management practices and resource use pattern, estimating farm level productivity gains/input savings, changes in resource use efficiency and change in quantity of pesticides used were studied using partial budgeting method as follows:

$$\Delta R = R_{BI} - R_{NB}$$

Where, ΔR = productivity gains/input saving, R_{BI} = per hectare net return on farm with adopted technology, and R_{NBI} = per hectare net return on farm without technology

Garrett's ranking technique was used to identify and rank the constraints to adoption of tissue culture technology. The following formula had used:

Where, R_{ij} = rank given for i^{th} factor by j^{th} individual, and N_j = number of fators ranked by j^{th} individual.

For each factors the scores of individual respondents was added together and divided by the total number of respondents. Percent position of each rank was then converted into scores referring to the table given by Garret (1952). These scores for all the factors were arranged in descending order, ranks were given and finally important factors were identified.

RESULTS AND DISCUSSION

Socio-economic characteristics of sample farmers

Understanding of general characteristics of sample farmers is expected to provide bird's eye view of the general features prevailing in the study area. Therefore an attempt has been made in the study to analyse some of the important characteristics of the sample farmers. The general characteristics on size of holding, age, education, size of family, popular varieties in study area and occupation of farmers was presented in Table 1. The average family size of tissue culture banana growers was 8 and the average family size of sucker propagated banana growers was 6. In case of sucker banana producing farmers 30% were illiterate, 26% had primary level education, 38% had secondary level education and only 6% of the people were having higher secondary and above level education. Similarly, in case of tissue culture banana producers 24% were uneducated, 26% were up to primary level education, 44% had secondary education level and only 6% of the farmers had higher secondary and above level education.

In the study area, 87% of families' main occupation was agriculture and 13% of the farmers were following non agricultural activities in sucker banana producing samples. But, in case of tissue culture banana producing

Table 1. Age, education, size of family and occupation pattern of Banana farmers.

S/N	Particulars	Jalgaon		Average total
		Sucker's banana	Tissue culture banana	
I	Average farm size (ha)	9	10	9
II	Average age of farmer (years)	50	48	49
	Average family size (No. of family labourer)	6	8	7
	Growing varieties	Basrai, Shrimanti and Ardhapuri	Grand Naine	-
III	Education levels:			
	Illiterate	9 (30)	7 (24)	16 (27)
	Primary	8 (26)	8 (26)	16 (27)
	Secondary	11 (38)	13 (44)	24 (40)
	Higher secondary and above	2 (6)	2 (6)	4 (6)
IV	Main occupation agriculture	26 (87)	25 (84)	51 (85)
V	Subsidiary occupation agriculture	4 (13)	5 (16)	9 (15)
	Total	**30**	**30**	**60 (100)**

Table 2. Socio-economic profiles of sample farmers in Maharashtra.

Particulars	Sucker's banana and tissue culture banana	Jalgaon
Banana farmers	Sucker's banana growers	30 (50)
	Tissue culture banana growers	30 (50)
Community of sample farmers	SC	2 (3)
	ST	11 (18)
	OBC	28 (47)
	Others	19 (32)
Type of family	Joint	37 (62)
	Nuclear	23 (38)
Total sample size		60 (100)

Figures in parentheses indicate percentage of sample farmers and percentage to the total.

samples, 84% of the respondents' main occupation was agriculture and 16 per cent of respondents were following non agricultural activities. Average size of farm in case of sucker propagated and tissue culture banana was 9 and 10 ha, respectively. The average size of banana farm in the study area was 9 ha. All the farmers were growing local variety called Basrai, Shrimanti and Ardhapuri in sucker banana production and Grand Naine was used in case of tissue culture banana.

The number of selected farmers in the Maharashtra state was 60, of which sucker's propagated farmers formed 50% viz., 30 and tissue culture banana propagated farmers formed the rest with 50% viz., 30 (Table 2). The farmers from SCs and STs comprised 3 and 18%, respectively in total sample size and the OBCs and others formed 47 and 32% in total sample size respectively. The sample farmers have joint and nuclear families in a proportion of 62 and 38%, respectively in total sample.

Cost structure of banana production in two methods

Per hectare cost of production of sucker propagated and tissue culture banana in the study area is presented in the Table 3. The total cost of production of tissue culture banana was Rs. 210035 per hectare which was higher than the cost of production of sucker propagated banana Rs. 163306 in case of main crop. Total material cost of sucker banana was Rs. 102487 which was lower than the total material cost incurred on tissue culture banana Rs. 140257. The major difference in the materials costs between the two methods of production was mainly due to difference in cost of suckers and plant protection chemical.

The cost of suckers was Rs. 13645 where as cost of tissue culture plantlets were Rs. 48451. The cost incurred on plant protection chemicals in sucker banana was Rs. 2827 where as it was Rs. 1822 in tissue culture banana. There was no large difference was observed in other

Table 3. Inputs use pattern for sucker's banana and tissue culture banana growers.

Particulars (Rs. /ha)	Jalgaon (Maharashtra) (N=60)	
	Sucker's banana	Tissue culture banana
	Mean	Mean
Land preparation	6762 (4)	6495 (3)
Plantlets	13645 (8)	48451 (23)
Plant nutrient	57151 (35)	57411 (27)
Irrigation	16966 (10)	16047 (8)
Human labour	37090 (23)	47236 (22)
Pesticide	2827 (2)	1822 (1)
Others cost (propping, wind break, bunching etc.)	28864 (18)	32573 (16)
Material costs (2+3+6+7)	102487	140257
Total cost	163306 (100)	210035 (100)

Figures within parentheses indicate percentage of total cost of production.

Table 4. Yield pattern in sucker's banana and tissue culture banana growers

Particulars	Jalgaon (Maharashtra) (N=60)	
	Sucker's banana	Tissue culture banana
	Mean	Mean
Production (MT/ha)	45.50	60.50
Gross returns (Rs./ha)	344522	524248
Net returns (Rs./ha)	181216	301605

(land preparation and irrigation) costs between the two methods of production. Total human labour cost incurred in sucker banana was Rs. 37090 and in tissue culture banana was Rs. 47236. Out of total labour cost, human labour cost was major item compared to machine and bullock labour.

Yield and returns pattern in banana production

Per hectare production, gross returns and net returns pattern of sucker banana and tissue culture banana have been presented in Table 4. The Per hectare yield in tissue culture banana was 60.50 metric tonnes which was higher than the yield of sucker banana (45.50 metric tonnes). By products of banana includes suckers and leaves. The gross returns obtained per hectare of tissue culture banana were Rs. 4, 58,991 which was higher than the gross returns obtained from sucker banana (Rs. 3, 44, 522). The net returns obtained in sucker banana were Rs. 1, 81, 216 and in tissue culture banana were Rs. 3, 01, 605.

Constraints faced by growers in production of tissue culture banana

The farmers were interviewed to elicit the problems faced by them relating to various aspects of banana production crop in the study area and it has been presented in Table 5. Out of Total sample size, 30 farmers were interviewed in sucker propagated and 30 in tissue culture plantlets propagated banana crop. The survey data on constraints of tissue culture banana cultivation analysis in Jalgaon districts of Maharashtra indicated that more than 76% farmers had faced the problem of higher price of tissue culture plantlets was limiting in its cultivation by the economically poor farmers and 74% farmers expressed that the lack of government subsidy on tissue culture plantlets. About 61, 55 and 52% farmers expressed that they were facing the problems of lack of government outlets for plantlets sale, pest and disease attacked on plantlets and lack in reliable market facilities, respectively. Finally, lack of knowledge about identifying the pests, diseases and knowledge of plant protection, no fruit price variation, non availability of genuine planting material and lack in availability of cheap irrigation facilities were also problems in cultivation of tissue culture banana plantlets.

Conclusion

In this research paper, we have analyzed the cost and yield benefits of tissue culture banana technology over

Table 5. Constraints faced by farmers in adoption of tissue culture banana in Jalgaon.

Constraints faced by farmers	Garrett's rank score (%)	Rank
Plantlets price variations	76	I
Government subsidy availability	74	II
Government retailer facility	61	III
Disease and pests attack on crop	55	IV
Reliable market facility	52	V
Fruit price variation	39	VI
Lack of expertise	38	VII
Desire quantity	34	VIII
Cheap irrigation facility available	22	IX

the sucker's propagated banana in Jalgaon district, using primary survey data. Based on found out socio-economically benefits of tissue culture technology over sucker propagated banana, the implementation of new technology should be carried out with local community participation and based on the existing capacity of its natural resources as well as its human resources both individually or in group. Last but not least, government interventions in technology spread is also play an important role in technology adoption.

Conflict of Interest

The authors have not declared any conflict of interest.

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Perceived impacts of climate change among rural farmers in Imo State, Nigeria

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The study analyzed the impacts of climate change among rural farmers in Imo State, Nigeria. Specifically, it ascertained the socioeconomic characteristics of the farmers, their level of awareness of climate change, the perceived causes of climate change and the perceived impacts of climate change. Multistage sampling technique was used to select a sample of 100 farmers for the study. Data were collected using structured questionnaire. The data were analyzed using frequency counts, percentages, mean score and bar charts. Results showed that a greater proportion (40.6%) of the respondents knew a little about climate change. Also, it showed that gas flaring (M = 2.07, S.D = 1.94), violation of local customs (M = 2.01, S.D = 1.83) and natural phenomenon (M = 2.00, S.D = 1.83) were perceived as causes of climate change. The result further showed that declining crop yields (49.0%), declining soil fertility (17.0%), drought events (17.0%) and increased heat wave (15.0%) were the perceived impacts of climate change on agriculture in the study area. It was recommended that relevant, timely and up-to-date information on climate change should be provided to the farmers to boost their adaptive capacity to climate change.

Key words: Climate change, impacts, rural farmers, Imo State, Nigeria.

INTRODUCTION

Climate change is one of the most topical environmental issues of this century. Though, it is not possible to predict precise future climate conditions, but the scientific consensus is that global land and sea temperatures are warming under the influence of greenhouse gases and will continue to warm regardless of human interventions for at least the next two decades (Intergovernmental Panel Climate Change, IPCC, 2007).

Climate change refers to any change in climate over time, whether due to natural variability or as a result of human activity (IPCC, 2007). It could also be defined as any significant change in measures of climate lasting for an extended period. This include changes in average weather conditions on earth, such as change in average global temperature, as well as changes in how frequently regions experience heat, droughts, storms, floods and other extreme events (Climate Change Information Resource, CCIR, 2004). According to the Ministry of

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Environment of the Federal Republic of Nigeria, MOE FRN (2003) climate change has become a global issue in recent times, manifesting in different climate parameters including cloud cover, precipitation, temperature ranges, sea level and vapour pressure.

The main causes of climate change have been attributed to anthropogenic (human-induced) activities (IPCC, 2007). Human activities such as the burning of fossil fuels and changes in land use like deforestation release greenhouse gases (GHGs) into the atmosphere which increases the already existing concentration of these gases. According to the South African Confederation of Agricultural Unions, SACAU (2009), the main GHGs are carbon dioxide, methane and nitrous oxide which account for 80, 14 and 6% of the total GHG emission, respectively.

Climate change is projected to have numerous and varied impacts on the environment and human lives. However, such impacts depend on the extent of adaptation, rate of temperature change and socio-economic conditions (IPCC, 2007). This implies that some sectors, systems and regions will more likely be affected than others but the impact will vary depending on system's sensitivity and adaptive capacity. Developing countries such as in Africa despite their least contributions to GHG emissions are increasingly expected to face climate-related threats. This according to Oli and Alec (2009) is as a result of their dependence on climate sensitive sectors (rain-fed agriculture, fishery), poverty and its history of resource, political and ethnic conflicts.

Such impacts include changes in wind patterns and precipitation, contraction of snow and sea ice, very likely increases in frequency of hot extremes, heat waves and heavy precipitation increases in high latitudes and likely decreases in most subtropical land regions (IPCC, 2007). These impacts are projected to affect agriculture, water availability, coastal areas and human health, thus affecting all natural and man-made systems to an extent (SACAU, 2009).

According to Ekong (2003), agriculture is the most dominant occupation in the rural areas. It constitutes the backbone of most African economies, making the largest contribution to GDP and the biggest source of foreign exchange, accounting for about 40% of the continent's foreign exchange earnings (Eva, 2009). However, it is considered as the sector most threatened by climate change in sub-Saharan Africa where there is low capacity to adapt (Shah et al., 2008). Eva (2009) maintains that climate change will exacerbate the existing challenges already facing the sector such as urbanization and industrialization, population increase, degradation of resources and insufficient public spending for rural infrastructure and services.

As posited by Ziervogel et al. (2006), impacts of climate change on agriculture include limited supply of rainfall and drying out of water sources, scarcity of grazing lands, shortage of dairy products, and loss of wild plants for gathering, migration of grazers, poor harvest and livestock losses among others. These problems would aggravate the stresses already associated with subsistence production, such as isolated location, small farm size, limited resources, informal land tenure etc (IPCC, 2007). These will make small-scale farmers in the rural areas particularly vulnerable to the risks of climate change.

It is important to identify the various impacts of climate change in vulnerable communities, which will enable the determination of the extent of risks posed by the changing climate on their dominant occupation (that is, agriculture) as well as enhance the development, streamlining and up scaling of adaptation strategies. This is further necessitated by the fact that impacts of climate change are location-specific and these have to be determined in order to develop comprehensive and suitable adaptation strategies which will capture vulnerable communities in developing countries. It is against this backdrop that the following research questions are being asked: what are the perceived causes of climate change and what are the perceived impacts of climate change in the study area?

Purpose and objectives

The overall purpose of the study is to identify the perceived impacts of climate change among rural farmers in Imo State, Nigeria. Specifically, the study sought to:

1. Determine the socio-economic characteristics of the farmers;
2. Ascertain the awareness of climate among the farmers;
3. Identify perceived causes of climate change in the study area; and
4. Identify perceived impacts of climate change in the area.

METHODOLOGY

The study was carried out in Imo State, which is among the five states in the Southeast geopolitical zone of Nigeria. It lies within latitude 4° 45' N and 7° 15' N and longitude 6° 50' E and 7° 25' E and covers an area of about 5100 km^2. It is divided into three political zones namely Owerri, Okigwe and Orlu and comprises 27 LGA. The population of the state stands at 4.8 million people (Federal Republic of Nigeria Official Gazette, 2007). Rainfall distribution is bi-modal with peaks in August and September. Variation in annual rainfall is between 1900 and 2200 mm. Temperature is uniform in the state with mean annual temperature of about 20°C. The annual relative humidity is 75% and the state lies within the rainforest agro-ecological zone. The major economic activity of the people is farming which confirms the predominance of rural communities in the state. Major crops grown include maize, cassava, yam and cocoyam while major livestock kept are goats, sheep and poultry (Umunakwe, 2011).

All farmers in the state constituted the population for the study. Multi-stage sampling technique was used to select the sample. The first stage comprised the purposive selection of one LGA from each of the three political zones in Imo state based on peculiar vulnerability factors which include flood, erosion, oil exploration and other natural disasters. The second stage comprised the purposive selection of one autonomous community from each of the three LGAs based on the vulnerability factors above. The third stage involved the purposive selection of three villages from each of the three autonomous communities based on vulnerability factors mentioned above. The fourth stage comprised the purposive selection of 12 farmers from each of the nine villages. Overall, 108 farmers were used for the study. Data for this study were collected using a structured questionnaire and interview schedule and were validated using face and content validity.

To ascertain the level of awareness of climate change, respondents were asked to indicate their knowledge of climate change and their responses were measured on a nominal scale of Know a lot = 4, know = 3, know a little = 2 and don't know = 1. The percentages of the observations on the scales were determined. To ascertain the perceived causes of climate change, a list of possible causes of climate change obtained from literature and field observation were provided and the respondents' perception were measured on a 5 point likert type scale. These are, To a Great Extent = 4, To Some Extent = 3, To a Little Extent = 2, To a Very Little Extent = 1 and To No Extent = 0. The mean score was determined by adding up all the values of the scale to obtain 10. Then, it was divided by the number of values, that is, (10/5) to obtain 2. Items with $M \geq 2$ were regarded as perceived causes of climate change in the study area. The perceived effects of climate change were ascertained by providing a list of possible effects of climate change obtained from literature and personal observations and respondents were asked to indicate the ones they perceive as effects. Data obtained were analyzed using percentage distribution, mean statistics and bar charts.

RESULTS AND DISCUSSION

Age

Data in Table 1 show that majority (61.5%) of the respondents were between the ages of 41-56 years while a very small proportion (1.8%) was 73 years and above. The mean age of the respondents was 50.51 years. This implies that the respondents were still within active and productive ages and can be efficient in agricultural production. In addition, their mean age suggests that they have sufficient knowledge and experience pertaining their environment and would be able to give vital information regarding climate change. According to IPCC (2007) any event is considered as climate change if it has occurred consistently for at least a period of one decade. Therefore, the mean age of the respondents which is above one decade would have enabled them observe such events.

Sex

Data in Table 1 show that majority (55.7%) of the farmers were male while the remaining 44.3% were female. This reflects the dominance of male farmers in the study area. Studies have revealed that women are marginalized in most societies in developing countries (Ekong, 2003; Igbokwe, 2005; Ani, 2004) and this limits their access to natural resources such as land thus affecting their involvement in agriculture. This marginalization could limit their access to agricultural services. For example, Holmes and James (2008) observed that in spite of the high ratio of agricultural extension staff to farmers in Ethiopia, female farmers still have limited access to extension services. This could increase the vulnerability of the farmers to climate change.

Marital status

Entries in Table 1 show that majority (70.7%) of the farmers were married while 20.8, 6.6 and 1.9% were widowed, divorced and single, respectively. Marriage could provide farmers with a supply of family labour, which is cheaper and readily available and also enhances the sharing of agricultural information and knowledge. According to Nnadi et al. (2012), marriage encourages complementarities of efforts among farming households. This could promote adaptation among farming households.

Educational qualification

Results in Table 1 show that majority (99.10%) of the farmers had one form of formal education or the other while the remaining 0.9% had no formal education. It could be inferred from this that literate farmers dominate the study area though the level of literacy differed. The acquisition of formal education will increase the receipt of information on climate change, leading to a broader knowledge of it. According to Agbamu (2008), the acquisition of formal education promotes the adoption of improved agricultural technologies.

Household size

Data in Table 1 show that majority (52.9%) of the farmers had household sizes of between 6-10 persons. Others were 39.6 and 7.5% for household sizes of 1-5 and 11-15 persons, respectively. The mean (M) household size was seven persons. This shows that the farmers had fairly large households which could supply them with cheaper family labour. Large households would encourage diversification of enterprises by farmers thus increasing their productivity and income. It would also minimize expenses especially on labour. A study by Nnadi et al. (2012) confirmed that households in Imo State, Nigeria are fairly large.

Secondary occupation

Entries in Table 1 further show that majority (50.0%) of the farmers engaged in trading as a secondary

Table 1. Distribution of respondents according to their socio-economic characteristics (n = 108).

Socio-economic characteristic	%	M
Ages (Years)		
25-40	21.2	
41-56	61.5	
57-72	15.5	50.51
73 and above	1.8	
Sex		
Male	55.7	
Female	44.3	
Marital status		
Single	1.9	
Married	70.7	
Widowed	20.8	
Divorced	6.6	
Educational qualification		
No formal education	18.5	
Primary school attempted	3.2	
Primary school completed	18.9	
Secondary school attempted	27.4	
Secondary school completed	19.8	
OND/NCE	11.3	
HND/First degree	0.9	
Household size (Persons)		
1-5	39.6	
6-10	52.9	
11-15	7.5	7
Primary occupation		
Farming	65.1	
Hunting	1.9	
Trading	24.5	
Government worker	8.5	
Secondary occupation		
Farming	9.4	
Trading	50.0	
Driving	3.8	
Masonry	1.9	
None	34.9	
Farming experience (Years)		
1-10	49.8	
11-20	27.4	
21 and above	22.4	14.30
Farming system		

Table 1. Contd.

Crop production	61.3	
Livestock production	7.5	
Mixed farming	31.2	
Major crops grown		
Cassava	65.2	
Maize	7.5	
Yam	22.6	
Cocoyam	4.7	
Major livestock kept		
Poultry	21.7	
Goat	12.3	
Sheep	8.5	
Pig	0.9	
None	56.6	
Farm size		
< 1 ha	66.5	
1-1.9 ha	32.0	1
2 ha and above	1.5	

Source: Field Survey Data, 2011.

occupation while 9.4, 3.8 and 1 .9% indicated farming, driving and masonry, respectively as their secondary occupations. Rural people have been observed to diversify their means of livelihood as a way of adapting to climate change (Roncoli et al., 2010). Diversification of livelihood activities helps farmers to cope with climate change especially in the event of collapse of on.

Farming experience

Data in Table 1 show that a greater proportion (49.8%) of the respondents had engaged in farming activities for a period of 1-10 years. Also, 27.4 and 22.4% had been into farming for 11-20 years and 21 years respectively and above respectively. The mean farming experience was 14.30 years. This means that the respondents have practiced farming for a fairly long period of time enough to observe changes attributable to climate change. The period is also enough for the farmers to observe the effects of the changing climate. According to IPCC (2007), any change should be observed for at least one decade before it could be said to be climate change.

Farming system

Data in Table 1 reveal that majority (61.3%) of the farmers were into crop production while the remaining 31.1 and 7.5% were mixed and livestock farmers, respectively. This finding still attests to the fact that farmers in the study area diversify their enterprises. As observed by Rancoli et al. (2010), farmers in rural Kenya integrate crop and livestock production as a measure to cope with the changing climate.

Crops grown

Data in Table 1 also show that majority (65.2%) of the farmers cultivated cassava while 22.6, 7.5 and 7.4% cultivated yam, maize and cocoyam, respectively. Fundamentally, this result shows that the farmers cultivate more than one crop. The dominance of cassava in the area could be as a result of its ability to withstand adverse climatic conditions. Manyong et al. (2000) reported that cassava has the ability to survive suboptimal conditions such as drought and low soil fertility. However, the cultivation of other crops by the farmers could be seen as measure taken to cope with adverse climatic conditions considering the risks and uncertainties facing agricultural production.

Livestock kept

Entries in Table 1 show that majority (56.6%) of the farmers kept no livestock while 21.7, 12.3, 8.5 and 0.9%

Table 2. Mean distribution of respondents according to perceived causes of climate change (n = 108).

Causes of climate change	M	S.D
Bush burning	1.14	1.404
Use of excessive chemicals in farming	1.24	1.583
Deforestation	1.20	1.444
Overgrazing	0.76	1.239
Burning of fossil fuels	1.22	1.615
Indiscriminate use of generators to provide electricity	1.22	1.537
Depletion of ozone layer	1.30	1.646
Gases released from industries	1.93	1.890
Natural phenomena	2.00*	1.830
Violation of local customs	2.01*	1.860
Crude oil spillage	1.67	1.803
Swamp rice production	0.87	1.388
Gas flaring	2.07*	1.938

* Perceived causes of climate change. Source: Field Survey Data, September 2011.

kept poultry, goat, sheep and pigs, respectively. Globally, livestock contributes about 40% to the agricultural GDP and constitutes about 30% the agricultural GDP of developing countries (World Bank, 2009). However, the non-participation of majority of the farmers in livestock production could be attributed to the increasing costs and risks involved in the enterprise. According to Moyo and Swanepoel (2010), the increasing risks and uncertainties related to climate change and associated shocks add another dimension to changes observed in livestock production.

Farm size

Data in Table 1 further reveal that majority (66.5%) had less than 1 ha farm size while 32.0 and 1.5% had 1 to 1.9 and 2 ha, respectively. The mean farm size was 1.0 ha. This shows that the farmers were predominantly small landholders which is in conformity with the assertion of the African Fertilizer Summit (2006) that small holder farmers cultivate between 0.8 to 1.2 ha of land. This could be attributed to the declining availability of land for agriculture due to urbanization and increasing population and consequently lower farmers' productivity.

Level of awareness of climate change

Data in Figure 1 show that a greater proportion (40.6%) of the farmers knew a little about climate change, 31.1 and 6.6% knew and knew a lot about climate change, respectively. The figure further shows that a significant proportion (21.7%) of the farmers does not know about climate change. The limited knowledge about climate change among the farmers could be as a result of their inability to access scientific information on climate change which could be attributed to inadequate number of extension staff in the area (Onyeneke and Madukwe, 2008). This may hamper their ability to adapt to climate change thus leading to poor agricultural harvests. According to Aklilu (2002), information about climate change is largely confined within the academia and research institutions thus limiting their availability to end-users.

Perceived causes of climate change

The mean scores of the respondents in Table 2 show that gas flaring (M = 2.07), violation of local customs (M = 2.01) and natural phenomena (M = 2.00) were perceived as main causes of climate change by the farmers in the study area. This perception may have been influenced by their limited access to scientific information on climate change which strengthens their reliance on personal observation and experience as sources of information on the phenomenon. As reported by (GoZ-UNDP/GEF, 2010) majority of the public in developing countries are yet to be properly and adequately informed about climate change. Similarly, Aklilu (2002) observed that information about climate change is still confined within the academia and research institutions in developing countries and consequently limiting local people's access to such information. The unavailability of proper information on climate change will hamper the adaptive capacity of rural farmers, consequently worsening the growing cases of food insecurity and famine in the region.

Combustion of fossil fuels has been reported as one of the major causes of climate change (SACAU, 2009). A study by Egbule (2010), reported that natural gas is still being flared in the Niger Delta region of Nigeria and local

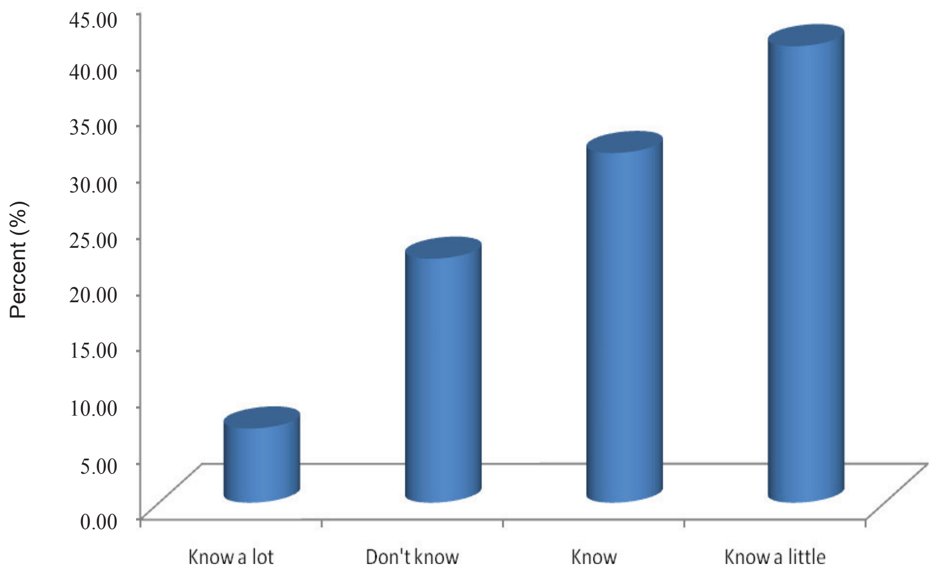

Figure 1. Level of awareness of climate change. Source: Field Survey Data, 2011

people there perceived it as among the causes of climate change. Flaring of natural gas emits GHGs into the atmosphere, thus increasing their concentration and leading to more warming effects.

The farmers also perceived climate change as a natural phenomenon. It is a common notion among local people that climate pattern varies naturally and they attribute it to the will of God. They strongly contend that climate is not stable so also are the weather elements like rainfall, sunshine and others. Though, climate variability is natural, scientific reports have shown that it is worsened by the emission of GHGs into the atmosphere (IPCC, 2007). This as a result exerts adverse influences on the ecosystem.

The farmers further perceived climate change to be because of the violation of local customs. This is common in the rural areas where people attribute any disaster or mishap in their environment to the anger of the gods. They hold the view that certain evil deeds incur on the people the wrath of the gods which sometimes can persist for generations. According to them, such wraths can alter the usual pattern of climatic and weather elements which will affect adversely the means of livelihood of the people especially agriculture which has a close relationship with climate. For example, some communities in the Igbo society believe that incest or adultery can cause the land in the area to be infertile thereby leading to poor harvests. Some others believe that failure to offer sacrifices to the gods of the land before planting can provoke the gods leading to the cessation of the rain. This however is in agreement with the findings of Kelbessa (2007) that African local people associate climate change to such issues as the violation of local customs, the wrath of gods, the end of the sinful generation and natural phenomenon.

Perceived impacts of climate change

Figure 2 shows that a greater proportion (49.0%) of the respondents perceived declining of crop yields was taken as the major impact of climate change on agriculture. Moreover, 17.0, 17.0, 15.0, 10.0, 5.0, 5.0, and 5.0% were perceived as declining soil fertility, drought events, increasing heat wave, high incidence of weed, changes in rainfall intensity and pattern, increasing incidences of pests and diseases on crops and excessive rainfall, respectively. This finding is in line with the report of a survey by Hassan and Nhemachena (2008) that increasing temperature, declining precipitation, changes in the pattern of rainfall and drought events are becoming more frequent.

The perception on declining soil fertility is in agreement with the model result which projects a reduction of suitable land for rain-fed agriculture and crop production. In Southern Africa, it is projected that this reduction could lead to net crop revenues dropping by as much as 90% (IPCC WGII, 2007).

Similarly, the perception on declining crop yields also is in line with the projection that climate change would reduce the production of maize in southern and western Africa, while decreases in North Africa's wheat yields could increase famine (Warren et al., 2006). Furthermore, Fischer et al. (2002) reported a general decline in the production of most subsistent crops e.g. sorghum in Sudan, Ethiopia, Eritrea and Zambia. In addition, climate change is expected to impact negatively on agricultural production by increasing risks of exposure to new pests and disease variants (Ensor, 2009). The knowledge of climate change impacts on agriculture will enable farmers adopt technologies that will reduce these impacts.

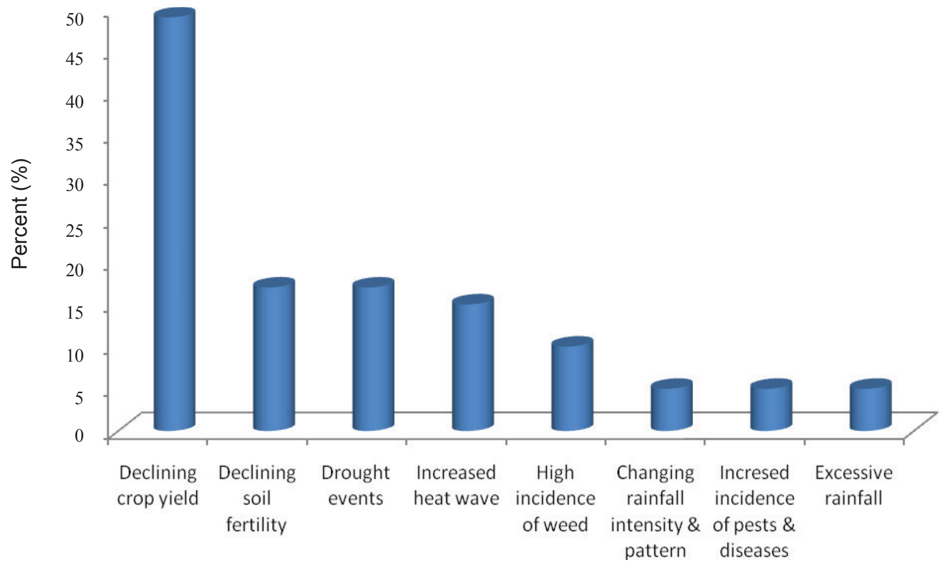

Figure 2. Perceived impacts of climate change.

Conclusion

Rural farmers have observed the occurrence of climate change. However, their knowledge on the causes is still limited in the sense that it is more local than scientific. For example, it was perceived as a natural phenomenon and a punishment by the gods. This implies that local farmers still have inadequate access to information on climate change and this could hamper their adaptive capacity, thus worsening food insecurity situations. Poor capacity of the extension organization to disseminate climate change information could have contributed to this. Furthermore, the impacts were mainly felt on agriculture which is their major occupation. Climate change has continued to exert negative influences on agricultural development in developing countries. Hence, the attainment of food security has remained very impossible. Agriculture relies on the climate more especially in developing countries. Therefore, any change in the climatic conditions will have significant effects on agricultural production.

RECOMMENDATIONS

Following the findings made in the study, the following recommendations are made:

1. Relevant and up-to-date information on climate change should be made available to farmers. However, to ensure the provision of relevant information to the farmers, their information needs regarding climate change should be first determined to ensure that information provided to them is useful. Furthermore, to ensure the speedy and timely provision of this information, devices that are fast, cover a wide range of information users and cost-effective like the ICTs should be incorporated into extension service delivery and climate change adaptation programmes. The provision of climate change information through mobile phones and telecentres will facilitate the accessibility of information by rural farmers.
2. Sensitization campaigns should be mounted in rural areas to educate farmers on climate change. ICTs such as radio and television considering their potentials such as speed, number of people covered and cost-effectiveness should widely be used in the campaign on climate change.
3. Systems of agriculture and improved agricultural packages designed to help farmers adapt to climate change should be made available to the rural farmers. This may include improved crop varieties and livestock breeds, farm inputs and credit facilities.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Growth and development of gabiroba [*Campomanesia adamantium* (Cambess.) O. Berg] fruits

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The Brazilian Savanna is one of the largest and richest tropical savannas in the world, possessing substantial biodiversity. However, little information is available about fruit development in Brazilian Savanna bushy species. Thus, the objective of this study was to physically and physico-chemically characterize the growth and development of gabiroba fruits, *Campomanesia adamantium* (Cambess.) O. Berg. After harvest, the fruits were analysed for acidity, density, volume, longitudinal diameter, cross-sectional diameter, fresh mass, dry mass, soluble solids, respiratory rate, firmness and soluble solids/acidity ratio. The data were then submitted to descriptive analyses. The developmental period of the gabiroba fruit comprised nine weeks (63 days) from the time of fruit set. The mass-accumulation curve of the gabiroba fruit resembled a double-sigmoidal pattern. The respiration rate of the fruit was low, and the climacteric phase occurred between 21 and 28 days after fruit set. Based on the analyzed attributes, authors conclude that gabiroba fruit can be harvested beginning 35 days after fruit set and extending to 56 days after fruit set. The optimum time for consumption occurs 49 days after fruit set, when fruit size, mass, SS/TA ratio and soluble solids reach their peak values, and acidity and firmness are reduced.

Key words: Cerrado, harvest, growth curve, fruit trees.

INTRODUCTION

The Brazilian Savanna is the second-largest biome in South America and covers an area of 2,036,448 km^2, approximately 22% of the Brazilian territory. This region of Brazil is known as the richest savanna in the world, harboring 11,627 cataloged native plant species. This biome also possesses great social importance because many human communities depend on its natural resources (MMA, 2013). However, few studies have examined the development of native Brazilian Savanna species, especially the growth stages such as pre-maturation, maturation, ripening and senescence (Silva et al., 2009).

One such species is gabiroba [*Campomanesia adamantium* (Cambess.) O. Berg], which belongs to the family Myrtaceae and is one of 33 species in its genus (Sobral et al., 2013). Gabiroba develops a shrubby

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shape, with many yellow branches, and can reach a height of 2 m. Its leaves are opposite, simple, ovate and entire, with translucent pits and acute to obtuse bases. The fruits are globular and 2 to 2.5 cm in diameter (Porto and Gulias, 2010). Fruits are consumed in nature or processed as juicy and ice cream (Baviati et al., 2004) and are appreciated by population considering the high Vitamin C content (Oliveira et al., 2011) and the medicine proprieties (Fernandes et al., 2014). Studies at refrigerate conditions (Campos et al., 2012) and modified atmosphere (Scalon et al., 2012) shown important to preserve the postharvest quality under 15 days of storage.

The demand for tropical fruits is increasing in both domestic and export markets due to increased awareness of the nutritional and therapeutic value of these fruits and of their diverse aromas and flavors. Brazil has a large variety of native and exotic fruit species with agro-industrial potential, which may provide future sources of income for local populations (Alves et al., 2008).

Plant growth and development depend on genetic and environmental factors, such as light, temperature, moisture and soil type. These factors promote a series of physiological and biochemical changes that ultimately determine the physico-chemical composition of the fruit (Guedes et al., 2008). Although most of these changes are well known, some still remain to be clarified (Bron, 2006). According to Bron and Jacomino (2006), understanding the regulation of the ripening process makes it possible to manipulate fruit development to obtain higher quality fruits, reduce postharvest losses and increase consumer acceptance.

Thus, the objective of this study was to characterize the growth and development of gabiroba fruits through morphological and physico-chemical analyses.

MATERIALS AND METHODS

Study area

The experiment was conducted on the Rio Doce Coqueiros farm in the town of Rio Verde, Goiás, Brazil. The farm is located at latitude 17° 56' 46'' S and longitude 51° 11' 50'' W, at an elevation of 694 m. The experiment took place from October to December 2012. The climate at study region is second to the Köppen climate classification, the type Aw (rain tropical savanna), characterized by a dry period during the winter and a rainy period during the summer, the average rainfall ranges between 1200 and 1800 mm. The temperature during experiment period change from 23.9 to 26.0°C, as the thermal amplitude was less than 4°C. The experiment took place from October to December 2012, during the rainy period with 127.9 µmol m^{-2} s^{-1} photosynthetically active radiation.

Morphophysiological evaluation

In October 2012, *C. adamantium* plants were selected, and its fruits were collected weekly from fruit set until full maturity, comprises nine weeks. Fruit set has been defined by petal fall and rapidly growing condition of the young fruit following ovary fertilization. The botanical material was herborized and deposited under 206 number in the Rio Verde Herbarium (IFRV).

Upon harvest, the fruits were immediately placed in plastic bags and then in styrofoam boxes containing ice packs and transported to the Plant Ecophysiology and Productivity Laboratory at the Federal Institute of Goiás - Rio Verde Campus. In the laboratory, the fruits were selected for the absence of mechanical damage and pest or pathogen attack.

Selected gabiroba fruits were evaluated for their respiratory rate, morphological characteristics (longitudinal diameter, cross-sectional diameter, density, fresh mass and dry mass) and physico-chemical characteristics (fruit firmness, soluble solids and acidity), as detailed as follow.

Respiratory rate

Ten fruits were placed into 250 mL glass flasks for a total of five replicates. To allow the temperature to stabilize, the flasks were kept at 25°C in an air-conditioned room for two hours. The fresh mass of the fruits was measured prior to measuring the respiratory rate.

To measure the respiratory rate, each flask was coupled to an open flow system using an infrared gas analyzer (IRGA, Qubit Systems Inc., Kingston, Ontario, Canada) under an air flow of 400 mL min^{-1}. The instrument was calibrated with a reference air concentration of 395 ppm (Naressi Neto, 2013). After measurement, the delta CO_2 value between the reference air and the analyzed air was used to calculate the respiratory rate of the fruits (Vines et al., 1965).

$$RCO_2 = \frac{(\Delta CO_2 x Flow(mL.min^{-1})\ x\ CF)}{(1{,}000{,}000\ x FW(Kg))}$$

Where: RCO_2 = respiratory rate, expressed in mg CO_2 kg^{-1} h^{-1}, ΔCO_2 = reference air – analyzed air,
Flow = air flow through the measurement chamber during analysis, CF = correction factor from mL CO_2 to mg CO_2* and FW = fresh mass of the fruits (kg) on the day of analysis.

$$* CF = \frac{grams\ of CO2}{22.415\ x\ \frac{(T+C)}{T}}$$

Where: T = temperature in Kelvin (273K), C = temperature in degrees Celsius (ºC) and 22.415 = gas constant.

Growth and physico-chemical characterization

The fruit growth was evaluated and the physico-chemical characteristics were analyzed after measuring the respiratory rate. The longitudinal diameter, cross-sectional diameter and density were measured for ten fruits in each replicate. The fruits were then divided into two lots: Lot 1 was used to assess the maturity attributes of soluble solids (SS), titratable acidity (TA) and fruit firmness, while Lot 2 was used to measure the fresh mass (g) and dry mass (g) of the fruits, as described as follow.

Diameter

The longitudinal and cross-sectional diameters were measured using a caliper with a precision of 2 mm (Worker brand). The

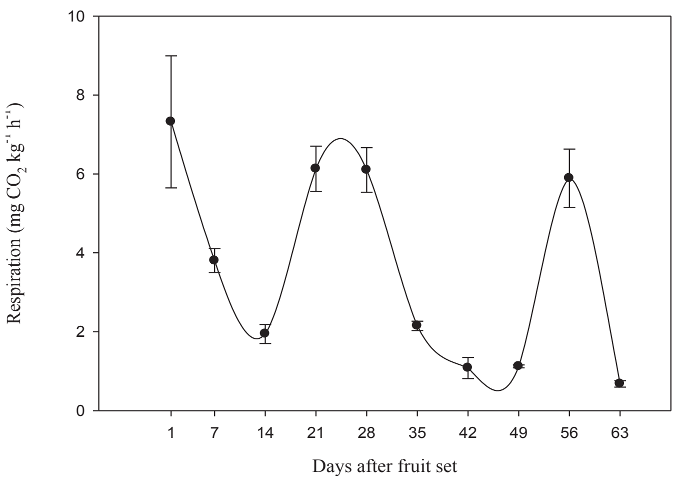

Figure 1. Respiration rate (mg CO_2 kg^{-1} h^{-1}) of gabiroba fruits from fruit set to full maturity.

longitudinal diameter corresponded to the portion of the fruit between the insertion of the pedicel and the insertion of the calyx. The cross-sectional diameter corresponded to the median portion of the fruit.

Density

The fruit density (g cm^{-3}) was obtained by first determining the volume of each fruit based on the displacement of a water column in a graduated cylinder. This value was used in the following equation: $D = \frac{mass}{volume}$

Soluble solids (SS)

The soluble solids (SS) content was determined from juice samples extracted from five fruits per replicate. Two drops of juice were placed on the prism of a manual refractometer (N-1E, Atago, Nagoya, Tokyo, Japan), and a refractive index reading, expressed in °Brix, was taken.

Titratable acidity (TA)

The fruit acidity was determined by neutralization titrations with NaOH (0.1 N) until the pH reached 8.2 (Instituto Adolfo Lutz, 1985). A 1 g sample from each of five fruits per replicate was extracted and macerated using a mortar and pestle. The sample was then transferred to an Erlenmeyer flask containing 50 mL deionized water and three drops of phenolphthalein before proceeding with NaOH titration.

The acidity was then calculated according to the following formula:

$$\frac{V * f * 100}{P * c} = acidity\ in\ molar\ solution, \%\ v/m$$

Where: V = volume in mL of NaOH solution (0.1 N) used for the titration, f = NaOH solution factor (0.1 N), P = mass in grams of the sample used in the titration, and c = correction value (here, 10 because the titration was performed with 0.1 N NaOH).

Fruit firmness

Changes in the fruit firmness of whole fruit were obtained using a pedestal applanation instrument, in which the fruit was placed on a vertical support and a glass bowl was set on the fruit. The firmness was measured as the ratio of the weight of the bowl to the deformed area (Calbo and Nery, 1995).

$$A = 0.784 * d1 * d2$$

$$N = \frac{P}{A} * 9.8$$

Where: N = firmness (N), P = applanation weight and A = area in cm^2. To convert the firmness (kgf) to (N), the formula is multiplied by 9.8.

Fresh mass

The fruits were weighed individually on a semi-analytical balance (AW220, Shimadzu model). The fresh mass was expressed in grams.

Dry mass

After measuring the fresh mass, the fruits were placed in a convection oven (Marconi brand, model MA035/5/10P) at 65°C for 72 h, until they reached a constant mass. The dry mass was expressed in grams.

Statistical design

The experiment utilized a randomized-block design. For the respiratory rate, longitudinal and cross-sectional diameters and density, five replicates of ten fruits each were used. For the soluble solids, fruit firmness, titratable acidity and fresh and dry mass, five replicates of five fruits per replicate were used. The data were analyzed using descriptive statistics, including the mean and standard error of the mean.

RESULTS

The developmental period of the gabiroba fruit comprised nine weeks, or 63 days after fruit set (DAF). The highest respiratory rates were observed at the first evaluation point, with a mean value of 7.32 mg CO_2 kg^{-1} h^{-1}. Over the course of fruit development, two respiratory peaks were observed: one between 21 and 28 DAF and another at 56 DAF (Figure 1).

The longitudinal and cross-sectional diameters of the gabiroba fruits showed similar behavior over the course of fruit development. During the first 14 days, the diameter increased from 4 to 6 mm. The diameter then increased rapidly, especially between 15 and 21 DAF. The maximum value of 14 mm was reached at 49 DAF. After that point, the fruit diameters decreased (Figure 2).

The fresh mass ranged from 0.77 to 9 g, increasing rapidly between 14 and 21 DAF. Between 22 and 28 DAF, the fresh mass increased slightly. After that point,

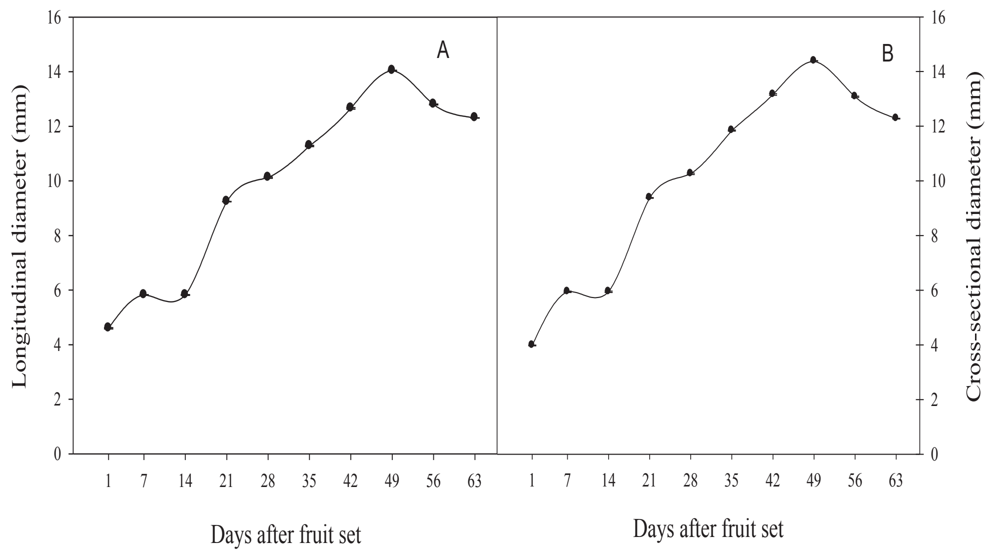

Figure 2. Longitudinal (A) and cross-sectional diameter (B) of gabiroba fruits from fruit set to full maturity.

the fresh mass increased markedly, reaching its peak at 49 DAF and decreasing thereafter (Figure 3A). The dry mass ranged from 0.17 to 1.81 g. This variable showed behavior similar to that of fresh mass, except that the peak value was reached between 49 and 56 DAF (Figure 3B).

The developmental curves of the longitudinal and cross-sectional diameters and the fresh and dry mass resembled double sigmoidal growth curves, characterized by three stages of development: an initial phase of rapid growth followed by a phase of slow growth and a final period of marked development.

At the first three evaluation points, which encompassed the first 14 days of fruit development, the fruit density was 0.80, 0.88 and 1.02 g cm^{-3}. At 21, 28 and 35 DAF these values decreased to 0.75, 0.83 and 0.90 g cm^{-3}. Subsequently, the density again increased to 1.0, 1.02, 1.02 and 1.11 g cm^{-3} at 42, 49, 56 and 63 DAF, respectively (Figure 3C).

The fruit firmness oscillated during the first 28 DAF.
The greatest firmness was observed at 14 DAF (28.84 N); subsequently, the firmness decreased at 21 DAF (20.56 N), then increased (27.96 N) and finally decreased abruptly after 28 DAF (Figure 4A). The acidity values decreased until 56 DAF (from 10.3 to 2.4%). After that point, the acidity increased slightly (to 4%) (Figure 4B). The soluble solids increased progressively over time. The initial value was 1.9 °Brix, and the final value was18.9 °Brix (Figure 4C).

The SS/TA ratio increased with fruit development. This ratio showed little variation until 35 DAF. Subsequently, the SS/TA ratio increased significantly, reaching a maximum value of 6.58 at 56 DAF and then declining (Figure 4D).

DISCUSSION

The period of fruit development can vary among species, varieties and cultivars. For *C. adamantium*, the focus of this work, and *C. pubescens* (Silva, 2009), the developmental period is similar: approximately 60 days. In *C. lineatifolia*, the developmental period is longer than 100 days (Balaguera-Lopez et al., 2012). During fruit growth and development, numerous metabolic changes occur, including changes in respiratory activity.

The high respiratory rates observed at the beginning of fruit development mainly reflect cell division during the active growth of the fruit (Gillaspy et al., 1993). The first respiratory peak of the gabiroba fruits most likely corresponds to the climacteric phase, which is marked by a sudden increase in autocatalytic ethylene production and respiration (Chitarra and Chitarra, 2005). This event triggers numerous changes during the development of gabiroba fruits, including seed formation, changes in density, decreasing acidity and firmness and increasing soluble solids and SS/TA ratio. The second respiratory peak at 56 days after fruit set most likely corresponds to the beginning of senescence, which leads to declines in fruit size, soluble solids and weight and an increase in acidity. An increased respiratory rate at the end of the developmental period has also been observed in jabuticaba fruit (Corrêia et al., 2007).

All of these changes during the growth and development of gabiroba fruits directly influence mass accumulation and consequently growth, as reflected by the changing longitudinal and cross-sectional diameters of the fruits. The fruits of most species exhibit characteristic growth curves, such as simple-sigmoidal or double-sigmoidal curves (Srivastava, 2002). According to

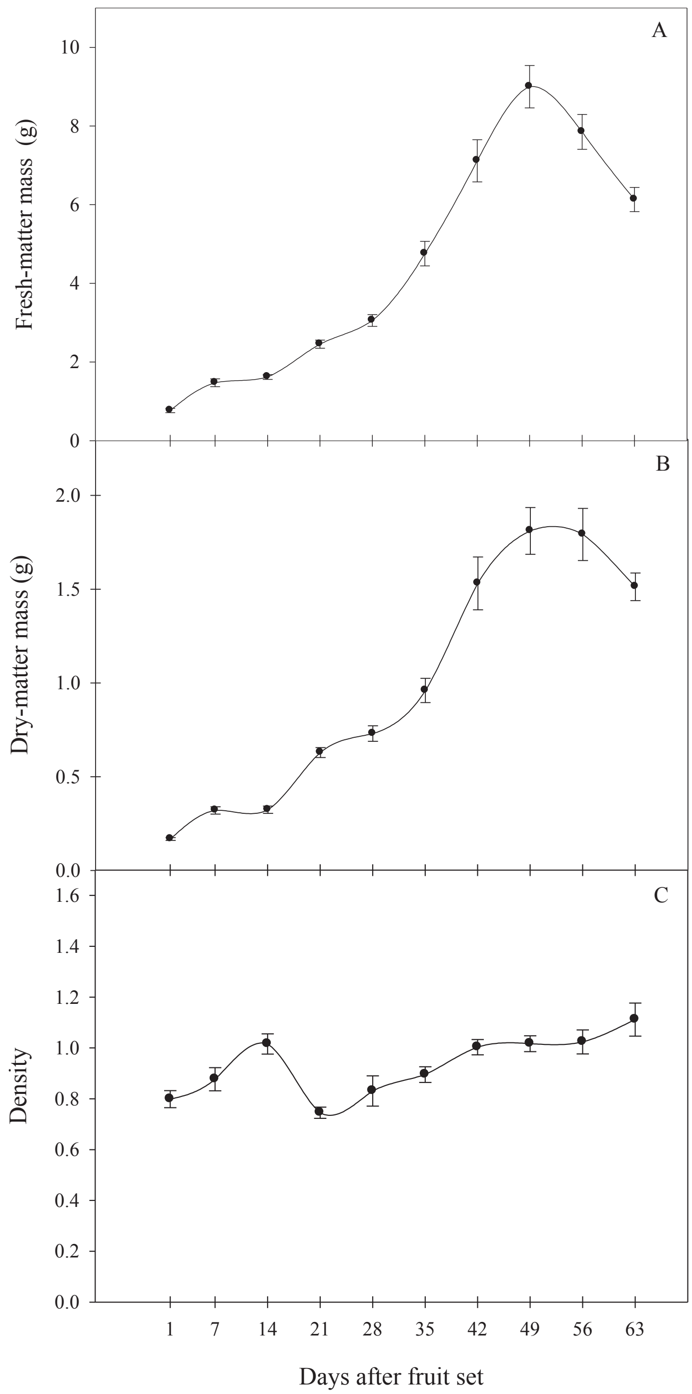

Figure 3. Fresh mass (A), dry mass (B) and density (C) of gabiroba fruits from fruit set to full maturity.

our results, the growth curve of gabiroba fruits fits a standard double-sigmoidal pattern. Double-sigmoidal curves have been observed in peach (Silva et al., 2013), guava (Serrano et al., 2008) and plum (Famiani et al., 2012) fruits, while simple-sigmoidal curves are characteristic of apple (Santos et al., 2011) and passion fruit (Alves et al., 2012).

The simple-sigmoidal curve is similar to a parabola and is divided into two phases: 1) slow growth and 2) rapid growth. The double-sigmoidal curve has three phases: 1) rapid growth due to cell division; 2) physiological and anatomical changes in the fruit, such as a decrease in the growth rate of the pulp, hardening of the pit and the final formation of the seeds, and 3) an accelerated increase in

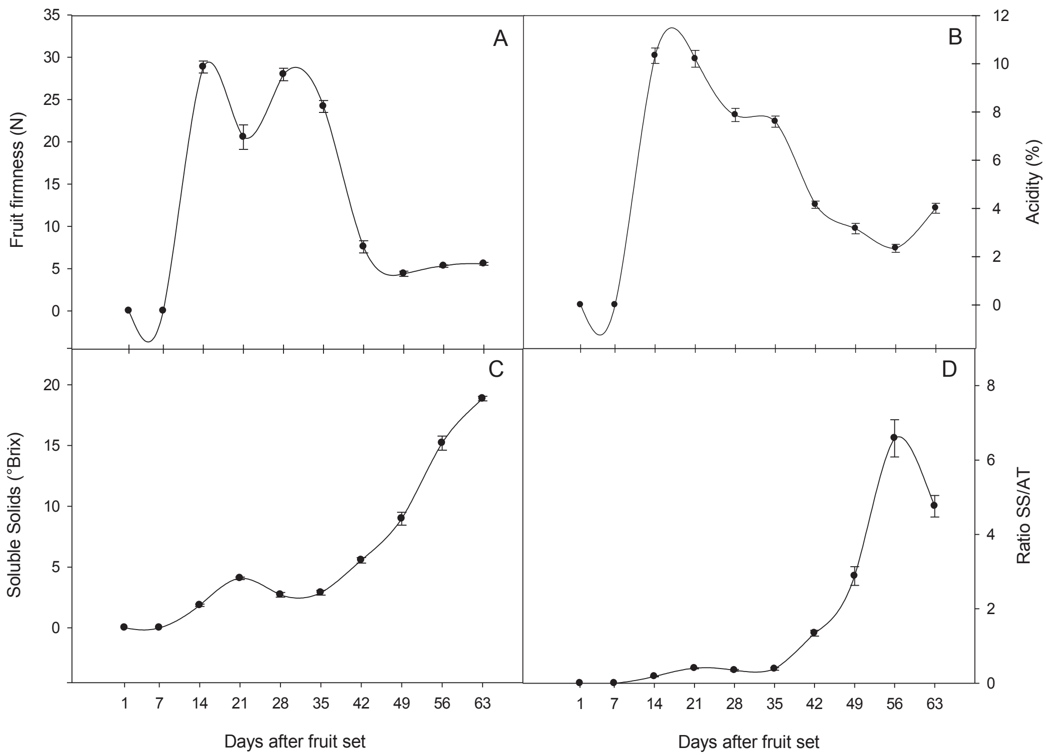

Figure 4. Fruit firmness (A), acidity (B), soluble solids (C) and soluble solids/acidity ratio (D) of gabiroba fruits.

cellular volumes and intercellular spaces, ultimately culminating in the ripening of the fruit (Barbosa et al., 1990; Chitarra and Chitarra, 2005).

The gabiroba fruits showed an initial phase of rapid growth, which was followed by a phase of slow growth and then a final period of rapid growth, as indicated by the accumulation of fresh and dry mass. The double-sigmoidal growth curve observed in the present study is similar to that observed during the flowering and ripening of *C. xanthocarpa* (Danner et al., 2010).

These events corroborate the observed results for *C. adamantium*. During Phase I, the increases in length, diameter, fresh and dry mass and density demonstrate rapid growth. The fruits exhibit stable firmness values, a maximum acidity level and low soluble solids and SS/TA ratio. At the end of this phase, between 14 and 21 DAF, the density and firmness values decrease.

This decrease in density can be explained by cellular elongation and increased intercellular spaces, which together increase the volume of the pulp. Firmness, as determined by the applanation method, reflects cell turgidity with respect to cell volume. As the cells become less turgid, the tissue sensitivity increases, resulting in a larger area of applanation and thus lower firmness values.

At the beginning of Phase II, the fruit mass increases slowly, and the fruits continue to exhibit high firmness and low SS and SS/TA. At the same time, acidity begins to decline. The fruit density increases during Phase II between 21 and 28 DAF, when the seeds undergo maturation (that is, hardening).

Longitudinal, cross-sectional diameters, and fresh and dry mass occur when Phase II transitions into Phase III, marked increases in mass after 28 DAF. In addition, significant declines in firmness and acidity increase in SS and SS/TA take place. These changes are related to climacteric respiration and thus to the fruit-ripening process. The observed changes in acidity are consistent with those found for uvilla/parilla (Arena and Coronel, 2011), pomegranate (Fawole and Opara, 2013a; Fawole and Opara, 2013b) and strawberry (Ornelas-Paz et al., 2013).

The decreases in fresh and dry mass and fruit size after 49 DAF and in SS/TA after 56 DAF are also related to source-sink relationships. As they spend more time attached to the mother plant, the fruits may begin to translocate their photoassimilated materials back into the plant, thus acting as source organs. Thus, the distribution of assimilated materials into the plant results from metabolic changes in the source-sink relationship.

Source organs produce photoassimilated materials that can be used as energy for respiratory processes or stored in reserve organs, known as sink organs. The main organs that export photoassimilated materials (sources) are adult leaves, while the main importers (sinks) are young leaves, roots, meristems and fruits (Duarte and Peil, 2010). However, if the fruit remains on the plant after its development and ripening are complete, it will most likely begin to act as a source organ, allocating its store of photoassimilated materials into new sink organs. The gabiroba fruit studied here show evidence of this process.

Understanding fruit growth curves is critical to preserving fruit quality after harvest. Density, fruit firmness, soluble solids content, titratable acidity and the SS/TA ratio are excellent indicators of fruit quality and are widely used in the harvesting and post-harvest treatment of horticultural produce.

No previous study in the literature has reported the optimum harvesting time for *C. adamantium*. Based on our data, the fruits of this species can be harvested beginning at 35 DAF, with their average density in 0.90 g cm^{-3}. For guava (*Psidium guajava*), the density at the time of harvest is 0.98 g cm^{-3} (Gouveia et al., 2003).

A decrease in acidity is commonly associated with an increase in soluble solids. The relationship between the sugar content and acidity of a fruit is expressed by the SS/TA ratio, which is used as a reference point for the flavor of many fruits. This variable further reinforces the ideal period for harvesting gabiroba fruit. The low SS/TA ratios during the first 35 DAF indicate that the fruit is not yet suitable for consumption due to its high acidity. Only after these values increase does the fruit become suitable for consumption; a higher SS/TA ratio contributes to the pleasant flavor of the fruit and indicates fruit ripening.

Considering all the measured attributes, we conclude that gabiroba fruits can be harvested upon reaching the following criteria: a longitudinal diameter of 11 to 14 mm, a cross-sectional diameter of 12 to 14 mm, a density of 0.90 to 1.02 g cm^{-3}, a fresh mass of 4.76 to 9.0 g or a dry mass of 0.96 to 1.81 g. Furthermore, the fruit should exhibit a firmness value of 7.58 to 4.40 N, an acidity level of 4.2 to 2.4%, a soluble solids content of 5.6 to 15.2 °Brix and an SS/TA ratio of 1.34 to 6.58.

Based on these criteria, the ideal harvesting period begins at 35 DAF and extends to 56 DAF. At 49 DAF, gabiroba fruit reaches its optimum quality for consumption, with the highest values of size, mass, SS/TA ratio and soluble solids and markedly reduced values of acidity and firmness.

Conclusions

The fruiting period of gabiroba, *C. adamantium* (Cambess.) O. Berg, comprises 63 days, begins in October and ends in December under the environmental conditions of the Rio Verde town, Goiás. The accumulation curves for the fruit fresh mass, dry mass, longitudinal and cross-sectional diameter resemble double-sigmoidal growth curves. Gabiroba fruits can be harvested between 35 and 56 days after fruit set.

Conflict of Interest

The authors have not declared any conflict of interest.

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Broadcast fertilizer rate impacts common bean grain yield in a no-tillage system

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Increasing fertilizer rates at sowing can provide significant increases in grain yield in vegetable crops. However, very high rates can impair root development because of increased soil salinization in the rows mainly because of KCl fertilizer. Broadcast fertilization without incorporation into soil may be a viable strategy to avoid this salinization. Therefore, we conducted a study to determine the effect of different fertilizer rates applied to the soil surface without incorporation on common bean grain yield and yield components in a no-tillage system. An irrigated field experiment with a randomized block experimental design with four replications was conducted in Brazil during the 2012 and 2013 growing seasons. The treatments consisted of four fertilizer rates of an N-P-K blend that were 0, 50, 100 (15 kg ha^{-1} of N, 90 kg ha^{-1} of P_2O_5 and 45 kg ha^{-1} of K_2O) and 150% of the recommended fertilizer rate for in-furrow applications. Increasing broadcast fertilizer application provided a significant increase in common bean grain yield. The application of 300 kg ha^{-1} of fertilizer (100% of the recommended fertilizer rate) on the soil surface without incorporation provided a similar result as the application of the same amount in the seed row. The results document that broadcast application of a fertilizer blend on the soil surface without incorporation is a viable management tool to increase common bean grain yield in no-tillage systems in soil with high fertility, which is based mainly on its content of organic matter, phosphorus, and base saturation.

Key words: *Phaseolus vulgaris* L., surface fertilization, Cerrado, no-tillage system.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important crop in many countries as a major source of protein in human diets. In 2012, 23 million Mg of dry common bean grains were produced worldwide, with the principal producers being Myanmar (3.9 Mg), India (3.7 Mg), Brazil (2.8 Mg), China (1.6 Mg), the USA (1.4 Mg) and Mexico (1.1 Mg) (FAOSTAT, 2013). However, despite its importance, farmers use low level of technology for common bean crops such as low fertilization rates, low quality of seeds, lack of improvement management

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Abbreviations: **NTS,** no-tillage system; **SOM,** soil organic matter; **NPP,** number of pods per plant; **NGP,** number of grains per pod; **M100G,** 100-grain weight; **GY,** grain yield.

practices, resulting in a global average grain yield of only 804 kg ha^{-1} (FAOSTAT, 2013). More specifically in Brazil, this crop represented 6.08% of the entire grain production area and 1.84% of the total grain production in the 2012/2013 growing season. Common bean is the 3rd largest agricultural crop in Brazil and occupies an area of 3.16 million ha, behind only soybean (*Glycine max*) and corn (*Zea mays*) (CONAB, 2013). The average Brazilian yield of common bean was 910 kg ha^{-1}, but there are farmers attaining grain yields greater than 3500 kg ha^{-1} (Nascente et al., 2012a; CONAB, 2013). To achieve a higher grain yield, crop fertilization management must be improved because a properly balanced supply of nutrients and the use of fertilizers can achieve significant increases in grain yield (Miranda et al., 2000; Andrade et al., 2004; Arf et al., 2011; Nascente et al., 2012a).

Foundation fertilizer can be applied during the sowing operation or broadcast independently of the sowing operation (Guareschi et al., 2008). The former operation consists of applying the fertilizer and seeds at the same time in the seed row. This is the most widespread application used by farmers in Brazil since the invention of the seeder (Malavolta, 1981). The latter operation consists of distributing the entire or partial amount of fertilizer prior to or soon after sowing. Broadcasting the fertilizer independently allows the seeding process to occur more efficiently and does not require refreshing the supply of the seeder with fertilizer (Bergamin et al., 2008; Castoldi et al., 2012), which is more representative in the seeder than the quantity of seeds.

Distributing the fertilizer in the sowing operation could lead to delays because it requires increased time and the number of individuals supplying the seeders, which influences the operational capacity. Using broadcast fertilization without incorporation allows a greater seeder operational efficiency because the seeder can operate longer without having to stop for additional fertilizer. Therefore, it will help reduce operational and total costs and also enable an increase in net revenue and economic sustainability compared to the traditional cultivation system (Matos et al., 2006). The common bean in Brazil is a crop where only 0.92% of farmers produce 51.2% of total grain production (Silva and Wander, 2013). Therefore, the efficiency and flexibility of sowing is essential. In this case, broadcast fertilizer application can help to improve the possibility of success of common bean cultivation.

Additionally, distributing the fertilizer by the broadcast method decreases the risk of damaging the germination and establishment of plants. When applying greater amounts of fertilizer in the seed row, the root system of the plants can be damaged by soil salinization. In the case of potassium (K), for example, when the source is the salt potassium chloride (KCl) and the rates are greater than 50 kg ha^{-1} of K_2O, application at sowing causes damage to the seed and radicle of the soybean plant (Bergamin et al., 2008). For the common bean, Kluthcouski and Stone (2003) also indicate that greater amounts of fertilizer closer to the seeds results in chlorosis on the primary leaves. These authors also note that mineral fertilizers primarily produce saline and osmotic effects, and these characteristics can affect the germination and development of plant seedlings and roots. In this sense, the application of mineral fertilizer broadcast without incorporation into the soil can be a viable and safe alternative for providing an adequate supply of nutrients to plants.

In common bean, studies of the application of nitrogen (N), phosphorus (P) and potassium (K) are usually performed in isolation (Arf et al., 2004, 2011; Barbosa Filho et al., 2005; Meira et al., 2005; Crusciol et al., 2007; Pelá et al., 2009; Valderrama et al., 2009; Zucarelli et al., 2011), and there are only few studies that review the mixed application of these nutrients using formulated fertilizers and even fewer studies of broadcast fertilization with plowing (Rodrigues et al., 2002; Kikuti et al., 2005; Viana et al., 2011). It is noteworthy that the application of fertilizer broadcast without incorporation has already been adopted by many farmers in Brazil, but few studies have reported its effectiveness in relation to other forms of fertilizer distribution (Guareschi et al., 2008; Castoldi et al., 2012). The objective of this study was to determine the effect of fertilizer rate under with broadcast application on the soil surface without incorporation on the yield of components and grain for the common bean.

MATERIALS AND METHODS

The experiments were conducted at Fazenda Capivara, Embrapa Rice and Beans, located in Santo Antônio de Goiás, GO, at 16°28'00" S and 49°17'00" W and 823 m above sea level. The climate is tropical savanna and considered Aw according to the Köppen classification. There are two well-defined, normally dry seasons from May to September (autumn/winter) and two rainy seasons from October to April (spring/summer). The average annual rainfall is between 1500-1700 mm, and the average annual temperature is 22.7°C, ranging annually from 14.2 to 34.8°C (Silva et al., 2010).

The soil was classified as a clay loam (kaolinitic and thermic Typic Haplorthox) acidic soil (Embrapa, 2006). Before initiation of treatment application in May 2012 and May 2013, chemical analyses were performed on soil samples taken from 0 to 20 cm depth to characterize the experimental area (Table 1). The chemical analyses were performed according to the methodology proposed by Claessen (1997). The soil pH was determined in a 0.01 mol L^{-1} $CaCl_2$ suspension (1:2.5 soil/solution), and the exchangeable Ca, Mg, and Al were extracted with neutral 1 mol L^{-1} KCl in a 1:10 soil/solution ratio and determined by titration with a 0.025 mol L^{-1} NaOH solution. Phosphorus and exchangeable K were extracted with a Mehlich 1 extracting solution (0.05 M HCl in 0.0125 M H_2SO_4).

The extracts were calorimetrically analyzed for P, and flame photometry was used to analyze K. The base saturation values were calculated using the results of the exchangeable bases and total acidity at pH 7.0 (H + Al). Micronutrients were determined in the Mehlich 1 extract by atomic absorption, and the organic matter was determined by the method of Walkley and Black (1934).

The experimental area has been cultivated in a crop-livestock no-tillage system (NTS) for seven consecutive years, which consists of following the crop rotation program with soybean (summer),

Table 1. Chemical characteristics of soil in the area prior to fertilizer application for two growing seasons.

Growing season 2012						
Ca	**Mg**	**Al**	**H+Al**	**K**	**CEC**	**pH ($CaCl_2$)**
	----------cmol$_c$ dm^{-3}----------					
3.4	1.5	0.0	3.1	0.16	8.16	5.6
BS	SOM[1]	P	Zn	Cu	Fe	Mn
%	g dm^{-3}		----------mg dm^{-3}----------			
62.01	27.0	7.7	3.8	2.3	27.1	34.3

Growing season 2013						
Ca	**Mg**	**Al**	**H+Al**	**K**	**CEC**	**pH ($CaCl_2$)**
	----------cmol$_c$ dm^{-3}----------					
3.2	1.4	0.0	3.7	0.38	8.68	4.9
BS	SOM[1]	P	Zn	Cu	Fe	Mn
%	g dm^{-3}		----------mg dm^{-3}----------			
57.37	35.7	15.8	5.4	2.0	32.0	26.0

[1]SOM – soil organic matter.

followed by rice (summer) and the common bean (winter), followed by corn and palisadegrass (*Urochloa brizantha*) [syn. *Brachiaria brizantha* (Hochst. Ex A. Rich) Stapf] (summer), followed by two years of grazing pasture.

The experimental design was a randomized block with four replications in both years (2012 and 2013). Treatments consisted of four fertilization rates: 0, 50, 100 and 150% of the recommended sowing fertilization rate for the crop (Sousa and Lobato, 2004). Thus, a fertilizer with the formula 5-30-15 (N-P_2O_5-K_2O) was broadcast on the soil surface at 0, 150, 300 and 450 kg ha^{-1} three days after sowing. The application of the fertilizer was performed manually over the entire surface of the plots without incorporation in the corresponding treatments. Additionally, the application of 300 kg ha^{-1} of 5-30-15 formulated (N-P_2O_5-K_2O in the sowing furrow at the same time of crop sowing was used as a control treatment with four replications in both years.

The sowing of the common bean cultivar 'Perola' was mechanically conducted on May 21st, 2012 and June 07th, 2013 in 0.50-m row spacing with a population of 240,000 seed ha^{-1}. Each plot consisted of 10 five-meter-long rows, with a useful area of eight central rows that disregarded 0.5 m from the ends of each row.

Seedling emergence occurred at nine and eight days after sowing in 2012 and 2013, respectively. In the V_4 vegetative stage of the common bean (four trifoliate leaves) (Vieira et al., 2006), a topdressing fertilization of 60 kg N ha^{-1} as urea was done for all plots. Other cultural practices were performed according to the recommendations of the crops to keep the area free of weeds, disease and insects (Vieira et al., 2006).

Supplemental water was applied to bean plots with a sprinkler irrigation system (linear move) was used, and three crop coefficients (K_c) were divided into four periods between emergence and harvest to manage the application of water. In the vegetative stage, a K_c value of 0.4 was used. For the reproductive phase, two values of K_c were used: 0.7 to 1.0 in the initial phase and the reverse of these values in the final phase of maturation, that is, 1.0 to 0.7. Therefore, the control of irrigation respective of the depth of root system exploitation at 0.2 m was initiated with the available water capacity (AWC) at its maximum and successively subtracting the value of crop evapotranspiration until the total water reached the minimum limit of 40% of AWC (Doorenbos and Pruitt, 1976).

Plots were harvested on August 22nd, 2012 and September 9th, 2013 by hand in the useful area (eight central rows that disregarded 0.5 m from the ends of each row) and was followed by mechanized stationary thresher. The harvested common bean grains were weighed and the yield expressed as 130 g kg^{-1}. In addition, plant population was measured counting the number of plants in 1 m² in the usable area and the following yield components were assessed: the number of pods per plant, number of grains per pod (evaluated in 10 plants per plot that were chosen at random), and weight of 100 grains (calculated from eight random samples per plot). Data were subjected to an analysis of variance (proc. GLM), and the means were compared by Tukey's test at $p \leq 0.05$. If the quantitative data (fertilizer rates) were significant, then the regression analysis was conducted (proc REG). Dunnett's test was performed at a significance of $p \leq 0.05$ to compare the fertilizer in the sowing row with each fertilizer rate applied. These analyses were done with SAS (SAS, 1999).

RESULTS AND DISCUSSION

The application of increasing doses of fertilizer broadcast on the soil surface without incorporation produced significant effects for all yield components and the grain yield of the common bean (Table 2), and the data fit a quadratic regression for the number of pods per plant (Figure 1) and grains per pod (Figure 2). These results corroborate the results presented by Fageria et al. (2004), Zucareli et al. (2006), Pelá et al. (2009) and Zucareli et al. (2011), who reported significant increases in yield components with increasing rates of fertilizer application. The only yield component that was not affected by the rate of fertilizer application was the 100-grain weight, and this variable is characteristic of cultivars with little variation as a result of environmental conditions (Pelá et al., 2009). Moreover, Valderrama et al. (2009) and Melém Júnior et al. (2011) also found no significant difference on the 100-grain weight with increasing rates of fertilizer application.

With respect to grain yield, a positive increase in yield

Table 2. Plant population (PP), Number of pods per plant (NPP), number of grains per pod (NGP) 100-grain weight (M100G), and grain yield (GY) of the common bean (*Phaseolus vulgaris*) as a function of the fertilizer rates broadcast on the soil surface without incorporation and differences of each fertilizer rate in relation to the control treatment (300 kg ha^{-1} of fertilizer applied at sowing furrow). Santo Antonio de Goias, GO, Brazil. Growing seasons 2012 and 2013.

Treatments	PP	NPP	NGP	M100G	GY
Fertilizer rate (kg ha^{-1})	**nº m²**	**nº**	**nº**	**(grams)**	**kg ha^{-1}**
0	20*	13.9	4.3	26.68*	3377
150	21*	15.3*[2]	4.7	27.39	3742
300	21*	15.8*	5.0*	26.83*	4024*
450	21*	16.0*	5.1*	26.50*	4167*
Control[3]	22*	15.9	5.1	26.59	4053
Growing season					
2012	21 a	13.6 b[1]	4.6 a	26.27 a	3584 b
2013	23 a	17.0 a	4.9 a	27.40 a	4070 a
Factors			ANOVA – Probability of F test		
Fertilizer rate	0.7984	<0.001	0.0459	0.8005	0.0089
Year	0.1147	<0.001	0.2501	0.0961	0.0229
Fertilizer rate x year	0.5149	0.4747	0.6555	0.5080	0.8048
Coefficient of variation (%)	7.2	8.4	14.9	6.8	16.1

[1]Means followed by the same letter in a column do not differ by Tukey's test for p≤0.05. [2]Means followed by * do not differ by the control treatment according to the Dunnett test at p≤0.05. [3](300 kg ha^{-1} in the sowing furrow).

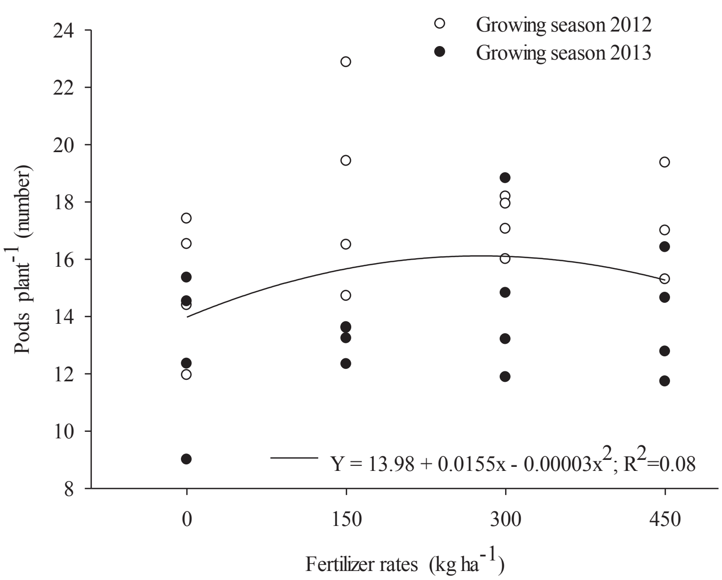

Figure 1. Number of pods per plant in the common bean crop as a function of the fertilizer rates broadcast on the soil surface without incorporation. Santo Antonio de Goias, GO, Brazil, growing seasons 2012 and 2013.

was found with increasing fertilizer rates, and the data fit a quadratic function (Figure 3). The grain yield of the common bean is a function of its yield components, such as the number of pods per plant, number of grains per pod and 100-grain weight (Araújo et al., 1996). The grain yield can be explained by the results obtained for the number of pods per plant and number of grains per pod because the other factors were fixed or unchanged.

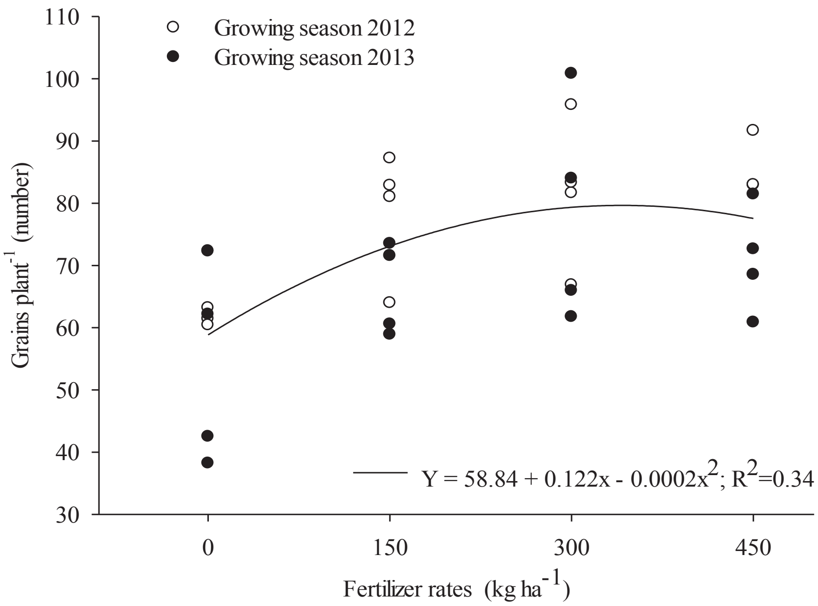

Figure 2. Number of grains per plant in the common bean crop as a function of the fertilizer rates broadcast on the soil surface without incorporation. Santo Antonio de Goias, GO, Brazil, growing seasons 2012 and 2013.

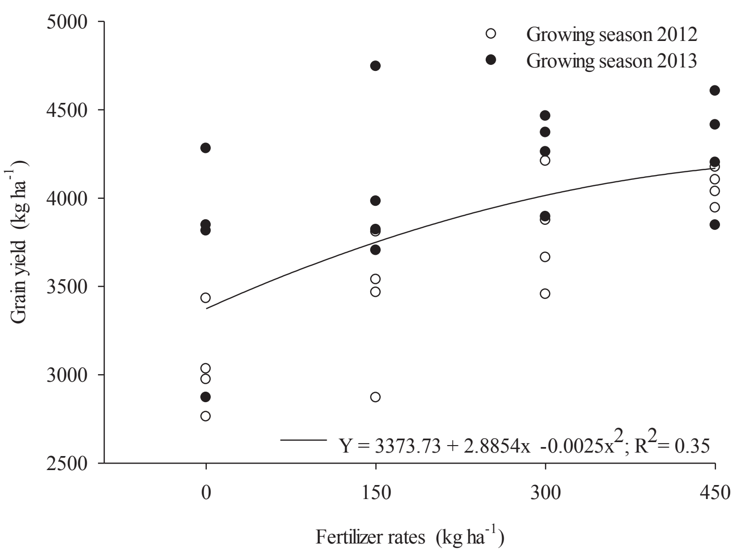

Figure 3. Grain yield in the common bean crop as a function of the fertilizer rates broadcast on the soil surface without incorporation. Santo Antonio de Goias, GO, Brazil, growing seasons 2012 and 2013.

Other authors also reported an increase in grain yield of the common bean because of the increased rate of fertilizer application (Pelá et al., 2009; Fageria et al., 2010; Zucareli et al., 2010, 2011; Melém Junior et al., 2011). It is noteworthy that these authors obtained their results by applying the fertilizer at sowing, and there are virtually no results (data) for the broadcast application of fertilizer to the soil surface without incorporation for the common bean.

Applying 300 kg ha^{-1} of fertilizer in the sowing furrow gave similar results to those for broadcasting 300 and 450 kg ha^{-1} of the fertilizer without incorporation (Table 2). In similar studies of soybean, Bergamin et al. (2008) and Gareschi et al. (2008) reported no differences between broadcast fertilizer application and application in seed row. Kurihara and Hernani (2013) also indicated that the application of fertilizer on the soil surface may favor the farmer because it produces increased operational income during the seeding process by eliminating the need to replenish with fertilizers, especially when using bagged products (60 kg bags), which require more spent not planting.

Kurihara and Hernani (2013) indicated that switching from banding adequate rate of fertilizer in the seed row to broadcast application with incorporation in the soil should provide medium to high fertility. These researchers reported significant reductions in grain yield for the soybean in soils with low fertility when the fertilizer was applied to the soil surface without plowing instead of in the sowing furrow, especially for P levels in the soil. In this sense, it appears that the experimental area in the present study had high fertility (Table 1), which is based mainly on its content of organic matter, phosphorus, and base saturation (Sousa and Lobato, 2004). Our results are appropriate for similar sites with medium or high fertility levels, but similar studies should be done on lower fertility sites before extrapolating our results to that type of site. There were higher values for the number of pods per plant^{-1} and grains per pod^{-1} in 2013 compared to 2012, which resulted in a higher grain yield (Table 2). These results may be a reflection of better soil fertility when the experiment was conducted in 2013 (Table 1) than in 2012.

A major concern when distributing fertilizer by the broadcast method has to do with concentrations of P, a nutrient that is immobile in soil and remains in the upper soil layers (0 to 0.05 m), which hinders its uptake by crops (Caires et al., 2003). However, the use of forage grasses that have root systems that explore a greater soil volume and can absorb nutrients at both the soil surface and at depths can provide greater nutrient cycling than may be possible by subsequent crops (Nascente et al., 2012b, 2013); thus, the use of forage grasses could be a viable alternative to minimize the problem of P concentrations at the soil surface. The area where the trials were performed was also used in the integrated crop livestock area; therefore, the rotation of crops and animal foraging could have provided a greater utilization of soil resources (Kluthcouski et al., 2003).

Another concern regarding the use of broadcast fertilization is in areas with inclines and where the indiscriminate removal of soil conservation practices, such as in terraced plots, could lead to significant nutrient losses (Bertol et al., 2007). Therefore, the adoption of soil conservation practices and the use of cover crops are important for minimizing potential losses from excessive rain and runoff (Bezerra and Cantalice, 2006).

Broadcast fertilization for common bean crops is a relatively new research area in Brazil and our results from two years can provide a viable alternative to farmers for improving their operating performance and increasing the grain yield of common bean. Special attention should be given when thinking in broadcast fertilization in very sloped ground. In this kind of soil it is necessary the use of appropriate soil conservation practices to prevent fertilizer loss, soil erosion, and potential environmental contamination. Besides, the use of broadcast fertilization is a more viable practice in soils with medium to high fertility (Kurihara and Hernani, 2013), and another advantage of using the distribution of fertilizer by broadcasting it on the soil surface without incorporation is the reduced of risk of damage that larger quantities of fertilizers, especially the K effects, can cause to the roots of crops (Kluthcouski and Stone, 2003).

Conclusion

The application of increasing fertilizer rates broadcast on the soil surface provides significant increases in the number of pods per plant and number of grains per pod and as a result the yield grain of the common bean. The application of 300 kg ha^{-1} of fertilizer on the soil surface without incorporation provided similar bean yield as the application of the same fertilizer in the sowing furrow (300 kg ha^{-1}). Our results indicate that broadcasting fertilizer on the soil surface without incorporation is a viable management technique that can increase yield of the common bean in no-tillage systems in soil with high fertility. Additional studies are required to determine efficacy of broadcast fertilization without incorporation on bean yield in soils with lower fertility.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Heat tolerance of Dwarf Cavendish banana (*Musa* AAA cv. Malindi) plants

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A study was undertaken to determine the thermo-tolerance of 4 month old dwarf Cavendish banana plants of cultivar "Malindi" planted using suckers. The electrolyte leakage procedure was used to generate sigmoidal curves from which the thermo-tolerance was determined at 50% electrolyte leakage. A completely randomized design (CRD) with three replicates was used. Leaflets (1.2 cm width × 5 cm length) from third fully opened leaves of plants fertilized by composted dairy cow manure and mineral fertilizers were exposed to heat regimes ranging from 30 to 80°C (at 5°C increments) for 30 min and 23°C (room temperature) as control. The results revealed that there was a positive strong correlation between heat regimes and electrolyte leakage percentage (r^2 = 0.73, p ≤ 0.01). Lethal temperature for Malindi banana plants was 58°C. The present investigation provided an accurate determination for heat tolerance of dwarf Cavendish banana cultivar "Malindi" and thus helps banana farmers to well manage their plants during such harsh weather conditions.

Key words: Dwarf Cavendish banana, electrolyte leakage, heat stress, thermo-tolerance.

INTRODUCTION

It is well known that heat stress is a major factor limiting the productivity and adaptation of many crops, especially when temperature extremes coincide with critical stages of plant development. Due to the global climatic changes, high temperatures become one of the main constraints threatening and retarding the growth and production of crops and thus agricultural sustainability. Recently, banana cultivation in the Sultanate of Oman is suffering from such stresses. According to McWilliams (1980), Ingram and Buchanan (1984) and Lester (1985) that tolerant of plants to heat stresses depends on the varieties, period of the thermal exposure and stage of growth of the exposed tissue (McWilliams, 1980; Ingram and Buchanan, 1984; Lester, 1985). One of the most important components of plant heat stress tolerance is its ability to adapt to high temperature under field conditions (Chen et al., 1982). According to Levitt (1988) direct heat injury results from short exposure to extreme temperature and causes changes in the native structure of the plasma membrane components (lipids and proteins). As a result, membrane losses its semi permeability and becomes leaky and more electrolytes could easily leak out. One of

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the effective procedures to measure cell membrane thermo-stability as well as indicator to direct heat-injury is electrolyte leakage (EL) technique (Sullivan and Ross, 1972; Furmanski and Buescher, 1979; Martineau et al., 1979; Ahrens and Ingram 1988; Ingram and Buchanan, 1981; Sullivan and Ross, 1972; Heckman et al. 2002; Shi et al., 2006). The validity of this method has been approved by stress physiologists and is relevant to the tolerance level in the field or in-situ on different crops including banana (Marcum, 1998). In this regard, Ingram and Ramcharan (1988) studied the effect of heat stress (temperature regimes 30 to 60°C for 30-300 min) on roots of banana and rooted cuttings of Dracaena marginata plants. They found that the critical temperature for each plant decreased exponentially as electrolyte leakage increased. Predicted critical exposure times at 48 and 52°C were >300 and 221 ± 51 min, respectively, for Dracaena, and 225 ± 36 min and 105 ± 14 min for banana, respectively. Ingram et al. (1987) aimed at detecting the critical high root zone temperatures for Carrizo citrange (*Citrus sinensis* × *Poncirus trifoliate*) seedlings that were grown for 9 weeks at root-zone temperatures of 28, 34 or 40°C for 6 h daily. The shoot: root ratio was significantly increased at 40°C. Also, Ahrens et al. (1988) employed electrolyte leakage procedure to determine the differences in tolerance to direct heat injury to leaves of some citrus cultivars and discover that the lethal temperature for a 20 min exposure was 54.3 ± 0.5°C for 'Glen' citrange citrus variety and 56.1± 0.4°C for 'Swingle' citrumelo variety. However, Ingram et al. (1984) found that roots of "Swingle" citromelo died at 53°C. Xiao and Zhao (1990) conducted studies on physiological and biochemical indices of heat tolerance of citrus leaves by using electrolyte leakage procedure. Leaves of 5 citrus species were treated with warm water at temperatures of 36 and 66°C for 20 min exposure time. Their results indicated that sour orange followed by lemon had the highest degree of heat tolerance. Zhonghai et al. (1999) exposed tissues to different temperature regimes of 46, 48, 50, 52, 54, 56, 58 and 60°C and compared them with leaves treated at 35°C (control) for 20 min by using electrolyte leakage procedure. They found that the plasma membrane was damaged and the rate of leakage increased with temperature also Anderson et al. (1990) determined the capacity of pepper leaves to acclimatize to high temperature by using electrolytes leakage procedure. Their results showed an interaction between exposure to temperature and duration, as well as lethal temperature which decreased linearly from 53 to 46°C as exposure duration increased exponentially from 5 to 240 min. Plants grown at 22/20°C day/night cycles and held 24 h at 38/30°C had increased their heat tolerance by 35°C, and from 51 to 54°C. There is limited research on the application of electrolyte leakage (EL) procedure on banana plants. On the other hand, according to studies on thermo-tolerance of dwarf Cavendish banana group plants through leaflets have not been reported so far. This laboratory experiment is aimed at closing this research gap. Additionally, providing such information will offer banana growers with accurate information about the favorable time of the year for transplanting banana plants and protecting them against heat in the field. The objective of this study was therefore, to determine the heat tolerance of Malindi banana cultivar under Omani weather conditions.

MATERIALS AND METHODS

Third leave from the top of banana plant locally named Malindi cultivar was used. Middle part of the leaf blade was cut and rinsed in deionized water to remove the dusts and electrolytes adhering to the surfaces and lightly cleaned with tissue papers. Leaf segments of five centimeter length and 1.2 cm width were cut. One segment was placed in each test tube, and 1 ml of deionized water (< 0.2 ds m^{-1}) was added to the test tube to prevent tissue desiccation and loosely covered with aluminum foil. All test tubes were placed in a water bath shaker equipped with thermostat and the tissues were exposed to a heat regime ranging from 30 to 80°C (5°C increments). The exposure time to each temperature was 30 min. Three tubes per treatment remained at 22°C as controls. At the end of each 30 min exposure, leaflet segments were removed from the water bath; cut into 1 mm strips to allow uniform diffusion of electrolytes, and returned to the tubes along with 40 ml of deionized water, and incubated in a refrigerator at 7°C overnight. This was followed by electrical conductivity determination of each solution. In the next day, leaf segments were taken out from the refrigerator, warmed up to room temperature (22 ± 2°C) and placed in a mechanical shaker (Gesellschaft Fur Labortechnik (GFL) mbh-model 3015-Germany) and shaken for one hour to diffuse the electrolytes. Thereafter, electrolytes leakage before final cells damage was measured with electrical conductivity meter (Orion-model 150-USA). Leaf segments were then fully damaged by autoclaving (Mediclave - Japan) (121°C) for 10 min and left on the shaker device for 1 h, then the total electrolyte leakage reading were taken by using the same conductivity meter. Percentage of electrolyte leakage before killing and after killing was calculated (Ahrens and Ingram, 1988) as follows:

EL% = [(electrolyte leakage before killing) / (electrolyte leakage before killing)] × 100

The three replicates of the control treatment leaflets went through the same procedure to find percentage of electrolyte leakage. The experiment consisted of twelve treatments, each treatment was replicated 3 times in a completely randomized design, and each leaflet represents one replication. Two-way analysis of variance was used to analyze the data of electrolyte leakage. Multiple comparisons among means were performed with Duncan's test (DMRT). Person's correlation analysis was used to examine relationships between heat regimes and electrolyte leakage percentage variables. All statistical analyses were carried out using GenStat Release 11.1. Sigmoid curves were illustrated by using Curve expert 1.3 programs.

RESULTS AND DISCUSSION

Exposure of plasma membrane to direct heat stress resulted in changes the membrane permeability and loss its semi-permeability and thus electrolyte leakage from cells can occur. Determination of cell membrane

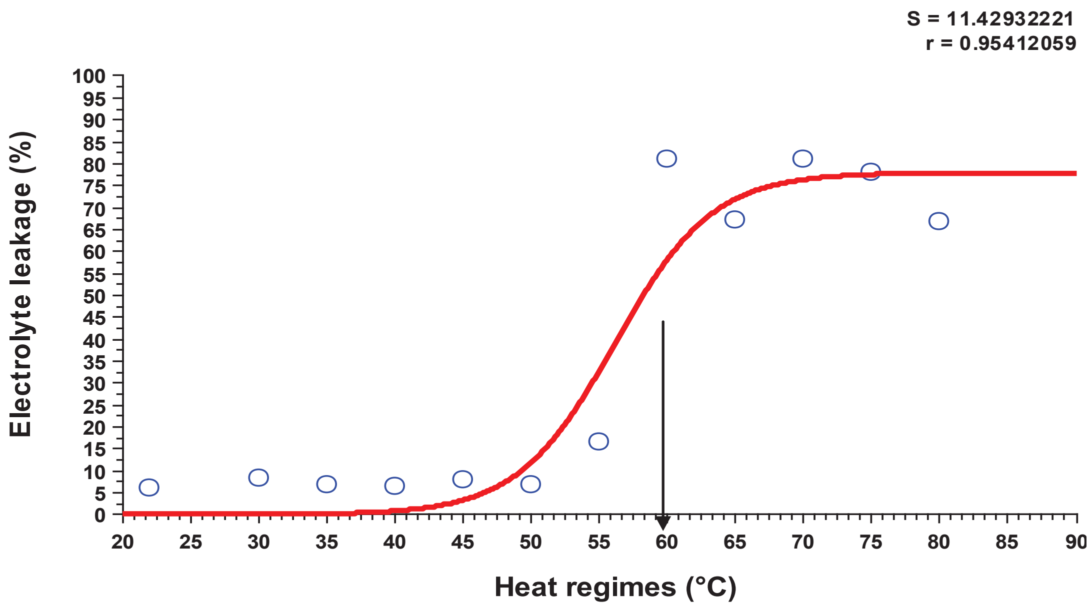

Figure 1. Heat tolerance of manured dwarf Cavendish banana plants cultivar "Malindi".

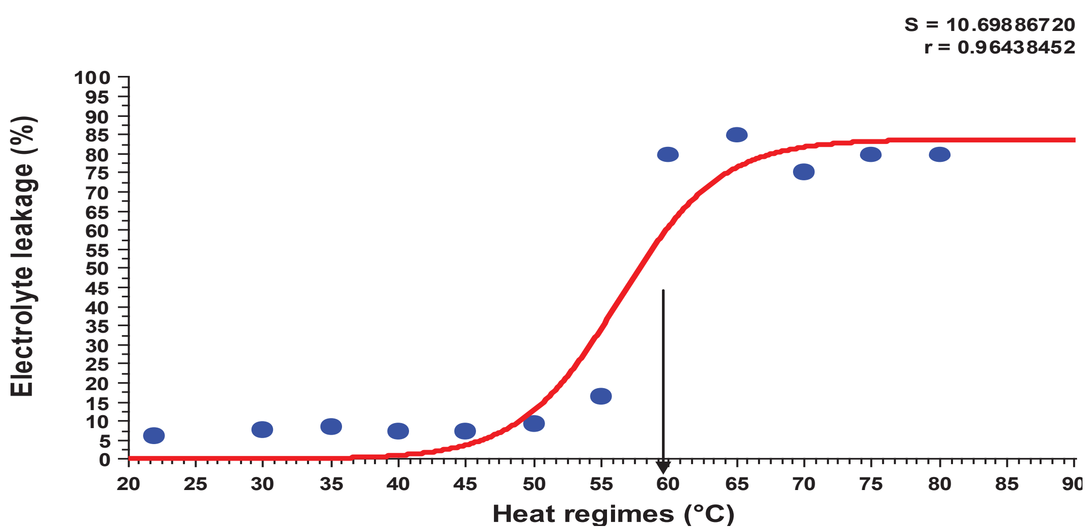

Figure 2. Heat tolerance of mineral fertilized dwarf cavendish banana plant cultivar "malindi" .

thermostability in plant tissues by using cell electrolytes leakage technique have been widely used (Levitt, 1980; Ingram, 1986). Under heat stress, proteins of the plasma membrane become denatured or aggregated according to the severity of stress and/or membrane lipids become hyperfluid. These changes resulted into increased leakage of electrolytes from the membrane (Levitt, 1980).

The results indicated that the injury occurred to plasma membrane of banana leaflet tissues as a result of direct exposure to heat regimes was gradually increased based on temperature degree. There were significant differences ($p \leq 0.05$) between heat regimes on the percent of cell electrolytes leakage. As shown in (Figures 1 and 2), the shape of the sigmoidal curve was consistent. The lethal temperature was determined at 50% electrolyte leakage. The sigmoidal curve started with slow increase in electrolyte leakage at low temperatures and then increased rapidly at 55°C onwards. Beyond 50% electrolyte leakage, there was leveling off with very minor changes in this leakage with increase in the temperature. The sigmoidal pattern of electrolyte leakage obtained in this study, for heat stress (Figures 1 and 2) is in line with other previous studies (Ingram and Buchanan, 1988; Ahrens and Ingram, 1988; Nielsen and Orcutt, 1996). The

Table 1. Effect of heat regimes on electrolyte leakage % of manured and mineral fertilized on dwarf Cavendish banana plant tissues cultivar "Malindi".

Heat regimes (°C)	Composted dairy cow manure♠	NPK + foliar spray♠
Control	6.1 c	6.1 c
30	8.3 c	7.6 c
35	7.1 c	8.4 c
40	6.6 c	7.4 c
45	8.1 c	7.4 c
50	6.9 c	9.5 c
55	16.6 c	16.6 c
60	80.9 a	79.6 a
65	67.2 b	84.9 a
70	80.9 a	75.2 ab
75	78.2 a	79.4 a
80	67.0 b	79.7 a

Probability values: Heat regimes: $P < 0.001$; fertilizer types: $P < 0.038$; temperature × fertilizer; $P > 0.131$; CV %: 16.7.

figures clearly show inflection of the line. Thus, based on the inflection point at 50% electrolyte leakage, it was evident that the lethal temperature for 4 months old dwarfs Cavendish banana plants was 58°C. Our results are not in agreement with the report of Taylor and Sexton (1972) that thermal danger point for banana is 47°C as well as the report of Ingram and Ramcharan (1988) who found 48°C to be the lethal temperature for banana root. However, our results is in consonants with other researchers (Ingram and Buchanan, 1984; Lester, 1985) that genotype, period of exposure and growth stage of tissue play a vital role in determination of the level of plant tolerance to heat stress. Although there was a variation between low temperatures on the percent of electrolyte leakage, but statistically there was no significant difference among them ($p \geq 0.05$). However, significant differences were detected between low and high temperature regimes ($p \leq 0.05$). Also, it was found that leakage of electrolytes was similar in leaflets segments irrespective of fertilizer types used even at relatively low temperatures such as control, 30, 35, 40, 45, 50 and 55°C (Table 1). Similar results were obtained at high temperatures of 60, 65, 70, 75 and 80°C. Also, there was no interaction between temperature regimes and fertilizer on the percentage of electrolyte leakage. Regardless of the type of fertilizer used, the injury point to leaflet tissues of different fertilized plants started at the same temperature (55°C). Although leaflet tissues taken from plants that was fertilized with manure compost had significantly less electrolyte leakage when compared with those under mineral fertilizer (7% lower) (Table 1). This may be attributed to the ability of compost to supply nutrients for long time especially calcium and potassium which are very important in strengthening cell membranes.

The sigmoidal pattern of electrolyte leakage obtained in this study, for heat stress is in the line with other previous studies (Ingram and Buchanan, 1988; Nielsen and Orcutt, 1996). The current study provided experimental evidence for heat tolerance of banana plants (Malindi cultivar). Perhaps this is the first study that provided a quantitative determination for heat tolerance of dwarf Cavendish banana plants by using leaflets segments through electrolyte leakage technique. Moreover, this information has very important implications since many banana plants could be affected or die if air temperature during the day reaches more than 40°C especially during summer months. Accordingly, in the present study, heat absorption factor has been considered because tissue temperature is usually higher than air temperature by at least 10 to 12°C (Levitt, 1988). These results would guide banana growers to the suitable time of the year before transplanting banana plantlets and protect their plants against extreme of temperatures.

Conclusion

Results presented in this investigation demonstrated that heat stress regime resulted consistently in generating a sigmoidal curve of electrolyte leakage. It provides evidence of the validity of electrolyte leakage technique in determining heat tolerance of banana plants (Malindi cultivar) throughout leaf tissues. This study showed perhaps for the first time a quantitative determination of the thermo-tolerance of Dwarf Cavendish banana plants (Malindi cultivar). Although average percent of electrolyte from plant tissues that received compost dairy manure was less than that fertilized by mineral fertilizers, but heat tolerance of leaflets tissues obtained from either plants receiving organic composted dairy manures or mineral fertilizers was quantitatively the same. Lethal heat tolerance for dwarf Cavendish banana plants was found to be 58°C. The duration factor in stress is very important and it should be taken in to consideration in future studies.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effectiveness of urea nanofertilizer based aminopropyltrimethoxysilane (APTMS)-zeolite as slow release fertilizer system

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In this study, nanofertilizer effectiveness of urea/APTMS-modified zeolite as a slow release fertilizer nitrogen from urea was evaluated. The natural zeolite is activated by 1% of HF with a temperature of 120°C and a zeolite surface has been modified with aminopropyltrimethoxysilane (APTMS) 5%. Zeolite was activated with APTMS-modified zeolite characterized by X-ray diffraction spectroscopy (XRD), surface area analyzer (SAA) and UV-Vis spectroscopy. Modification of activated zeolite with APTMS result in decrease in pore size as well as crystalinity. While the aminopropyl group was well defined with FTIR spectra, pore size measurenment using SAA obtained APTMS-modified pore size was 7.74 nm; while a maximum adsorption capacity of urea was 3.668 mg/g. Slow release urea of APTMS-modified zeolite was evaluated and observed by the increasing of urea absorbance at a wavelength of 190 nm in aqueous media. The experiment result showed that urea without zeolite was released up to 100% limit at 10 min observations, while the urea impregnated with APTMS-modified zeolite in 1 M NaCl media was released up to 100% at 120 min observation times. Meanwhile, the APTMS-modified zeolite in aqueous media was released only about 56.24% at 120 min. The result conclude that the APTMS-modified zeolite is capable of retarding the release of urea and potential to be developed as a control release of nitrogen from urea.

Key words: Zeolite, modified aminopropyltrimethoxysilane (APTMS), urea, slow release fertilizer.

INTRODUCTION

Indonesia is the largest consumer of fertilizer, one of which is nitrogen fertilizer. In 2012, total demand reached 11.1 million tons of fertilizer. Therefore, fertilizer is a major issue for Indonesia. The high demand for nitrogen fertilizer is due to its having the most important element for crops such as rice, namely nitrogen (N). Despite the relatively important element, nitrogen is an element with least efficient utilization as it is easily lost through leaching in the form of nitrate, evaporating into the air in the form of ammonia gas, and changed into other forms that cannot be utilized by the plant. One example of the type of fertilizer N often found in the market in Indonesia

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is in the form of urea ($CO(NH_2)_2$). Fertilizer is easily soluble in water and evaporates into the air so that nitrogen fertilizer is low fertilizer efficiency. When Nitrogen is applied into the soil, approximately 30 to 40% is taken by plants, and 60% is lost in the process of ammonia volatilization into the gas (De Datta, 1987).

Therefore, another innovation is required to improve the efficiency of fertilization. One attempt to reduce the nitrogen fertilizer by making it in a slow release form. Enterprises slow release of nitrogen fertilizer can reduce environmental pollution due to the release of nitrogen being controlled so that the amount of excess nitrogen in the form of nitrate that enters the water is reduced, thereby reducing water pollution. One of the materials that can be developed as a controller release nitrogen in fertilizer is a natural zeolite. Recently, several types of SRFs have been developed and tested, including SRFs using various zeolites. Bansiwal et al. (2006) was used to evaluate slow release phosporous intake in surfactant modified zeolite (SMZ). The results indicated that SMZ is a good sorbent for PO_4^{3-}, and a slow release of P was achievable. Li (2003) has used surfactant-modified zeolite as fertilizer carriers to control nitrate release. However, SRFs are often expensive and the release of nutrients is slow at the time of high nutrient need (Miller and Gardiner 1998).

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, with a three-dimensional lattice, furrowed by an inner network of pores and channels. Zeolites have a high cation exchange capacity and have often been used as inexpensive cation exchangers for various applications (Breck, 1974; Barrer, 1976). The studies on SRFs based on zeolites are limited to nutrients, which can be loaded in cationic forms such as NH_4^+ and K^+. However, if the nutrients are in anionic forms such as NO_3^- or PO_4^{3-}, the loading is negligible on unmodified zeolites.

Modification of natural zeolite with aminopropy ltrimethoxysilane (APTMS) aims to increase the affinity uptake of urea in the framework of the zeolite pores, so it can control the release of urea nitrogen according to the time and amount needed. It can occur because the active amine groups on the APTMS will bind the nitrogen of urea compound so that the release of urea can be controlled. Using the APTMS-modified zeolites as urea intake medium, we have slow release fertilizer that is expected to optimize the uptake of nitrogen by plants and maintains the presence of nitrogen in the soil with the amount of fertilizers applied smaller than the conventional method.

Based on the description above, this research focuses on testing the efficiency of the modified natural zeolite nanoporous as a mixture of urea which is expected to help release of nitrogen from fertilizers. Preparation of slow release fertilizer (SRF) zeolite materials with the right amount is expected to control the release of nitrogen in accordance with the time and number of plants needed and maintain the presence of nitrogen in the soil, so that a given amount of fertilizer is more efficient than conventional methods and can save costs.

METHODOLOGY

Natural zeolites activation

This is a method by Budi et al. (2013), in which natural zeolite is crushed and sieved to 60 mesh size to qualify. The zeolite was washed with distilled water and soaked in 1% HF for 10 min. Zeolite was thereafter washed with distilled water until neutral pH. Then, the zeolite was heated in an oven at 120°C for 4 h. After the acid activation procedure, the result was characterized using XRD (Bruker D8 Advance X-Ray Diffraction) to evaluate the difractogram pattern of no activation and activated zeolite. Also, SAA (Surface Area Analyzer) measurenment was used to determine the surface area of the activated zeolite.

Surface modification of activated zeolite

According to Zhao et al. (2012), activated zeolite was modified with silane agent of 5% APTMS, and then shaken for 8 h at room temperature with a speed of 150 rpm. It was thereafter washed with distilled water and dried. The APTMS-modified zeolite result was characterized by FTIR (Fourier Transform Infrared Spectroscopy) to determine the functional groups of aminopropyl attached to zeolite and SAA (Surface Area Analyzer) to determine the surface area of APTMS-modified zeolite.

Adsorption urea onto APTMS-modified zeolite

1.0 g of APTMS-modified zeolite was added to urea solution of 36.5 mg urea in 100 mL of distilled water. The mixture was mixed for 24 h at room temperature, and placed at a temperature of 40°C overnight. The obtained product was evaluated using FTIR (Fourier Transform Infrared Spectroscopy) to confirm the functional groups of urea attached to APTMS-modified zeolite.

Slow release fertilizer testing

Slow release fertilizer testing (SRF) was conducted with APTMS-modified zeolite, zeolite, urea and urea-APTMS without dissolving. 0.5 g of each sample was added to NaCl 1 M as well as to distilled water, and was then soaked at a speed of 140 rpm in room temperature. The solutions were measured using UV Vis spectrometer for each certain time (5 to 120 min) to calculate the urea released at λ_{max} 190 nm.

DISCUSSION

Urea is one example of fertilizer that has low absorption efficiency in soil which is in the range of 30 to 40%. Urea easily dissolves in water to form NH_4^+ cations. Therefore, one of the effort is how to improve the efficiency of urea fertilizer to temporarily bind into zeolite nanopore get the nanofertilizer approach. The first achievement of this research is activation of natural zeolite. The zeolite was destructed to about 60 mesh in size, to gain the homogenized size and enlarged surface area. Zeolite was activated through acidification using 1% HF solution

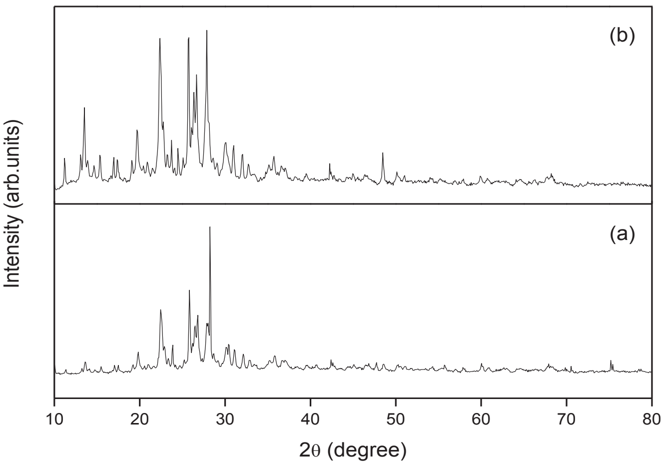

Figure 1. XRD patern of (a) activated zeolite (b) natural zeolite used for SRF.

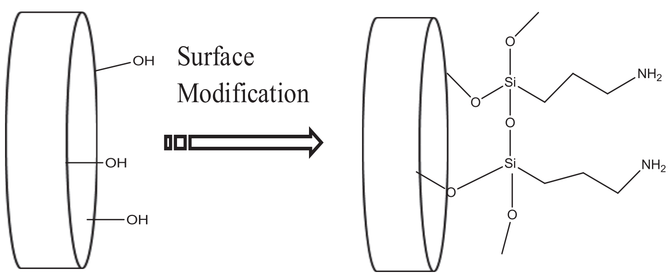

Figure 2. Mechanism of surface modification of zeolite by APTMS.

to dissolve the impurities of natural zeolite content. Acidified zeolite was then cleaned up until neutral condition was obtained and was thereafter dried at a temperature of 120˚C.

In the acid activation process, silica dissolution process was adopted, which is one component of the zeolite framework (Jozefaciuk and Bowanko, 2002). Silica dissolution will lead to changes in the structure of the zeolite and the decreasing ratio of Si/Al. The decrease of Si/Al ratio may affect the zeolite adsorption capacity and selectivity towards polar molecules such as water vapor (Bonenfant et al., 2008). Figure 1 shows the XRD pattern of the zeolite before and after activation by 1% HF solution.

Results of the XRD analysis showed the presence of activated zeolite reduction of Si/Al ratio. It can be seen in the decrease in peak of SiO_2 and Al_2O_3 (Figure 1b). Decrease in the Si/Al ratio influence the zeolite pores due to a higher electric field impact increased "charge site" on the surface of the zeolite. In addition, decrease of Si/Al ratio also aims to remove certain ions from the zeolite framework.

Modification of activated zeolite using APTMS successfully influence the chemical properties of the material. APTMS attach mechanisms into zeolite framework and can be assisted by zeolite surface activation in the previous stage. Aminopropyl group will attach to the pore zeolite surface to make monolayer formation. The purposed mechanism of surface modification of zeolite by APTMS was shown in Figure 2.

The FTIR analysis of the unmodified zeolite and APTMS-modified zeolite indicates that the modification process was successful. From the FTIR spectrum in Figure 3, it can be seen that there was a shift in the region of 1030 cm^{-1} which was the absorption of the Si-O-$(CH_2)_3NH_2$. This shift may occur due to the molecular weights of compounds increases, so it will shift to the smaller wave numbers. In addition, the characteristic absorption of the primary amine from modified zeolites in the region 3000 to 3330 cm^{-1} showed that the modification was successful. The intake of urea into APTMS-modified zeolite occur via chemical adsorption using aminopropyl group (Figure 3).

Urea is capable of interacting with aminopropyl to form linked amino. This have been observed by decrease in absorbance of UV Vis spectra which indicates the decrease of urea concentration in liquour medium. Based on the quantitative calculation from UV Vis spectroscopy data, the maximum adsorption capacity of urea by APTMS-modified zeolite was 3.668 mg/g, while the maximum adsorption capacity of unmodified zeolite reach only about 0.243 mg/g (Budi et al., 2013). It may be caused by differences in surface properties of zeolite and APTMS-modified zeolite. Absorption on zeolites without modification occur as a result of the interaction of the charge and the zeolite pores properties, wherein the urea in solution will be trapped by the pores of the zeolite surface both external and internal. While in APTMS-modified zeolite, absorption occurs by being trapped into pore zeolite via aminopropyl anchor as well as by electrostatic interactions on the surface of modified zeolite. The pore size and surface area measurements using SAA method were 7.74 nm in pore size and a surface area of 5.488 m^2/g. The pore properties should influence adsorption capacity of urea.

SRF testing of urea in APTMS-modified zeolite in different media compared with untrapped urea were evaluated. The result of those analyses was shown in Figure 4. SRF evaluations were observed by the increasing of urea absorbance at a wavelength of 190 nm in aqueous media. Based on the experiment, it was observed that urea without zeolite was released up to 100% limit in 10^{th} min observations, while the urea impregnated to APTMS-modified zeolite in 1 M NaCl media was released up to 100% at 120 min observation times. Meanwhile, the APTMS-modified zeolite in aqueous media was released for only about 56.24% at 120 min. It may be caused by ionic strength influence of salt content in aqueous medium. In the presence of NaCl solution, the system is more capable of making interaction than those without NaCl solution. The result concludes that the APTMS-modified zeolite is capable of

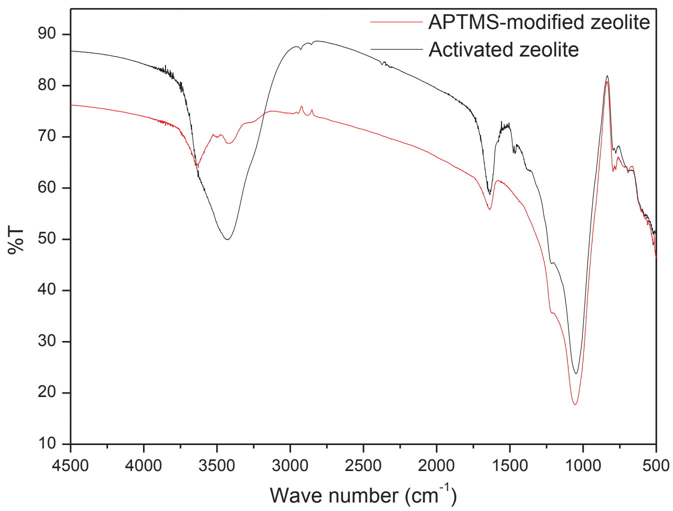

Figure 3. FTIR spectra between unmodified zeolite and APTMS-modified zeolite.

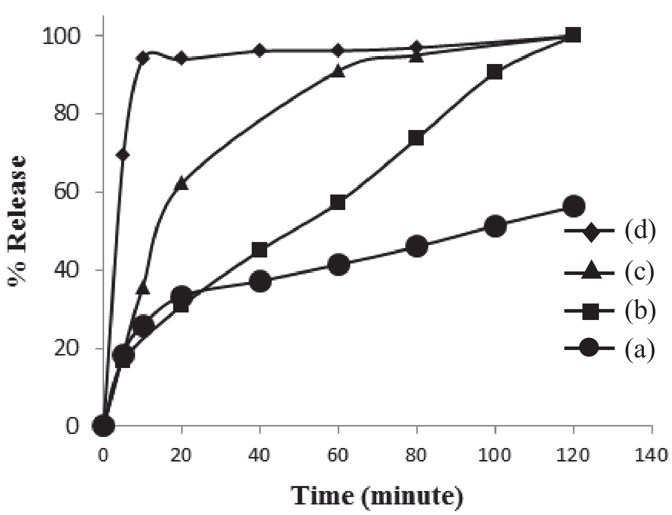

Figure 4. SRF test of urea with (a) APTMS-modified zeolite in aqueous media (b) APTMS-modified zeolite in 1M NaCl aqueous media (c) APTMS-modified zeolite without aqueous solvent (d) urea untrapped.

holding the release of urea and potentially to build a SRF system based urea fertilizer.

Conclusion

The study revealed that the SRF based urea system can be built by urea trapped in APTMS-modified zeolite. The APTMS-modified zeolite is capable of holding the release of urea by the chemical interaction between urea and APTMS-modified zeolite via amine linked. The ionic strength influence the capability of SRF. In the presence of NaCl solution, the system is more capable of interaction than those without NaCl solution.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Evaluation of the effects of current irrigation water pricing systems on water productivity in Awash River Basin, Ethiopia

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Awash basin is the only basin that operates with the concept of river basin management and irrigation water pricing in Ethiopia. The effectiveness and impacts of the current irrigation water pricing system in the basin has not been studied yet. Hence, the objective of this research is to evaluate effects of irrigation water pricing on scheme level water productivity in Awash River basin. Based on systematic selection criteria, 29 irrigation water users were selected from middle and upper Awash. Structured questionnaire and discussions have been used to generate the primary data. Scheme specific data such as area cultivated, amount of water diverted each year, water fee, service charge and operation and maintenance cost for primary irrigation canals of each legal water user in the basin for the last five consecutive production years (2005/2006-2009/2010) were collected from Awash Basin Authority. SPSS and CROPWAT were used to analyze the information gathered through questionnaires and irrigation water requirement respectively. Water productivity of cotton, sugarcane and onion was computed for total available water (excess rainfall + irrigation), irrigation water and water lost through crop evapotranspiration. The current irrigation water pricing in Awash basin seems to be low and does not encourage individual users in improving water productivity. Hence, it is resulted in low crop water productivity. Therefore, cost of irrigation water in Awash basin should have to be optimized with a well specified and revised pricing objective(s) if it has to influence the water productivity.

Key words: Irrigation, irrigation water pricing, water productivity, Awash basin.

INTRODUCTION

Irrigation development has been identified as an important tool to stimulate economic growth and rural development, and is considered as a cornerstone of food security and poverty reduction in Ethiopia. Irrigation is one means by which agricultural production can be increased to meet the growing food demands in Ethiopia (Awulachew et al., 2005). Robel (2005) also states that one of the best alternatives to consider for reliable and sustainable food security development is expanding irrigation development on various scales, through river diversion, constructing micro dams, water harvesting structures, etc. However, growing population with higher cultivation intensities, increasing urbanization, computation of sectors for water allocation and

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environmental concerns have all combined to put pressure on global water resources. Failure of attention to management aspect of irrigation has resulted not only in degradation of productive land but also caused other environmental externalities. This is evidenced by the degradation of irrigated areas like in Awash River basin.

In lower Awash, from the total area of 10285 ha of land which was brought under surface irrigation system in the year 1982, about 33% of that area was abandoned due to salinity after only 5-8 years of operation (Girma and Fantaw, 2005). Despite of their promise as engines of food security, irrigation projects typically perform far below their potential. Head-tail problems, leaky canals and malfunctioning structures because of delayed maintenances, leading water use efficiency and low yields are some of the commonly expressed problems. Large part of low performance may be due to inadequate water management system at system and field level (Cakmak et al., 2004). To make irrigation projects sustainable both economically and environmentally, users need to improve their agricultural productivity which requires changes in their institutional structures, water use management systems and policies, improve their service delivery systems, and proper management of farm lands.

Irrigation water pricing is an effective mechanism to generate revenue for sustainable management of irrigation system and at the same time enhance efficient water use and improve water productivity. Irrigation water pricing increases the production rate per unit use of water, reduce the loss of water to unusable sinks and reallocate water for higher priority uses (Howell, 2001). Reddy (2009) also state that irrigation water pricing has an important role in revenue generation for irrigation operating agencies, improve economic efficiency of water resource use, improve equity and fairness among water users, and to enhance water resource conservation. Hence, pricing of irrigation water can be used as an economic, a financial or an environmental tool (Molle and Berkoff, 2007).

Water pricing is seen as a key economic and policy instrument to improve the sustainability of water management, to encourage conservation and improvement of quantitative and /or qualitative status of water bodies (Johansson et al., 2002; Shajari and Soltani, 2008; Zoudmides and Zacharides, 2009; Thaler, 2010). Despite the fact that irrigation water pricing is considered as an important tool to improve efficiency in resources utilization, it is not a common practice in Ethiopia. Although at infant stage and constrained by many problems water pricing is practiced in Awash River basin. This study was conducted in 2011 to assess current status of water productivity (crop yield per water consumed) at scheme level for selected crops in Awash River basin and evaluate the effects of current water pricing system on irrigation water productivity in the basin.

METHODOLOGICAL APPROACH

A structured questionnaire survey with face to face interview method was used to collect a data about irrigation water pricing systems in Awash basin. In order to conduct the questionnaire survey and discussions representative irrigation water users were selected. The list of all legal irrigation water users, their annually cultivated area, location within the basin, means of water abstraction from the river and their farming system was collected from Awash basin authority (ABA). Representative legal irrigation water users were selected systematically with consideration of users' irrigation management institute (private farm, community farm and state farm), crops under cultivation and users location (upper and middle Awash).

Based on the above selection criteria 29 irrigation water users were selected from middle and upper Awash. No irrigation scheme is included from lower Awash, because most large and medium scale irrigation schemes in the area are currently abandoned due to construction of Tendaho and Kessem irrigation projects. 20 small scale (command area less than 200 ha), 5 medium (201 to 3000 ha) and 4 large scale (more than 3000 ha) irrigation schemes were included in the detail survey of the study. A structured questionnaire survey was used to collect all necessary primary data about irrigation water pricing from these selected individual irrigation water users in the basin. The questionnaire used for this study was designed in attempt to collect area cultivated by individual user, annual production, individual irrigators yearly water demand, impact of irrigation water pricing on users water demand, individual users response to irrigation water pricing. The questionnaire survey and related field observations were conducted for three months from March to June, 2011.

Water resources management policy, sector strategy and different regulations and proclamations were reviewed to state the legal frame work of water resource management and irrigation water pricing in Ethiopia. Discussions were also held with officials and experts from MoWRE and ABA about irrigation water pricing experiences and its impacts in Ethiopia and Awash River basin respectively.

Scheme specific data such as area cultivated, amount of water diverted each year, water fee, service charge in the basin for the last five consecutive production years (2005/2006-2009/2010) were also collected from ABA. Monthly climate data were collected from representative meteorological stations (stations near to the selected irrigation schemes) for the analysis of water productivity of selected crops in the basin.

Method of data analysis

The quantitative and qualitative data collected from the primary sources were analyzed using qualitative methods and descriptive statistics. Statistical Package for Social Sciences (SPSS) was used for the analysis of quantitative data to estimate the response of irrigation water users to irrigation water pricing and impacts of irrigation water pricing on water productivity. Data collected from key informant interviews, discussions and observations were qualitatively assessed to state the current irrigation water pricing system, its objectives and its practical application in Awash basin. Finally, outputs of the statistical analysis were presented using tabulation, cross-tabulation, means, frequencies and percentages.

Water productivity

Nowadays, there is a trend to call improving irrigation water productivity as a must (Molden et al., 2003). Molden and Theib (2007) defined water productivity as the ratio of the net benefits from crop, forestry, fishery, livestock, and mixed agricultural

Table 1. Physical and economic water productivity indicators.

S/No.	Water productivity indicator	Physical productivity (kg/m^3)	Economic productivity (ETB/m^3)
1	Total water productivity (WP_T)	$\frac{Y_a}{ER + DW}$	$\frac{Y_a}{ER + DW} * \alpha$
2	Irrigation water productivity (WP_I)	$\frac{Y_a}{DW}$	$\frac{Y_a}{DW} * \alpha$
3	Crop water productivity (WP_C)	$\frac{Y_a}{ET_c}$	$\frac{Y_a}{ET_c} * \alpha$

Where, Ya= actual harvestable yield (Kg), α = monetary value of harvestable yield (ETB/kg), ER = effective rainfall in mm or m^3, DW = Diverted irrigation water to individuals' command area in mm or m^3, and ETc = crop evapotranspiration.

systems to the amount of water required to produce those benefits. Water productivity may be generically defined as the ratio between the actual yield achieved (Ya) and the water used expressed in Kg/m^3. Water productivity (WP) can be expressed either in physical or economic terms (Kumar et al., 2009; Yokwe, 2009). Water productivity broadly denotes the outputs (physical or economic) derived from a unit volume of consumed or depleted water. Water productivity combines accounting of water with crop yield or its economic return to indicate the value of a unit volume of water. Depending on how the terms in the numerator and nominator are expressed, water productivity can be expressed in general physical or economic terms. In this study, the following physical and economic water productivity indicators were used for the estimation of water productivity (Dong et al., 2001) (Table 1).

In addition to the above water productivity indicators relative irrigation supply (RIS), the ration of amount of irrigation water supplied to the crop to the amount of crop irrigation water demand was also estimated for all surveyed irrigation water users to compare crop irrigation requirement and irrigation water supply.

$$RIS = \frac{Irrigation\ supply}{Irrigation\ requirement}$$

Where: RIS is relative irrigation supply.

Estimation of crop evapotranspiration and effective rainfall

The FAO computer programming model CROPWAT 8.0 was used for the estimation of crop evapotranspiration, crop irrigation water requirement and effective rainfall. The model carries out calculations for reference evapotranspiration, crop water requirements and irrigation requirements in order to develop irrigation schedules under various management conditions and scheme water supply. It allows the development of recommendations for improved irrigation practices, planning of irrigation schedules, assessment of production under rainfed conditions or deficit irrigation, drought effects and efficiency of irrigation practices (Kassam and Smith, 2001).
In this study effective rainfall was computed from the mean monthly rainfall data for estimation of total water productivity using the USDA soil conservation service method. The USDA soil conservation service method uses the following equation for calculation of effective rainfall;

$$PE = P_{tot} * \frac{125 - 0.2P_{tot}}{125}\ (For\ P_{tot} < 250\text{ mm})$$

$$PE = 125 + 0.1 * P_{tot}\ (For\ P_{tot} > 250mm)$$

Where: PE is effective rainfall (mm) and P_{tot} is total rainfall (mm). Estimated depth of excess rainfall and crop evapotranspiration was expressed in m^3 by multiplying the excess rainfall depth by the annually irrigated area of selected individual users during the last five production years.

RESULTS AND DISCUSSION

Irrigation water pricing practices in Awash River basin

Any significant water diversion from Awash River for irrigation purpose requires the approval of ABA. ABA has the mandate and power of controlling irrigation systems in the basin up to the primary irrigation canals. Controlling secondary, tertiary and on-farm irrigation canals is the mandate of respective individual irrigation users. ABA is the only one of its kind in Ethiopia that operates with the concept of river basin management.

The ABA collects water fees on volumetric basis with an initial objective of covering all cost expenses of the authority. Individual users are charged according to their annual consumption of irrigation water with a charging rate of 3 ETB per 1000 m^3. All legal irrigation water users in the basin are charged 78.18 ETB per hectare per year for the service rendered by the authority in addition to the water fee. Users abstracting water with gravity are additionally charged 5.9198 ETB per hectare per year to cover monthly salaries of gate operators.

Each year a contract is signed between the Authority and each of its clients, and irrigation water use permit is issued. The permit stipulates its expiration date, the amount of water required by each client, means of water abstraction, area to be irrigated, and irrigation period. Once the irrigation season starts, a water request format is prepared by the authority for legal irrigators diverting water from each primary off take structure.

Individual irrigators submit their irrigation water demand request to the authority on a weekly basis based on the request format prepared by the Authority. The amount of water diverted to individual users is measured at primary off take structures of the primary irrigation canals with

Table 2. Physical and economic total water productivity.

Crop	Physical TWP (kg/m^3)				Economic TWP (Birr/m^3)			
	Min.	Max.	Mean	S.D	Min.	Max.	Mean	S.D
Cotton	0.027	0.516	0.156	0.086	0.230	7.219	1.257	1.125
Sugarcane	4.380	13.500	7.891	2.669	0.044	2.159	1.08	0.474
Onion	1.018	1.811	1.385	0.234	1.628	10.323	5.179	2.801

water measuring staff gauges.

Some small scale irrigation schemes use one offtake structure (single recording gauge) in common and water fee is collected based on their annually irrigated area. Those small scale irrigators (especially in Amibara area) who do not have access to irrigation water directly from the primary irrigation canal, get access from other users' secondary or tertiary canals. These users face water scarcity problems during low flow or/and high irrigation demand season and excess water is released to their farm during low irrigation demand season.

Vertical water flow measuring staff gauges of standard enameled iron with plated sections located at every primary offtake (PO) structures are used to measure amount of water flow to individual irrigation users. The stage-flow relationship (rating curve) of these flow measuring staff gauges is adjusted every year after the maintenance of primary irrigation canals using current meter. Gate operators are available 24 h at every primary offtake structure to adjust amount of water flowing to individual users and to take staff gauge readings.

The current irrigation water pricing system in Awash River basin does not have limitations on the maximum extraction rate of irrigation water for upstream users. Consequently there is no way of restricting the amount of water being extracted during the periods of peak irrigation demand. As a result downstream irrigation users suffer from water shortage during low flow and high irrigation water demand periods. Such irrigation water shortage problems are common at Amibara area (middle Awash) for users diverting water through gravity flow located at the lower end tail of the main canal during months of April and May.

Many irrigation water users in the basin abstract water from Awash River either through motor pumps or gravity flow without any permit issuance from the authority. Some users get access to irrigation water either by tampering or abstracting through motor pumps from primary irrigation canals illegally. Such illegal irrigation water users in the basin are charged neither for services nor for the cost of water. At the end of each Ethiopian budget year letter is issued to individual legal irrigation water users in the basin from ABA including their annual irrigation area (ha), amount of water consumed (m^3), irrigation water charge (ETB), and service charges (ETB) and requested to pay their annual water and service charges through bank account of the basin.

Crop water productivity

Crop water productivity in this study was expressed in terms of the total available water (including both effective rainfall and diverted irrigation water), diverted irrigation water and water lost through crop evapotranspiration. Results of all water productivity values were expressed in physical (kg/m^3) and economic (ETB/m^3) terms. Minimum, maximum and average values of physical and economic total water productivity (TWP) of cotton, sugarcane and onion are presented in Table 2. Values of average total available water productivity for cotton, sugarcane and onion was 0.16, 7.80 and 1.38 kg /m^3 respectively. During 2005/2006-2009/2010 production years, sugarcane producers were able to get more production rate per unit of available total water than cotton and onion producers get. But, economic water productivity, measured in terms of gross value per unit of total available water was highest for onion producers with five years average value of 5.179 ETB/m^3.

Excluding the amount of water available from excess rainfall and considering only amount of water diverted to individual irrigation users' offtake structure, irrigators were able to produce 0.2, 11.84, and 2.01 kg of cotton, sugarcane and onion respectively from every m^3 of diverted irrigation water (Table 3). The economic return of every m^3 of irrigation water diverted to individual users' irrigated land was 1.70, 1.63 and 7.67 ETB on average from cotton, sugarcane and onion respectively. Table 4 shows physical and economic crop water productivity of cotton, sugarcane and onion from every meter cube of water lost through crop evapotranspiration during the surveyed five production years. For the production of 0.36 kg of cotton, 15.26 kg of sugarcane and 2.27 kg of onion, one meter cube of water was lost through crop evapotranspiration on average. Gross economic return per unit of crop evapotranspired water was highest for onion followed by cotton. Sugarcane producers' were with the highest physical and lowest economic productivity values from unit of total available, irrigation and crop evapotranspired water during the surveyed production years.

Table 5 indicates the annual and five years average relative irrigation supply (RIS) for cotton, sugarcane and onion crops during the surveyed production years. As it indicates, sugarcane producers were with the lowest five years average RIS value (1.281) compared to cotton and

Table 3. Physical and economic irrigation water productivity.

Crop	Physical IWP (kg/m^3)				Economic IWP (Birr/m^3)			
	Min.	Max.	Mean	S.D	Min	Max	Mean	S.D
Cotton	0.026	1.104	0.204	0.166	0.097	15.453	1.702	2.190
Sugarcane	0.600	29.505	11.836	6.463	0.060	4.101	1.626	0.967
Onion	1.528	2.674	2.039	0.329	2.444	15.244	7.674	4.142

Table 4. Physical and economic Crop water productivity.

Crop	Physical CWP (kg/m^3)				Economic CWP (Birr/m^3)			
	Min.	Max.	Mean	S.D	Min.	Max.	Mean	S.D
Cotton	0.139	0.853	0.357	0.146	1.194	5.659	2.545	0.89
Sugarcane	9.807	18.807	15.261	3.063	1.126	3.009	2.069	0.622
Onion	1.594	2.934	2.269	0.451	2.551	16.722	8.432	4.571

Table 5. Annual and five years average RIS for cotton, sugarcane and onion.

Crop type	Production year					Five years average
	2005/2006	2006/2007	2007/2008	2008/2009	2009/2010	
Cotton	1.653	1.477	1.804	1.510	1.552	1.599
Sugarcane	1.481	1.189	1.696	1.095	0.952	1.281
Onion	1.678	1.662	1.559	1.559	1.661	1.624

onion producers. This indicates that sugarcane producers divert irrigation water 28% more than the estimated crop water requirement. This result is an evidence that irrigation water users in Awash basin diverts more irrigation water that exceeds the amount of crop irrigation water requirement by 59.9, 28.1 and 62.4% for cotton, sugarcane and onion respectively. This additional excess irrigation water is beyond the irrigation water requirement of these crops and does not have contribution in production improvement; rather it could be a source of problems related to application of excess irrigation water like water logging and salinity. It also indicates the low irrigation water management level of irrigators in the basin.

To evaluate the effect of additional operational cost of motor pumps on water productivity or water use efficiency, relative irrigation supply was compared for the two state owned large scale sugar estates of Metehara and Wonji. These irrigation schemes are more or less under the same management conditions and they divert irrigation water through gravity and motor pumps respectively. Figure 1 indicates the relative irrigation supply comparison of Metehara and Wonji irrigation schemes. RIS of Wonji is almost the same for the surveyed five production years with an average value of 1.048, but that of Metahara varies from 1.218 during 2005/2006 up to 1.902 in 2007/2008 with an average five years value of 1.473. From this result it can be concluded that irrigation schemes with additional operational cost of motor pumps use irrigation water more efficiently than those schemes diverting irrigation water through gravity.

CONCLUSION AND RECOMMENDATIONS

Awash basin is the only basin that operates with the concept of river basin management in Ethiopia. Different organizations were established and subsequently replaced to manage land and water resources or only water resources of the basin. Currently ABA is legally delegated by MoWRE to manage water resources of the basin in an efficient, equitable and optimum manner. Irrigation water users in the basin are charged for their water consumption on volumetric basis with a charging rate of 3 ETB/1000 m^3 with an additional service charge of 78.18 ETB per hectare per year. The initial objective of pricing irrigation water in Awash basin was to cover the running cost of the authority including monthly salary of employees. The absence of maximum water abstraction limit for users in the basin affects lower end tails users to face water shortage problems during high irrigation demand seasons in middle Awash.

During the surveyed five production years, application of irrigation water and crop production per unit of irrigated

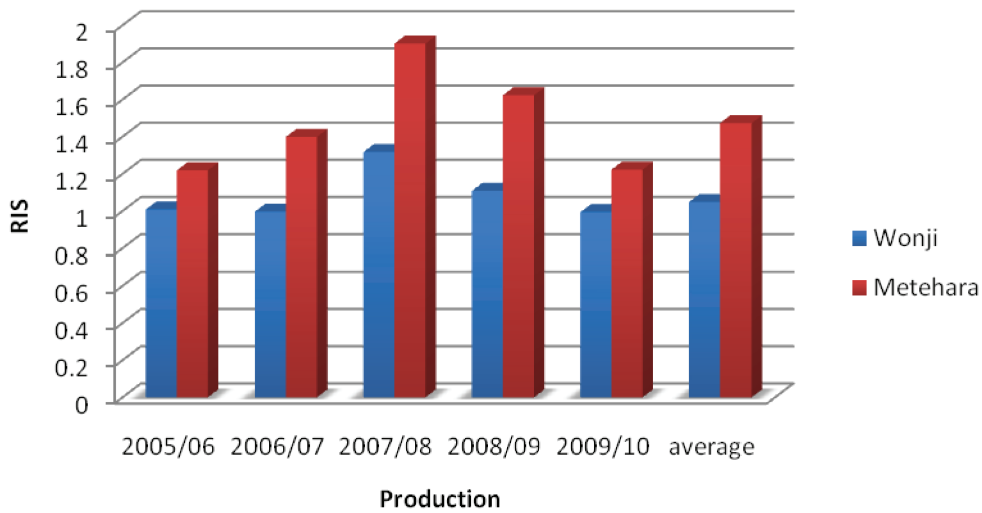

Figure 1. Annual relative irrigation supply of Metehara and Wonji sugar estate farms.

land was poorly related with R^2 value of 0.147 and 0.442 for sugarcane and onion crops respectively. In case of cotton, the productivity indicates a declining with increasing irrigation application rate. This kind of relation between crop production and irrigation water application indicates application of excess irrigation water than the amount required by the crops. This is also evidenced by the result of relative irrigation supply which was found to be more than one for all crops considered. Apart from water supply the conditions of irrigation management level also affects land productivity.

Since irrigation water in the basin is distributed and allocated by Awash Basin Authority there is no conflict between legal irrigation water users. Compared with experiences from other countries that are collecting water fees based on volumetric irrigation water pricing method, the charging rate in Awash basin is too low. As a result, the current irrigation water pricing level in the basin does not encourage irrigation water users to consider water price in users annual irrigation water demand, irrigation scheduling, water application rate, crop selection, change in cropping pattern, area expansion, and improve water productivity. This low price level is resulted in low water productivity level, low water management level and high water application rate compared to the irrigation water requirement of crops considered in the basin. Low water management level and high irrigation water application rate in turn may lead to problems related to excess water application like water logging and salinity.

The irrigation water price level currently practiced in Awash Basin was set in 1994 together with the water tariff determinations for other sectors. Since then, it has never changed. The pricing system must be flexible and subjected to changes depending upon socio-economic and environmental circumstances and management objectives. Cost of irrigation water in the basin should be optimized up to a certain level that can cover not only the operation and maintenance as well as the running costs of authority but also that can encourage water users to use water more efficiently (as irrigation water demand management option). Awash basin authority should have also to set maximum irrigation water extraction rate for upstream irrigation users based on crop type and irrigated land of individual users in order to leave enough water for downstream irrigation users during high irrigation demand periods.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effect of soil amendments on the nutritional quality of three commonly cultivated lettuce varieties in Ghana

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Effect of soil amended with poultry manure and NPK fertilizer on the quality of three cultivars of lettuce; Great Lake, Eden and Trinity cultivated in KNUST, were assessed and analysed. The cultivars were raised, harvested and stored at the Department of Horticulture of the Kwame Nkrumah University of Science and Technology, Kumasi. The harvested leaves were assessed for calcium, iron, vitamin C, chlorophyll, carotenoid level, weight loss and dry matter content. The treatments showed a significant effect (p≤0.05) on the quality parameters except weight loss and dry matter content. The cultivars also varied (p≤0.05) with regards to the quality indicators assessed. Similarly, the interaction of the treatments and cultivars showed significant differences.

Key words: Organic fertilizer, Inorganic fertilizer, quality, shelf life *Lactuca sativa*.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a native of Europe, Asia and Northern Africa and has been cultivated for over 5,000 years. Lettuce is a rich source of antioxidants, Vitamin A and C and phytochemicals which are anti-carcinogenic. It also provides some dietary fibre, carbohydrates, protein and a small amount of fat. Lettuce also provides calcium, iron and copper, with vitamins and minerals largely found in the leaf. Lettuce is usually consumed as a salad or shredded in a salad mix of onion, tomato, cheese and basil. In the market gardens of tropical regions including Ghana, early maturing iceberg type lettuce with three prominent cultivars (Eden, Trinity and Great lakes) are mostly cultivated. The productivity and quality of these lettuces depend on the growing conditions and soil amendments. Also, the difference in minerals and vitamins of the various cultivars of lettuce might also be due to the genotypic difference since they are grown under the same environment (Ojetayo et al., 2011).

In Ghana, commercial and subsistence farming has been and is still relying on the use of inorganic fertilizers for growing vegetable crops (Lampkin, 1990). This is because they are easy to use, quickly absorbed and utilized by crops. The widespread adoption of synthetic fertilizer and associated agricultural practices had a host of unintended consequences to our environment, the quality of our foods and health, and the sustainability of our food system. Organic manure can serve as alternative practice to inorganic fertilizers (Gupta et al., 1988; Wong et al., 1999; Naeem et al., 2006) for improving soil structure (Bin, 1983; Dauda et al., 2008). Organic fertilizers can be used to reduce the amount of

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toxic substances such as nitrates produced by conventional fertilizers in vegetables like lettuce, hence, improving the quality of leafy vegetables as well as human health. However, poultry manure has long been recognized as the most desirable of these organic fertilizers because of its high nitrogen content and availability in most part of the country. It decomposes in the soil releasing nutrients for crop uptake. In a research conducted to find the effects of animal manure on different crops, it was reported that poultry manure was appreciably richer in plant nutrients than other animal manures (FAO, 2008).

Public concern about food quality and safety is steadily increasing. The judgment of fresh vegetables depends on visual characteristics as well as on nutritional quality. The idea of nutritional quality includes beneficial and harmful ingredients, taste, fragrance, freshness and shelf-life as well as the risk of toxic pathogens (Sagoo et al., 2001). Regarding lettuce, the marketable and nutritional quality depends heavily on the agronomic strategy used. Fast release of nitrogen (N) from fertilizers or a surplus of N can lead to an increase in nitrate content of plant tissues, synthesis of N-containing compounds and a decrease in beneficial phytochemicals.

The productivity and quality of vegetable crops especially lettuce therefore depends on the growing conditions and fertilizer application. Despite many investigations in the area of the effect of fertilizers on growth and yield of lettuce, there is little information on the postharvest quality of the crop using various fertilizers to assess its nutritional components. With these ideas in view, it called for an experiment to be conducted to determine the effect of soil amendments on the nutritional on the nutritional quality of three commonly cultivated lettuce cultivars in Ghana. Specifically to determine the effect of soil amendments on the nutritional composition of lettuce.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Department of Horticulture experimental field, Kwame Nkrumah University of Science and Technology. The area is located between latitude 6°40"26' North and longitude 1°35" West and lies approximately 260 m above sea level. The climate of the area is tropical maritime, which is characterized by a wet and dry season with a double maxima rainfall regime. The major rainfall season occurs between March and September, peaking in June and August. November to March is the main dry season making rainfall weakly bimodal. The mean annual rainfall is 1,300 mm and the mean temperature is 28°C.The experiment was carried out in the dry season (October to December 2012).

Soil and manure analysis

Soil and manure analysis was performed before the commencement of field experiments and after cultivation. Total nitrogen was determined using Kjeldahl method. Available phosphorus was determined calorimetrically using Spectrophotometer. Potassium, calcium and Iron from soil, manure and plant tissues were determined by the use of Atomic Absorption Spectrophotometer (AAS). The soil pH was measured using pH meter. All tests were performed at Crop Science Laboratory-KNUST.

Fertilizers

Organic and inorganic fertilizers were used. The organic fertilizer specifically used was decomposed poultry manure and its application rate was 20 t ha^{-1} reported to 3.6 kg $plot^{-1}$ of 1.80 m^2. A rate of 300 kg ha^{-1} of inorganic fertilizer (N: P: K-15-15-15) equivalent to 0.054 kg per 1.80 m^2 was also applied. There was also a control plot with no treatment.

Lettuce cultivars

The cultivars of lettuce used in this experiment included Eden, Trinity and Great lakes and all belonging to the Crisp/Iceberg family. The choice of these cultivars was due to their prominence among most vegetable growers and consumers in the country. The seeds of the plant were purchased from Chinese woman's certified agrochemical shop in Kumasi. The lettuce plants were harvested after a normal growth period of 9 weeks.

Experimental design

The experimental design used was a 3×3 factorial in a Randomized Completely Block Design (RCBD) with three (3) replications. Total plots were 27 plots. Plot size was 1.5×1.2 m. Thus the experimental field was 48.60 m^2. The plots were separated with paths of 0.5 m, between replications and 0.5 m between treatments. The gross experimental area was 81.1 m^2 and 1m of borders was provided around the experimental field on which lettuce plants were grown.

Planting pattern

Seeds of lettuce were sown in a nursery and transplanted onto the field after three weeks. Healthy and vigorous seedlings were selected and transplanted to the field at a spacing of 30 × 30 cm with a total of 20 plants per plot. Periodic watering and all other cultural practices were carried out regularly on the field.

Lettuce sampling

The lettuce plants were harvested after a normal growth period of 9 weeks including period in nursery. Six lettuce heads from each plot were collected with sterile disposable gloves just before harvesting and were put into separate sterile polythene bags and transported on ice to the laboratory for nutritional analysis.

Chlorophyll and carotenoids

Chlorophylls and carotenoids were extracted using methanol 99.9% as solvent. Samples were kept overnight in a dark cold room at 4°C. Leaf pigments were immediately determined after extraction. Absorbance readings were taken at 665.2, 652.4 and 470 nm. Total chlorophyll and carotenoids levels were calculated by using Lichtenthaler's formula.

Calcium (Ca) determination

Calcium was determined by ashing accurately weighed 1 g of dried and ground sample into glazed, high-form porcelain crucible for 2 h in a muffle furnace at 500°C. The ashed sample was left to cool and 10 drops of deionised water followed by 3 to 4 ml of nitric acid were added to the sample. Excess nitric acid was evaporated by placing the sample on a hot plate set at 100 to 120°C. The sample was returned to furnace and ashed for additional 1 h and after being cooled, the ash was dissolved in 10 ml hydrochloric acid and transferred quantitatively to 50 ml volumetric acid. In order to counteract chemical interferences, which have been fairly documented to depress calcium absorbance, a releasing agent in form of lanthanum (10,000 µg mL^{-1}) was added in all replicates and standards to obviate combined interference effects.

Iron (Fe) Determination

Washed samples were fragmented and dried in an exhaust drier at 55°C. The dried material was ground in a laboratory mill. From each sample, 2.5 g of dry plant matter was weighed out and mineralized in a muffle furnace at 450°C. After complete mineralization, combusted samples were dissolved in 10% HCl and transferred to flasks of 50 cm^3 capacity. The concentration of iron was determined by the atomic flame absorption method using an AAS 3 Zeiss apparatus.

Vitamin C (ascorbic acid) determination

Ascorbic acid (AA) was extracted by homogenizing 7 g of tissue in 6% metaphosphoric acid. Ascorbic acid content was determined by using a column Inertsil 3 (5 µm, 4.6×250 mm) (Rizzolo et al., 2002).

Weight loss

This parameter was determined by observing the daily changes in weights of the sampled plants in the plastic bottles until their shelf-life were completed. This was carried out with the use of an electric sensitive balance.

Dry matter

Weight of oven-dry mass of edible leaves was taken after drying the harvested leaves at 65°C for 48 h. The dry matter after shelf- life was also done using the same procedure.

Shelf - life process

Lettuce plants were placed in plastic bottles filled with 100 ml of water and stored in a room of 30°C and atmospheric humidity of 71%. The weights of samples in the plastic bottles and the appearance of the leaves were observed each day and notices were made on leaf drooping, browning, and yellowing as these affects the products marketability quality. The produce shelf-life was determined by the number of days they were stored up to marketable or acceptable quality.

Data collection

Data were collected on nutritional composition, dry matter and weight loss. Nutritional parameters involved parameters such as Ca, Fe, Carotenoid, Chlorophyll and Vitamin C. Data was collected from the three central rows of each plot comprising six sample plants.

Data analysis

Data collected were subjected to statistical analysis, using Statistix Analytical Software version 9. Means differences were separated at 5% using LSD.

RESULTS

Soil and manure analysis for soil nutrients

The analysis for nutrient compositions in the soil and manure before planting as well as after harvest are shown in Tables 1 and 2. The result showed that, the soil was deficient in organic content, total nitrogen, calcium and potassium. It was slightly acidic while the manure, an alkaline. Soil analysis of the amended plots after the experiment showed that, plots amended with poultry manure performed the highest in all the parameters than the other amendments except with minimal level of total nitrogen due to the fact that, it was greatly used by the leafy vegetable for growth. This therefore suggests that poultry manure amended plots have greater chance of influencing the quality of the plant.

Quality parameters of lettuce after harvest

Interaction effect of treatments and cultivars on quality parameters assessed on lettuce after harvest

Calcium: There was a significant interaction effect ($p \leq 0.05$) of the treatments and cultivars on the calcium content of the lettuces. Great Lakes grown on soil amended with poultry manure recorded the highest calcium level (44.22) and was significantly different from N.P.K.-Eden (42.78), Control-Great Lakes (42.11), N.P.K.-Trinity (42.00), Control-Eden (41.67) and Trinity (41.11) in the decreasing order. These were statistically not different from each other. Likewise, Eden (43.56) and Trinity (42.89) cultivars grown on soil amended with poultry manure were significant not different and similar to the Great Lakes lettuce on the same soil (Table 3).

Iron: The interactions showed a significant difference ($p \leq 0.05$) on the iron content. Trinity (1.94) and Eden (1.90) grown on plots treated with poultry manure and Trinity (1.89) cultivated on plots treated with N.P.K. had a similar iron content. They were significantly different from the rest except Great Lakes lettuce (1.84) grown on plots treated with poultry manure. The three cultivars grown on the control plots and Great Lakes on N.P.K. treated plots were not different (Table 3).

Vitamin C: Likewise, the interactions showed a

Table 1. Soil and manure analysis before experiment.

Sample	Organic content (%)	Organic matter (%)	Total N (%)	Ca^{2+} cmol kg^{-1}	K^{+} cmol kg^{-1}	Fe mg kg^{-1}	Available P mg kg^{-1}	pH
Soil	0.72	1.24	0.11	5.2	0.14	20	154	6.1
Manure	15.96	-	2.29	11.84	4.47	11	2.46	8.12

Table 2. Soil analysis after harvest.

Sample	Organic content (%)	Organic matter (%)	Total N (%)	Ca^{2+} cmol kg^{-1}	K^{+} cmol kg^{-1}	Fe mg kg^{-1}	Available P mg kg^{-1}	pH
Control	0.83	1.43	0.05	8.73	0.11	23.33	255.08	6.78
P. manure	0.89	1.53	0.07	8.93	0.16	25.33	330.20	6.78
N.P.K	0.83	1.44	0.07	8.80	0.15	30.00	238.84	6.67

significant difference ($p \leq 0.05$) on the Vitamin C content of the harvested lettuces. Eden grown on plots treated with poultry manure recorded the highest vitamin C content, followed by Great Lakes and Trinity on poultry manure treated plots, Eden, Trinity and Great Lakes on N.P.K. treated plots and the least, by Eden, Trinity and Great Lake on the control plots in the decreasing order (Table 3).

Carotenoid: A significant interaction effect ($p \leq 0.05$) was recorded between the treatments and cultivars. Trinity (2.49), Eden (2.45) and Great Lakes (2.44) grown on poultry manure treated plots had a similar carotenoid level and were different from Trinity (2.34), Eden (2.28) and Great Lakes (2.23) harvested from plots treated with N.P.K. as well as Eden (2.12), Great Lakes (2.08) and Trinity (2.04) on the control plots in the decreasing order. The highest carotenoid level was recorded by the three lettuce cultivars grown on plots treated with poultry manure and the least, by the lettuce cultivars grown on control plots (Table 3).

Chlorophyll: Trinity (4.56) lettuce grown on plots treated with poultry manure contained the highest chlorophyll content and was significantly different ($p \leq 0.05$) from the rest except Great Lakes (4.25) grown on the same plots. Trinity (4.16) harvested from the control plots had the least chlorophyll content and this was significantly not different from N.P.K.-Eden (4.18), N.P.K.-Trinity (4.19) and Great Lakes (4.19) and Eden (4.18) on the control plots (Table 3).

Dry matter: The interaction of the soil amendments and the cultivars showed a significant difference ($p \leq 0.05$) on dry matter content. N.P.K.-Eden and Control-Trinity had 5.08 dry matter content which was the highest and different compared to the least, 4.91 recorded by Trinity grown on poultry manure treated plots. The highest was significantly not different compared to the rest except 4.97, 4.94 and 4.91 in the decreasing order recorded by Control-Eden, N.P.K.-Great Lakes and poultry manure-trinity, respectively (Table 3).

Interaction effect of soil amendment and cultivars on quality parameters assessed on lettuce after storage

Calcium: Eden cultivar grown on plots treated with poultry manure retained the highest level of calcium (42.67). It was significantly different ($p \leq 0.05$) from Trinity harvested from N.P.K. (40.89) treated plots as well as Eden (40.44) and Trinity (40.22) from the control plots in the decreasing order. Except for these, the rest of the interactions were not significant when compared against one another (Table 4).

Iron: Trinity harvested from poultry manure treated plots at storage end retained and recorded the highest level of iron of 1.90 mg g^{-1}. It was significantly different ($p \leq 0.05$) from all of the interactions except iron retained by the same cultivar grown on N.P.K. treated plots (1.86). Eden harvested from control plots had the least iron of 1.71 mg g^{-1} This was statistically not different Great Lakes from both N.P.K. treated (1.76) and control (1.75) plots as well as Eden from N.P.K. (1.76) treated plots (Table 4).

Vitamin C: Again, Eden harvested from plots treated with poultry manure recorded the highest level of vitamin C (20.78) at the end of shelf life. It was significantly different ($p \leq 0.05$) from all except Great Lakes (19.89) from the same plots. Great Lakes, Trinity and Eden grown on the control plots recorded the least vitamin C content of 15.44, 16.00 and 16.11 in the increasing order. They were significantly equal to Trinity and Great Lakes grown of N.P.K. treated plots (Table 4).

Carotenoid: Trinity grown on plots treated with poultry manure (2.48) at the end of storage maintained a significantly high level of carotenoid against the rest of

Table 3. Interaction effect of soil amendment and cultivars on some quality parameters of lettuces after harvest.

Treatment*Cultivars		Calcium	Iron	Vitamin C	Carotenoid	Chlorophyll	Dry matter
P. Manure	Gt Lakes	44.22^{a}	1.84^{bc}	20.89^{ab}	2.44^{ab}	4.25^{ab}	5.06^{ab}
P. Manure	Eden	43.56^{ab}	1.90^{a}	21.89^{a}	2.45^{a}	4.22^{bc}	5.01^{abcd}
P. Manure	Trinity	42.89^{abc}	1.94^{a}	20.22^{bc}	2.49^{a}	4.56^{a}	4.91^{d}
N.P.K.	Gt Lakes	43.89^{ab}	1.79^{cd}	17.56^{de}	2.23^{c}	4.21^{cd}	4.94^{cd}
N.P.K.	Eden	42.78^{bc}	1.83^{c}	19.11^{cd}	2.28^{c}	4.18^{de}	5.08^{a}
N.P.K.	Trinity	42.00^{cd}	1.89^{ab}	18.11^{d}	2.34^{bc}	4.19^{cde}	4.99^{abcd}
Control	Gt Lakes	42.11^{cd}	1.75^{d}	16.00^{e}	2.08^{d}	4.19^{cde}	5.02^{abc}
Control	Eden	41.67^{cd}	1.76^{d}	16.44^{e}	2.12^{d}	4.18^{de}	4.97^{bcd}
Control	Trinity	41.11^{d}	1.79^{cd}	16.44^{e}	2.04^{d}	4.16^{e}	5.08^{a}
LSD (5%)		1.41	0.06	1.57	0.11	0.04	0.10
CV (%)		3.52	3.47	9.00	5.04	0.96	2.10

Table 4. Interaction effect of soil amendment and cultivars on some quality parameters of lettuce after storage.

Treatment*cultivars		Calcium	Iron	Chlorophyl	Vitamin C	Carotenoid	Dry matter	Weight Loss
P. Manure	Gt Lakes	42.33^{abc}	1.80^{cd}	4.21^{a}	19.89^{ab}	2.34^{bc}	5.02^{d}	25.68^{b}
P. Manure	Eden	42.67^{a}	1.83^{bc}	4.20^{ab}	20.78^{a}	2.37^{b}	5.26^{abc}	28.68^{ab}
P. Manure	Trinity	41.67^{abcd}	1.90^{a}	4.24^{a}	19.00^{bc}	2.48^{a}	5.42^{a}	27.92^{ab}
N.P.K.	Gt Lakes	42.44^{ab}	1.76^{de}	4.17^{bc}	16.56^{de}	2.26^{cd}	5.11^{cd}	25.84^{b}
N.P.K.	Eden	41.67^{abcd}	1.76^{de}	4.15^{c}	18.00^{cd}	2.20^{d}e	5.30^{abc}	32.92^{a}
N.P.K.	Trinity	40.89^{cd}	1.86^{ab}	4.16^{c}	16.89^{de}	2.33^{bc}	5.22^{abcd}	27.53^{ab}
Control	Gt Lakes	41.11^{bcd}	1.75^{ef}	4.16^{c}	15.44^{e}	2.03fg	5.14^{bcd}	27.27^{ab}
Control	Eden	40.44^{d}	1.71f	4.16^{c}	16.11^{e}	2.11ef	5.36^{ab}	27.77^{ab}
Control	Trinity	40.22^{d}	1.78^{de}	4.14^{c}	16.00^{e}	1.96g	5.16^{bcd}	33.17^{a}
LSD (5%)		1.54	0.04	0.03	1.58	0.1	0.22	6.79
CV (%)		3.96	2.6	0.87	9.51	4.89	4.45	25.31

the interactions. It was significantly different from all of the interaction means. Eden (2.37) subjected to the same treatment was second to the highest. It was also different from the rest except Great Lakes (2.34) and Trinity (2.33) subjected to poultry and N.P.K. treatments respectively on the field. Trinity cultivar grown on control plots had the least carotenoid content (1.96). It was not different from Great Lakes (2.03) grown on the same control plots (Table 4).

Chlorophyll: Great Lakes and Trinity subjected to poultry manure treatment on the field recorded the highest chlorophyll content at the end of storage. They were significantly different from all of the cultivars subject to the N.P.K. treatment and the control that had the least, except Eden, the second highest subjected to the same treatment as shown in (Table 4).

Dry matter: Trinity subjected to poultry manure treatment on the field had the highest dry matter content (5.42) and was significantly different ($p \leq 0.05$) from Great Lakes grown on plots subjected to N.P.K. (5.11) and poultry manure (5.02) respectively in the decreasing order. The rest of the interaction means were significantly not different from one another (Table 4).

Weight loss: Trinity (33.17) and Eden (32.92) grown on the control and N.P.K. treated plots respectively suffered the highest weight loss while Great Lakes subjected to N.P.K. (25.84) and poultry manure (25.68) treatments suffered the least. Both categories were significantly different from each other. The rest of the interactions statistically recorded the same weight loss (Table 4).

DISCUSSION

Minerals and vitamin composition of lettuce

Lettuce like other leafy vegetable is an important source of minerals and vitamins needed in our diets for good functionality and repairs of the human body. Fresh vegetables like lettuce is judged on visual characteristics as well as nutritional quality (Rattler et al., 2006) such minerals and vitamins composition. Results of the experiment showed a significant impact of the soil amendment, varietal difference on the level of minerals and vitamin composition of lettuces studied and analysed. The three lettuce cultivars, Great Lakes, Eden and Trinity were rich in calcium, vitamin C and had a considerable good amount of iron in a significant different quantities. The difference in the minerals and vitamin level might also be due to the genotypic difference since they are grown under the same environment (Ojetayo et al., 2011).

Calcium composition of lettuce

The interaction of the soil amendments and the cultivars explains that, fresh Great Lakes with the highest calcium content was harvested from plots for which the soil was treated with poultry manure (organic fertilizer).The high calcium content from the plot treated with poultry manure can be attributed to relatively ample amounts of calcium in the chemical composition of chicken manure (Bokhtiar and Sakurai, 2005).

There was a significant drop in the level of calcium in the lettuce after shelf life. Yet, the nutritional lost was minimal to cause a drastic change in the level of significance. Regardless of the lost, Great Lakes cultivar maintained the highest calcium and the least, by Trinity. The results also show that Great Lakes performed well with either poultry manure or N.P.K. fertilization and even the control. The difference might also be due to the genotypic difference since they are grown under the same environment (Ojetayo et al., 2011).

Also reduction in calcium content of lettuce after storage shows that vegetables deteriorate immediately after harvest irrespective of the storage method adopted (Babarinsa, 2000). Again, temperature and relative humidity are critical factors in the maintenance of quality during storage, which reconfirms the inconsistency of the mineral quality of lettuce irrespective of the fertilizer type used. This agrees with the findings of Babatola and Olaniyi (1997) and Olaniyi et al. (2010).

Iron composition of lettuce

Freshly harvested Trinity had the highest iron content followed by Eden and the least, in Great Lakes. This is completely a reverse occurrence seen with calcium content in lettuce. It would be right to say based on the result that, cultivar of lettuce which contains significant higher calcium has a considerable low iron content. The poultry manure released a significant higher iron to the lettuce in the soil compared to N.P.K. and the least, by the control. The effect could be due to the fact that organic carbon acts as a source of energy for soil microorganism, which upon mineralization releases organic acids that decreased soil pH and improves availability of Fe (Bokhtiar and Sakurai, 2005). The effect of Fe on the cultivars as seen in the interaction showed Trinity had the highest iron content along with Eden. This outcome might have had a significant impact on the level of the iron in the three lettuce cultivars studied though much is influenced naturally by genetic makeup.

The analysis after shelf life however showed a slight change in the iron content. Trinity retained significantly a higher iron content compared to Eden and Great Lakes which had a significant equal iron. The effect of fertilization with the poultry manure and N.P.K. remained unchanged. The marginal losses in iron content incurred by the cultivars could mainly be due to external factors which triggers high water loss in the lettuce as well as the living produce falling back on the reserved nutrients to survive. Also reduction in Fe content of lettuce after storage shows that vegetables deteriorate immediately after harvest irrespective of the storage method adopted (Babarinsa, 2000).

Vitamin C

Vitamin C is known to serve as antioxidant that offers protection against some form of cancer. This along with some other phytochemicals and antioxidants reduce the risk of cancer of the respiratory system and intestinal tract (Wolford and Banks, 2013). Vitamin C may vary in concentration depending on the cultivar type. The results of the study showed varying concentration of vitamin C in the cultivars. Eden was richer in ascorbic content, followed by Trinity and the least in Great Lakes.The significant difference in vitamin C obtained among the cultivars might also be due to the genotypic difference since they are grown under the same environment (Ojetayo et al., 2011).

The poultry manure (organic fertilizer) and control acted differently from each other with poultry manure contributing to a significant higher amount of the vitamin especially on Eden while the control performing poorly. Schuphan (1974) found that spinach and lettuce grown organically were higher in ascorbic acid compared to those grown conventionally, using composted manure over organic fertilizers .The three cultivars also performed poorly on the control soil as they had the least vitamin C content.

The storage period and light had a significant impact on the lettuce by causing the three cultivars to suffer a loss in vitamin C though it is said that cut vegetables normally retain vitamin C content longer. Vitamin C like any antioxidants is susceptible to degradation when exposed to oxygen or light (Gil et al., 2006) after harvest. These could be a reason to why the lettuces; Great Lake and

Trinity different from Eden suffered a lost in vitamin C increasingly. According to Kader (2002) and Lee and Kader (2000), postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and the losses are enhanced by physical damage, extended storage duration, high temperatures and low relative humidity.

Phytochemicals

Carotenoid

The three lettuce cultivars showed no significant difference in the carotenoid level in terms of varietal effect. This could mean that they all have similar genetic traits responsible for carotenoids concentration. This is contrary to carotenoid concentration in some wild accessions of lettuce due to genetic variation reported by Mou (2005). Soil nutrients made available to the vegetable from different sources, that is organic and inorganic significantly had an impact on carotenoid level in the lettuces after harvest and shelf life. This effect was seen in the interaction of the cultivars and soil amendment materials. And numerous factors such as maturation stage (Rodriguez -Amaya, 2000), difference in the plant cultivars (Olson, 1999), soil type, effect of agrochemicals and cultivation conditions (Amaya-Farfan, 1999) may have interfered with the carotenoid content (Cardoso et al*.,* 2009) after harvest. The analysis after harvest showed that Great Lakes, Eden and Trinity when subjected to poultry manure application, recorded the highest level of carotenoids. This is similar to the outcome of Rattler et al. (2006) where they had increased in β-carotene content in lettuce, a form of carotenoid as result of using and increasing compost manure (organic fertilizer). The lettuce cultivars responded well to the inorganic fertilizer (N.P.K.) producing carotenoid levels close but different to the organic source. Rattler et al. (2006) also had a lower level of carotenoids (β-carotene) with application of mineral fertilizer. It thus means that, different fertilizer type (organic and inorganic) had a significant impact on the level of carotenoids. The least carotenoids level was produced by the lettuces when grown on the control soil as a result of limited nutrients in Table 4. That is, lettuce grown on soil lacking much nutrients and the effectiveness of a cultivar to respond to the soil nutrients from different nutrient source may affect the content of carotenoids.

A lost in the carotenoid levels in the three cultivars occurred and hence caused a slight difference in the quantities remained in them after shelf life. The results showed that, Trinity had the capability of retaining significantly, the highest carotenoid level with poultry manure fertilization, followed by Eden and Great Lakes with the same treatment. Meanwhile, Trinity without any fertilizer treatment performed poorly in retaining the lowest level of carotenoids. This outcome could mean that, type of fertilizer application can affect the rate at which lettuce loses carotenoids in storage. It is also possible that genetic makeup may slightly have an effect on carotenoids lost caused by damage through injuries, temperature and humidity variation at storage. Amaya-Farfan (1999), attributed carotenoids difference in plants to several factors but the most relevant to the study after shelf life is the presence of damage to plant structure, exposure to light and storage condition which might have been a responsible cause. And According to Azevedo and Rodriguez-Amaya (2005), carotenoids content is affected by climate alterations, with exposure to sunlight and higher temperatures, increasing the biosynthesis of carotenoids but, at the same time, inducing photo-degradation, thus reducing their levels in plants. This phenomenon could have taken place before end of shelf life.

Chlorophyll content

Chlorophyll is a plant pigment which appears green and gives the green colour of plants. The treatments had a significant effect on the chlorophyll content of the harvested lettuces. The lettuce cultivar that had poultry manure treatment as fertilizer recorded significantly, a higher chlorophyll content than those with N.P.K. and no treatment application. This is in support of the argument by Kempraj (2012) that, the presence of nitrogen in soil and chlorophyll in plants are directly related. He further stated that, chlorophyll could be used as an indirect indicator of nitrogen levels in fertilizer management. Based on this claim, it is possible that the poultry manure released more nitrogen to the lettuces than N.P.K.

Trinity cultivar responded well to the poultry manure fertilization by producing the highest chlorophyll content and was not significantly different from Great Lakes when subjected to the same treatment. Trinity cultivar however performed poorly when subjected to N.P.K. and the control treatment to produce the lowest chlorophyll contents. This is due to inadequate nutrients especially nitrogen to be made available to crops for absorption.

There was a significant drop in chlorophyll due to the interaction of the treatments and cultivars at the end of shelf life. Trinity together with Great Lakes retained the highest chlorophyll content different from the chlorophyll recorded by the other interactions except with Eden, subjected to poultry manure application. Also, all cultivars retained very low chlorophyll content when subjected to N.P.K. and the control except Great Lakes subjected to N.P.K., which did fairly good by retaining second highest amount of chlorophyll. It is as result of the organic fertilizer (poultry manure) releasing enough nutrients to the various cultivars.

Also, the drop in the chlorophyll level at the end of shelf life might be due to decomposition. It is claimed by

Webexhibits (2013) that, chlorophyll decomposed in presence of sunlight and hence plants replenish by producing more. This claim could therefore support this outcome as the three cultivars of lettuce maintained their chlorophyll level immediately after harvest and had a fall due to the fact that, they were taken of their growth medium and parent stalk which helped in the replenishing process. Also, storage period and adverse condition within the storage area might have affected the lettuce and aided in the decomposition of the chlorophyll.

Dry matter content

The soil amendment materials and the cultivars showed no significant individual effect after harvest. This could mean that, the different cultivars show no genetic variation in terms of dry matter and thus have similar moisture composition. And probably, the fertilizer type does not contribute to the dry matter content of the lettuce and any variations may be due to other factors. The analysis of dry matter content after shelf life was similar to that recorded after harvest except that, Great Lakes cultivar recorded a significant low dry matter that differed from Eden and Trinity which were similar. It is possible that the formation of the crisp head by Great Lakes reduced the surface areas of the leaves and hence, interfered with rate of respiration which results in moisture loss.

The interaction between the cultivars and treatments had a significant impact on the dry matter of lettuce both after harvest and end of shelf life. Eden and Trinity subjected to NPK and no treatment respectively had the highest dry matter content after harvest whiles Trinity grown with poultry manure fertilization recorded the least dry matter. It is generally reported that, leafy vegetables as well as root vegetables and tubers have the trend for higher dry matter contents when organically grown (Woëse et al*.,* 1997; Bourn and Prescott, 2002; AFSSA, 2003). The study revealed that, difference may occur in vegetables that have similar or same genetic traits if organically grown. That is, after storage, Trinity and Great Lakes fertilized with poultry manure, recorded the highest and lowest dry matter content respectively. The result also shows that, Great Lakes have the tendency to retain enough or probably have a high moisture content compared to Trinity. It also explains that, moisture content and dry matter content relate in the opposite direction.

Weight loss

The three cultivars of lettuce recorded no significant difference in terms of weight loss. It could be attributed to the fact they have similarities in moisture (water) composition regardless with difference in other characteristics as well. Likewise, type of fertilizer as a treatment also did not singly caused any significant effect. The absorption of water from the soil to other parts of the crop depends on the roots and basically, the three cultivars might have the same rooting system.

Contrary to the above, the interaction between the cultivars and the soil treatment slightly showed a significant impact on the weight loss of lettuce. That is, Eden together with Trinity suffered the highest weight loss fertilized with N.P.K. and the control compared to Great Lakes subjected also to N.P.K. and poultry manure which suffered the least weight loss under such circumstances. They were not different when compared against the rest.

CONCLUSION AND RECOMMENDATION

The soil treatment had a significant impact on the mineral composition, vitamin C content and chlorophyll concentration but not the carotenoids. The cultivars varied in the levels of calcium, iron, vitamin C and chlorophyll content due to variation in the expression of inherent genes with the cultivars and amount of nutrients made available to the lettuces. These minerals, vitamin C as well as the chlorophyll and carotenoids were relatively in high quantities. However, there was a significant drop in the mineral composition, chlorophyll, carotenoid and vitamin C.

Based on the results of the experiment, highly decomposed and treated poultry manure could be encouraged for use as an alternative for other sources of fertilizers for the cultivation of lettuce as it records higher levels of minerals, vitamin C as well chlorophyll irrespective of the cultivar type.

Also, in homes of no refrigerators, freshly harvested lettuce plants should be kept in vessels of considerable amount of water as the process prolongs the produce shelf life for three to four days.

Conflict of Interest

The authors have not declared any conflict of interest.

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