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Full Length Research Paper

Effect of lupine *(Lupinus* Spp.*)* intercropping and seed proportion on the yield and yield component of small cereals in North western Ethiopia

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Wheat, barley and finger millet as a major crop and lupine as a companion crop are food crops often traditionally grown in an intercropping in North Western Ethiopia. The experiment was conducted on intercropping of lupine (*Lupinus albus* L.) with wheat *(Triticum aestivum),* barely *(Hordeum vulgar)* and finger millet *(Eleusine coracana)* in 2009 at Adet Agricultural research station. The treatments were sole wheat at a seed rate of 175 kg/ ha, sole barley at a seed rate of 125 kg/ ha, sole finger millet at a seed rate of 30 kg/ ha, sole lupine at a seed rate of 90 kg/ h and 25, 50 and 75% of the sole lupine seed rate combined with each full cereal seed rate to determine the effect of lupine intercropping and seed proportion on the growth, yield and yield component; and lodging of wheat, barley and finger millet. The trial layout was a completely randomized block design with three replications. SAS software's were used to compute the analysis of variance. Increasing in lupine seed proportion in a mixture, delay in finger millet days to heading and maturity also significantly increased. The yield and yield component of most cereals were not significantly affected when they were intercropped with lupine in all seeding ratios except finger millet plant height, harvest index and wheat total biomass yield. Hence, growing cereals in association with lupine was not showed its yield reduction and the farmer's primary objective of maintaining a 'full' cereal yield was attained. Intercropping lupine with cereals gave physical support for cereals particularly in high lupine seed proportion. The combined yield advantage was greater than one in the cases of lupine-wheat followed by lupine-finger millet mixtures at all seeding ratios. Hence, two of the best combinations which were gave higher land use efficiency are the lupine-wheat mixture at the 75:100 seeding ratio (49.4%) followed by the lupine-finger millet mixtures at the 75:100 seeding ratio (29.4%).

Key words: Wheat, barley, finger millet, lupine, intercropping, seed proportion.

INTRODUCTION

Intercropping is the cropping system involving the growing of two or more crops in the same piece of land at the same time or relayed which could compute for growth resources for certain growth period. This farming practice

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is a popular crop production system used in subsistence tropical agriculture and is very common in the semi arid areas of Africa (Connolly et al*.,* 2001). It is also a cropping practice that possess the potential of providing valuable ecosystem services such as improved pest control (Mitchell et al*.*, 2002), increased resource use efficiency (Hauggaard-Nielsen et al*.,* 2001), lowered weed infestation levels (Liebman and Dyck ,1993) in crop livestock mixed farming system.

In many parts of Ethiopia, farmers traditionally harvest only once in a year on sole crop basis even in high rain fall areas. Moreover, in the past much research efforts have been directed towards improving technology for sole cropping. Such traditional farming did not insure the production of adequate food for a family especially under conditions where average land holding is very small (Nigusei, 1994). In Ethiopia, different crops are grown traditionally in mixtures by small farmers to satisfy dietary needs, spread the period of peak demand for labor and minimize the risk associated with climate conditions. Thus, the most important intercrop mixtures used by farmers in Ethiopia can be grouped in to four broad categories: cereal-cereal; cereal-legume; tree-annual crop and legum-legum associations' (Yayeh et al*.*, 2014). Intercropping cereal with a legume, however, is relatively the most common in most parts of the country.

Cereals are the major food sources in Ethiopia and farmers regard the cereal as the major component of an intercrop (EIAR, 1992). Indeed, the traditional objective has been to produce a full yield of cereal (as much as with a sole crop) while the associated legume yield is considered as additional yield (Yayeh et al*.,* 2014).

Lupine *(Lupinus* Spp*.)* is one of the major highland food legumes grown in Ethiopia (Yayeh et al*.*, 2014). Its production is limited in North West Ethiopia and mainly used to prepare local drinks (Ali et al*.,* 2006). It is grown on an area of 25,526 ha with an annual average production and yield of 287, 17.3 t/ha and 1100 kg/ha (CSA, 2004), respectively. Out of this, 37% of the total land was cultivated by West Gojam. Farmers use intercropping different legumes with other crops as one of the strategies to overcome the shortage of arable land and attribute several crops for diversification of crop products, high productivity per unit area and for maintenance and improvement of soil fertility (Aleligne and Steven, 1987). Lune have been traditionally gown as intercrop with cereals and oil crops by low input farmers and is restricted to low-income classes, to times of drought (Jansen, 2006). They grow it as traditional additive system of intercropping in which lupine used as minor crop and cereals as major crop (Yayeh et al*.*, 2014).

The current trend in global agriculture is to search for highly productive, sustainable and environmentally friendly cropping systems (Crews and Peoples, 2004). One of the strategies to improve food security would be the inclusion of grain legumes either intercropped with cereal or in rotation with it. Farmers in West Gojam are seriously constrained by small farm size of 1.42 ha/household due to increase human population (CSA, 2007). Thus, intercropping lupine with cereals is cultivated to a greater extent than before because of its adaptability, stability and feasibility of production under low soil fertility status and biotic. It is also an annual legume, and non climbing growth habit and has high levels of protein (Jansen, 2006).

Moreover, the tape root system of lupine could exploit water and nutrients from deeper soil layers than cereals (Jansen, 2006). Jansen (2006) and Gardner and Boundy (1983) also point out wheat intercropped with lupine has access to a larger pool of Phosphors, Manganese and Nitrogen than sole-cropped wheat. Production cereals in intercrop with lupine could also provide a rotational yield response to main season crops (Petch and Smith, 1985). However, management of cereals intercropped with lupine follows simple natural principles, and its practice is limited only by the imagination of farmers. They used less than 25% lupine seed rate with full cereal seed rate (Yayeh et al*.,* 2014). No published studies have been made in research areas to improve the productivity of this kind of cropping system. As a result, the yield of cereal crops vary considerably among farmers and in most cases the yield advantage is unknown. Therefore, the objective of this paper was to estimate the effect of lupine and seeding proportion on major cereal crops in lupine-cereal intercropping systems.

MATERIALS AND METHODS

Description of the study area

The study was conducted in the 2009 rain fed cropping season at Adet Agricultural Research station (AARC), North Western Ethiopia. It is located between 11°17' N latitude and 37°43′ E longitude with an altitude of 2240 m.a.s.l (AARC, 2002).

According to Gonder soil testing laboratory center (2009), the soil characteristics of experiment site were clay as shown Table 1.The study area receives a uni-modal rainfall which extends early June to late September with regard to its monthly distribution June, July and August are the three important months with high rain fall and more or less uniform spatial distribution (Aleligne and Steven, 1987). According to Adet Metrological station (2009), the total annual rainfall during the experimental growing season was 975.3 mm which is less than the 23 year average total annual rainfall (1253.4 mm) (Figure 1). The mean monthly minimum and maximum temperatures during the growing season were 11 and 27.2°C which is greater than the 23 year average mean monthly minimum (9.1°C) and maximum (25.7°C) temperatures (Figure 2).

Field experimental design

Plots were laid out in randomized complete block design (RCBD) with three replications. Spacing between plots and replications were 0.5 and 1 m respectively. There were nine intercropping in additive series (25, 50 and 75% of recommended lupine seed rate with full cereal seed rates) and four sole cropping systems (pure stands of lupine, wheat, barley and finger millet). The plot size was 12 m^2 (2*6 m). Sole lupine was common to all lupine-cereal combinations

Table 1. Physico-chemical properties of the soil at Adet research station.

Chemical soil properties		Mechanical properties	
PH	6.06	Sand (%)	28.00
OC (%)	2.47	Clay (%)	46.72
Total N (%)	0.18	Silt (%)	25.28
Av.P (ppm)	1.98	Class	Clay
CEC	37.97		

CEC: Cation exchange capacity measured in cmol (+)/kg soil (NHAc), Av.P: Available phosphors in ppm and OC: organic carbon.

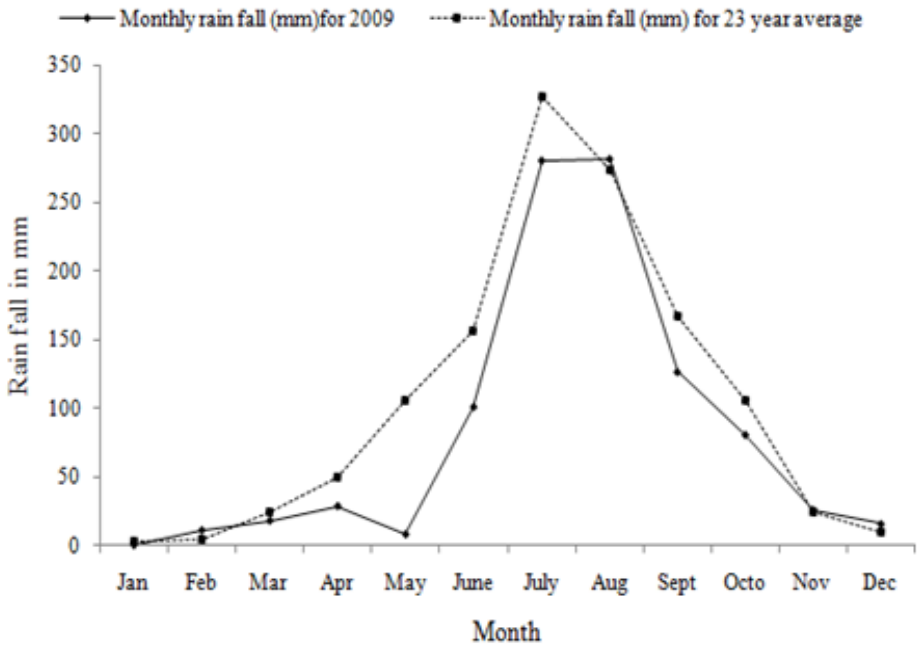

Figure 1. Mean monthly rainfall (mm) of the study area for 23 year average and 2009 cropping season.

for comparison purpose.

Sowing method and management practices

The experiment was conducted in rainfed season (2009). Additive series intercropping system was used which is cropping of the base crop/cereals at optimum level and the addition of a proportion of the minor crop/ lupine with the main crop being the one of primary importance because of economic or food production reasons in the area. Pure stands of lupine, wheat, barley and finger millet as well as nine lupine-cereal mixtures in three seeding ratios in additive series (25, 50 and 75% of recommended lupine seed rate with full cereal seed rates) were planted. Sole cropping of lupine, wheat, barley and finger millet were planted at a recommended seeding rate of 90, 175, 125 and 30 kg/ha, respectively. In sole cropping, lupine was planted in an inter-row space of 30 cm; and wheat, barley and finger millet were broadcasted.

In the intercropping system, first lupine row was established in the inter-row spacing of 120, 66 and 35 cm for the 25, 50 and 75% seed proportion, respectively, and full cereal components were broadcasted. Lupine was planted after establishments of cereal crops. For all intercropping systems space between lupine plants were 5 cm. All plots were received a basal application of Diammonium phosphate (DAP) at the rate of 100 kg/ha at planting. For cereal components, 100 kg/ha Urea was applied except the sole lupine treatment assuming the lupine was benefit from self-fixed nitrogen. One third basal and two third top-dressed application of UREA were applied during planting time and at tillering stage of sole and intercropped cereals, respectively.

Data collected

Agronomic attributes of cereals: Plant height in cm, spike length (cm) of barley and wheat and finger length (cm) of finger millet, seed per spike of barley and wheat, tiller per plant, finger per plant, stand cover per meter square, thousand seed weight, biomass and grain yield; and harvest index (%) and lodging index (%). Moreover, land use efficiency was also determined by land equivalent ratio (LER) which was calculated using the formula developed by Willey and Osiru (1972):

$LER = (Y_{AB}/Y_{AA}) + (Y_{BA}/Y_{BB})$

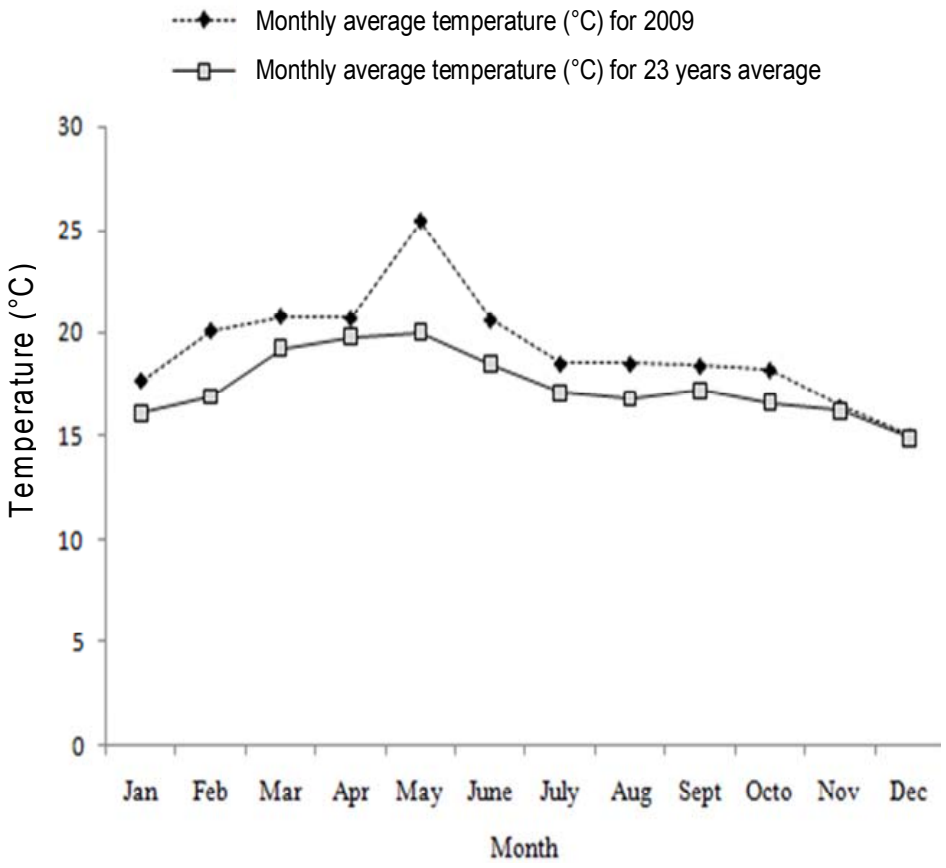

Figure 2. Mean maximum and minimum air temperature (T°C) of the study area for 23 year average and 2009 cropping season.

Y_{AB}= Yield of crop A when mixed with crop B
Y_{BA}= Yield of crop B when mixed with crop A
Y_{AA}= Yield from sole planted crop A
Y_{BB}= Yield from sole planted crop B

Lodging /index /percentage was proposed to be calculated using the formula developed by Caldicott and Nuttall (1979). Lodging scale was from 0 to 5 where, 0 mean no lodged plants and 5 mean plants completely lodged.

$$\text{Lodging Index} = \frac{\sum (\text{lodging score x their respective \% of area lodged})}{5}$$

Data analysis

Data were statistically subjected to analysis of variance (ANOVA) using JMP-5 (SAS, 2002). Separate Analysis of variance was performed over the three lupine-cereal combinations to determine yield and yield component of each cereal crops. However, combined analysis of variance was conducted over the three lupine-cereal combinations to determine land use efficiency using land equivalent ratio of each cropping system. In all the comparisons, the level of significance was set at $\alpha = 0.05$. Mean comparison for the treatments were computed using each pair Turkey-HSD test for parameters found to be significantly different at a given level of significant.

RESULTS AND DISCUSSION

Phenology

The influence of intercropped lupine crop proportion on cereal days to 50% heading and 50% maturity in lupine-cereal intercropping is presented in Table 2. The analysis of variance indicated that these treatments significantly influenced ($P<0.01$) only days to maturity of finger millet in the case of lupine-finger millet intercropping (Table 2). Days to maturity for sole finger millet were 158 as compared to 162 for 50:100 seeding ratio which took longer duration to maturity (Table 2). Result of this investigation also showed that increase in companion crop proportion in a mixture, delay in finger millet days to heading and maturity also significantly increased.

Intercropping of lupine with finger millet delays the days to maturity of finger millet as compared to sole finger millet perhaps due to competition for light in which the two crops were growing together for a long period of time. The second reason for delayed in maturity date of finger millet across increasing seeding ratio could also be attributed to as the minor crop proportion increases, the intra-specific competition between lupine stands hastens

Table 2. Effect of lupine-cereal intercrops on date of heading and maturity of cereals at Adet Agricultural research center, West Gojam in 2009.

Treatments and statistics	Mean	
	50 % DH	50 % DM
Lupine–wheat intercropping		
Sole wheat	62.00[a]	128.33[a]
25% Lupine+100% wheat	61.33[a]	129.00[a]
50% Lupine+100% wheat	61.67[a]	128.33[a]
75% Lupine+100% wheat	61.67[a]	128.33[a]
CV (%)	1.208	0.55
Lupine–Barely intercropping		
Sole barely	50.67[a]	85.67[a]
25% Lupine+100% barley	50.67[a]	85.33[a]
50% Lupine+100% barley	51.00[a]	86.00[a]
75% Lupine+100% barley	51.33[a]	86.00[a]
CV (%)	0.98	0.47
Lupine–Finger millet intercropping		
Sole finger millet	104.33[a]	158.67[c]
25% Lupine+100% f/millet	104.67[a]	160.00[b]
50% Lupine+100% f/millet	103.33[a]	162.33[a]
75% Lupine+100% f/millet	103.67[a]	163.00[a]
LSD (0.05)	-	0.23
CV (%)	2.60	0.25

Values (means) connected by different superscript letters are significantly (P<0.05) different within columns according to Tukey-HSD tests. DH: 50 % date of heading and DM: 50 % date of maturity. F/millet: finger millet.

efficient utilization of the growth resources thereby increases yield component parameters of lupine (Gabatshele et al*.,* 2012).

The result also in parallel with the findings of Gabatshele et al*.* (2012), who found that Maize planted in Maize-cowpea intercropping, had longer flowering data as compared to maize planted in sole maize. Lupine growth causes high shading effect over the finger millet and then delayed maturity period. On the other hand, non significant differences (P>0.05) were observed to barley and wheat days to heading and days to maturity in intercropping in any change in companion crop proportion when compared to sole cropped (Table 2). This is probably because barley uses growth resources without lupine crop competition throughout all growth stages. Hence, barley has rapid and short growing period (85 days) as well as tillering ability and early germination (6 days). Barley dominates the minor crop (lupine) in all proportions in the system. Similarly, wheat had the second fast and short growing period (128 days) in lupine-wheat intercropping but much less than barley and used growth resources earlier than the minor crop (lupine) with a high competitive ability.

Growth and yield components

The plant height of finger millet was significantly affected by intercropped lupine proportion (P<0.05) in the case of lupine-finger millet intercropping system (Table 3). Maximum plant height was recorded at 75:100 seeding ratio (114.0 cm) due to struggle for light in such very dense stands while minimum plant height was observed in finger millet pure stand (102.7 cm) which did not differ statistically from 25:100 seeding ratio probably due to lower inter-specific competition for growth resources especially light between the component species (Table 3). However, finger length (cm), number of finger per plant and tiller per plant of finger millet were not significantly (P>0.05) affected by studied treatments (Table 3).Likewise, the plant height (cm), spike length (cm), seed per spike, tiller per plant, population per m^2 and 1000-seed weight (gram) of barley and wheat were not significantly affected by the same treatments (P>0.05) when each crop was intercropped with lupine in three seeding ratios (Table 3).

Intercropping lupine with barley and wheat in three seeding ratios did not show different response as compared to respective sole cropped (Table 3). This was probably because of early sowing of cereals which helps the crop to express its potential and makes favorable condition in utilization of growth resources in lupine-cereal intercropping. The result is in agreement with Gabatshele et al*.* (2012), who stated that maize growth and yield component were not significantly affected by maize-cowpea intercropping in different seeding ratios.

Table 3. Effect of lupine-cereal intercrops in additive series on growth and yield component of cereals at Adet Agricultural research center, West Gojam in 2009.

Treatments and statistics	Mean					
	HP(cm)	SP (F)L (cm)	SE/SP (F/PL)	TI/PL	ST/m^2	TSW (gram)
Lupine–wheat intercropping						
Sole wheat	153.22^a	7.73^a	17.00^a	16.33^a	317.11^a	21.25^a
25% Lupine+100% wheat	156.33^a	7.73^a	16.73^a	15.87^a	363.77^a	22.28^a
50% Lupine+100% wheat	151.00^a	7.93^a	17.13^a	15.13^a	339.11^a	22.51^a
75% Lupine+100% wheat	151.00^a	7.80^a	16.00^a	13.47^a	325.55^a	22.07^a
CV (%)	4.63	4.76	3.9	18.77	6.29	3.61
Lupine–barely intercropping						
Sole barely	113.00^a	9.13^a	21.27^a	17.40^a	1160.89^a	39.06^a
25% Lupine+100% barley	115.73^a	9.20^a	22.60^a	17.83^a	1220.89^a	38.82^a
50% Lupine+100% barley	114.53^a	9.07^a	20.60^a	18.07^a	1666.66^a	39.01^a
75% Lupine+100% barley	116.53^a	10.07^a	21.47^a	18.27^a	1450.63^a	39.00^a
CV (%)	2.44	6.75	8.21	2.99	29.62	0.92
Lupine–finger millet intercropping						
Sole f/millet	102.67^b	9.37^a	8.13^a	12.20^a	147.33^a	***
25% Lupine+100% F/millet	103.79^b	8.99^a	8.40^a	10.73^a	212.67^a	***
50% Lupine-+00% F/millet	111.47^{ab}	8.89^a	8.27^a	9.00^a	181.67^a	***
75% Lupine+100% F/millet	114.03^a	8.81^a	8.20^a	10.60^a	198.67^a	***
LSD (0.05)	7.10	-	-	-	-	-
CV (%)	3.29	3.76	7.13	26.04	16.85	-

Values (means) connected by different superscript letters are significantly (P<0.05) different within columns according to Tukey- HSD tests.HP: Plant height in cm; SP (F) L: Spike length (cm) of barley and wheat and finger length (cm) of finger millet; SE/SP: Seed per spike of barley and wheat; TI/PL: Tiller per plant; F/PL: Finger per plant; ST/m$^{2:}$ Stand cover per meter square and TSW: Thousand seed weight.***Difficult to measure.

Competitive ability of barley in particular and wheat in general for growth resources was higher than lupine in all seeding ratios which was also confirmed by Yayeh et al. (2014). This result was in agreement with the conclusion of Brandt et al. (1989), who found that no effect of intercropping clover cultivars on wheat yield components and phenological parameters.

Biomass, grain yield and harvest index

Biomass yield

Wheat biomass yield: The results showed that the biomass yield of wheat in lupine-wheat combinations significantly (P<0.05) influenced by intercropped seeding proportions (Table 4). Although, there was a general reduction in the biomass yield of wheat as a result of intercropping as compared to sole cropped wheat, lowest biomass yield was recorded in lupine-wheat intercropping at 75:100 seeding ratio (3666 kg/ha) (Table 4). This could be due to competition for light and nutrients. In the same experiment, the highest biomass yield was recorded in sole cropped wheat (7000 kg/ha) as compared to lupine-wheat combinations at all seeding ratios (Table 4) due to absence of inter-specific competition. Generally, at high crop proportion, lupine reduced the biomass yield of the wheat component. This result corroborates with Hauggaard-Nielsen et al. (2005), who found that wheat biomass yields falling with increased plant density in wheat-pea intercropping.

Barely biomass yield

The biomass yield of barely revealed a non significant effect of companion crop proportions (P>0.05) in the case of lupine-barely intercropping (Table 4). This could be explained due to nearly complete dominance of barely over lupine in all proportions at the early stage of lupine, and so no inter-specific competition of growth resources between component species that reduces the biomass yield of barely.

This is a common observation that one species grows faster than the other(s) in intercrops. A faster initial growth, that often leads progressively to dominance in terms of resource capture and thus to prospects of greater biomass growth and yield (Fukai and Trenbath, 1993).

Table 4. Effect of lupine-cereal intercrops on grain yield, biomass yield and harvest index of cereals at Adet Agricultural Research Center, West Gojam, in 2009.

Treatments and statistics	Mean			
	GY	BY	HI	LI
Lupine–wheat intercropping				
Sole wheat	2030[a]	7000[a]	29.77[a]	18.13[a]
25 % Lupine+100 % wheat	2494[a]	4667[ab]	54.08[a]	17.45[a]
50 % Lupine+100 % wheat	2127[a]	5667[ab]	39.99[a]	13.67[a]
75 % Lupine+100 % wheat	1935[a]	3667[b]	52.79[a]	11.90[a]
LSD (0.05)	NS	19.98	NS	NS
CV (%)	9.85	23.32	19.49	35.6
Lupine–barely intercropping				
Sole barely	3805[a]	10667[a]	35.57[a]	22.00[a]
25 % Lupine+100 % barley	2845[a]	8417[a]	33.79[a]	21.43[a]
50 % Lupine+100 % barley	2912[a]	9400[a]	31.17[a]	11.13[a]
75 % Lupine+100 % barley	3301[a]	9267[a]	35.85[a]	16.70[a]
LSD (0.05)	NS	NS	NS	NS
CV (%)	16.30	11.48	10.73	39.5
Lupine–finger millet intercropping				
Sole finger millet	2936[a]	18667[a]	15.58[a]	20.32[a]
25 % Lupine+100 % f/millet	2323[a]	22000[a]	10.44[b]	16.00[ab]
50 % Lupine+100 % f/millet	2389[a]	22000[a]	10.97[ab]	15.87[ab]
75 % Lupine+100 % f/millet	1935[a]	23333[a]	8.50[b]	11.63[b]
LSD (0.05)	-	-	3.60	5.31
CV (%)	24.29	16.55	15.86	16.68

Values (means) connected by different superscript letters are significantly (P<0.05) different within columns according to Tukey-HSD tests. GY: Grain yield in kg/ha; BY: Biomass yield in kg/ha; LI: Lodging index in % and F/millet: finger millet.

In the present study, this is true particularly for lupine-barley intercropping systems. However, due to the same reason to the reduction of wheat biomass yield in lupine-wheat intercropping, there was a general decrease in barley biomass yield in lupine-barley intercrops from sole barley (10667 kg/ha) to 50:100 (9400 kg/ha) ,75:100 (9267 kg/ha) and 25:100 (8400 kg/ha) seeding ratios (Table 4).

Finger millet biomass yield

Biomass of finger millet was not significantly affected by intercropped seeding proportions (P>0.05) (Table 4). However, the highest biomass yield was recorded over the highest cropping proportion (75:100) (23333 kg/ha) as compared to sole finger millet (18666 kg/ha) (Table 4). This means, as the added proportion of the companion cop increase, finger millet biomass yield also increased (Table 4). This could be attributed to reduction of lodging due to intercropping across increasing cropping proportions. Lupines usually hold up lodging of finger millet when they were grown together and in turn protect reduction of finger millet yield. Increased in plant height of finger millet in line with seeding ratios might be also contributed to increase in biomass yield.

Grain yield

The intercropped lupine-cereal seeding proportions did not affect grain yield of cereals (P>0.05) (Table 4). The present results in agreement with Rudnicki and Galezewski (2007), who reported that lupine presence in lupine-oat intercropping in different seeding proportions, did not affect the grain yields of oat. This situation allows the cereal to be maintained at or near the optimum monocrop population and yield which is similar to the existing farmers' practices. It is similar to Natarajan and Willey (1980), who reported that 2 sorghum: 1 pigeon pea seeding ratios, sorghum growth was not affected by the presence of pigeon pea, and the farmers' primary objective of maintaining a 'full' sorghum yield was achieved if the density of the intercropped sorghum was equivalent to the sole crop optimum. Though, there were no significant difference between seeding ratios, grain yield reduction was pronounced in lupine-wheat (from 2400 to 1935 kg/ha) and lupine-finger millet (from 2300 to 1935 kg/ha) intercrops from a lower to a higher seeding ratios, while the reverse is true for barley (from 2800

to 3301 kg/ha) in lupine-barley intercropping (Table 4).

Sole cropped grain yield of barley (3805 kg/ha) and finger millet (2936 kg/ha) were higher than each intercropped with lupine perhaps due to the fact that absence of inter-specific competition in sole cropping, though, uneven rainfall distribution during the growing period and other factors (Figure 2) considerably reduced over all grain yields of cereal species. This was in agreement with the findings of Gardner and Boundy (1983), who noted that yield depression of cereal by lupine in intercropping. Similarly, Chetty (1983) reported that little depression of the yield of finger millet by fodder legumes, field beans, Dolichos lablab and Lucerne.

However, the reverse is true for wheat in lupine-wheat intercropping. Maximum wheat grain yield in lupine-wheat combinations at 25:100 seeding ratios (2494 kg/ha) than sole cropped wheat (203 kg/ha) could be due to lower septoria infestation and differences with respect to resource use in both time (e.g. crops of differing growth phenologies), space (e.g. crops of different rooting depth) and physiology (e.g. legume and non-legume crops differing in source of N) could gave rise to more efficient resource capture and/or use in intercrops than corresponding sole crops.

In terms of competition, this means that crops grown in mixture do not compete for exactly the same ecological niche and that competition between crop species is therefore weaker than between plants of the same species (Yayeh et al*.,* 2014). This was similarly reported by the competitive production principle in which if the two species cannot occupy the same niche, which is to say they cannot compete with one another intensely (Vandermeer, 1989).

This is in agreement with Sarunaite et al*.* (2009), who reported that the wheat intercropped with lupine, bean and pea produced significantly higher grain yield than wheat in sole crop. Similarly, Chen et al. (2004) reported that increased cereal seed yield in legume-cereal mixture may be attributed to nitrogen fixing ability of legumes and extensive root system of cereals. This result inconsistent with Gardner and Boundy (1983), who reported that high lupine seed proportion, causes reduction in wheat yields in lupine-wheat intercropping**.**

Harvest index

Low crop harvest index is the major cause of less crop yield (Murray et al*.,* 2010). Analysis of variance indicated that harvest index of finger millet was significantly (P<0.05) influenced by intercropped seeding proportions in lupine-finger millet intercropping systems (Table 4). The highest harvest index was recorded in sole cropped finger millet (15.58%) followed by 50:100 seeding ratio (10.97%) while the lowest harvest index was recorded in 75:100 (8.50%) and 25:100 (10.4380%) seeding ratios (Table 4). In general, lowest harvest index was recorded in intercropping system than sole cropping system probably due to higher competition from the intercropped lupine.

Reduction in plant height lowered the dry weight of the vegetative parts and thereby lowered the straw yield which resulted in an increased harvest index. Harvest index was positively correlated with grain yield but negatively correlated with vegetative growth (Murray et al*.,* 2010; Yayeh et al*.,* 2014). However, intercropped seed proportion in lupine-wheat and lupine-barley intercropping did not significantly (P>0.05) affect harvest index of wheat and barley as compared to the respective sole cropped (Table 4).

Lodging

Generally, two types of lodging were occurring in cereals during this experiment. These are: wheat and barley root lodging in the case of lupine-barley and lupine-wheat combinations early in the season and finger millet stem breakage in the case of lupine-finger millet later in the season as the stalk becomes more brittle due to maturation (Table 4). Lodging in barely was often a result of the combined effects of a tall standing and large head crop, diseased plant (net blotch and scald) and wind. Lodging in wheat was often a result of the combined effects of diseased plant (Septoria Infestation) and wind. Likewise, lodging in finger millet was caused by the weight of the higher internodes of the stems plus leaves and heads and wind.

Wheat and barley lodging did not affected significantly by lupine-wheat and lupine-barley intercropping in three seeding ratios (P>0.05) (Table 4). This is might be due to lupine at the early stage in all lupine-barely intercropping was near to completely dominated by barely, and so barely did not physically supported by lupine. Though, statistically not significant, lodging was more pronounced under barley and wheat sole cropping as compared to intercropping (Table 4). Highest lodging percentage was recorded in sole barley (22%) and sole wheat (18.13%) as compared to all lupine-barely and lupine-wheat intercropping system. Moreover, as seeding ratios increases in the combination, barley and wheat lodging was reduced (Table 4).

This corroborate with Beyenesh (2009), who reported that barley was sensitive to lodging under sole cropping than mixtures. Nonetheless, finger millet lodging was significantly (P<0.05) affected due to intercropping in different seeding ratios in the case of lupine-finger millet intercropping (Table 4). The present study indicated that lodging was highly reduced in all lupine-finger millet combinations as compared to sole cropped finger millet (20.32%). Moreover, lupine-finger millet combination at 75:100 seeding ratio (11.63%) highly reduced lodging as compared to 25:100 (16.00%) and 50:100 seeding ratios (15.87%) which were statistically on par with each other

Table 5. Land use efficiency of lupine-cereal intercrops at three seeding ratios at Adet Agricultural Research Center, West Gojam in 2009.

Cropping system	Seed proportion (%)	Land use efficiency (%)
Sole lupine	100	0
Sole wheat	100	0
Sole barely	100	0
Sole finger millet	100	0
Lupine: wheat	25:100	33.4
Lupine: wheat	50: 100	31.3
Lupine: wheat	75: 100	48.9
Lupine: barley	25: 100	-24.0
Lupine: barley	50: 100	-21.0
Lupine: barley	75: 100	-11.0
Lupine: f/millet	25: 100	9.7
Lupine: f/millet	50: 100	23.4
Lupine: f /millet	75:100	29.4

Values (means) connected by different superscript letters are significantly (P<0.05) different within columns according to Tukey-HSD tests. LER: Land equivalent ratio.

(Table 4). In other words, finger millet was physically supported by lupine particularly in high lupine seed proportion. This result was in agreement with Putnam (1993), who reported that in lupine-pea combination the lupine prevent lodging of pea, and the pea provides an earlier canopy closure for weed control in the lupine. Barley culms (stem) were regaining their upright position and gave optimum yield due to lodging before flowering and prevailing favorable weather conditions. Similarly, finger millet lodging did not much affect the yield probably due to lodging occurs after the plant had matured and finger millet was physically supported by lupine but it might reduce the amount of harvestable grain.

Land use efficiency

In assessments of crop productivity of sole cropping systems, a useful expression is mass yield (mass per unit area). However, in intercropping systems, direct comparison is difficult because products are different for the different plant species growing on one piece of land (Beets, 1982). In this case, crop productivity should be evaluated using a common unit. A widely used method to know land use efficiency in terms of hectare of land saved due to intercropping or in terms of percentage of yield advantage or disadvantage is the land equivalent ratio (LER) (Beets, 1982). Total land equivalent ratio (LER) was significantly higher than 1.00, which shows an advantage from intercropping over pure stands in lupine-wheat and lupine-finger millet combinations in terms of the use of environmental resources for plant growth.

The combined land use efficiency was greatest in the cases of lupine-wheat mixture at the 75:100 seeding ratio (48.9%), followed by the same combination at the 25:100 seeding ratio (33.4%) and at 50:100 seeding ratio (31.3%) (Table 5). This indicates that 0.489 ha, 0.334 ha and 0.313 ha more area would be required by a sole cropping system to equal the yield of intercropping system.

The second crop combination which gave higher land use efficiency was lupine-finger millet at 75:100 seed ratio (29.4%) followed by the same combination at 50:100 (23.4%) and 25:100 (9.7%) seeding ratios which causes, 29.4, 23.4 and 9.7% higher yield than sole cropping (Table 5). These findings were in agreement with Caballero et al. (1995), who reported a mixed stand advantage at lower oat seeding proportions in common vetch-oat combination. Similarly, compared with corresponding sole crops, yield advantages have been recorded in pearl millet-cluster bean (Yadav and Yadav, 2001). On the other hand, total LERs below 1.00 were found in all lupine-barley combinations, which gave a disadvantage of these mixtures over pure stands (Table 5). This result was in agreement with Ghosh (2004), who reported that common vetch–barley and common vetch-triticale mixtures shows a disadvantage over pure stands. This could be due to competitive ability of barely was higher than lupine.

SUMMARY AND CONCLUSIONS

The present study demonstrated that, except days to maturity of finger millet, intercropping of lupine with wheat, barley and finger millet at three different seeding ratios had no effect on phenological attributes of cereal species. The agronomic attributes of most cereals were not significantly affected when they were intercropped with lupine in all seeding ratios except finger millet plant

height, harvest index and wheat total biomass yield. Cereal growth was not affected by the intercropped lupine, and the farmers' primary objective of maintaining a 'full' cereal yield was attained.

The maximum lupine seed proportion was superior to the lowest when intercropped with wheat and finger millet. Intercropping higher proportion of lupine with wheat and finger millet did help much in increasing total grain yield and biomass yield without affecting main crop yield. Intercropping lupine with cereals gave physical support for cereals particularly in high lupine seed proportion. The combined yield advantage was greater than one in the cases of lupine-wheat followed by lupine-finger millet mixtures at all seeding ratios. Hence, two of the best combinations which were differed from what farmers currently use and gave higher land use efficiency were the lupine-wheat mixture at the 75:100 seeding ratio (49.4%) followed by the lupine-finger millet mixtures at the 75:100 seeding ratio (29.4%). These mixtures seem promising in the development of sustainable crop production with a limited use of external inputs.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Head and lateral length on water distribution uniformity of a PVC drip irrigation system

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Characteristics of emitters under low pressure are essential for the design of low pressure drip irrigation systems. Few data is provided by the manufacturers for drip emitter operating under low pressures. No guidelines regarding the optimum combination of operating pressure head and lateral length available either. A laboratory test was conducted to evaluate the effects of pressure head and lateral length on water distribution uniformity of a PVC drip irrigation system. Five different lengths of lateral were considered under a pressure head of 55 kPa (8.21 psi / 5.6 m). The five lengths were 20, 40, 60, 80 and 100 m. Drip tape tested in this study is a non-pressure compensating (NPC) emiiter with 3.00 L/h discharge rate, 16 mm diameter and 35 cm emitter distance. Evaluation of 10 sampled emitters from the 20 m lateral showed a flow variation (Qvar) of 5%, uniformity coefficient (UC) of 99%, with a coefficient of variation (Cv) of 0.016. The 40 m lateral length showed a flow variation (Qvar) of 9%, UC of 98% with a Cv of 0.024. The 60 m lateral length also showed an average flow variation (Qvar) of 16%, UC of 95% with a Cv of 0.060. The 80 m lateral length showed a flow variation (Qvar) of 23%, UC of 93% with Cv of 0.08 and the 100 m lateral length showed a flow variation (Qvar) of 39%, UC of 89% with a Cv of 0.138. EU for 20, 40, 60, 80 and 100 m were 92, 90, 80, 75 and 58% respectively. Flow discharge vs. sampled emitter points (Q-E curves) was also developed for each length. Q-E curves were fitted to the data resulting in R^2 values of 0.1566, 0.1202, 0.8607, 0.7904 and 0.8998 respectively for 20, 40, 60, 80 and 100m. The operating pressure was 55 kPa (8.21 psi / 5.6 m) for all the tested length. From the statistical analysis, it was observed that as the lateral length increase invariably decreases the average discharge (Qvar). It is therefore recommended to use the 60 m lateral length with a low pressure head of 55 kPa (8.21 psi / 5.6 m), since the 60 m length satisfied the uniformity distribution criteria under a low pressure.

Key words: Pressure head, lateral length, irrigation uniformity.

INTRODUCTION

Water for agricultural use is becoming scarce, both in quantity and quality, not only in the traditionally prone arid and semi-arid zones, but also in regions where rainfall is abundant. Agriculture represent the major user worldwide, and a general perception that agriculture water use is often wasteful and has less value than other

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uses is widespread (Postel, 2000; Jury et al., 2005). Furthermore, energy analysis of agricultural operations has shown that irrigation system consumes a significant amount of energy as compared to other operations (Topak et al., 2005). For these reasons, there is an urgent need to use water resources efficiently by enhancing crop productivity per unit of water.

The drip irrigation systems require intensive capital due to sophisticated technology. Therefore, it is beyond the capacity of the most farmers under small scale farming. If the drip system could be made affordable and within the reach of small and marginal farmers, it will definitely increase the productivity and income of the farmers and also, conserve the scarce precious water resources of the country. International Development Enterprises (IDE) has developed a low cost drip irrigation system, which has been extensively field tested to advance this technology accessible to small and marginal farmers. It has pressure head of 0.5 - 3.0 m with 73 - 84 percent distribution uniformity (Polak et al., 1997). In addition to substantial water saving, the advantage of drip irrigation is that water can be applied where it is most needed in a controlled manner according to the crop requirements.

Drip irrigation has advantages over conventional furrow irrigation as an efficient means of applying water, especially where water is limited. Vegetables with shallow root systems and some crops like corn respond well to drip irrigation with increased yield and substantially higher seed or cob quality with smaller water applications, justifying the use of drip over furrow irrigation (Camp, 1998). However, high initial investment costs of these systems need to be off-set by increased production to justify investment over furrow irrigation systems. The main components of a PVC drip irrigation system are the drip polyethylene tubes with emitters attached to the inside wall and equally spaced according to the crop characteristics of the crop (corn).

Characteristics of emitters (pressure compensating) under low pressure are essential for the design of gravity drip irrigation systems. Few data is provided by the manufacturers for drip emitter operating under low pressures. No guidelines regarding the optimum combination of operating pressure head, lateral length and land slope are available either. The inlet pressure head gained by the attractive flow should be balanced by the total head loss due to friction and emitter insertions along the drip line. If the inlet pressure head becomes greater than the required pressure head at the lateral for uniform emitter flows through the lateral, it may cause back-flow from the lateral downstream closed end to the inlet upstream. On the other hand, if the inlet pressure head becomes lower than the total required pressure head along the lateral, it may yield negative pressure at the emitters at any section of the lateral and it will affect the distribution uniformity (since the inlet pressure and the total required pressure should be the same). The friction loss within the lateral which is a function of the inlet pressure, diameter of laterals, spacing of emitters, and slope of laterals, plays a vital role in the distribution uniformity in drip systems.

The distribution uniformity (DU) of water is one of the most important parameters to characterize drip emitters and design of a drip irrigation system. It is a measure of the uniformity of water application to the area being irrigated, expressed as a percentage between 0 and 100%, although it is practically impossible to attain 100%. DU of less than 70% is considered as poor, 70 to 90% is good, and greater than 90% as excellent. The common measure of DU is the low quarter DU, which is the ratio of the average of the lowest quarter of samples to the average of all samples. Distribution Uniformity in a drip irrigation system is dependent upon manufacturing variation of emitters, operating pressure head, lateral length and land slope. In order to obtain a better DU when designing an efficient drip irrigation system, the combination of operating pressure, lateral length and land slope must be considered. Therefore, all of these factors should be included in designing a drip irrigation system in order to have acceptable distribution uniformity within a certain length of the lateral at a low pressure head.

MATERIALS AND METHODS

Description of study area

The experiment was conducted at the College of Water Conservancy and Civil Engineering at South China Agricultural University, Guangzhou-P. R. China, from November, 2012 to April, 2013.

The following parameters were used to evaluate different drip irrigation products operating under high and low pressure head:

Average emitter discharge rate (Qvar):

$$Q_{var} = \frac{qmax - qmin}{qmax} x100$$

Standard deviation of emitter flow rate (Sq)

$$s_q = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(q_i - q_{var})^2}$$

The variation coefficient of emitter flow (Cv) $c_v = \frac{s_q}{q_{var}}$

Uniformity coefficient (UC)

$$UC = 100\left[1 - \frac{1}{nq_{var}}\sum_{i=1}^{n}|q_i - q_{var}|\right]$$

Emission uniformity (EU)

$$EU = \left[1.0 - \frac{1.27c_v}{\sqrt{n}}\right] \times \left(\frac{q_{min}}{q_{var}}\right) \times 100$$

Where: q_{max}- maximum emitter flow rate, q_{min}- minimum emitter flow rate, n-number of emitters, q_{var}- average discharge, q_i- flow discharge.

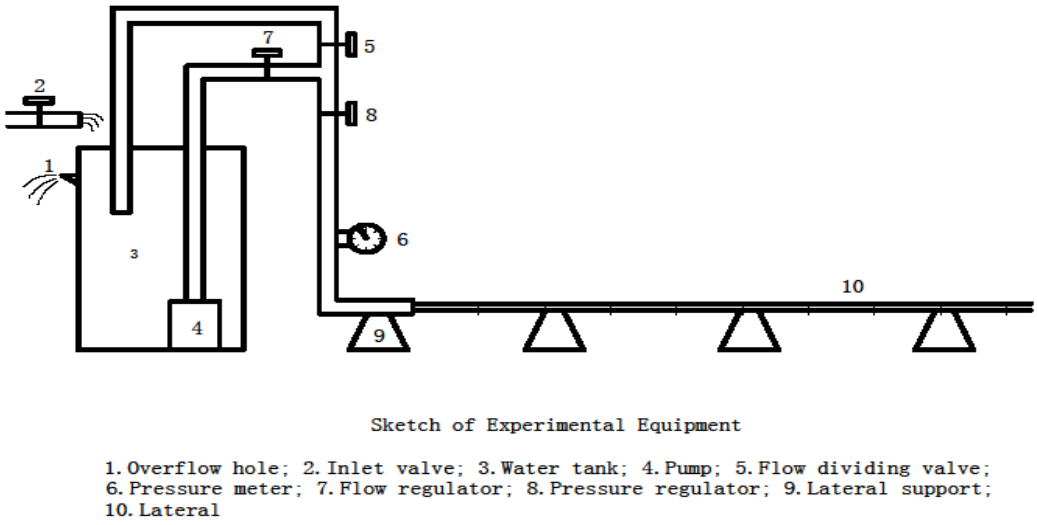

Figure 1. Layout of the test apparatus for discharge (Q) testing.

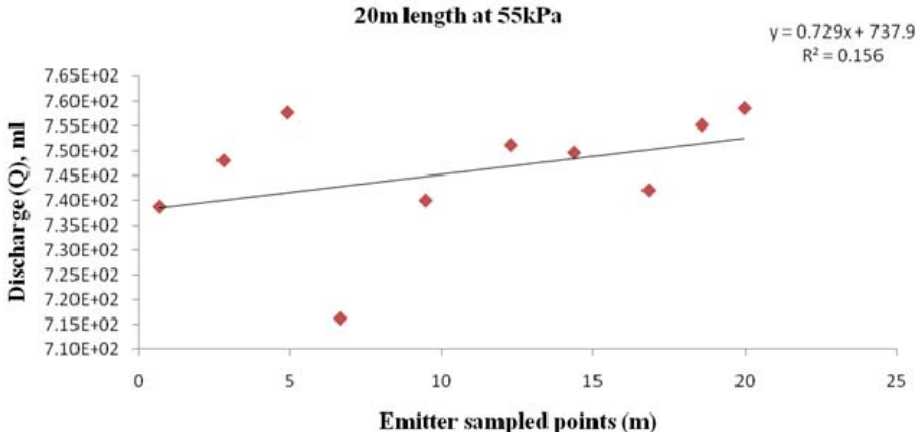

Figure 2. Average discharge under 20 m length at 55 kPa (8.21 psi / 5.6 m).

Computations followed the methodology proposed by Keller and Bliesner (1990) and Kang and Nishiyama (1996).

Testing the apparatus

A laboratory based experiment was set up for this study. A 200 L capacity container was used for the water supply. An immersible pump of HX 8670/8670A was used to supply pressure to the lateral of 16 mm internal diameter in Figure 1.

In this experiment; 10, 20, 30, 30, 30 sampled emitters were randomly selected for five different lateral lengths, 20, 40, 60, 80 and 100 m respectively. The emitters were kept dripping for about 30 min for all the five lengths during the samples collection. On the other hand, after turning on the pump, emitters were allowed to drip for approximately 5 to 10 min to allow for air to escape from the tubing. Samples were collected only after making sure that the last emitter at the laterals end drip out and no air was exiting from the tubes. Water collection period was set in such a way that approximately 700 to 750 ml water samples could be collected to calculate discharge rate per minute. The testing operating pressure was 55kPa (8.21 psi / 5.6m) for all five tested lengths considered.

RESULTS AND DISCUSSION

Performance evaluation on 20 m length

The variation in average discharge of a 16 mm diameter PVC pipe of 20 m long lateral at 55 kPa (Figure 2). For a total emitter, the R^2 value was 0.1566 and the discharge (Q) relationship was Q= 0.7292x + 737.93. From the R^2 value, it can be said that the developed discharge vs. sampled emitter point's relationship did not describe the emitter in terms of lateral length accurately (Figure 2). It was observed that as the pressure was kept constant at 55 kPa (8.21 psi / 5.6 m) along the lateral, and the

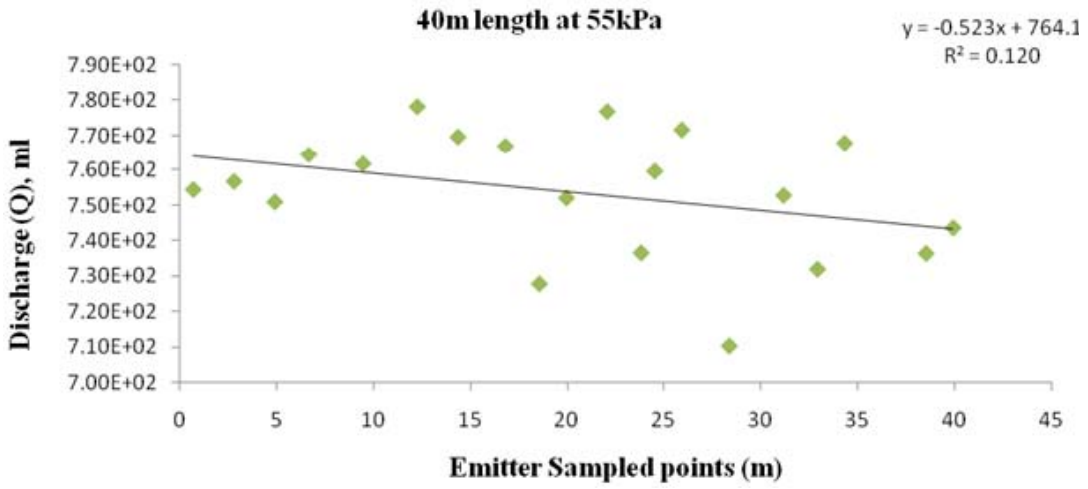

Figure 3. Average discharge under 40 m length at 55 kPa (8.21 psi / 5.6 m).

discharge (Qvar) was 5%. It was reported by Bralts et al. (1987), that emitter flow variation of 10% or less is generally considered desirable. Values of Cv are statistically determined from measured flow rate from a large (>50) sample set of emitters depending on the lateral length all subjected to the same reference pressure head and is the ratio of the standard of deviation of the measured flow rate the mean flowrate. The 20 m long at 55 kPa gave an excellent Cv of 0.016 which was reported by ASAE EP405.1,-(2000), that for point-source emitters, values of Cv less than 0.05 are considered excellent. EU of 92% was also desirable as reported by ASAE EP405.1,-(2000), that it is desirable for most microirrigation systems when design emission uniformity ranges from 85% to 95%. UC was acceptable at 99% as reported by Bralts et al. (1987), that an acceptable UC should be greater than 90%.

Performance evaluation on 40 m length

For a total emitter the R^2 value was 0.1202 and the discharge (Q) relationship was Q= -0.523x + 764.18 (Figure 3). From the R^2 value, it can be said that the developed flow discharge vs. emitter relationship did not describe the emitter terms of lateral length accurately. The discharge (Qvar) of a 16 mm internal diameter PVC lateral of 40 m at 55 kPa (8.21 psi / 5.6 m) was 9%, which confirms what was reported by Bralts et al. (1987), that emitter flow variation of 10% or less is generally considered desirable. The coefficient of variation (Cv) was 0.024, confirms the report by ASAE EP405.1, (2000), that for point-source emitters, values of Cv less than 0.05 are considered excellent. A 90% of desirable EU agrees to the report by ASAE EP405.1, (2000), that it is desirable for most microirrigation systems when design emission uniformity ranges from 85 to 95%. An acceptable UC of 98% confirms the reported by Bralts et al. (1987), that an acceptable UC should be greater than 90%. The variation in average discharge of 40 m lateral length at 55 kPa is shown in Figure 3.

Performance evaluation on 60 m length

For a total emitter, the R^2 value was 0.8607 and the flow discharge (Q) relationship was Q = -2.0745x + 719.96. From the R^2 value, it can be said that the developed flow discharge vs. emitter relationship did not describe the emitter in terms of pipe length accurately (Figure 4) since the slope was negative. The variation in average discharge of a 16 mm diameter PVC lateral of 60 m long lateral at 55 kPa (Figure 4). The 60 m length at 55 kPa (8.21 psi / 5.6 m) gave a 16% average discharge (Qvar), which makes it acceptable as reported by Bralts et al. (1987), that emitter flow variation between 10 to 20% is considered acceptable. An average Cv of 0.06 was obtained which confirm that Cv between 0.05 to 0.10 are considered average as reported by ASAE EP405.1, (2000), for line-source emitters. EU of 82% which is acceptable as reported by ASAE EP405.1, (2000), EU between 80 to 85% is considered to be acceptable. UC was 95% which confirms the reported by Bralts et al. (1987),that an acceptable UC should be greater than 90%.

Performance evaluation on 80 m length

For a total emitter, the R^2 value was 0.7904 and the flow discharge (Q) relationship was Q= -1.9648x + 691.89. From the R^2 value, it can be said that the developed flow discharge vs. emitter relationship, it is also observed that there was a slight reduction in flow discharge in the emitter terms of lateral length (Figure 5). The variation in

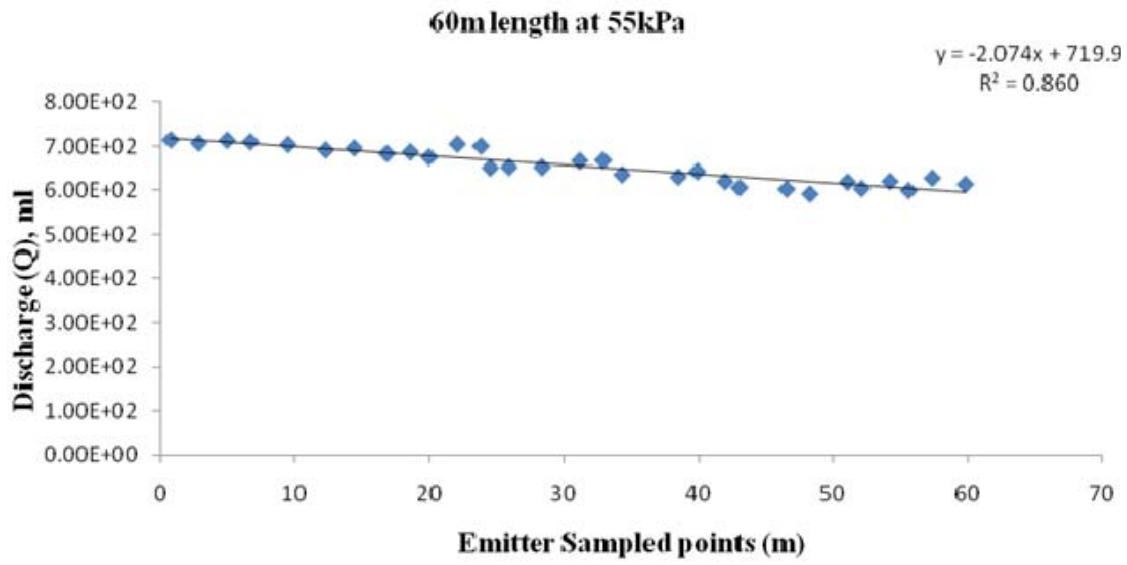

Figure 4. Average discharge under 60 m length at 55 kPa (8.21 psi / 5.6 m).

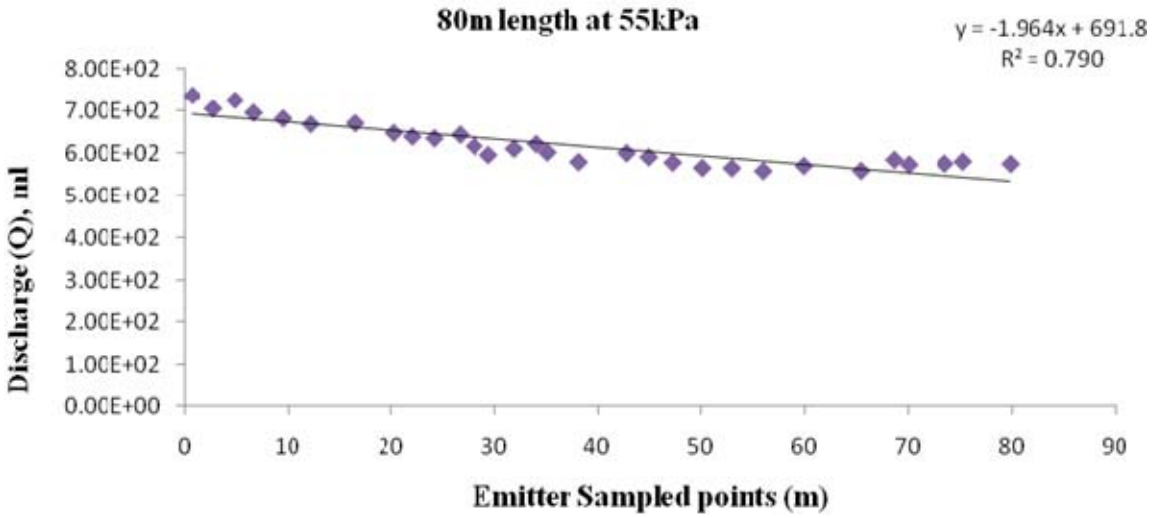

Figure 5. Average discharge under 80 m length at 55 kPa (8.21 psi / 5.6 m).

average discharge of a 16 mm diameter PVC lateral of 80m long lateral at 55kPa (Figure 5). It was observed that as the pressure was kept constant at 55 kPa (8.21 psi / 5.6 m), an unacceptable discharge (Qvar) of 23% was obtained as reported by Bralts et al. (1987), that Qvar greater than 20% is considered unacceptable. There was an average coefficient of variation (Cv) of 0.08, which confirms the report by ASAE EP405.1, (2000), that for point-source emitters, values of Cv between 0.05 to 0.10 are considered average. EU of 75% was also unacceptable as reported by ASAE EP405.1 (2000), that emission uniformity which ranges from 80 to 85% is considered acceptable and below 80% is also considered unacceptable. An acceptable UC was 93% which confirms the report by Bralts et al. (1987), that an acceptable UC should be greater than 90%.

Performance evaluation on 100 m length

For a total emitter, the R^2 value was 0.8998 and the flow discharge (Q) relationship was Q = -2.2404x + 672.01. From the R^2 value, it can be said that the developed flow discharge vs. emitter relationship describes the emitter slightly terms of lateral length (Figure 6). An unacceptable discharge (Qvar) of a 16 mm diameter PVC lateral of 100 m

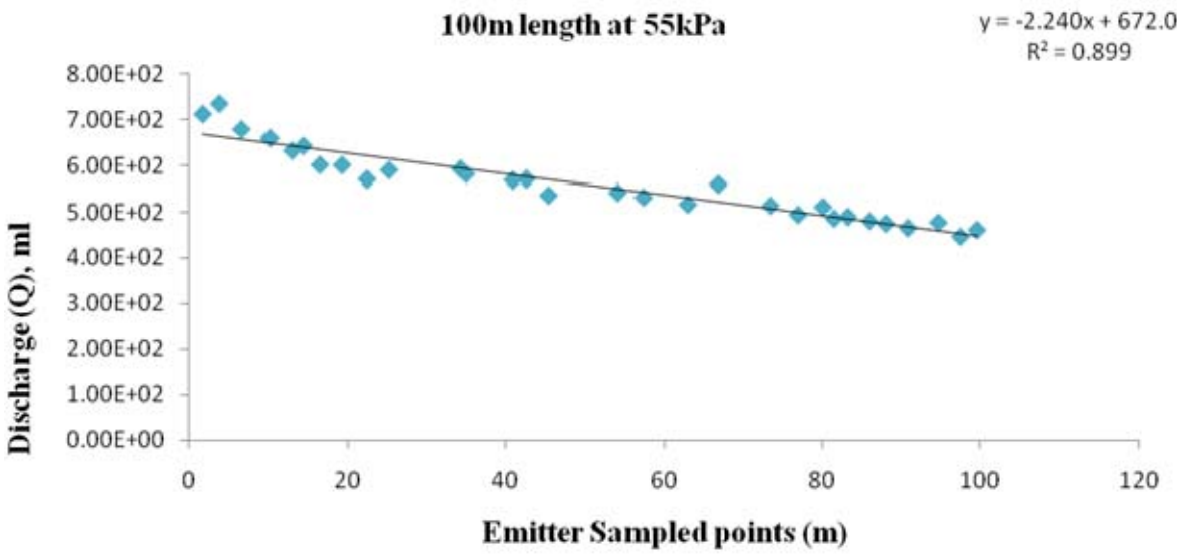

Figure 6. Average discharge under 100 m length at 55 kPa (8.21 psi / 5.6 m).

at 55 kPa (8.21 psi / 5.6 m) was 39% was recorded, which confirms the report by Bralts et al. (1987), that Qvar greater than 20% is considered unacceptable. A marginal Cv of 0.138 was obtained which confirm that Cv between 0.10 to 0.15 are considered marginal as reported by ASAE EP405.1 (2000), for line-source emitters. An unacceptable 58% EU agrees to the report by (ASAE EP405.1, 2000), that emission uniformity which ranges from 80 to 85% is considered acceptable and below 80% is also considered unacceptable. The variation in average discharge of 100 m lateral at 55 kPa (8.21 psi / 5.6 m) (Figure 6) an unacceptable UC of 89% was recorded, which confirms the report by Bralts et al. (1987), that an acceptable UC should be greater than 90% and below 90% makes it unacceptable.

Average discharge (Q) under different lateral length

Irrigation efficiencies of the systems are shown in Table 1. The hydraulic design and component selection of affordable drip system offers satisfactory hydraulic performance. Discharge variation (Qvar) tested under a hydraulic pressure of 55 kPa (8.21 psi / 5.6 m) of a 16 mm diameter PVC lateral was better in terms of shorter length of the pipes 20, 40, 60, 80 and 100 m as 5, 9, 16 23 and 39%, respectively. Table 1 shows that shorter lateral gives desirable flow discharges (Qvar). For the purpose of uniformity of distribution, it is recommended to use shorter laterals under low pressure heads to achieve a desirable average discharge flow as reported by Bralts et al. (1987) (Figure 7).

The Cv obtained for 20, 40, 60, 80 and 100 m are 0.016, 0.024, 0.06, 0.08 and 0.138 respectively under a hydraulic pressure of 55 kPa (8.21 psi / 5.6 m). It can be inferred that, the shorter the lateral the better the coefficient of variation (Cv) as reported by Bralts et al. (1987). Since 20, 40, 60, 80 and 100 m produced; excellent, excellent, average, average and marginal Cv's, respectively. 92, 90, 82, 75 and 58% was obtained for 20, 40, 60, 80 and 100 m respectively for the emission uniformity EU of the pipe. This can also be inferred that, the shorter the lateral, the better the emission uniformity (EU) as reported by Bralts et al. (1987) (Table 1).

It was also observed that in the 20 and 30 m, the average discharge (Qvar) along the lateral form the 1st emitter was not far from the Qvar sampled at the 10 and 20th emitter respectively. This showed a uniform distribution along the later in terms of the length considered at a pressure head of 55 kPa (8.21 psi / 5.6 m). But for the 60, 80 and 100 m, the Qvar along the lateral from the 1st emitter were far from the Qvar sampled at the 30th emitter respectively for 60, 80 and 100 m at a pressure head of 55 kPa (8.21 psi / 5.6 m). This shows that as the lateral length increases, it invariably decreases the average discharge (Qvar).

Conclusion

This experiment characterized five length of lateral of 16 mm diameter product by measuring their flow rates under a pressure of 55 kPa (8.21 psi / 5.6 m). From evaluation of 10, 20, 30, 30, 30 emitters from 20, 40, 60, 80 and 100 m respectively, the 20 m showed a flow variation (Qvar) of 5%, uniformity coefficient (UC) of 99%, with a coefficient of variation (Cv) of 0.016, 40 m showed flow variation (Qvar) of 9%, UC of 98% with a Cv of 0.024, 60 m showed an average flow variation (Qvar) of 16%, UC of 95% with a Cv of 0.060, 80 m showed a flow variation

Table 1. Micro-irrigation system uniformity classification based on tested performance evaluation of 16 mm diameter lateral at 55kPa (8.21 psi / 5.6 m).

Pipe Length (16 mm θ)	Pressure (kPa)	Qvar %	Sq	Cv	UC%	EU%
20	55	5	12.49	0.016	99	92
40	55	9	17.86	0.024	98	90
60	55	16	39.93	0.060	95	82
80	55	23	52.24	0.080	93	75
100	55	39	76.77	0.138	89	58

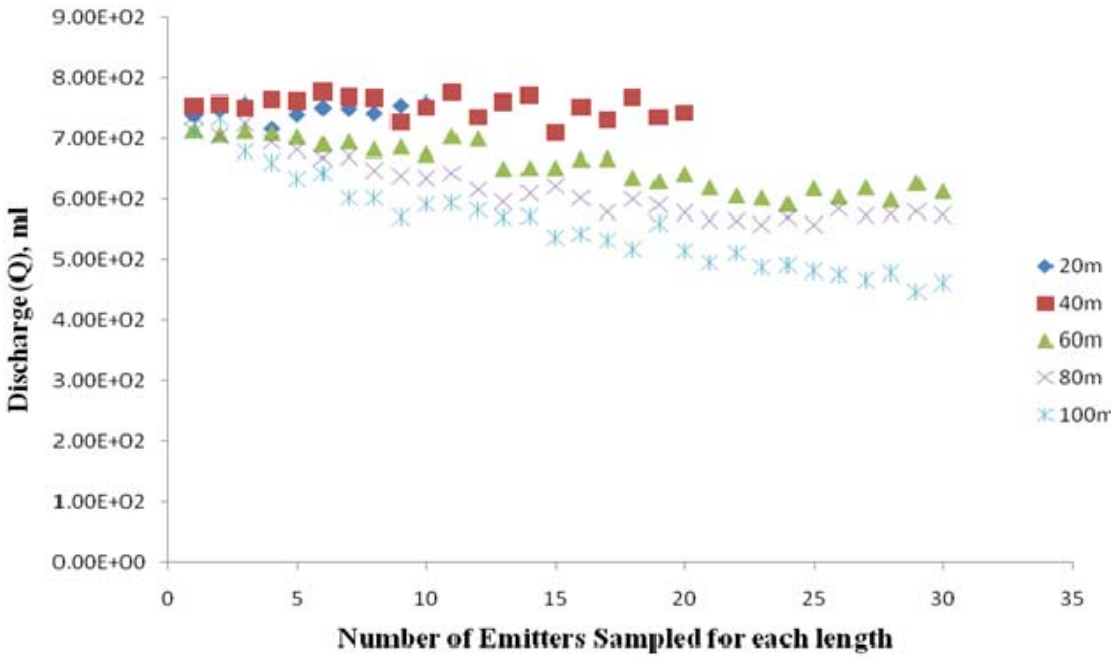

Figure 7. Comparism of average discharge (Q) under different lateral length at 55 kPa.

(Qvar) of 23%, UC of 93% with Cv of 0.080 and 100 m showed a flow variation (Qvar) of 39%, UC of 89% with a Cv of 0.138. EU for 20, 40, 60, 80 and 100 m were 92, 90, 80, 75 and 58% respectively.

Flow discharge vs. emitter (Q-E curves) was also developed for each length. Q-E curves were fitted to the data resulting in R^2 values of 0.1566, 0.1202, 0.8607, 0.7904 and 0.8998 respectively for 20, 40, 60, 80 and 100 m. The operating pressure was 55 kPa (8.21 psi / 5.6 m) for all the tested length.

From the statistical analysis, it was observed that as the lateral length increases, it invariably decreases the average discharge (Qvar).

Conflict of Interest

The authors have not declared any conflict of interest.

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Short Communication

Analysis of the effect of non-formal credit sources in enhancing the income of small scale rubber farmers in Edo State, Nigeria

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The effect of non-formal credit sources in enhancing the income of small-scale rubber farmers in Edo State were studied by purposive selection of ninety respondents drawn from rubber growing local government areas of Edo state. Data collected were analyzed using the production function to examine the influence of some variables on the income of respondents. Empirical result showed that 65% of variations in income of respondents were explained by age, farm size, access to credit, level of education and leadership role. The analysis indicated farm size (X_2) was positive and significantly related to income of the farmers while access to credit (X_3) and leadership role (X_5) were significant and inversely related to income of the rubber farmers. The study recommends that rubber farmers should form cooperative societies to enable them benefit from production credit from agricultural and commercial banks.

Key words: Income, non-formal credit, small-scale, production function, Edo state, Nigeria.

INTRODUCTION

Natural rubber tree belongs to the family of latex producing plant called *Euphorbiaceae* .The rubber tree is of the genus *Hevea* of which eleven species have been documented. Among these species, *Hevea brasiliensis* Muell Arg is the major source of natural rubber due principally to its superior latex yield over other species of *Hevea*. Natural rubber was introduced into Nigeria in 1895 from the Wick ham collection of 1876. To date, twenty-four high latex yielding clones have been developed in Nigeria. These clones have latex yield of 2000 – 3500 kg/ha/yr (Omokhafe and Nasiru, 2004).

Natural rubber was ranked as the fourth most valuable agricultural export commodity in Nigeria after cocoa, groundnut and palm kernel, with 92 percent of natural rubber production exported, making rubber essentially a foreign exchange earner for the national economy. It is a dependable source of raw material for local industries; it also provides employment opportunities for farmers, tappers, manufacturers and other personnel in marketing. Natural rubber has diversity of uses. Latex and coagula are important in automobile industries for the manufacture of tyres and tubes. Latex is useful in the

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manufacture of surgical gloves while the rubber seeds are processed into rubber seed oil and alkyd resins for industrial uses. Furthermore, rubber is environmentally friendly and helps to protect the soil from erosion (Abolagba and Giroh, 2006). Nigeria has 247,100 ha of land under rubber cultivation and majority of these hectares are owned by small-scale farmers (Aigbekaen et al., 2000; Delabarre and Serier, 2000)

Lack of credit facilities and high cost of credit are among the major constraints faced by rubber farmers in Nigeria. The rubber industry is dominated by small-scale rubber farmers who utilize about 85% of the rubber growing areas in the country. Production of natural rubber is concentrated in Edo State where about 90% of the country's output is obtained. Of this output, about 75% was from the small holdings. Most of the small-scale farmers having little or no access to credits may be because they are not organized into recognizable entities for the purposes of attracting such credit facilities. For rubber farmers, the situation is even worse because of the fact that rubber seedling and even budded stumps take about five to seven years to attain maturity. Because of this, no credit organization or commercial bank is normally willing to extend loan repayment for that length of time and farmers have to devise means of obtaining credit from non formal sources.

The low productivity which characterizes Nigeria agriculture stems in part, from the state of poverty of rural farmers which constitute about 80.78% of the Nigerian farmers (Ewuola, 1985). Farmers requirement for credit have direct implication on innovation. Most of the improved technologies (like hybrid seeds, chemicals etc.) extended to farmers demand that they have the means by which they will procure them. Credit will therefore facilitate innovation adopted by farmers, which will eventually lead to higher output from the agricultural sector (Abalu et al., 1981; Imoudu, 1986). Asika and Nwachukwu (1985) stated that credit is equally needed by farmers to expand their farm size, hire more labour to supplement the limited and fixed supply of farm labour, transporting inputs to the farm and evacuating of farm produce to the market and stores and for processing of produce to other forms desired by the people. In fact, credit to farmers would ensure reduction in produce spoilage and wastage.

The objective of this study is to determine the effect of non-formal credit on income of small-scale rubber farmers in Edo State.

METHODOLOGY

The study area

The study was conducted in Edo State, Nigeria. Edo State lies between Latitudes 5° 44´´ and 7° 34´´ N of the equator and between Longitudes 5° 04´´ and 6° 43´´ E of the Greenwich Meridian. It shares boundary with Kogi State in the south by Delta State, in the West by Ondo State and in the East by Kogi and Anambra States.

The state covers a land area of about 17,902 km^2 with a population of 2,159,848. Edo State is divided into 18 Local Government Areas. The State is characterized by a tropical climate which ranges from humid to sub humid at different parts of the year. Three distinct vegetation identified in the State are Mangrove forest, Fresh swamp and Savannah vegetations. The mean annual rainfall in the northern part is 127 to 152 cm while the southern part of the State receives about 252 to 254 cm respectively. Mean temperature in the state ranges from a minimum of 24°C to a maximum of 33°C .The people of the state are mostly farmers growing varieties of crops such as cassava, rice, yam, plantain, pineapple and tree crops such as rubber, oil palm and cocoa. Other occupations of the state include small and medium scale businesses and jobs done by artisans and civil servants who engage in farming on part time basis (Emokaro and Erhabor, 2006).

Data collection procedure and analysis

The multi-stage sampling procedure was used to select the respondents. Six Local Government Areas (LGAs) that are dominantly noted for rubber production in the state were purposively selected. Twenty (20) rubber farmers were randomly selected in each of the six local governments which gave a sample size of one hundred and twenty (120) small scale rubber farmers in the study area and served with structured and validated questionnaire consisting of open and closed – ended questions to elicit information from the targeted respondents. Out of the number 90 were correctly filled and used for analysis. The production function postulated for rubber farmers in the study area is implicitly presented by Equation (1)

$$Y = F(X_1, X_2, X_3, X_4, X_5, \mu_1) \quad (1)$$

Where: Y = Farm income per annum in Naira; X_1 = Age of the farmers (years); X_2 = Farm size (hectares); X_3 = Access to credit (N); X_4 = Educational attainment; X_5 = Leadership role and μ_1 = The error term (was assumed to have zero mean and constant variance).

The Linear, Semi-log, Exponential and Cobb-Douglas functional forms were tried using ordinary least square technique (OLS). The estimated functions were evaluated in terms of the statistical significance of R^2 as indicated by F-value, the significance of the coefficients as given by the t-values, the signs of the coefficient and the magnitude of standard errors. Based on these statistical, economic and econometric criteria, semi log functional form was selected as the lead equation which is explicitly represented by Equation (2):

$$Y = \log \beta_0 + \log \beta_1 X_1 + \log \beta_2 X_2 + \beta_3 X_3 + \log \beta_4 X_4 + \log \beta_5 X_5 \quad (2)$$

Where: β_0 = A constant, β_1, β_2 …. B_3 are regression coefficients to be estimated while other variables are as previously defined.

RESULTS AND DISCUSSION

The sources used by the farmers were non-formal sources which include Esusu, money lenders and private savings. Disbursements to borrowers were timely unlike the formal credit institutions (Agricultural Cooperative and Rural Development Bank (NACRDB), Cooperative and Commercial Banks, like First Bank, Union Bank etc) that are usually marred by bureaucratic delays, collateral security and untimeliness in release of fund.

Table 1. Influence of selected variables on the income of respondents.

Variable code	Variable name	Coefficients	Standard error	T-value
	Constant	19716.930	44859.757	0.440
X_1	Age of Respondents in years	22024.095	26110.062	0.844
X_2	Farm size of respondents in hectares	133247.6***	15537.080	8.576
X_3	Access to credit by the respondents in Naira	-60365.5***	18851.600	-3.202
X_4	Educational attainment of respondents measured by the number of years of formal schooling	-5472.461	10327.799	-0.530
X_5	Leadership role of respondents	-19721.2*	9387.045	-2.101

Source: Data analysis 2007, R^2 = 0.666, Adjusted R^2 = 0.646, F-value = 34.243 *** ,***, * Indicate Significance at 1and 5%.

Result of the analysis, Table 1, shows that the coefficient of multiple determination (R^2) was 0.646 (about 65%) which implied that 65% of the variation in the income of rubber farmers explained the variables included in the model. The difference (35%) may be attributed to error and non inclusion of other variables in the model. The entire model is well fitted to the data as evidenced by F value of 34.243 and significant (p>0.01).

The result indicated that farm size (X_2), access to credit (X_3) and leadership role (X_5) were significant, which implies that a unit increase in farm size will increase the farmers income *ceteris paribus*. The coefficient for farm size (X_2) was positive and significantly related to income of the farmers. This shows that land is a critical factor in production and adoption of improved farm practices and can be used as a collateral security in obtaining credit from formal sources. Access to credit (X_3) is significant and inversely related to income of the Nigerian rubber farmers. This implies that farmers may refuse to borrow or were not opportune to obtain credit as the case may be. So any unit increase in the independent variable (X_3) access to credit, the income of the farmer will decrease equal to the value of the coefficient of the variable. Leadership role (X_5) was also significant but negative, this may be possible because leaders are not using the opportunity they had in obtaining loan to invest on their farms, rather give more time to their functions in the society like attending meetings, settling cases and also pride of leadership and also underrating farm work as a business.

CONCLUSION AND RECOMMENDATIONS

The study has identified factors that enhance the income of small scale rubber farmers in Edo State. The factors were age, educational attainment, access to credit, leadership role and farm size. Based on the findings of the study, it is hereby recommended that in order to enhance access to agricultural loan to farmers, it is recommended that farmers should join cooperatives so as to reap other benefits such as easier access to other farm inputs, possible reduction in cost of production, learning of improved farm practices and increased bargaining.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Managing national poverty eradication programme for food security among all farmers Association in Nigeria

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The study evaluated the impact of National Poverty Eradication programme (NAPEP) on food security among the All Farmers Association of Nigeria (AFAN), using data from Kogi State of Nigeria. The study is borne out of the curiosity to address the problems of poverty and hunger in Kogi State of Nigeria. The z-test statistics was used to test the hypothesis at 5% level of significance. The study showed that beneficiaries of NAPEPs intervention had significantly greater output in crops and that their income significantly exceeded that of non-beneficiaries. One of the two major problems that hindered the success of NAPEP is that only a few beneficiaries selected among the numerous individuals are within the low income bracket. The second problem is inadequate loan granted to beneficiaries. Based on this, the paper recommended that NAPEP should increase the number of beneficiaries, increase their loans, intensify provision of central productive input and focus on the processing of agricultural produce. This will add value to the produce, command high prices, increase varieties, generate more employment opportunities, enhance food security and reduce poverty incidence.

Key words: National poverty eradication programme, food security, all farmers association of Nigeria, small scale farmers, industrialists.

INTRODUCTION

The Federal Government of Nigeria has at various times committed millions of naira to programmes aimed at increasing agricultural production and reducing poverty. Yet, contemporary studies and the government itself attest that poverty and hunger still prevail in the country. As noted by Jibril et al. (2009), poverty is currently one problem in Nigeria whose level contradicts the immense resources in this country.

Several studies on agricultural development implication and evaluation of government programmes as well as poverty have been carried out. No study has been conducted to evaluate the impact of National Poverty Eradication programme (NAPEP) on All Farmers Association of Nigeria (AFAN) in Kogi State. This study is carried out to refocus and make NAPEP more proactive and consequently to make its objective more realistic.

There is no universally accepted definition of poverty but some countries have established poverty line that demarcates the poor from the non-poor. The first conceptual approach to poverty was based chiefly on monetary poverty measured in terms of household income and consumption (Kankwenda, 2000). Poverty

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has been seen as lack of money and material resources as well as lack of access to economic and social opportunities (Alegieuno and Attah, 2005).

The multidimensional nature of poverty is increasingly being acknowledged. It is now a concept that transcends the absence of sufficient monetary income. The characteristics of poverty comprehensively include lack of employment opportunities, lack of land and production credit, food insecurity and malnutrition, lack of shelter, physical isolation and vulnerability to external shocks and diseases (Coote et al., 2000). Due to the multidimensional nature of poverty and its grave consequences on both humans and the economy, government have embarked on public expenditure to eliminate poverty. For most Saharan African countries, it is believed that agriculture offers the best and most sustainable way out of poverty (Fan, 2008). It was in line with this school of thought that successive Nigerian governments adopted several programmes aimed at tackling poverty, using small scale farmers as a focus group.

Government's focus on small scale farmers is predicted on two major reasons. Idachaba (1993) and Ajakaiya (1993) gave these two reasons. Firstly, poverty in Nigeria is rural in nature and small scale farmers dominate the population of rural areas in Nigeria. Secondly, small scale farmers produce about 75% of the food consumed in the country.

Therefore, any measure that reduces the pervasive poverty among the rural populace will result in the reduction of the level of poverty in the nation.

The proceeding concept has held sway among policymakers in Nigeria because the dominant poverty characteristics relate to agriculture. It is no longer news that agriculture was the main stay of Nigerian economic before it was replaced by oil.

Also, it is a common knowledge that agriculture is the chief employer of both skilled and non-skilled labour in Nigeria (IFPRI, 2007). In order to increase food productivity and reduce poverty, several programmes have been launched. Agricultural programmes and policies have become institutionalised as far back as 1900. Since then, numerous programmes have emerged to tackle low crop productivity and poverty. Some of these programmes include: Agricultural Development Project, National Accelerated Food Production Programme, Operation Feed the Nation, National Special Programme on Food Security, National Fadama Development Project, Family Economic Advancement Programme and Better life Programme.

The NAPEP was established in 2001 as the primary agency of the Federal Government of Nigeria to eradicate extreme poverty in Nigeria. The functions of NAPEP are to:

(1) Coordinate all poverty eradication efforts in the Federation.

(2) Monitor all poverty eradication activities of the federal government.

(3) Maintain a comprehensive and detailed databank on all activities aimed at eradicating poverty in Nigeria.

(4) Carry out impact assessment of all efforts meant to eradicate poverty in Nigeria, and suggest the necessary reviews and policies required to enhance effectiveness.

(5) Directly intervene in key sectors or critical needs periodically by implementing scaled key priority projects.

Between 2001 and 2005, NAPEP has trained 142,112 youth participants across the nation generating a multiple of three- spi apprentices to a participant. This gives a total of 711400 trained youths within the period, with an average of 177850 trained annually drawing from the experience of the implementation of capacity acquisition.

Coordinated Agricultural Programme (CAP) after the first phase was repackaged in 2004 to provide for the collaboration of 12 State governments. In 2005, up to 247,1999999.80 naira was released for the setting up of participants. It is expected that this arrangement would create about 140,000 jobs in the affected states (NAPEP, 2007). In 2002, Kogi State NAPEP received 5,000,000.00 naira for Farmers Empowerment Programme (FEP). This facility was extended as loan to poultry keepers, fish farmers in the state. Sixteen cooperate groups across Kogi State benefited from this facility (Gunwa, 2007).

According to NAPEP (2007), one strategy for alleviating poverty is to empower the poor masses to leverage resources and tools for well being and sustainable livelihood. NAPEP identifies access to micro credit to attacking one of the principal challenges facing the poor. All along, poor people have been unable to access credit facilities which are usually available to entrepreneurs operating in both the formal and informal sectors. The simple reason has been the lack of requisite collateral by the poor.

Subsequently, the failure of the formal financial system to extend credit facilities and other vital services to small and micro enterprises in the informal sector has short-changed a large number of people from activity participating in the economic development process. To a large extent, this trend has increased the number of people defined as poor, thereby exacerbating the poverty rate in Nigeria.

The provision of micro-credit presupposes the existence of micro-entrepreneurs or low-income borrowers who are already mobilised to use their loans with the view to repaying promptly and with ease. In other words, micro-credit cannot be practiced in a vacuum (Gunwa, 2007). The capacity of the prospective borrowers must be built, their skills developed and strengthened and right environment should be created for the repayment of loans.

In line with the above concept, Kogi State NAPEP recognised the need to partner with institutions that have poverty contents in their activities across the State. Thus,

Kogi NAPEP selected credible non-governmental organisations and community banks referred several viable cooperative groups, with at least ten members each, which are into micro-economic activities. The selection of the beneficiaries spread across the entire Kogi State; it involved up to 11 micro financing institutions. One of the Kogi State NAPEP major micro finance institutions is the All Farmers Institutions of Nigeria (AFAN).

In 2002, for instance, AFAN disbursed about 50% of the Micro-Partner Matching Fund (MPMF) to 105 farmers' cooperative groups across the 21 Local Government Areas in the State (Gunwa, 2007).

In line with its predecessors as well as its mandate, NAPEP identifies agriculture as one of its key sector and focuses on those who organise themselves into cooperative societies that benefit from NAPEP intervention in Kogi State, which is All Farmers Association of Nigeria (AFAN).

The AFAN is both microfinance institute and a group of cooperative societies. The Kogi State chapter of AFAN comprises 118 cooperative societies which are spread across the 21 Local Government Areas of the State. The cooperative societies are involved in agricultural activities raising from arable cash crops enterprises to poultry and processing. Loans which these groups have benefited range from N200,000 to N500,000 per cooperative society. AFAN in Kogi State is a conglomerate of various cooperative groups whose type of grade of farming under the auspices of AFAN, the various component cooperative groups benefit from the loan facilities extended by NAPEP in the State (Gunwa, 2007). The basis of this loan facility is to mitigate the financial constraint which is the bane of small scale farmers in Nigeria.

Programme evaluation studies are carried out at three stages: re-evaluation, on-going and post-evaluation studies. Primarily, post-evaluation studies aim at identifying the impact which a certain intervention has made on the beneficiaries (Jitinger, 1987). The subject of evaluation in this study is the impact of NAPEP's intervention on the output and income of AFAN members of Kogi State.

Several studies on agricultural development implications and evaluation of government programmes have been carried out. For instance, Ejembi and Ejembi (2005) analysed the production decisions of female livestock producers in Makurdi Local Government Area and its implication for agricultural Development. Also, Ogbanje (2008) evaluated the special programme for food security in Benue State. Furthermore, Apoju et al. (2006) appraised the participation of women in Kano State Agricultural and Rural Development Authority Extension Delivery Programmes. In addition, Abeng and Onunuugbo (2006) reviewed the impact of ICT for agricultural development on poverty alleviation. No study has been conducted to evaluate the impact of NAPEP on AFAN members of Kogi State of Nigeria.

The overall objective of this study is to evaluate the impact of NAPEP'S intervention on AFAN members in Kogi State. The specific objectives are to:

(i) Determine the membership profile of AFAN in Kogi State;
(ii) Analyse the socio- economic characteristics of the respondent;
(iii) Assess NAPEP'S agricultural programmes in Kogi State;
(iv) Evaluate the implication of NAPEP'S intervention in Kogi State; and
(v) Evaluate the impact of NAPEP's agricultural programmes on AFAN members in Kogi State.

In order to achieve specific objective, the following hypotheses were stated and tested:

(i) There is no significant difference between the total output value of NAPEP beneficiaries and non-beneficiaries;
(ii) There is no significant difference between the income of NAPEP beneficiaries and non-beneficiaries.

Evaluation studies mostly aim at refocusing the attention of programmes and agencies on their set goals and objectives. The study benefits the operators of NAPEP, the stake holders, the economy, members of AFAN, peasant farmers etc. The on-going evaluation study will help to strengthen the focus of the operators of NAPEP in Kogi State in particular and Nigeria at large. The findings of the study will draw the attention of NAPEP stakeholders and collaborators/partnerships to further areas of intervention. Thus more beneficiaries will come on board, and existing ones will receive further boost.

For a nation like Nigeria, agriculture remains the surest way out of poverty, the best attentive sources of foreign exchange, employment and food sufficiency. In the light of this fact, finding of this study will increase the scope of NAPEPs intervention in agriculture.

Members of AFAN as well as other peasants farmers in Kogi State will benefit from the findings of this study with more micro-credits extended to them; hence the farmers will witness increased income level and productivity. With these key variables of poverty under control, it is expected that sustainable poverty reduction will be attained.

METHODOLOGY

The study area is Kogi State located in the North central Zone of Nigeria. It is the confluence of River Niger and River Benue; its capital is Lokoja, which is the first administrative capital of modern-day Nigeria. Kogi State was created in 1991 from parts of Kwara State and Benue State. It comprises the peoples of the default Kabba Province of Northern Nigeria. The four main ethnic groups in

Kogi State are Igala, Ebira, Okun (Yoruba), Bassa and Yaba (Yoruba). It has a population of 3,595,789 (National Census, 2006).

Agriculture is the mainstay of the economy. The principal cash crops are notably coffee, cocoa, palm oil, cashews, peanuts, maize cassava, yam rice and melon. The mineral resources include coal, limestone, iron, petroleum and tin. The state is home to the largest iron and steel industry in Nigeria and one of the largest cement factories in Africa is currently being built there. Up to 118 cooperative societies have so far registered as members of AFAN, which are spread across the 21 Local Government Area of Kogi State, consisting of beneficiaries and non-beneficiaries of NAPEP's intervention. They are engaged in various agriculture and agro-allied ventures.

The study is limited to members of AFAN in Kogi State who have benefited from the intervention of NAPEPs and members of AFAN in the State who have not benefited from the intervention of NAPEPs. Emphasis was laid on the income and total output value of these respondents to determine the impact of NAPEP programme on the beneficiaries. Furthermore, the focus of this study was limited to evaluating the impact of NAPEP on its beneficiaries with respect to four key agricultural services offered to NAPEP beneficiaries (AFAN members): loan facility, agrochemical supplies, provision of improved crop varieties and marketing assistance rendered.

The population for the study comprises all small scale farmers who are members of All Farmers Association of Nigeria (AFAN). A two-stage stratified random sampling technique was used to select respondents for the study. In the first stage, two local government areas were randomly selected from each of the 3 Senatorial Zones in the State. In the second stage, small scale farmers who are AFAN members were selected from each local government area based on an unbiased sampling proportion of 0.021%. The sampling proportion was applied to the population of AFAN members in each local government area. Based on this, the sample size for the study was 199.

Data for the study were collected mainly from both primary and secondary sources. The primary data were obtained through the use of structured questionnaires that were given to the selected participants. Secondary data were collected from the records of AFAN and NAPEP.

The adequacy of the research instrument was determined using content validity. In the process, the instrument was thoroughly examined by appropriate experts independently. The observations of the experts were harmonised and necessary corrections effected on the instrument before field survey commenced. This is in line with the recommendation of Kerlinger (1973).

The test–retest method was used to ascertain the reliability of the instrument. In this case, the instrument was administered to 50 farmers in the study area twice within an interval of two weeks. The results of these surveys were correlated using Pearson product moment correlation which was found to be 0.75 at the significant level of 0.1. Thus, the instrument was adjudged reliable.

The data for the study were analysed using both descriptive and inferential statistics. The descriptive statistics was used to analyze objectives i-iv of this study while income change comparison and t-test were used to analyze specific objective v. The statements of hypotheses were tested using t-test.

In order to evaluate the impact of NAPEP's agricultural programmes on AFAN members in Kogi State, a comparison of the income change of respondents with NAPEP intervention to the income change of respondents without NAPEP intervention was made as follows.

Income change of respondent with NEPEP intervention = Income of Beneficiaries AFTER NEPEP intervention ─ Income of Beneficiaries BEFORE NEPEP intervention.

Assuming income change without intervention is the same for Beneficiaries and Non-Beneficiaries, then,

Income change of respondent without NEPEP intervention = Income of Non-Beneficiaries AFTER NEPEP intervention ─ Income of Non-Beneficiaries BEFORE NEPEP intervention.

RESULTS AND DISCUSSION

The membership profile of AFAN is presented in Table 1. Findings revealed that majority of the respondents acquired their membership of AFAN through cooperative societies (72.4%). In other words, they first registered with a cooperative society as a prerequisite for acquiring the membership of AFAN where as 27.6% of the respondents joined AFAN as individuals that is, they joined AFAN directly. This analysis shows that membership of AFAN can be acquired directly (as an individual) and indirectly (through cooperative societies).

As seen in Table 1, the major enterprise of most of the respondents was arable crop production (45.7%), whereas, 21.1% of them were mainly involved in tree crop production. Furthermore, 14.1, 8.0, 6.0 and 5.0% were engaged in livestock production, fishery, trading and processing enterprises, respectively. Majority of the respondents were involved in one form of agricultural activity or the other. This finding is in conformity with the report of the International Food Policy Research Institute (IFPRI) (2007) that agriculture is the major occupation of most rural Nigerians. Fans (2007) held that agriculture offers the most sustainable way out of poverty in most developing countries. Gunwa (2007) indicated that the Phase II of farmers Empowerment Programme (FEP), that includes Kogi State, was designed to boost the production of arable crops as well as livestock.

Furthermore, findings in Table 1 showed that most of the respondents (67.3%) benefited from the agency's intervention programme. According to NAPEP (2007), financial assistance from the agency is expected to mitigate critical production constraints faced by small scale farmers. This finding is in line with Gunwa (2007) that AFAN is one of the groups targeted to benefit from NAPEP.

The socio-economic characteristics of the respondents are presented in Table 2. Findings revealed that males constituted 63.8% whereas females constituted 36.2% of the respondents. Thus males constituted the majority of the respondents. Although females account for a high proportion of agricultural activities (Adepoju et al., 2006), they are usually discriminated against by agricultural development agents. According to Mohammed et al. (2009), men tend to benefit more than women from development programmes.

Majority of the respondents (37.2%) were aged between 40 and less than 50 years old. Those aged 30 and less than 40 years, 50 and less than 60 years, 20 and less than 30 years, and greater 60 years represented 33.20, 19.60, 7.0, 2.50 and 0.50% respectively, of the respondents. The age of most of the respondents was

Table 1. Profile of AFAN membership.

Options	Frequency	Percentage
Acquisition of membership		
Individual	55	27.6
Trough cooperative society	144	72.4
Total	**199**	**100**
Major enterprise		
Arable crop	91	45.7
Tree crop	42	21.1
Livestock	28	14.1
Fishery	16	8.0
Processing	10	5.0
Trading	12	6.0
Total	**199**	**100.0**
Beneficiary status		
Beneficiary	134	67.3
Non-beneficiary	65	32.7
Total	**199**	**100.0**

Source: Field Survey, 2010.

Table 2. Socio-economic characteristics of respondents.

Variable	Frequency	Percentage
Sex		
Male	127	63.3
Female	72	36.7
Total	199	100.0
Age (years)		
0 and below	1	0.5
20 < 30	14	7.0
30 < 40	66	33.2
40 < 50	74	37.2
50 < 60	39	19.6
≥ 60	5	2.5
Total	199	100.0
Level of Education		
Post graduate	14	12.1
Post secondary	76	38.2
Secondary	69	34.7
Primary	27	13.6
Non- formal	3	1.4
Total	**199**	**100.0**
Level of Income (N)		
1,000 < 50,000	28	14.1
50,000 < 100,000	30	15.1
100,000 < 150,000	17	8.5
150,000 < 200,000	78	39.2
≥ 200,000	46	23.1
Total	**199**	**100.0**

Source: Field Survey, 2010.

Table 3. Reported NAPEP'S provision of agricultural services in Kogi State.

Services	Frequency	Percentage
Provision of loan facilities	55	27.6
Provision of agrochemicals	58	29.1
Provision of improved crop varieties	43	21.6
Provision of marketing assistance	43	21.6

Source: Field Survey, 2010.

more than the 37 years which was found by Obinne et al. (2009). This was probably due to the fact that this population for the study comprised members of a social group other than those solely engaged in farming.

Findings in Table 2 further showed that majority of the respondents had post-secondary education such as National Diploma and National Certificate of Education. Holders of Secondary School Certificate constitute 34.7%, where as those with first school leaving certificate and post-graduate certificate were 13.6 and 12.1%, respectively. Respondents who had non-formal education were 1.4%. This analysis shows that the respondents attained various levels of education and can, thus give reliable opinions on the subject matter. The level of education of the respondents was above the literacy level used to characterize small scale farmers in Nigeria. The result shows farmers with a level of education which is higher than the secondary reported by Balogun et al. (2007). This is appropriate for successful interaction at the social level of farming membership of a formal cooperative society.

Income level distribution showed that majority of the respondents (39.2%) earned between N150, 000.00 and less than N200, 000.00 from their agricultural enterprises, whereas income class of greater than or equal to N200,000.00 constituted 23.1% of the respondents; 15.1, 14.1 and 8.5% of the respondents comprised those who earned between N50,000.00 and less than N100,000.00; N1,000.00 and less than N50,000.00, and N100,000.00 and less than N150,000.00, respectively. Those farmers earn an average income of N196, 685.00. Higher farm income encourages farmers to increase their level of productivity.

NAPEP's provision of Agricultural Services in Kogi State is presented in a multiple response format in Table 3. Findings revealed that 27.6% of the respondents were assisted with loans by NAPEP. Lack of financial capital has been the major constraint faced by small scale farmers in developing countries. Asogwa et al. (2007) noted that farmers are poor and cannot invest adequately in agricultural productivity. Thus, the provision of loan to farmers by NAPEP is an appropriate strategy to mitigate a major constraint faced by farmers. In addition, up to 29.1% of the respondents indicated that NAPEP assisted them with agrochemicals such as fertilizer, herbicide and insecticide. This assistance is also appropriate.

The great pressure on land fast depletes soil fertility. This is where the use of inorganic fertilizer for soil fertility restoration and subsequent increased production become a valuable succour. Igwe et al. (2009) corroborate this axiom when they noted that the security of fertilizer is the bane of crop productivity among small scale farmers. In addition, the use of herbicide has been found to reduce the arduous labour associated with weeding.

Up to 21.6% of the respondents indicated that NAPEP assisted them with improved varieties of their various crops. Improved varieties of crops have various potentials including early maturity, higher productivity and resistance to pest and disease attack. Therefore, the provision of improved varieties of crops is an appropriate strategy for increasing agricultural productivity. Umeh (1998) reported that most farmers use farmers-saved-seed to assuage scarcity of seed for planting. Similarly, Anozie et al. (2008) indicated that improved varieties of crops are not easily accessible by small scale farmers.

Equally, 21.6% of the respondents showed that NAPEP assisted them with the marketing of their produce. Lack of easy access to market affects farmers in two major ways: one, where farmers do not have easy access to markets, they obtain low prices for their produce; and two, where farmers cannot easily sell their produce, and there can be large scale post harvest loss. Damiyal et al. (2007) maintained that post-harvest losses have been the bane of Nigerian's agricultural sector.

The implications of the NAPEP intervention in Kogi State are presented in Table 4. Findings revealed that majority of the respondents (34.2%) repaid NAPEP's loan with income from their farm enterprises. Whereas 33.2% indicated that they repaid their loans with income from other sources, 32.6% of respondents were indifferent. This analysis shows that NAPEP has no specific policy on loan repayment with respect to income source which would have served as a basis for monitoring by NAPEP. The situation could lead to large scale default in loan; loans are not often repaid and, hence, not recycled. This trend can cripple the sustainability of NAPEP. Findings in Table 4 also show that the expansion of the enterprise of most respondents (44.7%) was due to NAPEP intervention. While 22.6% of the respondents disagreed with this opinion, 32.7% of the respondents were simply indifferent. This finding implies that the impact of the NAPEP intervention has not been felt by all members

Table 4. Implications of NAPEP intervention in Kogi State.

Variable	Frequency	Percentage
Loan repayment sources		
Income from farm enterprise	68	34.2
Income from other Sources	66	33.2
Not Applicable	65	32.6
Total	**199**	**100.0**
Effect of NAPEP		
Enterprise expansion due to NAPEP	89	44.7*
Increased in income due to NAPEP	71	35.7*
NAPEP's capacity to reduce poverty	104	52.3*
NAPEP's capacity to reduce unemployment	48	62.3*
Sector NAPEP should refocus		
Agro processing	98	49.2
Storage of agricultural produce	68	34.2
Produce transport	7	3.52
Feeder road construction	9	4.52
Agro- processing and produce	17	8.54
Total	**199**	**100.0**

Source: Field Survey, 2010.*Majority's response.

of AFAN. Similarly, majority of the respondents (35.7%) agreed that NAPEP's intervention accounted for their increase in income, although, 31.6% disagreed and 32.7% were indifferent. Expansion of enterprise and income of beneficiaries are critical to poverty reduction. One of NAPEP's strategies for alleviating poverty is to empower the poor to utilize resources for their well being and sustainable livelihoods through enterprise expansion and increased income (NAPEP, 2007).

The foregoing attests positively to the impact of NAPEP. It is therefore not surprising that majority of the respondents indicated that NAPEP's intervention has the capacity to reduce poverty (52.3%) as well reduce unemployment (62.3%). Nzekwu (2006) held that one of the basic causes of poverty in a developing country is lack of access to employment opportunities. Coote et al. (2000) too noted that the characteristics of poverty comprehensively include lack of employment opportunities, access to social services, land and production credit. Therefore, reduction of unemployment as a result of NAPEP's intervention is credible.

There are varying opinions on the sector which NAPEP should re-focus, probably, having concentrated on health and education in the ongoing phase. Findings revealed that most of the respondents were in favour of agro-processing (49.2%) and storage of agricultural produce (34.2%). Post harvest losses have been the bane of agricultural productivity. The panacea to this problem revolves around processing of produce as well as good storage of the produce so as to minimise the losses incurred by farmers annually. Abdullahi (2001) put the annual post harvest losses at 25 million metric tonnes of agricultural commodities with an estimated value of 500 billion naira.

The analysis of the major problems of NAPEP is presented in Table 5. Findings revealed that the major problem of NAPEP in Kogi State was that just a few beneficiaries were selected (32.7%). Other serious problems which respondents associated with NAPEP in Kogi State were inadequate loan (20.7%) and a combination of inadequate loan and few beneficiaries (16.1%). The general perception is that there are relatively few beneficiaries of the intervention of NAPEP Programme. The lack of loan and credit facilities identified by KHAN (1994) as one of the critical characteristics features of the poverty and rural under-development actually indicate there are few beneficiaries of intervention packages. Duru (2002) pointed out that loan facilities often find their way into the hands of unintended beneficiaries. This is why Olaitan (2005) suggested that micro-finance institution enables the poor to create, own and accumulate assets. Sustainable access to micro-finance helps alleviate poverty by generating income, creating jobs, and generally empowering the people.The income change of beneficiaries and non-beneficiaries of the programme is presented in Table 6. The result shows that mean income change of beneficiaries is 11,945 naira while the mean income change of non-beneficiaries is 2,429 naira. This represents 99.09% change in income for beneficiaries

Table 5. NAPEP'S Major Problems.

Problem	Frequency	Percentage
Inadequate loan	41	20.7
Few beneficiaries	65	32.7
Inadequate training	2	1.0
Inadequate input	4	2.0
Untimely loan	6	3.0
Short moratorium	5	2.5
Few beneficiaries and untimely loan	12	6.0
Inadequate loan and few beneficiaries	32	16.1
Inadequate loan and inadequate training	13	6.5
Inadequate loan and input	7	3.5
Inadequate loan and untimely loan	6	3.0
Few beneficiaries inadequate training	6	3.0
Total	**199**	**100.0**

Source: Field Survey, 2010.

Table 6. Income change among the respondents.

Variables	Beneficiaries	Non-Beneficiaries
Income before NAPEP intervention	1,615,340	820,004
Mean income before NAPEP intervention	12,054.78	12,615.45
Income after NAPEP intervention	3,216,000	977,900
Mean income after NAPEP intervention	24,000	15,044.62
Income change	1,600,660	157,896
Mean Income change	11,945.22	2,429.17
Mean percentage change	99.09	19.26

Source: Field Survey, 2010.

and 19.26% change in income for non-beneficiaries, indicating the high impact of the programme on the beneficiaries who are mostly poor small scale farmers.

The difference in total output value (in 00,000 naira) of crops of beneficiaries and non-beneficiaries is presented in Table 7. Out of the 10 crops used for this test of mean difference in total output value of beneficiaries and non-beneficiaries, the t-ratios of yam (3.397) and cassava (8.971) were positive. This implied that beneficiaries had output in yam and cassava than non-beneficiaries. The t-ratios were statistically significant at 0.01% level of significance, showing the positive impact which NAPEP's intervention made on the beneficiaries. On the other hand, non-beneficiaries of NAPEP's intervention had more output in maize and guinea corn than the beneficiaries.

The t-ratios of maize (-1.814) and guinea corn (-1.961) were statistically different at 10% level of significance.The implication of this result is that NAPEP's intervention in Kogi State probably did not focus directly on the production of these food crops. The difference in income of beneficiaries and non- beneficiaries is presented in Table 8. The result of the t-test of difference of means of income showed that beneficiaries of NAPEP intervention had mean income of N535.970.00, where as non-beneficiaries had mean income of N371,293.00. The mean difference in income of beneficiaries and non-beneficiaries was as large as N164, 677.00. The t-ratio of the difference is positive (2.85) and is significant (0.006) at 0.01 level of probability. Thus, the null hypothesis is rejected in favour of the alternative hypothesis. The implication is that there is significant difference in income of beneficiaries and non-beneficiaries of NAPEP services being evaluated in this study. This result, thus, underscores the successful impact of NAPEP's intervention on poverty reduction among the beneficiaries in Kogi State.

Conclusion

The study evaluated the effect of the intervention of NAPEP on the members of AFAN in Kogi State. Membership of AFAN can be acquired both as an

Table 7. Differences in output of cropsof beneficiaries and non-beneficiaries of NAPEP.

Crop	Group	Output value	Mean difference	t-ratio	Sig.
Yam	Beneficiaries	4.9125	1.2234	3.397	0.001*
	Non-Beneficiaries	3.6891			
Cassava	Beneficiaries	18.8277	10.8230	8.971	0.000*
	Non-Beneficiaries	8.0046			
Maize	Beneficiaries	1.3739	-8.8693	-1.814	0.077**
	Non-Beneficiaries	2.2432			
Cowpea	Beneficiaries	2.2667	-0.6833	-0.364	0.751
	Non-Beneficiaries	2.9500			
Groundnut	Beneficiaries	1.4115	-0.2654	-0.687	0.505
	Non-Beneficiaries	1.6769			
Rice	Beneficiaries	2.6400	-0.62000	-2.134	0.100
	Non-Beneficiaries	3.2600			
Guinea corn	Beneficiaries	0.6077	0.4308	-1.961	0.074**
	Non-Beneficiaries	1.0385			
Millet	Beneficiaries	0.4500	-0.2500	-1.667	0.344
	Non-Beneficiaries	0.7000			
Soybean	Beneficiaries	1.2500	0.2000	0.667	0.626
	Non-Beneficiaries	1.0500			
Cashew	Beneficiaries	6.3333	5.5167	1.155	0.300
	Non-Beneficiaries	0.8167			

Source: Field Survey, 2010, *t-ratio is significant at the 1% level of probability (2-tail test), ** t-ratio is significant at the 10% level of probability.

Table 8. Difference in income between beneficiaries and non-beneficiaries.

Beneficiary status	Mean income	Mean difference	Standard deviation	t-ratio	Sig.
Beneficiaries	535,970	164,677	439.9876	2.85	0.006
Non-Beneficiaries	371,293				

Source: Field Survey, 2010, *t-ratio is significant at 0.01 level of probability (2-tail test).

individual and through a cooperative society. A great majority of the respondents were involved in one agricultural enterprise or the other, but mostly arable crop production. The respondents were largely educated and were adults engaged in income generating activities.

Majority of the respondents benefited from NAPEP'S intervention in one form or the other, and hence attributed the expansion of their enterprises in incomes and number of employees to NAPEP'S intervention. The phase one of NAPEP in Kogi State concentrated more on health and educational sectors of Kogi State economy. Hence, the sectors which NAPEP could re-focus, especially in the subsequent phase of the programme, are processing and storage of agricultural produce.

The t-test for outputs in crop indicates that beneficiaries of NAPEP's intervention had significantly greater output in crops like cassava, yam and maize than non-beneficiaries. On the other hand, non-beneficiaries

had significantly greater output in guinea corn than the beneficiaries. Furthermore, the income of the beneficiaries significantly exceeded that of the non-beneficiaries. Thus, the intervention of NAPEP had positive impact on members of AFAN who were beneficiaries of NAPEEP intervention in Kogi State.

However, some major problems hindered the success of NAPEP. One of the problems was that only a few beneficiaries were selected among the numerous individuals who fall under the low-income bracket. Another problem was that the loan granted to the people was inadequate.

RECOMMENDATIONS

Based on the findings of the study, the following recommendations are made:

(1) NAPEP should select more beneficiaries. In this way, the impact can spread wider.
(2) NAPEP's loan to beneficiaries should also be increased. This will enable beneficiaries to make more meaningful investment with the fund.
(3) Since agriculture is the major occupation of rural people in Kogi State, NAPEP should intensify the provision of critical productive inputs. This will enhance productivity, especially of the beneficiaries.
(4) In line with the popular community driven development strategy, NAPEP's intervention should focus on the processing of agricultural produce. This will add value to the produce, make the produce to command higher prices, increase varieties and generate more employment opportunities.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Growth, nodulation and yield components of mung bean (*Vigna radiata*) as affected by phosphorus in combination with rhizobium inoculation

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Mungbean (*Vigna radiata* L.) is capable of fixing atmospheric nitrogen through bacteria living in its root nodules. To study the influence of phosphorus (P) fertilization and *Rhizobium* inoculation on the nodulation, growth and yield of mungbean (*Vigna radiata*), a pot experiment was conducted in wire house, during spring season of 2010 at University of Agriculture, Faisalabad. A composite soil sample was collected from the research area and analysis of physical and chemical properties of the soil was done. Seeds of mungbean cv, NM-92 were inoculated with *Rhizobium* and sown in pots containing 14 kg soil. Phosphorus was applied at 1.2, 2.4 and 3.6 g per 14 kg soil in each pot alone and along with inoculation of Rhizobium phaseoli except in control pot. The source of P was single super phosphate that was mixed with soil before filling the pots. It was noted that combined use of P and inoculant enhanced the number of nodules per plant. The maximum nodules, 8.67 per plant, were recorded where 3.6 g P along with *Rhizobium* inoculation was applied. Phosphorus application along with *Rhizobium* inoculation increased the plant height significantly (13.2%) over control. Maximum increase in plant height at maturity, total number of pods and number of grains per pod were also recorded where 3.6 g of phosphorus along with *Rhizobium* inoculation was applied.

Key words: Mungbean, growth, nodulation, phosphorus, inoculation.

INTRODUCTION

Mungbean (*Vigna radiate* L), commonly known as green gram, is an important conventional pulse crop of Pakistan. In most of the Asian countries, the diet is cereal based. It is a rich source of protein (23%) (Hussain et al., 2010). It has the ability to fix atmospheric nitrogen (N) through *Rhizobium* species, living in nodules on its roots. The area sown under this crop in the year 2008-2009 was 231.1 thousand hectares with a production of 157.4 thousand tons (Govt. of Pakistan, 2009). The rate of nodulation is very low in most of the mungbean growing

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area of Pakistan. There are numerous reasons but imbalanced nutrition appears to be one of them. Therefore, artificial inoculation of mungbean with effective *Rhizobia* is a useful practice for improving its yield (Guozan et al., 1985). Seed inoculation significantly increased the growth parameters of mungbean (Khan and Kounsar, 2000). Besides this, according to Gowda and Gowda (1978), due to the ability of fixing atmospheric nitrogen, mungbean requires phosphorus to improve the yield and quality of grain. Phosphorus also has favourable effects on the number and weight of effective nodules on the root system of leguminous crops (Brady, 1984). The present study was planned to determine the effect of *Rhizobium* in combination with different levels of P on nodulation, growth and yield of mungbean cultivar NM-92 under irrigated conditions at Faisalabad.

MATERIALS AND METHODS

Pot experiment was conducted during March – May 2010 in wire house of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Surface soil (15 cm depth) was collected from the research area and air dried, mixed thoroughly and passed through 2 mm sieve. A representative sample was taken for physical and chemical analysis of the soil. Characteristics of experimental soil are given in Table 1.

Seeds of mungbean cv, NM-92 were inoculated with *Rhizobium* phaseoli according to standard methods and sown in pots containing 14 kg soil in each. The inoculum was purchased from Ayub Agriculture Research Institute (AARI), Pakistan. It was sold as Nitorgen Ka Teeka. 10% sugar solution was made and added with the black powder, the material provided in the standard pack, and mixed thoroughly. When the sticky paste or solution was ready it was mixed well with the seed of mungbean and sown according to different treatments immediately after drying for one hour under the shady place. The experiment was laid out in 2 factorial completely randomized design (CRD) consisted of different treatments like P_0 (control treatment that was without P and *Rhizobium* inoculation), $P_{50\%}$ of recommended P (1.2 g P), $P_{100\%}$ of recommended P (2.4 g P), $P_{150\%}$ of recommended P (3.6 g P) per pot, P_0 (*Rhizobium* inoculation without P), $P_{50\%}$ (*Rhizobium* inoculation+1.2 g P), $P_{100\ \%}$ (*Rhizobium* inoculation + 2.4 g P), $P_{150\%}$ (*Rhizobium* inoculation + 3.6 g P). These treatments were replicated three times to minimize error.

Recommended doses of N and K fertilizers were applied at 25 and 62 kg ha^{-1}, by using urea of 38 g per 14 kg soil (w/w) and MOP of 72 g per 14 kg soil (w/w) for the sowing, respectively.

The pots were irrigated with fresh water, using sprinkler bucket in seven days interval. Plant height was recorded after 15 days of germination and at the time of maturity. After harvesting, length of roots, number of nodules per plant, number of pods per plant, number of grains per pod, weight of grains per plant and 1000-grain weight were recorded. NP uptake was determined by the following formulae:

N uptake = {(Conc. of N in grain × Oven dry wt. of grain) + (Conc. of N in straw × Oven dry wt. straw)} / 100

P uptake = {(Conc. of P in grain × Oven dry wt. of grain) + (Conc. of P in straw × Oven dry wt. straw)} / 100

The unit is g $plant^{-1}$, for conversion of g $plant^{-1}$; g $plant^{-1}$ will be multiplied by 1000. The data collected for various characteristics were subjected to the analysis of variance and means were compared by using Duncan's Multiple Range Test (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

Plant height at maturity (cm)

The comparison of inoculated means with uninoculated revealed that average height of inoculated plants was increased by 10.19% as compared to average height of uninoculated plants (Table 1). The overall comparison of means resulted in the increase in plant height by 3.9 to 13.2% over its respective control; the interaction between graded P levels and rhizobium inoculation was significant. The maximum plant height (40.35 cm) was recorded in the inoculated plants when it was supplemented with 150 % of the recommended dose of phosphorus. These results were also supported by Thakur and Panwar (1995) who reported an increase in plant height by seed inoculation and fertilizer application.

Number of nodules $plant^{-1}$

In uninoculated plants, no nodulation was observed (Table 1). The inoculated plants were infected by *Rhizobium* for nodulation and the average number of nodules per plant was 6.0 which further increased aggravately with application of increased P levels. The average number of nodules per plant was 11.96 in case of inoculated compared with uninoculated plants as it was 0.00 nodules $plant^{-1}$.The comparison means of different P levels with *Rhizobium* treatments indicated that all the three levels increased nodulation from 78-190% over their *Rhizobium* inoculation alone. These results were quite in line with those of Bar and Lal (1991).

Number of pods $plant^{-1}$

Rhizobium inoculation along with graded levels of phosphorus significantly affected the pod formation (Table 2). In case of uninoculated plants, maximum number of pods was observed where the highest dose of phosphorus (150%) of the recommended phosphorus) was applied which further increases to 23.5% with combined application of P and rhizobium inoculation. Among the inoculated plants, the maximum pod formation (24.16 pods $plant^{-1}$) was observed in P_{100} which was 42.11% higher over its respective control. Inoculation strongly promoted the average pod formation as compared to uninoculated plants (50.32% higher than uninoculated plants). The comparison of the overall means of different P levels indicated that P rates of recommended and 150% of the recommended were statistically non significant but they were 17.59 and

Table 1. Effect of phosphorus and Rhizobium inoculation on plant height and no. of nodules plant^{-1}.

Phosphorus levels	Plant height (cm)			Number of nodules plant^{-1}		
	Uninoculated	Inoculated	Mean	Uninoculated	Inoculated	Mean
P_0 [no phosphorus(control)]	32.05^{f}	34.71^{d}	33.38^{B}	0.00^{e}	6.00^{d}	3.0^{C}
P_{50} [50% of recommended P(1.2 g)]	33.15^{e} (3.4)*	36.19^{c} (4.3)*	34.67^{B} (3.9)*	0.00^{e}	10.17^{c} (69.5)*	5.1^{B} (78)*
P_{100} [100% of recommended P(2.4 g)]	36.27^{d} (13.2)	39.28^{b} (13.2)	37.78^{A} (13.2)	0.00^{e}	14.33^{b} (138.8)	7.2^{A} (140)
P_{150} [150% of recommended P(3.6 g)]	35.11^{b} (9.5)	40.35^{a} (16.2)	37.73^{A} (13.0)	0.00^{e}	17.33^{a} (188.8)	8.67^{A} (189)
Mean	34.15^{B}	37.63^{A} (10.19)*		0.00^{B}	11.96^{A}	

Table 2. Effect of phosphorus and Rhizobium inoculation on no. of pods plant^{-1} and no. of grains pod^{-1}.

Phosphorus levels	**No. of pods plant^{-1}**			**No. of grains pod^{-1}**		
	Uninoculated	**Inoculated**	**Mean**	**Uninoculated**	**Inoculated**	**Mean**
P_0 [no phosphorus(control)]	12.33f	17.00c	14.66C	4.20b	5.58b	4.89C
P_{50} [50% of recommended P(1.2 g)]	13.66e (10.8)*	19.00d (11.7)*	16.33B (11.4)	5.28b (25.7)*	6.63ab (18.8)*	5.95B (21.7)*
P_{100} [100% of recommended P(2.4 g)]	14.50de (17.6)	24.16a (42.1)	19.33A (31.9)	6.18ab	7.53a (34.9)	6.85A (40.1)
P_{150} [150% of recommended P(3.6 g)]	15.16d (23.0)	23.50a (38.2)	19.33A (31.9)	6.18ab (47.1)	7.14a (28.0)	6.66A (36.2)
Mean	13.91B	20.19A (50.3)*		5.46B	6.72A (23.1)*	

22.95% higher over their respective control. Similar results were recorded by Shukla and Dixit (1996) and Ashraf et al. (2003).

Number of grains pod^{-1}

In uninoculated plants the increasing P levels enhanced the grain formation as compared to control (without phosphorus) and P_{100} and P_{150} were statistically non significant (Table 2). In inoculated plants the P levels increased the grain formation as compared to control and P_{100} and P_{150} were statistically non significant. Overall comparison of means of P level indicated that recommended P enhanced the grain formation which was 40.08% higher than its respective control. The comparison of average of inoculated plants to uninoculated was also highly significant and it was 23.08% higher than uninoculated plants. The interaction of rhizobium inoculation and phosphorus inoculation was found non- significant. Shaheen and Rahmatullah (1996) and Perveen et al. (2002) also observed similar types of results in their findings with different treatments of Phosphorus with Rhizobium.

Weight of grains plant^{-1} (g)

The data (Table 3) regarding the grain weight of mungbean (g plant^{-1}) as affected by various levels of phosphorus alone and in combination with *Rhizobium* were recorded after harvest and analysis of variance indicated the significant difference in grains' weight of mungbean due to different treatments. Among the overall means for different P levels, the maximum grain weight (3.32 g plant^{-1}) was observed in P_{100} and it was statistically non-significant with P_{150}. Average fresh weight of grains in the inoculated plants was 105.23% greater than the average fresh weight of grains of uninoculated plants. The interaction between phosphorus levels treatments and *Rhizobium* inoculation was also highly significant. These results are in accordance with Khan and Kounsar (2000) and Perveen et al. (2002).

1000- Grain weight (gm)

The analysis of variance of the data regarding the effect of phosphorus and *Rhizobium* inoculation on the 1000-grain weight (Table 3) indicated that difference in 1000-grain weight due to different phosphorus levels and inoculated treatments were highly significant but at the same time the interaction of P rates along with Rhizobium inoculums was non significant. Among the overall means for graded P levels, maximum 1000- grain weight (25.6 g) was recorded in case of P_{150} which was 16.89% higher than its respective control. At the same time, this P_{150} was statistically at par with P_{100}. The average mean (24.8 g) for inoculated plants resulted in 5.1% greater than the average mean (26.3 g) in uninoculated plants. Interaction of both graded P levels and *Rhizobium* inoculums was non- significant. Response of mungbean to phosphorus along with *Rhizobium* inoculation was also studied by Singh et al. (1993), Khan and Kounsar (2000) and

Table 3. Effect of P and *Rhizobium* inoculation on weight of grains plant^{-1} and 1000- grain weight.

Phosphorus levels	Weight of grains plant^{-1} (gm)			1000- grain weight (gm)		
	Uninoculated	Inoculated	Mean	Uninoculated	Inoculated	Mean
P_0 [no phosphorus(control)]	1.39 ^{e}	1.92 ^{c}	1.66 ^{C}	21.6^{b}	22.2^{b}	21.9 ^{C}
P_{50} [50% of recommended P(1.2g)]	1.65 ^{d} (18.7)*	2.84 ^{b} (47.9) *	2.25 ^{B} (35.5)*	23.8^{b} (10.2)*	24.5^{ab} (10.4)*	24.2 ^{B} (10.5)*
P_{100} [100% of recommended P(2.4)]	1.93 ^{c} (38.8)	4.72 ^{a} (145.8)	3.32 ^{A} (100.0)	24.5^{ab} (13.4)	26.1^{a} (17.6)	25.3 ^{A} (15.5)
P_{150} [150% of recommended P(3.6)]	1.92 ^{c} (38.1)	4.65 ^{a} (142.1)	3.28 ^{A} (97.6)	24.7^{ab} (14.4)	26.5^{a} (19.4)	25.6 ^{A} (16. 9)
Mean	172 ^{B}	3.93 ^{A} (105.2)*		23.6 ^{B}	24.8 ^{A} (5.1)*	

Table 4. Effect of phosphorus and Rhizobium inoculation on nitrogen and phosphorus uptake by plant.

Phosphorus levels	Nitrogen uptake (mg plant^{-1})		Mean	Phosphorus uptake (mg plant^{-1})		Mean
	Un inoculated	Inoculated		Un inoculated	Inoculated	
P_0 [no phosphorus(control)]	64.85^{g}	97.68^{e}	81.27^{D}	2.43^{f}	3.93^{e}	3.18^{D}
P_{50} [50% of recommended P(1.2g)]	83.27^{f} (28.40)*	152.79^{b} (56.41)*	118.03^{C}(45.23)*	3.65^{e} (50.20)*	6.81^{d} (73.28)*	5.23^{C} (64.46)*
P_{100} [100% of recommended P(2.4)]	112.38^{d} (39.29)	282.09^{a} (188.78)	197.23^{B} (142.19)	7.36^{d} (203.29)	19.47^{b} (395.41)	13.41^{B} (321.69)
P_{150} [150% of recommended P(3.6)]	127.99^{c} (97.36)	290.06^{a} (196.94)	209.02^{A} (157.19	10.98^{c} (351.85)	25.19^{a} (540.96)	18.09^{A} (468.86

Perveen et al. (2002) and they noted the results which are in line with this study

Nitrogen uptake in plant (mg plant^{-1})

In inoculated plants the maximum nitrogen uptake (196.94 % higher than the respective inoculated control) was the highest dose of P (150% of the recommended P). The comparison of over all means of P treatments indicated that maximum uptake of N was at the P levels of 150% of the recommended dose and it was 157.19 % greater than the control where no P was applied. The other rest P levels P_{100} and P_{50} followed the P_{150}.Average N uptake in inoculated plants was significantly higher than the uninoculated plants. It was 111.74% higher than its respective uninoculated plants. The interaction between P rates and rhizobium inoculation was also found highly significant. Shah et al. (1996) and Shaheen and Rahmatullah (1996) also reported that treated plots with rhizobium inoculums improved the N and P uptake by plants.

Phosphorus uptake in plant (mg plant^{-1})

The analysis of variance of the data given in Table 4 showed that in uninoculated plants the highest dose of P increased the P uptake by 351.85 % over its respective control P_0.The other rest two levels of Phosphorus, P100 and P50 followed the P150 in the inoculated plants. The comparisons of overall means of uninoculated and inoculated plants showed that P treatments had significant effect on P uptake and maximum uptake was 18.09 mg plant^{-1}. This was 468.86% higher than its respective control where no P was applied. Inoculation significantly promoted the average P uptake by the plants as compared to uninoculated plants which were 127.04% higher than uninoculated plants. The interaction between P levels and rhizobium inoculation was also highly significant. Bar and Lal (1991) studied the same observations in their experiments.

Conclusion

Phosphorus along with *Rhizobium* inoculation application increased plant height, total number of pods, number of grains per pod, and 1000- grain weight significantly. Maximum increase was noted where 150% of the recommended phosphorus along with *Rhizobium* inoculation was applied but it was statistically at par with the results obtained

by applying 100% of the recommended phosphorus along with *Rhizobium* inoculation. So 100% of the recommended phosphorus along with *Rhizobium* inoculation is the best treatment in the experiment.

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Full Length Research Paper

Changes in morphological and physiological characteristics of sunflower (*Helianthus annuus* L.) hybrids in relation to the water deficit stress

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Sunflower is one of the most cultivated oil crops in the world. In Middle East environments where water deficit frequently occurs, tolerant cultivars and irrigation are used to overcome environmental constraints due to water scarcity. Since, in the literature, the data on the effect of these techniques on morphological and physiological characteristics of sunflower are scarce and contrasting, the aim of this paper was to evaluate the effect of the water deficit stress on morphological and physiological characteristics of sunflower hybrids in a Middle East environment. For this purpose, four hybrids (Azargol, Alstar, Hysun 33 and Hysun 25) sowed under three water regimes [normal irrigation, mild water deficit stress (WD2) and intense water deficit stress (WD3)] in 2009. The results of this study indicated that the highest and least seed yield in normal irrigation was found on Azargol (3448 kg ha^{-1}) and Hysun 25 (1688 kg ha^{-1}), respectively. However, in the mild and intense water stress conditions the seed yield with cultivar Alstar having the highest value (2121 and 829 kg ha^{-1}, respectively) and Azargol and Hysun 33 had the lowest value (893 and 263 kg ha^{-1}, respectively). The result of this study indicated that application of WD2 and WD3 caused a decrease in leaf area index (LAI) and total dry weight (TDW) of all sunflower hybrids that are studied in this research.

Key words: Sunflower, morphology, physiology, leaf area index (LAI), water deficit stress.

INTRODUCTION

Sunflower (*Helianthus annuus* L.), with a world production of grain and oil, respectively over 28.5 × 10^6 and 10.5 × 10^6 Mg achieved on around 22.6 × 10^6 ha with a seed yield of 1.3 Mg ha^{-1} (FAO-STAT Agriculture, 2009), is one of the most important sources of edible oil, widely grown in many countries throughout the world (Perry, 1978). In recent years, sunflower planted area has increased because of moderate cultivation requirements and high oil yield. Due to the sunflower ability to tolerate short periods of water deficit (Hattendorf et al., 1988; Me´rrien and Grandin, 1990), the potential exists for it to become an important crop also in sub-arid environments and wherever available irrigation water is limited (Boyer, 1982). The term stress is most often used subjectively and with various meanings. Stress is the altered physiological condition caused by factors that tend to alter equilibrium. Strain is any physical and chemical change produced by a stress (Gasper et al.,

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Table 1. Physicochemical characteristics of field soil.

Soil Characteristics	Results
Soil texture	loam
Sand (%)	36
Silt (%)	39
Clay (%)	25
Saturation percentage	33
Organic matter (%)	1.1
NH_4–N (mg kg^{-1} dry soil)	0.11
Available phosphorus (mg kg^{-1} dry soil)	3.1
Potassium (mg kg^{-1}dry soil)	245
Calcium (mg kg^{-1} dry soil)	62.81
Soil pH	7.62
Electrical conductivity (dSm^{-1})	1.8

2002; Chaves et al., 2002; Chaves et al., 2003; Hu et al., 2006).Some evidences have indicated that water stress deficit causes considerable decrease in yield of sunflower (Stone et al., 2001; Angadi and Entz, 2002). Growth is one of the most drought-sensitive physiological processes due to the reduction of turgor pressure. Cell expansion can only occur when turgor pressure is greater than the cell wall yield threshold. Water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure (Karthikeyan et al., 2007; Jaleel et al., 2007). The reduction in plant height is associated with the decline in the cell enlargement and more leaf senescence in the plant *Abelmoschus esculentum* under water stress (Manivannan et al., 2007). During water stress, the total leaf area per plant decreased significantly in *Eragrostis curvula*, *Oryza sativa*, *A. esculentum*, and *Asteriscus maritimus* (Rucker et al., 1995; Sadras et al., 1993; Shubhra and Ooswami, 2003). Reduction in leaf area by water stress is an important cause of reduced crop yield through reduction in photosynthesis (Rucker et al., 1995). The reduction in plant height and leaf area under water stress may be associated with the decline in the cell enlargement and more leaf senescence in *A. esculentum*. A decrease in total dry matter may be due to the considerable decrease in plant growth, photosynthesis and canopy structure, as indicated by leaf senescence during water stress in *A. esculentum*. Morphological parameters like fresh and dry weights have a profound effect in water-limited conditions. There was a one-third reduction in fresh and dry weights of the *Ziziphus rotundifolia* plant under drought conditions (Tsialtas et al., 2001). Progressive drought resulted in a significant reduction in early allocation of dry matter and decreased fresh and dry weight in all plant parts in *Populus davidiana*. Under water-deficit stress, the biomass production was decreased in *Populus cathayana* and drought severely affected all growth parameters. There was a significant reduction in shoot dry weight due to water-stress treatments in sugar beet genotypes (Zhang et al., 2004), and mild stress affected the dry weights of shoots, while shoot dry-weight loss was greater than root dry-weight loss under severe stress. Reduced biomass production due to water stress has been observed in almost all genotypes of sunflower (Jaleel et al., 2007; Martinez et al., 2003).

However, some genotypes showed better stress tolerance than others did. Tahir et al. (2003) evaluated 25 inbred lines of sunflower for drought tolerance. They reported a decrease in plant height, leaf area, head diameter, 100-achene weight, yield per plant, and plant biomass due to water stress. They further suggested that these traits could be used as a selection criterion for higher yield per plant under water deficit. The effect of water deficits on the harvest index of sunflower is complex due to the interactions between the timing and intensity of the stress relative to the developmental processes that determine the components of yield.

The objectives of this research were to evaluate the effect of the water deficit stress on morphological and physiological characteristics of sunflower hybrids in a Middle East environment.

MATERIALS AND METHODS

The experimental factors were irrigation regimes consisting of tree levels of irrigation after 50 (normal irrigation), 100 (mild stress) and 150 (intense stress) mm cumulative evaporation from evaporation pan Class A, respectively, and genotype represented by four sunflower hybrids (Azargol, Alstar, Hysun 33 and Hysun 25). Sunflower seeds were obtained from the Plant Improvement Institute in Karaj, Iran. All combinations of the above treatments were laid out in 2009 in the field according to a split-plot randomized complete block design (RCBD) with three replicates, assigning water supply treatments to the whole units and genotypes to the subunits. The soil used was loam. The soil texture was determined with the hygrometer method (Dewis and Freitas, 1970). The physiochemical characteristics are presented in Table 1. Electrical conductivity, pH and ions of saturation extract were determined according to Jackson (1962). The available

Table 2. Mean square values from analysis of variance (ANOVA) of leaf number, plant height, head diameter, harvest index and yield of sunflower hybrids (H) in relation to the water deficit stress (WD).

S.O.V	df.	Leaf Number	Plant height (cm)	Head diameter (cm)	Harvest index (%)	Seed yield (kg ha^{-1})
Rep	2	0.49 Ns	272.91^{NS}	0.75*	79.22**	147177.33**
Water deficit (WD)	2	22.77**	5724.63**	117.547**	1238.43**	12825106.54**
Error a	4	0.24	59.18	0.10	15.88	16140.67
hybrid (H)	3	89.75**	523.80**	28.896**	188.06**	1130703.75**
WD × H	6	4.39^{NS}	1180.99**	2.10**	90.78**	840906.04**
Error b	18	3.41	91.60	0.21	14.32	48939.56
CV		7.36	8.7	4.22	14.98	15.01

* = p ≤ 0.05; ** = p ≤ 0.01; NS = non-significant.

phosphorous was determined from saturated paste extract (Olsen and Sommers, 1982). The ammonium was estimated by acid digested material (Bremner and Mulvaney, 1982) and organic matter through sulphuric acid using the Walkley-Black Method (Sahrawat, 1982). The pre-planting irrigation was applied 15 days before sowing. When the soil came into condition of field capacity, then it was well ploughed for sowing. Seeds were hand drilled on 14 May, 2009 with row to row spacing of 65 cm. Thinning the plants was done 15 days after germination to keep plants at a spacing of 20 cm. The plant density was 70000 plant ha^{-1}. Water deficit treatments were applied at the vegetative stages of plant growth (Chimenti and Hall, 1993). All dry weights were expressed on a unit area basis after drying samples in a forced air oven at 70°C for at least 72 h. At maturity, yield plant^{-1} was recorded. The plants of a 5.2 m^2 area in the middle of each subplot were harvested and their seed were separated manually from heads to determine their yield. Harvest index was calculated as the ratio of seed yield to aboveground biomass (carbohydrate equivalent) at maturity. Leaf area index (LAI) and dry matter were measured from each Phenological stage to calculate LAI and total dry weight (TDW). Analysis of variance (ANOVA) of the data from each attribute was computed using the SAS package (SAS Institute, 1985) and MSTAT Computer Program (MSTAT Development Team, 1989). The Duncan's New Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel and Torrie, 1980). Microsoft office Excel (2007) was used for figures drawing.

RESULTS AND DISCUSSION

The ANOVA for the studied traits shows that water stress had significant effect (P ≤ 0.01) on leaf number, plant height, head diameter, harvest index and seed yield of all sunflower hybrids (Table 2). Differences among hybrids were also found significant (P ≤ 0.01) for the studied traits (Table 2). The water treatment-hybrid interaction (WD-H) were significant for plant height, head diameter, harvest index and seed yield. Results also revealed non-significant role of WD-H in leaf number (Table 2). The decrease in seed yield was more pronounced in intense water deficit stress (WD3, 150 mm cumulative evaporation from evaporation pan Class A) than that in the mild water deficit stress (WD2, 100 mm cumulative evaporation from evaporation pan Class A), which may be due to decrease in some morphologic and physiologic traits (Boyer, 1982).

Application of WD2 and WD3 caused a 51 and 79% decrease in seed yield of water stressed plants, respectively, as compared with normally irrigated ones (Table 3). The highest and least seed yield in normal irrigation was found on Azargol (3448 kg ha^{-1}) and Hysun 25 (1688 kg ha^{-1}), respectively (Table 4). However, in the mild and intense water stress conditions the seed yield with cultivar Alstar having the highest value (2121 and 829 kg ha^{-1}, respectively) and Azargol and Hysun 33 had the lowest value (893 and 263 kg ha^{-1}, respectively) (Table 4). The means comparison between the three irrigation treatments for the studied traits shows that water deficit stress (WD) has significant adverse effect on all traits (Table 3). The mean values of the leaf number, plant height, head diameter and the harvest index are lower in water deficit conditions (WD2 and WD3) compared with the normal irrigation (Table 3). Hybrids exhibited different response toward water stress conditions. Thus, within hybrid the Alstar showed the highest head diameter and harvest index, (13.30 cm, and 32% respectively), while Hysun 33 exhibited the least values of head diameter and the harvest index (9.50 cm, and 21%), respectively, also Hysun 33 showed the highest leaf number (29.18) and Azargol showed the highest plant height (116.78 cm) (Table 3). Ashraf and Mehmood (1990) reported that even a short term water deficit stress can cause substantial losses in crop yield that is in agreement with our results. The means comparison for the WD-H is summarized in Table 4. In the normal irrigation treatments, Hysun 33 exhibited the highest leaf number and Alstar exhibited the least value of leaf number, but there is non-significant difference in term of plant height between Hysun 33 and Azargol, but the highest amount of plant height was obtain by Azargol (153.50 cm). The results of this study indicated that in normal irrigation conditions Alstar having the highest and Hysun 33 having the least amount of head diameter and harvest index, respectively (Table 4). In WD2 and WD3 Alstar had much higher head diameter (13.50 and 10.76 cm, respectively) and the harvest index (34 and 21%), respectively (Table 4). Our result is also in agreement

Table 3. Effect of water deficit stress and hybrids on studied attributes of sunflower hybrids.

Treatments[†]		Leaf number	Plant height (cm)	Head diameter (cm)	Harvest index (%)	Seed yield (kg ha^{-1})
Water stress (WD)[1]	WD1	26.36^{a}	130.91^{a}	14.30^{a}	37^{a}	2591^{a}
	WD2	25.23^{b}	111.75^{b}	10.30^{b}	22^{b}	1274^{b}
	WD3	23.62^{c}	87.33^{c}	8.20^{c}	17^{c}	552^{c}
Hybrid (H)[2]	H1	25.84^{b}	116.78^{a}	9.60^{c}	25^{b}	1585^{b}
	H2	23.12^{c}	105.31^{b}	13.30^{a}	32^{a}	1914^{a}
	H3	29.18^{a}	116.17^{a}	9.50^{c}	21^{c}	1284^{c}
	H4	22.14^{c}	101.72^{b}	11.40^{b}	24^{bc}	1107^{c}

[†] WD1 = Normal irrigation, WD2 = Mild water deficit stress, WD3 = Intense water deficit stress, H1 = Azargol, H2 = Alstar, H3 = Hysun 33, H4 = Hysun 25. [† a, b, c, d] Within columns, means followed by the same letters are not significantly different ($P \leq 0.05$).

Table 4. Effect of irrigation treatment-hybrid interaction on seed number per head, 1000 seed weight, head diameter, harvest index and seed yield sunflower hybrids.

Treatments[†]		Leaf Number	Plant height (cm)	Head diameter (cm)	Harvest index (%)	Seed yield (kg ha^{-1})
WD1[1]	H1	26.30^{ab}	153.50^{a}	13.90^{b}	43^{a}	3448^{a}
	H2	23.56^{bc}	112.30^{bc}	15.63^{a}	39^{a}	2793^{ab}
	H3	31.86^{a}	151.60^{a}	13.53^{b}	34^{ab}	2437^{b}
	H4	23.73^{bc}	106.30^{bcd}	14.40^{ab}	30^{abc}	1688^{cd}
WD2[1]	H1	26.06^{ab}	121.20^{ab}	8.43^{d}	18^{cd}	893^{ef}
	H2	23.03^{bc}	103.00^{bcd}	13.50^{b}	34^{ab}	2121^{bc}
	H3	28.56^{ab}	122.30^{ab}	8.70^{d}	18^{cd}	1154de
	H4	23.26^{bc}	100.50^{bcd}	10.73^{c}	20^{bcd}	929^{ef}
WD3[1]	H1	25.16^{bc}	75.67^{cd}	6.53^{e}	14^{d}	413^{ef}
	H2	22.76^{bc}	100.70^{bcd}	10.76^{c}	21^{bcd}	829^{ef}
	H3	27.13^{ab}	74.67^{d}	6.27^{e}	11^{d}	263^{f}
	H4	19.43^{c}	98.33^{bcd}	9.23^{cd}	21^{bcd}	704^{ef}

[†] WD1 = Normal irrigation, WD2 = Mild water deficit stress, WD3 = Intense water deficit stress, H1 = Azargol, H2 = Alstar, H3 = Hysun 33, H4 = Hysun 25. [† a, b, c, d, e, f, g] Within columns, means followed by the same letters are not significantly different ($P \leq 0.05$).

with the results obtained in sunflower by Stone et al. (2001) and Angadi and Entz (2002).

In normal irrigation, the highest and the least LAI was obtain by Azargol (3.61) and Hysun 25 (1.82), respectively (Figures 1, 2, 3 and 4). However, in the irrigation after 100 and 150 mm evaporation (WD2 and WD3, respectively) the LAI ranged from 1.9 to 0 and 1.4 to 0, respectively with Azargol having the highest value in R6 stage (1.9 and 1.4, respectively) and Hysun 25 had the lowest value in R9 stage (43.59 and 36.47%), respectively (Figures 1, 2, 3 and 4). The result of this study indicated that application of WD2 and WD3 caused a decrease in LAI of all sunflower hybrids that studied in this research.

The normal irrigation treatments generally had the highest TDW in R6-R8 stages (range from 385 to 423 g), while the water deficit stress conditions (irrigation after 100 and 150 mm evaporation) decreased the TDW for all the sunflower hybrids studied (Figures 5, 6, 7 and 8). This suggests that water stress treatments significantly decreased TDW in all the sunflower hybrids, although result of this study indicated that WD3 condition (irrigation after 150 mm evaporation) caused the severe decreased in TDW (Figures 5, 6, 7 and 8). However, in normal irrigation there are significant differences in the TDW in the phenological R6-R7 stages among the various genotypes with Azargol having the highest TDW (408 g) in stage R7, while Hysun 25 had the least values of TDW

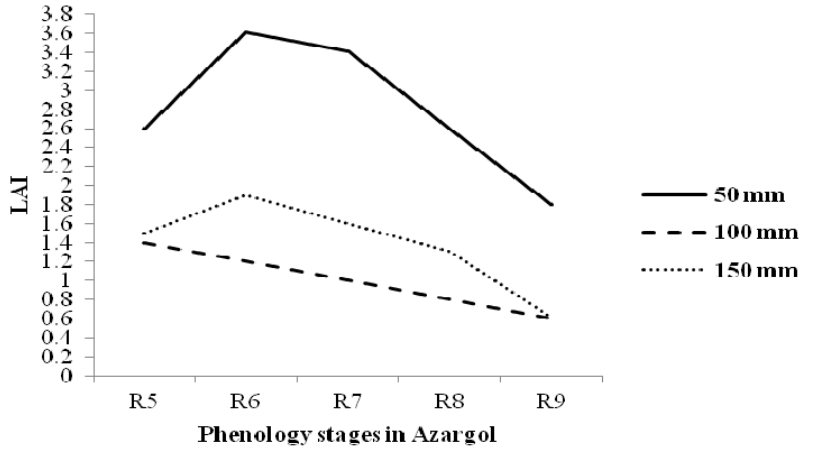

Figure 1. Effect of irrigation treatments on the leaf area index (LAI) of Azargol.

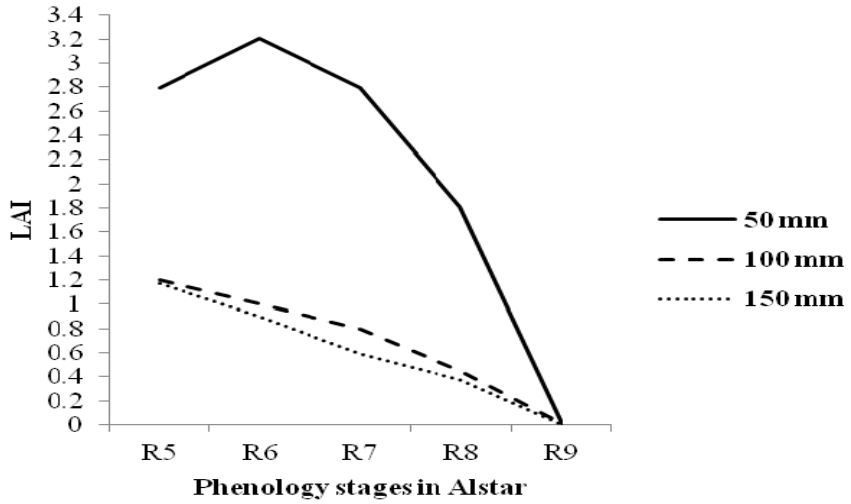

Figure 2. Effect of irrigation treatments on the leaf area index (LAI) of Alstar.

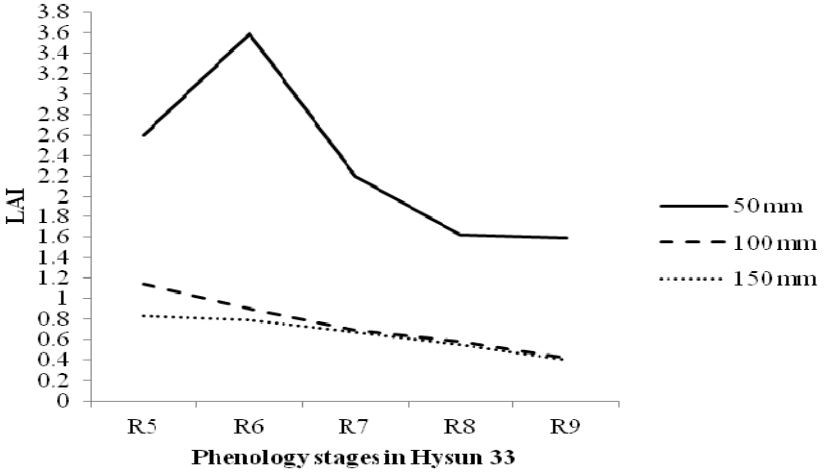

Figure 3. Effect of irrigation treatments on the leaf area index (LAI) of Hysun 33.

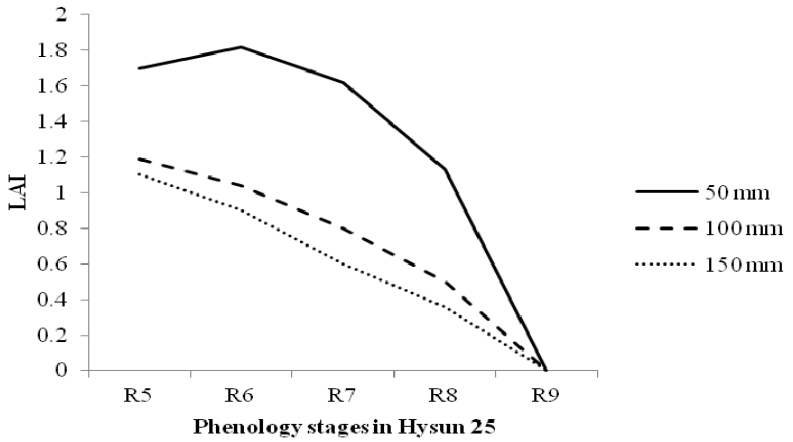

Figure 4. Effect of irrigation treatments on the leaf area index (LAI) of Hysun 25.

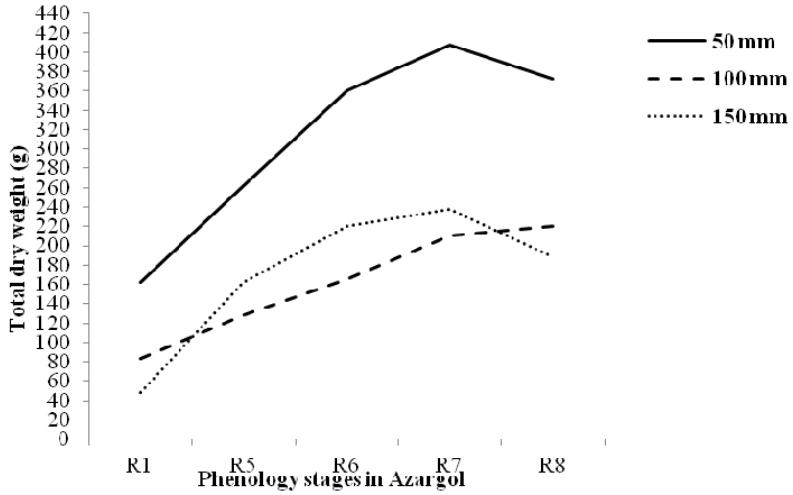

Figure 5. Effect of irrigation treatments on total dry weight of Azargol.

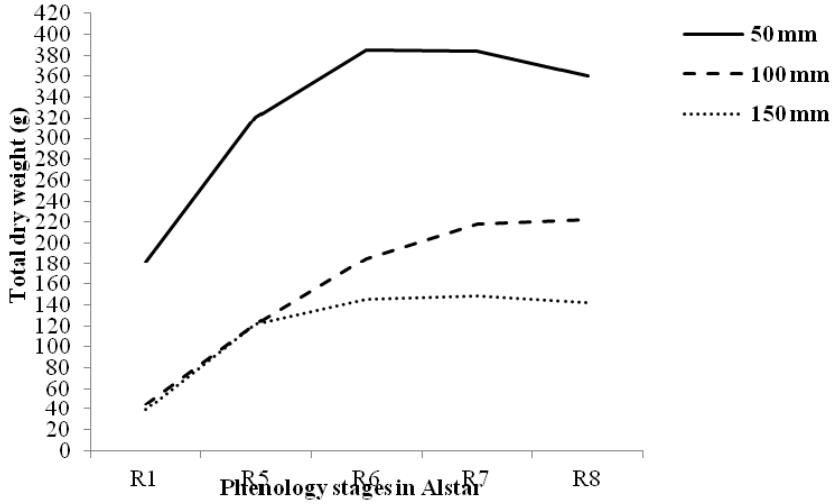

Figure 6. Effect of irrigation treatments on total dry weight of Alstar.

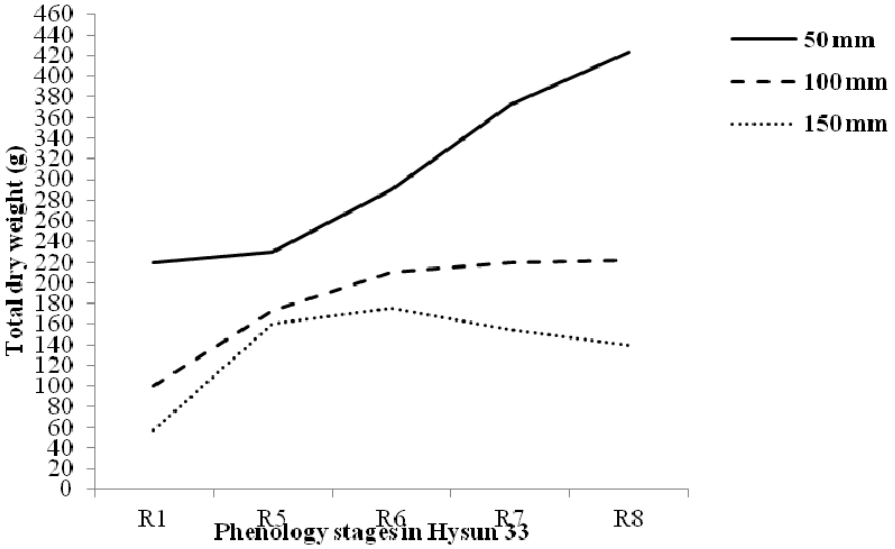

Figure 7. Effect of irrigation treatments on total dry weight of Hysun 33.

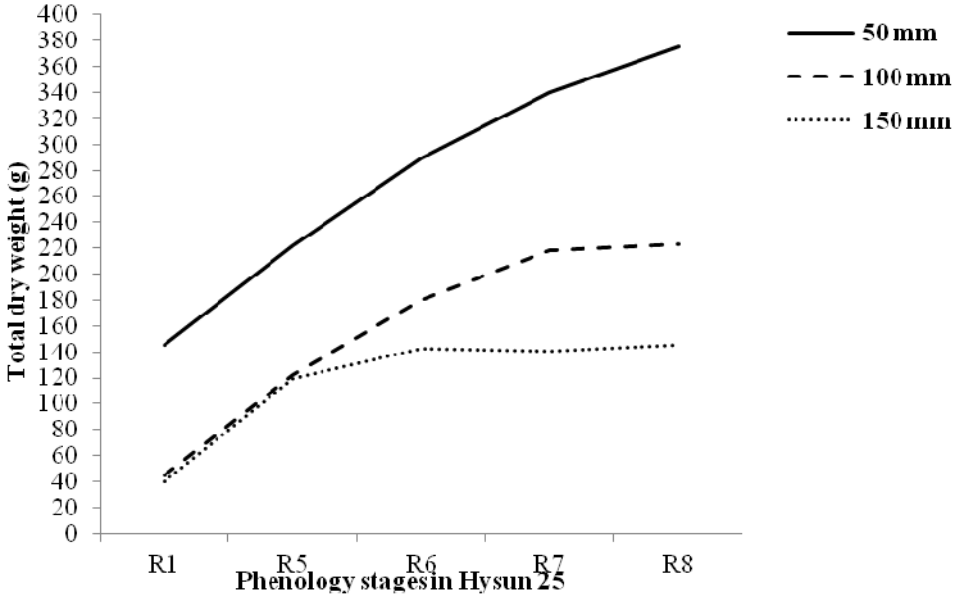

Figure 8. Effect of irrigation treatments on total dry weight of Hysun 25.

(340 g) in this phonological stage (Figures 5, 6, 7 and 8). In the irrigation after 100 mm evaporation (WD2) and among R1-R8 stages, the TDW ranged from 45 to 223 g with Hysun 25 having the highest value and Azargol had the lowest value in R8 stage and in the WD3 (irrigation after 150 mm evaporation) and among R1-R8 stages the TDW ranged from 39 to 57 g with Azargol having the highest value (238 g) and Hysun 25 having the lowest value (141 g) in R7 stage (Figure 2). It is evident that in the all water treatments and among all the cultivars studied, sunflower dwarf cultivars such as Alstar and Hysun 25 have the lowest LAI and TDW compared with tall cultivars such as Azargol and Hysun 33.

The result of this study suggests that variety and irrigation treatments significantly influence the leaf number, plant height, head diameter, harvest index, seed yield, LAI and TDW. It appears from the present and the previous studies that water stress has adverse effect on all studied traits in this research. A large genetic variation was observed for leaf number, plant height, head diameter, harvest index, seed yield, LAI and TDW under well watered and water deficit conditions. In our study

dwarf cultivars especially Alstar under water stress conditions have the highest amount of seed yield.

Conclusion

Drought is a worldwide problem, constraining global crop production and quality seriously, and recent global climate change has made this situation more serious. Drought is also a complex physical-chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals, mineral elements. Currently, drought study has been one of the main directions in global plant biology and biological breeding. The result of this study suggested that seed yield, morphological and physiological characteristics of sunflower are dependent on cultivars and irrigation treatments. Also, our study showed that dwarf cultivars under water stress conditions have the highest amount of seed yield.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Utilization of common carp fish surimi in baby food products

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This work was conducted to increase the nutritional value of some baby food namely Cerelac (rice-base) widely distributed in the Egyptian markets. Raising protein and minerals of baby food (Cerelac) increases its nutritional value. Dried surimi from common carp fish (*Cyprinus carpio*) fortified at 10, 20 and 30% levels. Chemical composition, microbiological, quality aspects and sensory evaluation were determined. Results indicated that moisture; protein, fat, ash, carbohydrates and energy values of surimi were 12.71, 60.77, 0.35, 4.29 and 21.88%, 333.75 kcal/100 g w/w, respectively. Aerobic bacterial counts detected *Staph.* spp., Coliform group, *Salmonella* and *Shigella,* while anaerobic bacteria and mold and yeasts did not detect. Increasing levels of surimi result an increment of moisture, protein, and ash contents. Fat, fiber and carbohydrates contents were reduced. Grams daily requirement (GDR) and percent satisfaction (PS/150) for protein decreased, while GDR and PS/150 for energy value increased. Fortification with 30% dried surimi leads to a maximum improvement of all tested sensory evaluation by different rates. This work strongly recommend that the fortification of Cerelac with 30% dried common carp fish surimi due to a maximum improvement of all tested sensory evaluation and nutritional value by different rates.

Key words: Surimi, fortification, Cerelac, nutritional value.

INTRODUCTION

The important sections of less developed countries of the world are believed to be facing problem of malnutrition due to deficiencies in protein. Fish protein has been proposed as a possible solution to this problem. Meat and fish protein provide approximately one-third of the dietary protein requirements (Buffa, 1971). With the decrease in the availability of traditional caught fisheries products, the demand for other sources of protein appears to be growing.

Carp fish is considered the main type of fish widely produced in fish farms all over the world. Their advantages are likely due to faster of their growth and easy to breed. Common carp fish is unacceptable to the consumer in the fresh form due to numerous species penetrating the flesh. However, ready to eat products (processed fish) were developed in an attempt to

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increase the acceptability and utilization of carp fish (Ganesh et al., 2006).

Surimi is a Japanese term for mechanically deboned fish flesh that has been washed with water and mixed with cryoprotectants for good frozen shelf-life (Toyoda et al., 1992). It is used as an intermediate product for a variety of seafood derivatives (Park et al., 2005), such as the crab legs and flakes. Minced fish, on the other hand, is a mechanically separated flesh that has not been washed and does not have good freeze storability. Washing not only removes fat and undesirable materials, such as blood, pigments and odor substances, but more importantly, increases the concentration of myofibrillar protein (actomyosin), thereby improving gel strength and elasticity, being the essential functional properties for surimi-based products (Guenneugues and Morrissey, 2005).

Surimi is made from minced meat, providing opportunities to use different sources of protein in its production, such as underutilized species with little or no commercial value, including non-fish species. The surimi process, parts of it or modified versions, could be a way of exploiting resources that otherwise would be neglected by the food industry and consumers. Fortunately, the use of novel species for the production of surimi is increasing. Besides fish, the potential for other resources, such as cephalopods (Cortés-Ruiz et al., 2008) and giant squid (Campo-Deaño et al., 2009). In addition, crabs are being studied for surimi production with the incorporation of new methods and technologies according to Luo et al. (2004).

Surimi has a high protein (14%) and low fat content (0.21%) as determined by proximate chemical analysis. The addition of surimi to food systems has been suggested as a way of improving of myofibrillar proteins to polymerize and entrap water in a network structure (Lanier, 1984).

Abd El–Aal and Ibrahim (2001) reported that minced fillet of silver carp fish had 78.97% moisture, 18.39% protein, 0.42% fat and 1.15% ash, while surimi had 79.39% moisture, 11.68% protein, 0.28% fat and 0.55% ash. Garcia et al. (1993) found that boar surimi-like material tended to have lower microbial counts and less lipid oxidation than unwashed counterparts.

As for fish meat, it is characterized by its high nutritional value due to its content of protein, minerals and vitamins. It is also easy to be digested and its aroma is attracting for consumer. Yet, the catfish and carp fish have fewer acceptances because of its low eating qualities and toughness, in addition the catfish was dark colored muscles and carp fish was more spines (fishbone) between flesh. Accordingly, in present study, it is suggested that minced meat of catfish and carp fish may be used to produce an intermediate raw material (surimi) which is characterized by its high nutritional value. This raw material should have good functional properties and may be used to improve the nutritional value of variety of food products. Surimi has a white color and no fish odor. It can also be added to baby foods as a supplement to raise the nutritional value according to Luo et al. (2004).

Hence, the objective of this work is to increase the nutritional value of some baby food namely Cerelac (rice-base) using dried surimi from common carp fish (*Cyprinus carpio*) fortified with 10, 20 and 30% levels.

MATERIALS AND METHODS

Source of materials

Male and female common carp fish (*C. carpio*) was purchased from the local wholesale market at Cairo City, Cairo Governorate. Samples were put into an ice box and transported to the laboratory for Faculty of Home Economics, Minufiya University.

Baby food (Cerelac) was purchased from a pharmacy at Sheben El-Kom City, Minufiya Governorate. Sugar (sucrose) was obtained from the local market at Sheben El-Kom City, Minufiya Governorate. Sorbitol and sodium tripolyphosphate (STPP) were purchased from El-Gomhoria Co., Cairo, Egypt. This work has been carried out in Minufiya University, Egypt starting at April, 2010.

Preparation of fish samples

Common carp fishes (about 5 kg of each) were washed, packed in polyethylene bags and stored at -20°C for 2 weeks until used. After thawing (overnight at 4°C.) the fish were eviscerated, headed, skinned, cleaned, washed and filleted by hand. The fillets without skin were ground using a meat grinder (Moulinex, HV2, Model A14, Moulinex, France) with 4 mm whole plate.

Preparation of surimi from common carp fish

Surimi was prepared from fillets using the methods described by Park et al. (1990) with some modification. The minced fish meats were immediately washed three times in stainless steel container (25 L) with water 1 part and crushed ice. 2 parts (iced water) at a ratio of 1 part minced fish to 3 parts iced water (W:W). Hand whipped was used to stir slurry 5 min, and excess water was removed between washing using cheese cloth. After the final washing cycle the minced fish was put in a cheese cloth bag and water removed by compression. Raw surimi was either directly packaged or mixed with cryoprotectants (4% sucrose, 4% sorbitol and 0.25% STPP) and chopped for 2 min using a meat blender (Moulinex, HV2, Model A14, Moulinex, France) and then packaged in polyethylene bags and stored at -4°C until analysis.

Dried surimi production

Frozen surimi from carp fish was thawed over night at 5°C in a refrigerator. Samples distributed in pan and put in vacuum oven to dry for 35 min at 55°C to obtain dried surimi, samples were weighted (dried weight). Preliminary dehydration trails were conducted to determine the most suitable time and temperature for the treatment.

Supplementation of baby foods (Cerelac)

Dried carp fish meat surimi was crushed to obtain homogenized dried surimi (surimi supplement) and added to Cerelac supplemented at 10, 20 and 30% to obtain supplemented formulas (W.W.). These supplemented formulas with dry surimi were

Table 1. Chemical composition of dried surimi from common carp fish.

Chemical composition (%)	Fish	
	Dried common carp fish surimi	
	WW (g)	DW (g)
Moisture (%)	12.71 ± 0.00115	-
Protein (%)	60.77 ± 0.00107	69.62 ± 0.0022
Fat (%)	0.35 ± 0.15744	0.40 ± 0.0206
Ash (%)	4.29 ± 0.00056	4.91 ± 0.0021
Carbohydrates (glycogen) (%)	21.88 ± 0.00156	25.07 ± 0.0017
Energy value (K.cal/100 g)	333.75 ± 0.00012	-

WW, Wet weight; **DW,** dry weight.

analyzed for chemical and microbiological characteristics. Preliminary supplement baby foods (Cerelac-rice base) with dried surimi were evaluated to determine the suitable treatment required for these baby foods.

Analytical methods

Moisture, protein (N × 6.25 Keldahl method), fat (hexane solvent, Soxhiet apparatus), fiber and ash were determined according to the method recommended by AOAC (2003).

Carbohydrates (as glycogen) and energy value

Carbohydrate was calculated by differences as follows:

% Carbohydrates = 100 - (% moisture + % protein + % fat + % fiber + % ash).

Energy value was estimated by multiplying protein and carbohydrates by 4.0 and fat by 9.0 (AOAC, 1995).

Microbiological methods

Preparation of fish samples and supplemented baby food samples for microbiological investigation

Total aerobic plate count (TAPC) determined on nutrient agar media according to the method described by Oxide Manual (1979), *Staphylococcus aureus* determined on Paird parker agar base media **(**ICMSF, 1996), while molds and yeast, enumerated in potato dextrose agar (ICMSF, 1996)**,** Coliform bacterial (Oxoid) enumerated on Endo agar media (WHO, 1988), *salmonella* sp. and *Shigella* SS agar modified Oxoid according to Bryan (1991) and anaerobic bacteria was examined using nutrient agar media (Difco Manual, 1970).

Organoleptic evaluation

Baby foods (Cerelac) supplemented were subjected to organoleptic tests (by 10 judges) according to Watts et al. (1989). Judging scale for color, aroma, taste, texture and overall acceptability was as follows: Very good 8 - 9, Good 6 - 7, Fair 4 - 5, Poor 2 - 3 and Very poor 0 - 1.

Statistical analysis

Statistical analysis were performed by using computer program statistical package for social science (SPSS), and compared with each other using the suitable tests. All obtained results were tabulated. Significant differences between treatments means were determined using Duncan's multiple test (1955).

RESULTS AND DISCUSSION

Chemical composition of dried surimi from common carp fish

The chemical composition of dried surimi from common carp fish is shown in Table 1. On the other hand, the energy value of dried surimi from common carp fish was 333.75 kcal/100 g on wet weight basis. These results are in agreement with Abd El-Aal and Latif (2002) and Ibrahim et al. (2005).

Microbiological aspects of dried surimi from catfish and common carp fish

Data presented in Table 2 show the microbiological aspects of dried surimi from common carp fish. The results showed that the TAPC was the only detected microorganisms in dried surimi from common carp fish. The Value was 1.7×10^1 cfu/g. On the other hand, *Staphylococcus* spp., Coliform group, *Salmonella* and *Shigella,* anaerobic bacteria and mold and yeasts were not detected in dried surimi of common carp fish. Results from the same table revealed that drying process had tremendous effect on the number of microorganisms. This observation may be due to the flow chart of processing of dried surimi on the microorganisms by destroying their tissues. Niki et al. (1982) published the results microbial tests of spray dried surimi made from Alaska Pollock which were as follows: bacterial count 1×10^4 cfu/g, Coli-aerogenes group not detected, Psychrotrophic bacteria 8×10^3 cfu /g, *Salmonella* not detected, Yeasts 30 cfu /g, Mold 20 cfu/g and *Vbrio*

Table 2. Microbiological aspects of dried surimi from common carp fish (cfu/g).

Test of microorganisms	Dried common carp fish surimi
Total aerobic plate count (TAPC)	1.7×10^1
Staphylococcus spp.	N.D.
Coliform group	N.D.
Salmonella and *Shigella*	N.D.
Anaerobic bacteria	N.D.
Mold and yeast	N.D.

Table 3. Chemical composition of baby food (Cerelac).

Chemical composition (%)		Cerelac of baby food
Moisture	WW	2.92
	DW	-
Protein	WW	7.10
	DW	7.32
Fat	WW	1.70
	DW	1.75
Ash	WW	1.66
	DW	1.71
Fiber	WW	2.10
	DW	2.16
Carbohydrates (glycogen)	WW	84.49
	DW	87.06
Energy value (k.cal/100 g)	WW	381.66
	DW	-

WW, Wet weight; **DW,** dry weight.

paratheempolyticus not detected.

Chemical composition of baby food (Cerelac)

Data given in Table 3 shows the chemical composition of baby food (Cerelac), these results are close to that reported by Egyptian Organization for Standardization and Quality Control (1990) which noted that the chemical composition's standard for weaning food mixture are as follows: moisture % not increases than 7% and ash not increases than 3%; but protein (7.10) was less than mentioned reference, being not less than 15%. Data of Table 3 for Cerelac, however similar to that reported by Sidky (1995). These results are in agreement with that reported by Bowes and Church (1983) and Thomokinson and Mathur (1985).

Chemical composition of baby food (Cerelac) as influenced by addition different levels of dried common carp fish surimi

The chemical composition of baby food (Cerelac) as influenced by addition different levels of dried common carp fish surimi is shown in Table 4. The obtained results indicated that the increasing fortification levels of dried common carp fish surimi in baby food (Cerelac) resulting a markedly increase of moisture content (%). In case of protein and ash contents (%), it could be noted that increasing the fortification levels of baby food (Cerelac) with dried common carp fish surimi result a significant increase in protein and ash contents. On the other hand, fat, fiber and carbohydrates content (%) showed a markedly reduction with increasing fortification levels by dried common fish surimi. Also, energy value recorded a

Table 4. Chemical composition of baby food (Cerelac) as influenced by addition different levels of dried common carp fish surimi.

Sample		Chemical composition (%)						
		Moisture	Protein	Fat	Ash	Fiber	Carbohydrates	Energy value (kcal/100 g)
Control (0%)	WW	2.95	7.10	1.70	1.66	2.10	84.49	381.66
	DW	-	7.32	1.75	1.71	2.16	87.06	-
With 10% dried common carp fish surimi	WW	4.85	11.50	1.59	1.90	1.79	78.37	373.79
	DW	-	12.09	1.67	2.00	1.88	82.36	-
With 20% dried common carp fish surimi	WW	5.67	15.52	1.45	2.17	1.58	73.61	369.57
	DW	-	16.45	1.54	2.30	1.68	78.03	-
With 30% dried common carp fish surimi	WW	6.10	18.45	1.32	2.43	1.37	70.33	367.00
	DW	-	19.65	1.45	2.59	1.46	74.90	-

WW, Wet weight basis; **DW.** dry weight basis.

Table 5. Nutritional evaluation of baby food (Cerelac) as influenced by addition different levels of dried common carp fish surimi.

Sample	Protein		Total calories	
	GDR (g)	PS /150%	GDR (g)	PS/150%
Control (0%)	197	25	223	22.45
With 10% dried common carp fish surimi	122	41	227	21.99
With 20% dried common carp fish surimi	90	55	230	21.24
With 30% dried common carp fish surimi	76	66	232	21.59

markedly reduction with increasing dried common carp fish surimi. These results are in agreement with the findings of Saad (2006).

Nutritional evaluation of baby food (Cerelac) for infant as influenced by addition different levels of dried common carp fish surimi.

Data given in Table 5 show the nutritional evaluation of baby food (Cerelac) for infant (6-12 months of age) as influenced by addition different levels of dried common carp fish surimi. It is clear to notice that grams daily requirement (GDR) is for protein decreased, while GDR for energy value increased with increasing fortification levels of dried common carp fish surimi in baby food (Cerelac).

On the other hand, PS/150 for protein increased, while PS/150 for energy value decreased with increasing fortification levels by dried common carp fish surimi in baby food (Cerelac).

Sensory evaluation of baby food (Cerelac) as influenced by addition different levels of dried common carp fish surimi

Data presented in Table 6 show the sensory evaluation of baby food (Cerelac) as influenced by addition different levels of dried common carp fish surimi. It is clear to mentioned that the scores of all tested sensory evaluation (color, aroma, taste, texture and overall acceptability) of control baby food (0% dried common carp fish surimi). There is fortification of baby food (Cerelac) with 20% dried common carp fish surimi due to a markedly improvement of all tested sensory evaluation by different rates. On the other hand, the maximum sensory evaluation of baby food was recorded with 30% dried common carp fish surimi. .

Conclusions

In the current study, the TAPC was the only detected microorganisms in dried surimi from common carp fish. On the other hand, *Staphylococcus* spp., Coliform group, *Salmonella* and *Shigella,* anaerobic bacteria and mold and yeasts were not detected. The results also showed the increasing fortification levels of dried common carp fish surimi in (Cerelac) resulting a markedly increase of moisture, protein, ash contents (%), while fat, fiber and carbohydrates content (%) showed a markedly reduction with increasing fortification levels by dried common fish surimi. GDR for protein decreased; GDR for energy value increased with increasing fortification levels in (Cerelac).

Table 6. Sensory evaluation of baby food (Cerelac) as influenced by addition different levels of dried common carp fish surimi.

Panel test	Type of formulas			
	Control (0%)	With 10% dried common carp fish surimi	With 20% dried common carp fish surimi	With 30% dried common carp fish surimi
Colour	7^{a}	8^{ab}	9^{b}	9^{b}
Aroma	6^{a}	7^{ab}	8^{bc}	9^{c}
Taste	7^{a}	8^{ab}	9^{b}	9^{b}
Texture	9^{a}	8^{a}	8^{a}	8^{a}
Overall acceptability	7^{a}	8^{ab}	9^{b}	9^{b}

Mean under the same line bearing different superscript letters are different significantly ($p < 0.05$).

On the other hand, PS/150 for protein increased, while PS/150 for energy value decreased with increasing fortification levels in Cerelac. This work strongly recommend the fortification of Cerelac with 30% dried common carp fish surimi due to a maximum improvement of all tested sensory evaluation and nutritional value by different rates.

Conflict of Interests

The authors have not declared any conflict of interests.

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Wood ash effect on the productive characteristics of Marandu grass in Cerrado soils

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The nutrient dynamics in soils are directly influenced by fertilizer application. Wood ash is an alternative fertilizer and soil corrective that provides enhanced fertility and increased crop production. This study aimed to evaluate the productive characteristics of Marandu grass and the functions of levels of plant ash in two Cerrado soils. The experiment was conducted in a greenhouse with a completely randomized design in a factorial 2 × 6 plot: two soils (oxisol and ultisol) and six doses of wood ash (0, 3, 6, 9, 12 and 15 g dm^{-3}) were tested in six replicates. The soil samples were collected in areas: the Cerrado, and wood ash from the ceramics industry was used as a fertilizer. Each experimental unit consisted of a plastic pot with up to 5 dm^3 of soil and 5 plants of *Brachiaria brizantha* Marandu grass. Three cuts were made in the shoots at 30 days intervals to evaluate the productive characteristics, such as the number of leaves, number of tillers, and dry mass of shoot and root. The wood ash fertilizer influenced the production Marandu grass in the two Cerrado soils (ultisol and oxisol) and provided the maximum mass production of shoots (third cut), number of leaves (first and second cuts), number of tillers (first cut) and root mass (third cut) in typic oxisol.

Key words: *Brachiaria brizantha*, oxisol, ultisol.

INTRODUCTION

Wood ash is a solid material that remains after the complete burning of woody biomass (Coelho and Costa, 2012) and from a heated boiler (Nkana et al., 2002) for the purpose of energy production. As an alternative to landfilling, the application of wood ash to soil may become a promising method of waste removal (Pita, 2009) because of its large-scale production in large urban centers. The application of plant ash in agriculture is presented as an important opportunity for returning a portion of the nutrients removed by crops. The recycling of ash reduces the need for commercial fertilizers and contributes to the reduction soil of acidification and increase of the supply of calcium (Zimmerman and Frey, 2002).

The elemental composition of ash is determined mainly by the quality of the incinerated material (Augusto et al., 2008). Some studies have shown that gray woody biomass is an alkalizing soil corrective (Osaki and Darolt, 1991; Cabral et al., 2008; Ferreira et al., 2012) and an important source of nutrients, such as phosphorus,

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potassium, magnesium, and calcium (Nieminen et al., 2005; Lima et al., 2006; Sofiatti et al., 2007) .

The dynamics of nutrients in the soil are influenced by the incorporation of wood ash (Ferreira, 2012) because of its high alkalinity (Loué, 1978), which is shown by the neutralization of available aluminum (Al^{3+}) soil. Lima et al. (2009) suggested that wood ash has a greater capacity to increase the soil pH compared to cattle manure and can provide increased levels of phosphorus, potassium and magnesium (Obernberger et al., 1997).

Soils of the Cerrado biome are generally low in available nutrients, and wood ash can be an interesting alternative to improving fertility because it contributes directly to crop production. The pastures usually contain crops with tolerance to acid soils of low fertility.

However, the absence of corrections and fertilization of the soil cannot be justified in pastures; these management practices are necessary because soil fertility greatly limits the crops' ability to achieve maximum productivity.

To use wood ash as a fertilizer, certain criteria must be met, such as attaining knowledge in the area of soil science, especially in relation to its effects on soils of different classes. The application of wood ash can reduce the use of mineral fertilizers and, consequently, production costs, and it promotes the safe disposal of wood ash in the environment (Bonfim-Silva et al., 2014).
Thus, considering the beneficial use of wood ash as a soil fertilizer, this study aimed to evaluate the productive characteristics of Marandu grass cultivated in two classes of Cerrado soils when fertilized with wood ash.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse during the period from December, 2012 to May, 2013 in Rondonópolis City, Mato Grosso States (MT), Brazil. The forage grass *Brachiaria Brizantha* was used as the Marandu grass grown in pots with a capacity of 5 dm^3 of soil. The experimental design was completely randomized in a 2 × 6 factorial design, with two Cerrado soils (oxisol and ultisol) and six doses of wood ash (0, 3, 6, 9, 12 and 15 g dm^{-3}) in six replicates. The two soils were collected at the 0 to 20 m layer in an area under Cerrado vegetation in the region of Rondonópolis City, and chemical and textural characterizations were performed according to EMBRAPA (1997).

The oxisol had the following chemical and textural characteristics: pH in $CaCl_2$ = 4.1; M.O. = 19.7 g dm^{-3}; P = 1.1 mg dm^{-3}; K = 47.0 mg dm^{-3}; Ca = 0.2 cmolc dm^{-3}; Mg = 0.1 cmolc dm^{-3}; Al = 1.0 cmolc dm^{-3}; H = 4.7 cmolc dm^{-3}; CTC = 6.1 cmolc dm^{-3}; V = 6.9% m = 70.4%. In addition, the oxisol had the following physical characteristics: sand = 575 g kg^{-1}; clay = 375 g kg^{-1} and silt = 50 g kg^{-1}.

However, the ultisol presented the following chemical and textural characteristics: pH in $CaCl_2$ = 4.9; M.O. = 6.2 g dm^{-3}; P = 4.8 mg dm^{-3}; K = 25.0 mg dm^{-3}; Ca = 1.0 cmolc dm^{-3}; Mg = 0.6 cmolc dm^{-3}; Al = 0.1 cmolc dm^{-3}; H = 1.5 cmolc dm^{-3}; CTC = 3.3 cmolc dm^{-3}; V = 50.9% and m = 5.7%. In addition, the ultisol had the following physical characteristics: sand = 830 g kg^{-1}; clay = 120 g kg^{-1} and silt = 50 g kg^{-1}.

The wood ash used in this study was derived from the region Rondonópolis, MT ceramic industry, and it is characterized as fertilizer (Darolt et al., 1993) with the following chemical characteristics: pH in $CaCl_2$ = 7.85; N = 0.17%; P_2O_5 (neutral ammonium citrate + water) = 1.42%; K_2O = 0,32%; Zn = 0.00%; Cu = 0.01%; Mn (CNA + water) = 0.00; Ca = 0.90% and S = 1.60%.

Both soils were incubated with wood ash for a period of 30 days. The powdered wood ash had soil amendments; thus, the soils did not have to be limed.

After the incubation period, 15 seeds of Marandu grass were sown, and after the plants reached an average height of 10 cm (which occurred at 12 days after sowing), the plants were thinned to five plants per pots and treated with nitrogen fertilization. Plants were fertilized after thinning and after each cut was performed with nitrogen (source: urea) at 200 mg dm^{-3}. Nitrogen fertilization was performed in all of the experimental units, and soil moisture was maintained at 80% of the gravimetric maximum water retention in the soil during the driving period of the experiment.

The interval between the Marandu grass cuts (three total) was 30 days, and after each cut, an assessment was made of the dry mass of shoots and roots (only on the third cut), number of leaves and number of tillers. The plant material was collected at each cut, dried in an oven with forced air circulation at 65 for 72°C h until a constant weight, and after drying, it was weighed on a balance scale to determine the mass.

Data were subjected to an analysis of variance by F-test, and for significant results, the qualitative factors for soil type were submitted to Tukey's test and quantitative factors for the dose of wood ash were submitted to a regression analysis, both at a 5% probability using the statistical program Sisvar (Ferreira, 2008).

RESULTS AND DISCUSSION

The application of wood ash as a fertilizer to the two classes of soils provided increments of productive characteristics of the Marandu grass with and interaction effects at the first and second cuts of the forage grass. At the third cut, there was an isolated effect, with an increased dry mass of shoots observed in the ultisol (Table 1).

In the first cut of the forage grass, the dry mass of the shoots was adjusted by a linear regression model for ultisol and oxisol, and an increase was found of 81.49 and 69.43%, respectively, when comparing the highest dose of wood ash (15 g dm^{-3}) to the treatment without fertilization with wood ash (Figure 1A).

In the second cut of the Marandu grass, there was interaction between the doses of wood ash and soil types in the dry mass of shoots. The oxisol was adjusted by a linear regression model, and an increase was found of 21.61% when the higher dose of wood ash (15 g dm^{-3}) was compared to the treatment without fertilization with wood ash. The forage grass grown in ultisol had a minimal production of dry mass of shoots in the wood ash dose of 7.06 g dm^{-3} (Figure 1B).

In the third evaluation, there were no significant differences in dry mass of the aerial parts of the Marandu grass grown ultisol. For the oxisol, the dry mass of the shoots was fit with a quadratic regression model, and the mass production of shoots was 6.15 g pot^{-1} at a wood ash dose of 11.80 g dm^{-3} (Figure 1C).

In both soils, there was a differential effect of wood ash in the production of dry mass, so at the first cut of the

Table 1. Isolated effect of soil on the dry mass of shoots (DMS) the of Marandu grass, the third cut fertilized with vegetable ash (g DMS g cinza^{-1}).

Soils	Dry mass of shoots
Oxisol	5.10^{b}
Ultisol	6.22^{a}
CV%	25.29

Means followed by the same letter in the line do not differ by Tukey's test at a 5% probability. CV% = Coefficient of variation.

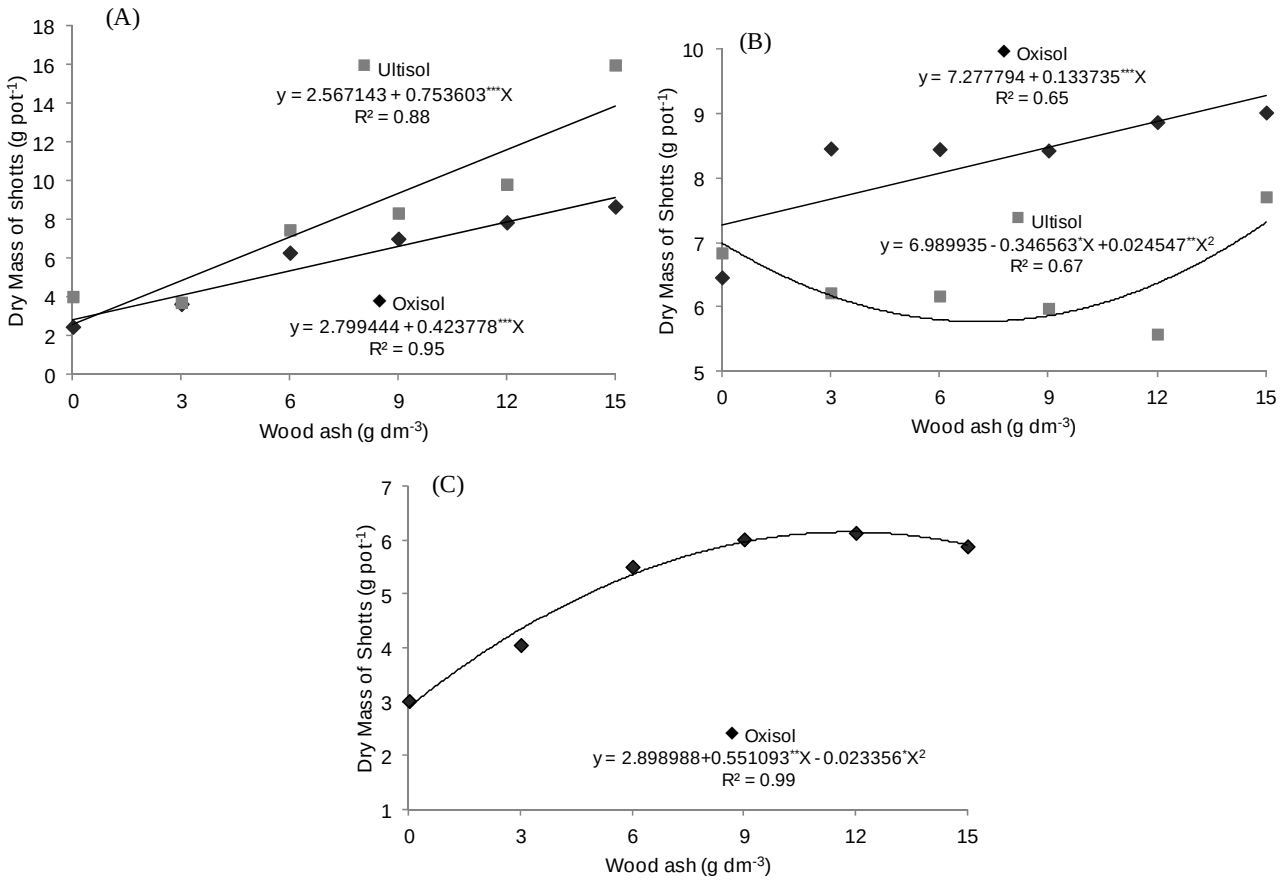

Figure 1. Dry mass of shott of Marandu grass subjected to doses of wood ash in oxisol and ultisol soil: the first (A) and second (B) cuts, and Oxisol in the third cut (C). *, ** and *** represent 5, 1 and 0.1% probability, respectively.

grass, the linear fit of this variable revealed that even when grown in different soils, the forage grass was not distinguished in its responses to fertilization with wood ash. The fit by the linear regression model can be justified by the need to prioritize the forage grass root development in the establishment phase, which allocated the forage grass assimilates to the roots of plants.

However, at the second cut, the fit to a quadratic regression model of the dry mass of shotts of the forage grass that was observed in ultisol may have shown an effect of the wood ash in the availability of nutrients in the soil, which would reflect the inherent characteristics of each soil in the ability to react with this residue. It is noteworthy that the oxisol soil showed an increased fertility during the characterization.

In the third section, the quadratic fit of the mass of shoots of the Marandu grass grown in oxisol may have been related to the residual effect of fertilization by the wood ash because there was no reapplication of this residue in the treatments. Thus, the mass production of

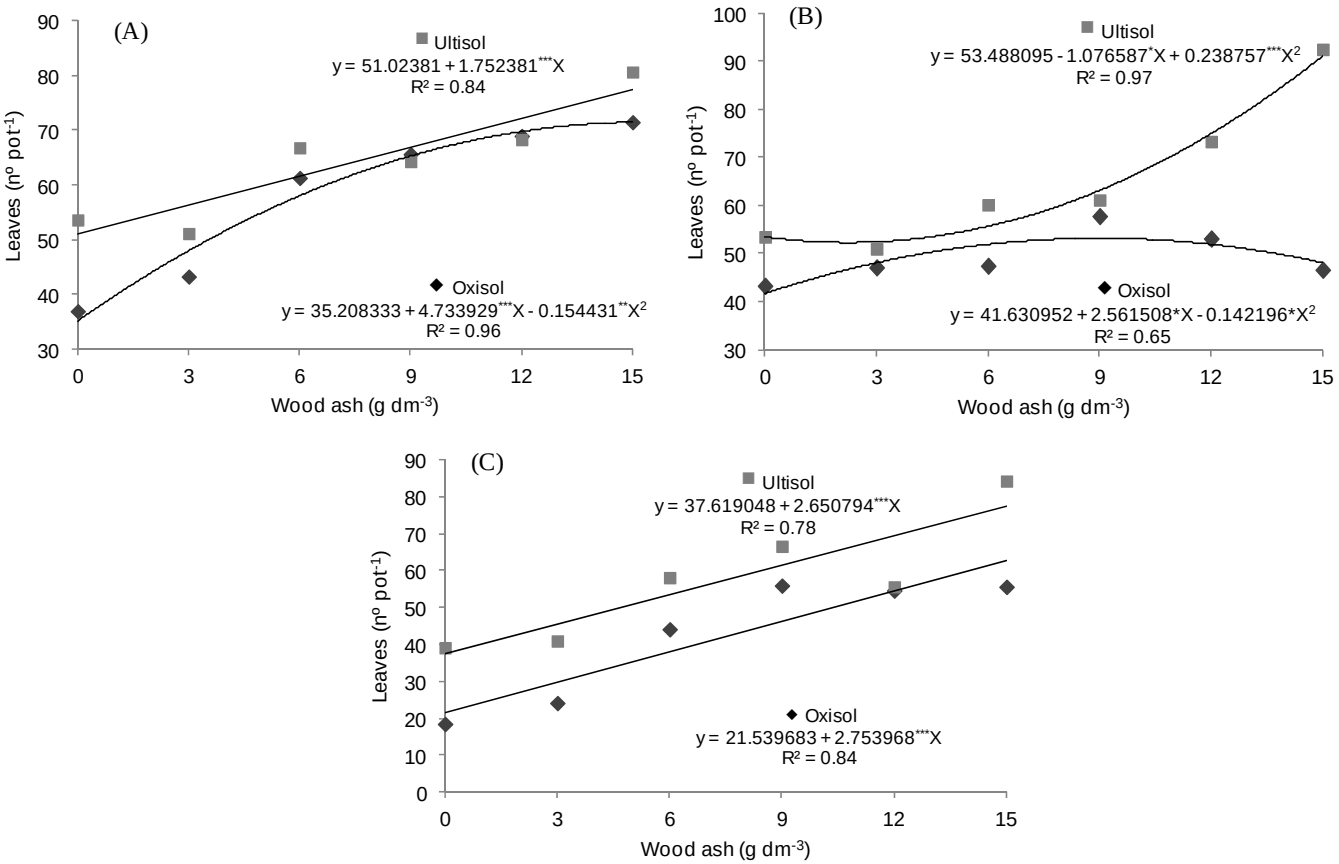

Figure 2. Number leaves in Marandu grass subjected to doses of wood ash in Oxisol and Ultisol soils at the first (A), second (B) and third (C) cuts. *, ** and *** represent 5, 1 and 0.1% probability, respectively.

shoots in the Marandu grass ultisol was not significant, which indicates that mass production likely tended to stabilize, so no significant differences were found.

Research conducted with the wood ash in the Cerrado oxisol soil showed that Marandu grass did not reach the maximum mass production of its shoot because of the low presence of minerals in the chemical composition of the ash wood (Bonfim-Silva et al., 2013). The mineral nutrients present in the plant ash directly influenced the production of the plant.

Santos (2012) studied the Marandu grass fertilization with wood ash in Cerrado oxisol and set the observed quadratic regression model according to the dry mass of the shoots fertilized with wood ash, which emphasized that the wood ash had concentrations of nutrients that could satisfy the nutritional demand of the crop.

In the present study, the Marandu grass grown in ultisol produced 13.87 g pot^{-1} of shoot mass, which was consistent with the results of Abreu and Monteiro (1999), Maciel et al. (2007) and Cabral (2011), who used mineral fertilizers; however, it is evident that the use of wood ash as a soil fertilizer improves soil fertility by increasing the production of this forage grass.

At the first cut, leaf production was adjusted to a quadratic regression model for the Marandu grass grown in field experiments, and the highest number of leaves (71.49) occurred at a wood ash dose of 15.33 g dm^{-3}. However, when the Marandu grass was grown on ultisol, the number of leaves was adjusted to a linear regression model and showed an increase of 34.0% in the number of leaves when the dose at 15 g dm^{-3} was compared to a treatment without fertilization with wood ash (Figure 2A).

The number of leaves of forage grass at the second cut was fit to a quadratic regression model, and the largest number of leaves of grass grown in the field experiment was 53.16 leaves in the wood ash treatment with a dose of 9 g dm^{-3}. The lowest number of leaves (52.27) of Marandu grass grown in ultisol was obtained at a wood ash dose of 2.25 g dm^{-3} (Figure 2B).

The production of Marandu grass leaves at the third cut was adjusted by a linear regression model for the oxisol and ultisol soils, and it showed an increment of 65.73 and 51.38%, respectively, at the highest dose of wood ash compared to the treatment without fertilization by wood ash (Figure 2C).

This study confirms the results by Bonfim-Silva et al. (2011), who applied doses of wood ash to Marandu grass in oxisol and observed an increase in the number of

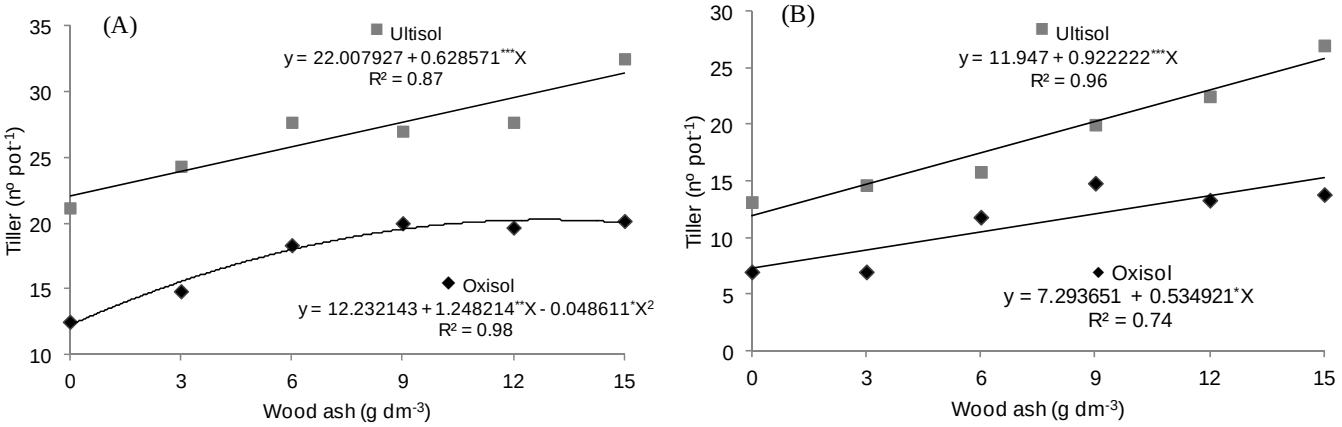

Figure 3. Tiller number in Marandu grass subjected to doses of wood ash in Oxisol and Ultisol soil at the second (A) and third (B) cuts. *, ** and *** represent 5, 1 and 0.1% probability, respectively.

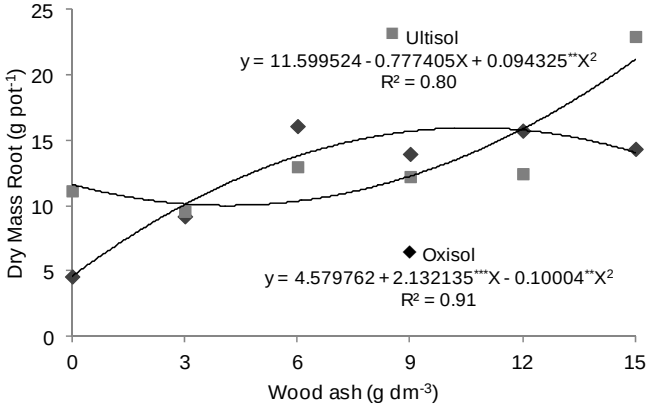

Figure 4. Dry mass root of Marandu grass subjected to doses of wood ash in oxisol and ultisol soils. ** and *** represent 1 and 0.1% probability, respectively.

leaves at the first and second cuts. Similarly, Santos (2012) studied the effect of wood ash in two cultivars of *B. brizantha* and noted that the production of Marandu grass leaves was positively influenced, which confirms the importance of using this waste as a fertilizer.

For the production of tillers, there was no significant effect at the first cut of Marandu grass. At the second cut, the maximum production of tillers set by the quadratic regression model when grown on oxisol was 20.24 tillers at a dose of 12.84 g dm^{-3} wood ash. Ultisol was adjusted by a linear regression model, and there was an increase of 29.99 and 53.66% at the first and second cuts, respective, when the highest dose of wood ash was compared to the treatment without wood ash (Figure 3A and B).

It was observed that at the second cut of the Marandu grass, the highest dose of wood ash applied to the ultisol soil was similar to the production at an equivalent amount of mineral fertilizer (nitrogen and sulfur) in the tiller of Marandu grass studied by Batista and Monteiro (2006). Thus, we emphasize the potential use of wood ash fertilization on forage grass.

Bonfim-Silva et al. (2011) observed linear effects in the number of tillers of the first two Marandu grass intervals, which had 2.5 to 2.0-fold increases, respectively, at higher doses of wood ash (3.37 g dm^{-3} reapplied every cut forage) compared to the control. According to Santos (2012), the use of wood ash as a fertilizer was also shown to produce significant number of tillers of Marandu grass in Cerrado oxisol soil.

The dry mass root of Marandu grass was set by the quadratic regression model at the minimal production amount observed in the wood ash treatment at a dose of 4.12 g dm^{-3} in ultisol. Moreover, we observed a higher production of 15.94 g pot^{-1} at a wood ash dose of 10.65 g dm^{-3} when the grass was grown in oxisol (Figure 4).

When the Marandu grass is grown on typic oxisol, it produces a higher root dry mass than when grown in ultisol. Considering that the soil fertility of the oxisol had a higher nutrient limitation, the nutrients provided through the application of wood ash improved the relative proportion of these nutrients in the soil, which was shown by the maximum root production, demonstrating the importance of using this residue, especially in soils of low fertility.

Bonfim-Silva et al. (2013) observed a maximum production of roots in Marandu grass at 21.03 g pot^{-1} in the oxisol fertilized with a wood ash dose of 3.29 g dm^{-3}. It is noteworthy that these authors reapplied doses of wood ash to the surface after the first cut. Santos (2012) observed that the production of this grass root was 60.96 g pot^{-1} at a wood ash dose of 15 g dm^{-3}.

The application of the wood ash residue thus increases the production potential of oxisols because the wood

ash promotes the rapid release of nutrients and there is a subsequent increase in soil fertility (Guariz et al., 2009).

Conclusions

Wood ash as a fertilizer influences the productive characteristics of Marandu grass in Cerrado soils. In the oxisol soils, Marandu grass presents its maximum production of the dry mass of shoots, number of leaves, number of tillers and root mass in doses of wood ash of 11.80, 9.0, 12.84 and 10.65 g dm^{-3}, respectively.
In ultisol, a linear fit and minimal production were observed for the productive characteristics of Marandu grass; however, the dry mass of the shoots did not differ between the two soil types.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Induced genetic variability for yield and yield traits in aromatic rice (*Oryza sativa* L.)

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The nature of induced mutation for polygenic variability was studied in two traditional aromatic rice genotypes, viz., *Kalanamak* and *Badshah Bhog.* Induced variability was observed in both the M_2 and M_3 generations indicated the possible selection for quantitative characters. The shift in mean was not found to be unidirectional nor equally in both directions in all the treatments. Most of the mutagenic families in different treatments showed shift in mean toward negative side coupled with high range and CV as compared to their respective control. In general, combination treatments of 30kR gamma-ray+ 0.2% EMS and 40 kR gamma-ray+ 0.2% EMS induced maximum variability for most of the traits. The use of physical and chemical mutagens or a combination of both has been an important tool for the increase of variability in agronomic traits. In general, there was reduction in variability, as judged from range and CV, in M_3 as compared to M_2 in all the treatments and traits in both the genotypes. The present investigation had clearly demonstrated the high potentials of 40 kR gamma-ray +0.2% EMS followed by 40 kR gamma-ray and 30 kR gamma-ray +0.2% EMS were found to be more useful in releasing desirable variability for yield and component traits in desired directions for most of the characters in both the genotypes in both the generations.

Key words: Kalanamak, Badshah Bhog, polygenic variability, micro-mutations.

INTRODUCTION

India has largest area under paddy in the world and ranks second in the production after China (Anonymous, 2007). The primary objective of the mutation breeding is to enhance the frequency and spectrum of mutations and also to increase the incidence of viable mutations. Since genetic variability is a pre-requisite for any successful breeding programme, the creation and management of genetic variability becomes central base for crop improvement programme. Rice is a diploid and self-pollinated crop and it possess enormous possibilities of improvement through mutation breeding; significant achievements have been made in developing new rice varieties with desirable characters through mutation breeding (Baloch et al., 2002). The present investigation has been taken up with two genotypes of rice which included *Kalanamak* (Medium Slender Grain) and

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Badshah Bhog (Short and flattened grain).

MATERIALS AND METHODS

Two thousand pure, healthy and dry seeds (moisture, 12%) of the two rice varieties, namely, *Kalanamak* and *Badshah Bhog* were irradiated with 10, 20, 30 and 40 kR doses of ^{60}Co. gamma rays at National Botanical Research Institute, Lucknow, Uttar Pradesh. Irradiated and unirradiated seed lots of each variety were divided into two equal parts (one thousand each). First lot was used as gamma rays treatment alone and other for combined treatment of gamma rays + Ethyl methane sulphonate (0.2%) and EMS (0.2%) alone. For chemical mutagen treatment, seeds were submerged for six hours in distilled water to insure complete hydration of the seeds at 30°C in incubator. Soaked seeds were blotted for removing surface water before transferring them into Ethyl methane sulphonate (0.2%) prepared with phosphate buffer solution having the pH 7.0 for a period of 6 h. in incubator (25°C) and were given intermittent shaking throughout the period of treatment to maintain uniform concentration. After EMS treatment, the seeds were thoroughly washed in running tap water for one hr to remove residual chemicals.

For micro mutational studies, all the M_1 plants having 60% pollen fertility or more (minimum being twenty plants per treatment) were advanced to raise M_2 generation following the procedure adapted by Gaul (1964). Seeds of twenty M_1 plants, selected on the basis of pollen fertility as described above, were sown separately in the nursery during rainy season of 2006. Twenty one days old seedlings of all the twenty M_1 plants selected from each treatment were transplanted in the well puddle field in Randomized Block Design with three replications. Agronomic practices were the same to that of M_1 generation. Data on five competitive normal looking plants from each M_2 families were taken randomly to record the observations on nine quantitative characters, namely, days to 50% flowering, days to maturity, plant height (cm), panicle length (cm), number of panicle bearing tillers/plant, number of grains/panicle, 100-seed weight (g) and grain yield/plant (g). Since the minimum number of promising micro-mutants in M_2 families for any one of the treatments was twenty, 5 normal looking plants were selected at random from each of these 20 families. Promising micro-mutants exhibiting higher grain yield from the family coupled with higher coefficient variation compared to their respective control were selected to grow M_3 generation treatment-wise. Since numbers of micro- mutations were variable in each treatment, only top twenty micro mutants were sown on raised nursery beds during the rainy (Kharif) season 2007. The 21 days old seedling of all the twenty M_2 plant progenies were transplanted in well puddle field at the distance of 20 × 10 cm from row to row and plant to plant, respectively as has been done in M_2 generation. Randomized Block Design with three replications was followed for transplanting. Each micro mutant was transplanted in three rows of 4 m in length. Recommended agronomic practices were followed to raise good crop. The quantitative and quality traits studied in the M_2 were also studied in the M_3 on five normal looking plants selected at random from each mutant families in each treatment in both the varieties.

RESULTS AND DISCUSSION

The primary objective of the mutation breeding is to enhance the frequency and spectrum of mutations and also to increase the incidence of viable mutations. Many physical and chemical mutagens have been used for inducing viable mutants in rice. In mutation breeding the choice of the mutagen is most important, and various methods have been developed to ascertain the efficiency of mutagen(s) and mutagenic treatments for the induction of desirable characters in a cultivated crop. Since genetic variability is a pre-requisite for any successful breeding programme, the creation and management of genetic variability becomes central base for crop improvement programme. Selection and hybridization are conventional methods for improvement of qualitative and quantitative traits. In this context, it is quite desirable to opt for induced mutagenesis which is recognized as a quick and successful method in creating genetic variability and bringing about desirable improvement. Mutations may be artificially induced by a treatment with certain physical or chemical agents; such mutations are known as induced mutations.

Plant height

In *Kalanamak* and *Badshah Bhog* (Table 1 and Figure 1), all but 10 kR, EMS, and 10 kR gamma-ray+ EMS treatments showed significant reduction in mean values coupled with high variability, as evident from range and CV in both the M_2 and M_3 generations. Remarkably, treatment with 30 kR gamma-ray caused shift in mean towards positive side in both the generations as compared to the control. The magnitude of variability, as judged from range towards desirable side (negative shift in mean values), was very high at 40 kR gamma-ray and 40 kR gamma-ray+ EMS in both the generations. Induction of mutants with short plant height in the treatments with 40 kR gamma-ray and 40 kR gamma-ray +EMS were very remarkable in both the generations. In *Badshah Bhog*, all the treatments except 10 and 20 kR gamma-ray alone and their combination treatments with EMS caused significant reduction in plant height in both M_2 and M_3 generations. The shift in mean towards negative direction that is, towards short stature was more evident in the treatments 30 kR gamma-ray+ EMS and 40 kR gamma-ray, as judged from the lower values of range in both the generations. EMS treatment significantly increased the mean values (shift towards positive side) for plant height in both the generations.

Days to 50% flowering

In *Kalanamak* and *Badshah Bhog* (Table 2 and Figure 2), all but 10, 20 and 10 kR gamma-ray + EMS treatments showed significant decline in mean values coupled with high variability, as evident form range and CV, in both M_2 and M_3 generations. The combination treatments produced more variability as compared to single treatments. The magnitude of variability, as judged from range, towards desirable side (negative shift in mean) was very high in treatments 20 kR gamma-ray +EMS

Table 1. Range, mean and coefficient of variation (CV) for Plant height (cm) in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	162-184	170.25	6.24	156-174	167.56	4.21	160-185	170.46	6.50	157-173	167.64	4.51
						Gamma rays						
10kR	151-186	168.18	8.10	154-179	166.20	7.98	150-184	169.17	7.23	155-180	166.31	7.04
20kR	150-188	167.78*	14.15	150-180	165.19	10.45	152-188	167.98*	11.98	153-185	165.82	8.91
30kR	148-189	172.78*	11.69	149-188	164.41*	13.66	150-185	173.20*	9.19	154-187	164.53*	11.19
40kR	89-188	164.87*	18.69	100-189	163.73*	17.10	104-189	165.23*	15.11	109-188	163.88*	15.01
						EMS						
0.2%	150-184	168.34	10.19	148-183	170.67*	9.12	151-185	168.88	9.52	150-184	170.82*	7.13
						Gamma-ray +EMS						
10kR+0.2%	153-185	167.90	12.78	145-185	165.47	11.53	155-186	169.09	10.68	149-186	166.01	10.03
20kR+0.2%	150-186	166.31*	14.21	140-185	165.37	12.35	157-186	166.67*	12.24	145-188	165.45	11.13
30KR+0.2%	148-188	165.19*	16.27	90-187	163.69*	14.19	150-187	166.35*	14.67	96-187	164.17*	12.16
40kR+0.2%	92-189	164.02*	20.54	133-189	162.32*	18.98	102-189	165.16*	16.98	136-186	163.74*	16.76
S.Em±	1.21			1.35			1.18			1.37		
CD at 5%	2.54			2.83			2.47			2.87		

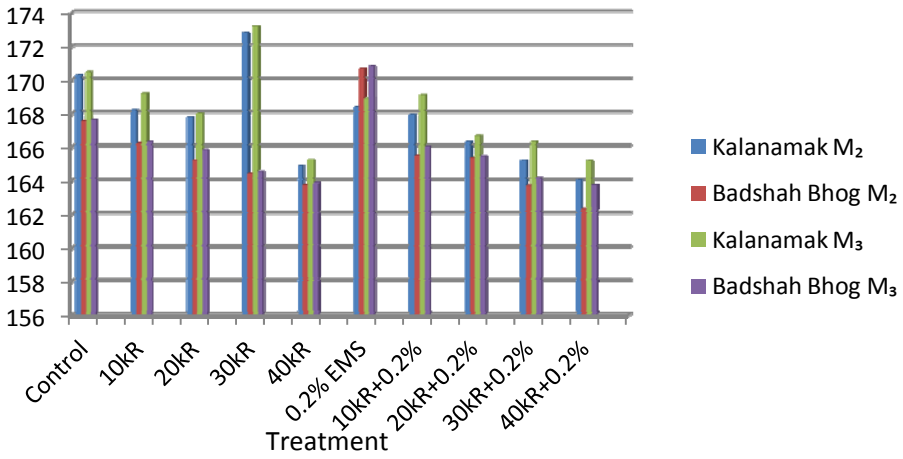

Figure 1. Plant height.

Table 2. Range, mean and coefficient of variation (CV) for Days to 50% flowering in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	138-150	141.60	4.48	136-149	141.15	3.34	140-151	141.89	4.57	135-148	141.23	3.30
						Gamma-ray						
10kR	136-152	140.84	7.04	133-152	139.77	5.89	135-155	141.14	6.80	133-151	139.93	5.16
20kR	133-155	139.66	8.66	129-153	138.64*	6.48	134-156	140.22	7.65	130-152	138.80*	6.01
30kR	129-156	138.79*	9.78	126-153	140.68	8.79	129-155	139.74*	8.44	126-153	140.76	7.71
40kR	128-157	137.97*	12.51	119-155	138.02*	10.16	124-155	138.45*	9.19	120-155	138.10*	9.18
						EMS						
0.2%	137-159	143.61*	6.90	132-151	143.55*	6.13	138-156	143.98*	6.02	131-152	143.59*	5.01
						Gamma ray+ EMS						
10kR+0.2%	129-153	139.68	7.08	126-153	139.86	7.49	130-154	141.09	6.91	128-152	140.06	6.11
20kR+0.2%	108-156	138.45*	9.39	122-154	137.57*	8.81	110-154	139.85*	8.86	124-153	137.93*	7.17
30kR+0.2%	128-155	136.96*	10.55	120-155	138.16*	9.71	127-153	138.90*	8.94	121-155	138.26*	8.61
40kR+0.2%	98-150	134.78*	14.55	115-156	136.31*	11.26	110-154	135.33*	8.94	117-156	136.58*	10.02
S.Em±		0.95			0.79			0.92			0.82	
CD at 5%		1.99			1.67			1.94			1.73	

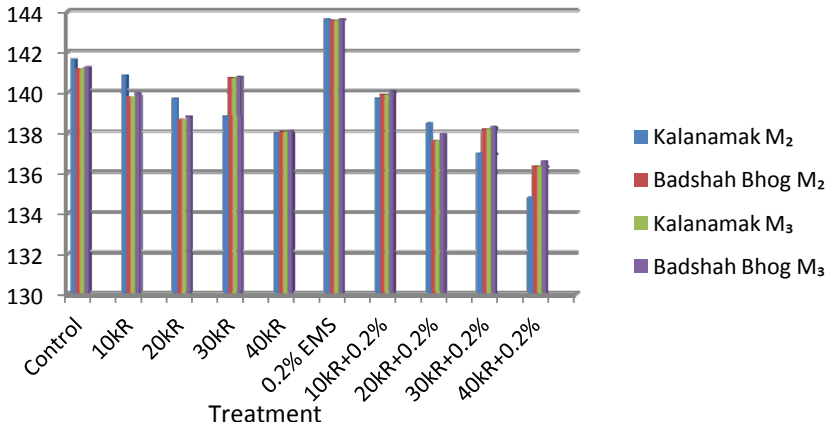

Figure 2. Days to 50% flowering.

and 40 kR gamma-ray+EMS in both the generations. Induction of mutants with as early in the treatments 20 kR gamma-ray + EMS and 40 kR gamma-ray + EMS were very remarkable in both the generations in the genotype *Kalanamak*. In the genotype *Badshah Bhog,* all the treatments except 10, 30 and 10 kR gamma-ray + EMS caused significant reduction in days to flowering in both M_2 and M_3 generations. The shift in mean towards negative direction (early flowering) was more evident in the treatments 30 kR gamma-ray + EMS, 40 kR gamma-ray + EMS and 40 kR gamma-ray, in both the generations. In this genotype, also, combined treatments (30 kR gamma-ray + EMS and 40 kR+ EMS) were found to be more promising as compared to single treatments with gamma-ray and EMS alone in both the generations. Single treatment with EMS alone significantly increased the mean days to flowering in both the genotypes and generations.

Days to maturity

In *Kalanamak* and *Badshah Bhog* (Table 3 and Figure 3), all but treatments `10, 20 and 10 kR gamma-ray + EMS showed significant reduction in days to maturity in both the generations in the genotype *Kalanamak*. The shifts toward negative side that is, early maturing were more pronounced in the treatment 40 kR gamma-ray + EMS as evident from the lower value of the range. While in other genotype, *Badshah Bhog* all the treatments except 10 kR gamma-ray, 30 kR gamma-ray and 10kR gamma-ray + EMS caused significant reduction in mean days to maturity in both the generations. Early maturing mutants were noted in the treatments 40kR gamma-ray + EMS, 30 kR gamma-ray + EMS and 40 kR gamma-ray. It was remarkable to note the induction of very early maturing (130-138) mutants in *Kalanamak* as compared to *Badshah Bhog* (induction of early mutants with 140-145 days). Single treatment with EMS alone caused significant positive shift in mean days to maturity in both genotypes and generations.

Panicle length

All but treatments 10 kR gamma-ray, 20 kR gamma-ray, EMS and 10 kR gamma-ray + EMS showed significant reduction in panicle length in both the generations in the genotype *Kalanamak* (Table 4 and Figure 4). The significant shift in the mean values towards positive side for panicle length was noted in treatment 30 kR gamma-ray in both the generations. From the upper values of range, treatments 40 kR gamma-ray, 30 kR gamma-ray + EMS and 40 kR gamma-ray + EMS were important in the M_2, while in the M_3 only treatment 40 kR gamma-ray + EMS was remarkable. In *Badshah Bhog,* all the treatments except 10, 20 and 10 kR gamma-ray +EMS caused significant reduction in mean values for panicle length in both the generations. The significant shift in mean in positive side in panicle length was noted in the treatment with EMS in both M_2 and M_3 generations, while 30kR gamma-ray caused significant increase in mean values in M_2 generation only.

Panicle bearing tillers per plant

In *Kalanamak* (Table 5 and Figure 5), all but 10 kR gamma-ray, 20 kR gamma-ray, EMS and 10 kR gamma-ray + EMS treatments showed significant decline in mean values from the control coupled with high variability, as evident from the range and CV, in both M_2 and M_3 generations. The magnitude of variability, as judged from the range towards desirable side (positive shift in mean), was very high in 40 kR gamma-ray +EMS in both the generations. Induction of mutants in the treatment with 40 kR gamma-ray +EMS was remarkable in M_2 generation in the genotype *Kalanamak*. In *Badshah Bhog* (Table 5 and Figure 5), all the treatments except 10 kR gamma-ray, EMS, 10 kR gamma-ray + EMS and 30 kR gamma-ray + EMS caused significant reduction in panicle bearing tillers per plant in both M_2 and M_3 generations. The shift in mean toward positive direction that is, higher panicle bearing tillers per plant was noted in treatment with 30 kR gamma-ray in the both M_2 and M_3. The mutants with increased panicle bearing tillers per plant were noted in most of the treatments in both the generations.

Number of grains per panicle

In *Kalanamak* (Table 6 and Figure 6), all but 10 kR gamma-ray, EMS and 10 kR gamma-ray + EMS treatments showed significant shift in mean values of number of grains per panicle as compared to control coupled with high variability, as evident from the range and CV, in both M_2 and M_3 generations. The treatments 20 kR gamma-ray and 30 kR gamma-ray + EMS induced significant shift in mean toward positive side in both the M_2 and M_3. Looking at higher values of range, combined treatments 30 kR gamma-ray + EMS and 40 kR gamma-ray + EMS were most promising, in both the generations as compared to control (113-199). Drastic effects of the treatments as judged from lower values of range, were very high in all the treatment as compared to control in both the generations. In *Badshah Bhog* (Table 6 and Figure 6), all the treatments except 10 kR gamma-ray, EMS and 20 kR gamma-ray + EMS in M_2 and 10 kR gamma-ray, EMS, 10 kR gamma-ray + EMS and 20 kR gamma-ray + EMS in M_3 showed significant shift in mean. The positive shift in mean was noted only in treatment 30kR gamma-ray in both M_2 and M_3 generations. Combined treatments (30 kR gamma-ray + EMS and 40 kR gamma-ray +EMS) were most promising,

Table 3. Range, mean and coefficient of variation (CV) for Days to maturity in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	161-179	168.85	4.33	163-178	170.88	3.61	160-179	168.93	4.39	164-177	170.90	3.63
						Gamma ray						
10kR	160-181	168.28	5.83	154-182	169.38	4.76	158-182	168.37	5.19	155-180	169.42	4.12
20kR	154-183	167.15	6.09	153-184	167.51*	6.31	149-182	167.59	5.99	152-183	167.58*	5.18
30kR	152-185	166.48*	9.66	148-186	169.31	7.66	147-186	166.81*	8.89	149-185	169.38	6.99
40kR	149-188	165.95*	11.14	144-188	166.83*	8.31	150-189	166.55*	10.81	146-187	166.85*	7.82
						EMS						
0.2%	158-185	170.94*	6.18	155-181	173.65*	5.17	156-186	171.87*	5.91	154-182	173.82*	4.61
						Gamma ray+ EMS						
10kR+0.2%	156-186	168.82	8.41	151-183	170.96	6.87	155-187	168.95	7.89	152-182	171.07	5.66
20kR+0.2%	145-188	166.72*	10.15	149-184	166.43*	7.91	146-189	166.83*	9.75	150-185	166.64*	6.84
30KR+0.2%	150-189	166.45*	12.42	145-186	168.65*	9.89	151-188	166.78*	11.06	147-187	168.87*	8.71
40kR+0.2%	130-189	163.04*	16.69	140-189	165.30*	10.42	138-189	164.08*	14.81	144-188	165.38*	9.82
S.Em±		0.97			0.71			0.96			0.72	
CD at 5%		2.03			1.66			2.01			1.69	

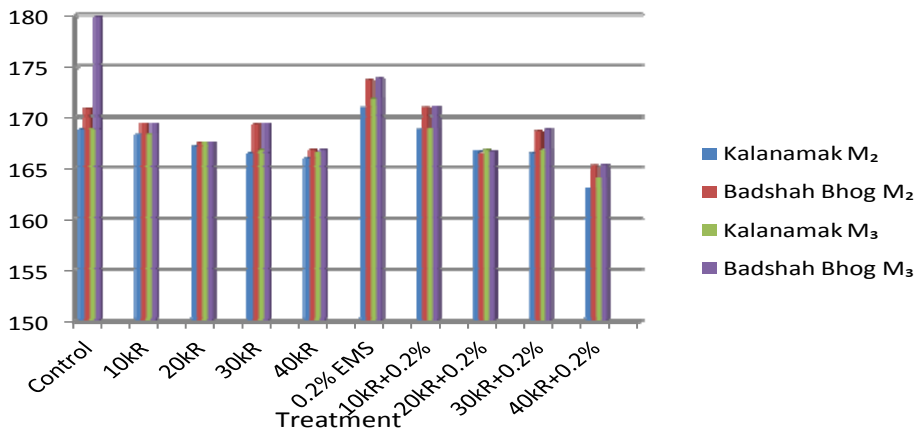

Figure 3. Days to maturity.

Table 4. Range, mean and coefficient of variation (CV) for panicle length in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	24-30	26.62	6.23	24-29	25.93	6.49	25-31	26.69	6.31	25-29	25.95	6.39
						Gamma ray						
10kR	23-31	26.34	7.05	22-29	25.40	7.96	24-30	26.50	6.84	23-28	25.82	7.16
20kR	21-31	25.92	10.80	21-30	25.17	8.87	20-31	26.15	9.04	23-29	25.26	8.19
30kR	22-30	27.63*	8.53	21-31	24.89*	11.50	23-29	27.69*	8.14	22-30	24.95*	10.51
40kR	20-32	24.46*	14.99	19-31	24.37*	12.84	19-31	24.53*	11.61	20-31	24.79*	11.78
						EMS						
0.2%	24-29	26.59	8.61	23-29	26.72*	9.72	24-30	26.69	7.19	22-30	26.68*	8.42
						Gamma ray+ EMS						
10kR+0.2%	22-30	25.97	9.81	22-31	25.35	8.91	21-31	26.04	8.21	22-31	25.39	7.61
20kR+0.2%	20-31	25.82*	10.98	21-30	24.83*	10.13	22-30	25.97*	8.99	21-31	24.93*	9.10
30KR+0.2%	20-32	25.13*	13.49	20-30	24.75*	11.15	21-31	25.37*	11.84	19-31	24.96*	10.06
40kR+0.2%	19-32	23.91*	15.94	19-31	23.78*	11.68	20-30	24.11*	12.94	20-30	23.96*	10.81
S.Em±		0.34			0.37			0.32			0.33	
CD at 5%		0.72			0.77			0.68			0.70	

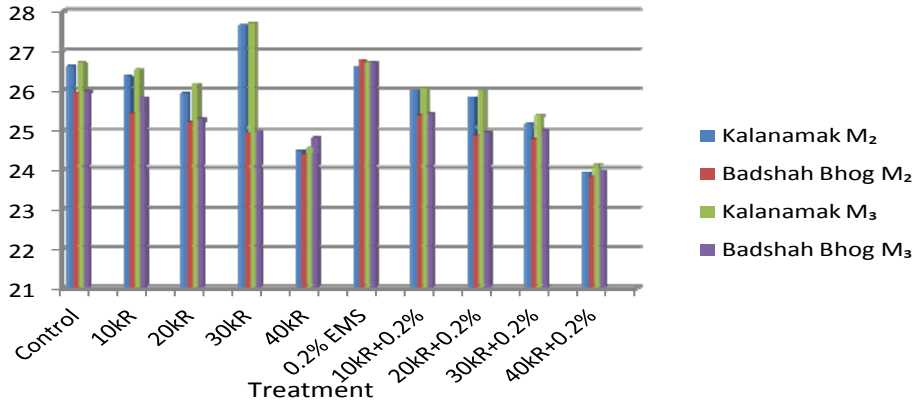

Figure 4. Panicle length.

Table 5. Range, mean and coefficient of variation (CV) for panicle bearing tillers per plant in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range†	Mean	CV	Range†	Mean	CV	Range†	Mean	CV	Range†	Mean	CV
Control	5-10	6.86	15.16	4-9	6.84	15.14	4-10	6.94	15.29	4-9	6.86	14-91
						Gamma-ray						
10kR	3-11	6.77	23.04	4-12	6.86	23.32	3-12	6.83	22.16	4-11	6.95	22.98
20kR	3-14	6.64	28.15	3-13	6.32*	27.61	2-14	6.72	27.91	4-12	6.39*	26.15
30kR	2-15	6.33*	30.80	2-13	7.31*	31.14	2-15	6.44*	30.02	3-13	7.34*	30.12
40kR	1-15	5.87*	33.99	2-15	5.84*	35.02	1-15	5.93*	32.36	2-14	6.04*	34.02
						EMS						
0.2%	3-12	6.57	27.12	4-11	6.89	26.19	4-12	6.73	26.19	4-11	6.91	25.14
						Gamma-ray +EMS						
10kR+EMS	2-14	6.59	25.81	3-12	6.85	23.38	3-11	6.66	24.49	3-10	7.06	22.31
20kR+EMS	2-15	7.76*	28.04	3-14	6.65	29.41	3-14	7.84*	27.31	3-13	6.75	28.18
30KR+EMS	2-13	6.35*	32.89	2-15	6.34*	33.27	2-14	6.40*	31.46	2-13	6.40*	32.14
40kR+EMS	2-16	5.72*	35.66	1-15	5.55*	37.43	2-15	5.88*	34.29	1-14	5.77*	35.21
S.Em±		0.22			0.22			0.22			0.21	
CD at 5%		0.45			0.46			0.44			0.44	

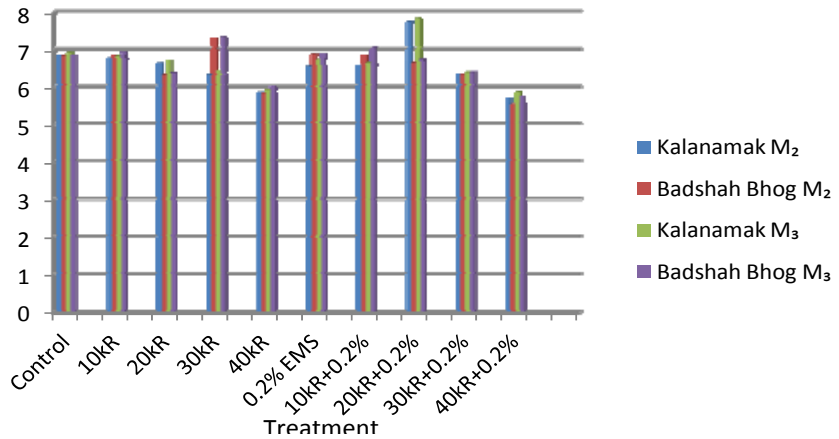

Figure 5. Panicle bearing tillers per plant.

Table 6. Range, mean and coefficient of variation (CV) for Number of grains per panicle in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range]	Mean	CV
Control	119-193	130.48	17.25	132-219	136.55	16.21	122-199	131.65	16.69	139-218	137.16	16.01
Gamma-ray												
10kR	81-218	128.67	25.71	89-226	134.66	25.62	89-220	130.77	24.91	75-228	136.45	25.04
20kR	72-224	135.25*	29.03	52-230	131.87*	26.94	76-220	138.93*	27.78	59-230	133.17*	25.82
30kR	65-231	124.07*	33.65	49-236	141.45*	34.39	62-237	127.42*	30.15	57-239	142.21*	31.15
40kR	56-238	126.41*	35.94	41-245	127.97*	38.15	60-240	128.11	32.34	59-251	130.98*	30.06
EMS												
0.2%	69-221	127.62	26.12	60-230	135.78	27.88	56-218	128.45	25.14	71-231	136.82	26.02
Gamma-ray +EMS												
10kR+EMS	67-226	128.05	28.10	56-225	131.17*	30.43	61-226	130.94	27.29	68-223	135.64	28.09
20kR+EMS	58-237	125.25*	35.68	54-236	138.09	33.19	50-234	126.88*	29.98	51-234	139.79	30.12
30KR+EMS	50-247	142.36*	36.02	45-249	129.57*	35.02	61-250	145.16*	30.25	49-246	132.29*	33.31
40kR+EMS	47-251	115.03*	38.73	41-261	125.73*	41.42	55-249	119.39*	35.10	51-260	127.77*	36.13
S.Em±		1.86			1.72			1.85			1.80	
CD at 5%		3.90			3.67			3.88			3.64	

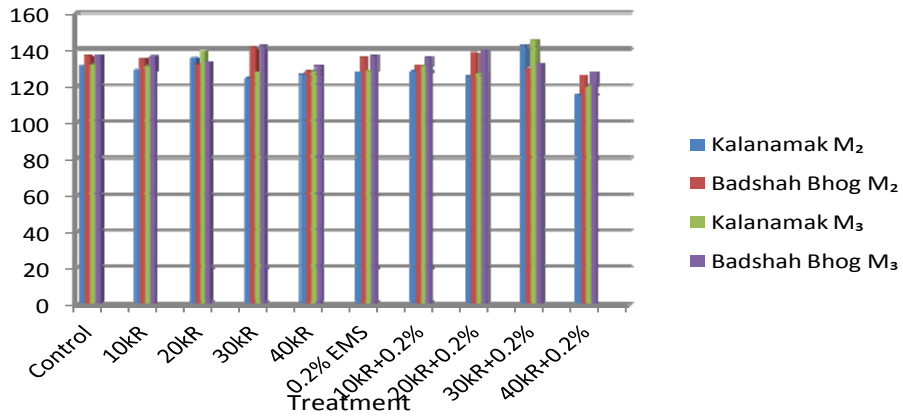

Figure 6. Number of grains per panicle.

as judged from high CV and isolation of mutants with increased number of grains per panicle as compared to control in both M_2 and M_3 generations.

100-seed weight

The significant shift in mean values as compared to control coupled with high variability, as evident from the range and CV, in both M_2 and M_3 generations were noted in *Kalanamak* (Table 7 and Figure 7), all the treatments except 10 kR gamma-ray, 20 kR gamma-ray, EMS and 10kR gamma-ray + EMS. While positive shift in mean was noted only in treatment 20 kR gamma-ray + EMS in both the M_2 and M_3. Mutants with high seed weight as compared to control were noted in all the treatments except 10 kR gamma-ray, 20 kR gamma-ray, EMS, and 10 kR gamma-ray + EMS in both the generations. In *Badshah Bhog* (Table 7 and Figure 7), all the treatments except 10kR gamma-ray, EMS, 10 kR gamma-ray + EMS and 20 kR gamma-ray +EMS caused significant shift in mean 100-seed weight in M_2 and M_3 generations. The shift in mean toward positive direction i.e., higher 100-seed weight was noted in treatment with 30 kR gamma-ray in both M_2 and M_3 and 20 kR gamma-ray + EMS in only M_3. The mutants with increased 100-seed weight were noted in treatments 40 kR gamma-ray and 40 kR gamma-ray + EMS in both the M_2 and M_3, while in treatment 30 kR gamma-ray + EMS in M_3 only.

Grain yield per plant

In *Kalanamak* (Table 8 and Figure 8), all the treatments except 10 kR gamma-ray, 20 kR gamma-ray, EMS and 10 kR gamma-ray + EMS caused significant shift in mean values as compared to control in both M_2 and M_3 generations; positive shift in mean was noted in the treatments 20 kR gamma-ray in M_3 only and 20 kR gamma-ray + EMS in both M_2 and M_3. It was remarkable to note mutants with increased grain yield per plant as judged from upper values of range, in most of the treatments in both M_2 and M_3 generations. While in *Badshah Bhog* (Table 8 and Figure 8), all treatments except 10 kR gamma-ray, EMS, 10 kR gamma-ray + EMS and 20 kR gamma-ray +EMS caused significant shift in mean grain yield per plant as compared to control in both the generations; positive shift in mean was noted only in treatment 30 kR gamma-ray. Combined treatments with 30kR gamma-ray and 40 kR gamma-ray were more drastic in reducing grain yield per plant in both the generations in both the genotype, *Kalanamak* and *Badshah Bhog*. Mutants with high grain yield as compared to the control, in most the treatments were remarkable to note.

The use of physical and chemical mutagens or a combination of both has been an important tool for the increase of variability in agronomic traits (Bansal et al., 1990; Agrawal et al., 2000; Sharma et al., 2008). The potentiality of ionizing radiation and chemical mutagens is different and their ability to induce mutation varies from crop to crop and genotype to genotype. Therefore, it is desirable to have the appropriate treatment schedule before under taking the mutagenesis.

On the other hand, Gupta and Swaminathan (1967) suggested that different M_2 families should be analysed for their mean and variance and families showing superior mean and increased variance over the control should be selected. Similar selection process was applied in the present study and also advocated by various workers in rice as well as other crops (Singh, 2003; Singh and Singh, 2003). In general, mutagenic treatments had resulted in decreased mean coupled with enhanced variability in both genotypes and generations as compared to their respective control, though the magnitude of shift in mean varied with the treatment, genotype and trait. The decline in means of treated population was demonstrated in rice (Jana and Roy, 1973; Awan et al., 1980; Siddiqui and Singh, 2010).

The decrease in means of mutagen treated population might be due to greater frequency of mutations with detrimental effects or due to difference in magnitude of induced individual change. The shift in mean was not found to be unidirectional nor equally in both directions in all the treatments. For days to flowering and maturity and plant height shifts in mean toward negative direction, as also noted in the present case, were of great significance as they yielded high frequency of mutants with early flowering and maturing and short stature. The induction and isolation of short statured with early maturing mutants having other desirable traits of the parental genotypes would be quite rewarding. In rice several workers (Singh and Singh, 2003; Domigo et al., 2007; Siddiqui and Singh, 2010) also found induced variability in desired directions for earliness and short stature.

The unidirectional variability towards positive side for grain yield and its component traits, as noted in the present case, were of great significance. This had yielded large number of mutants with improved grain yield per plant as well as other component traits irrespective of genotypes. Several workers also noted similar results after mutagenic treatment in rice (Singh and Singh, 2003, Siddiqui and Singh, 2010). Auxiliary traits like, panicle bearing tillers/plant, panicle length, number of grains per panicle and 100-seed weight on an average, showed positive shift in mean coupled with enlarged variability towards both sides. These auxiliary traits, as expected because of their positive correlation with grain yield, contributed significantly towards grain yield. Similar results were also noted in several crops, such as, rice (Baloch et al., 2002; Elayaraja et al., 2005; Domingo et al., 2007; Bughio et al., 2007; Siddiqui and Singh, 2010).

Hence, in the present study, most of these auxiliary traits were studied to screen out the high yielding mutants

Table 7. Range, mean and coefficient of variation (CV) for 100 -seed weight in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	1.69-1.99	1.85	6.65	1.66-2.11	1.83	5.68	1.70-2.01	1.87	6.91	1.66-2.09	1.84	5.63
Gamma-ray												
10kR	1.65-2.01	1.80	7.07	1.63-2.13	1.79	9.09	1.66-2.03	1.82	6.87	1.66-2.10	1.82	8.96
20kR	1.64-2.09	1.91	8.98	1.56-2.14	1.71*	10.17	1.65-2.12	1.92	8.03	1.58-2.13	1.72*	9.82
30kR	1.41-2.19	1.73*	11.87	1.58-2.19	1.95*	12.07	1.49-2.21	1.74*	10.89	1.60-2.16	1.99*	11.08
40kR	1.52-2.21	1.74*	12.08	1.55-2.26	1.70*	13.50	1.55-2.24	1.75*	11.99	1.55-2.20	1.71*	12.48
EMS												
0.2%	1.64-2.09	1.79	8.16	1.65-1.99	1.74	11.19	1.61-2.02	1.80	7.69	1.65-1.98	1.76	10.12
Gamma-ray +EMS												
10kR+EMS	1.58-2.06	1.77	9.19	1.45-2.16	1.75	9.10	1.51-2.19	1.78	8.96	1.60-2.13	1.78	8.14
20kR+EMS	1.53-2.17	1.96*	11.64	1.50-2.16	1.92	11.50	1.59-2.24	1.98*	10.21	1.55-2.14	1.95*	10.16
30KR+EMS	1.50-2.17	1.74*	12.81	1.49-2.21	1.66*	12.21	1.50-2.33	1.75*	11.69	1.52-2.16	1.71*	10.09
40kR+EMS	1.43-2.29	1.73*	15.97	1.42-2.36	1.65*	13.41	1.45-2.26	1.74*	13.87	1.51-2.25	1.69*	12.01
S.Em±		0.048			0.048			0.049			0.049	
CD at 5%		0.100			0.101			0.103			0.102	

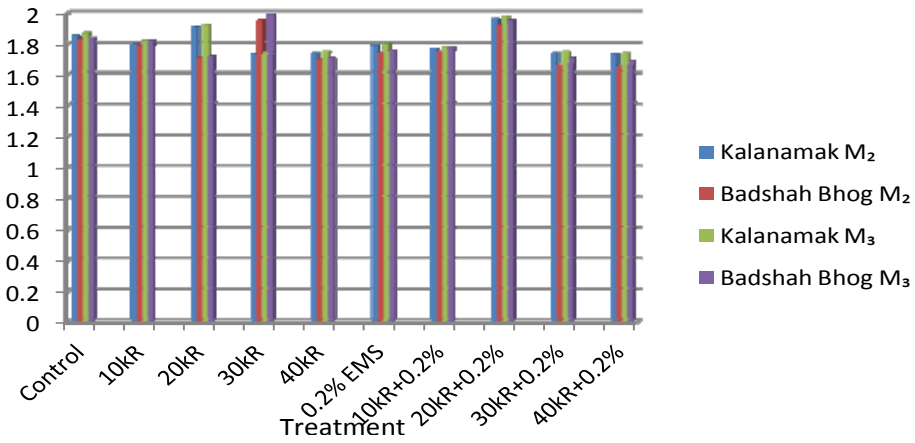

Figure 7. 100-seed weight.

Table 8. Range, mean and coefficient of variation (CV) for Grain yield per plant in M_2 and M_3 generations.

Treatment	M_2 generation						M_3 generation					
	Kalanamak			Badshah Bhog			Kalanamak			Badshah Bhog		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Control	11.98-16.41	12.76	15.77	11.21-17.99	13.06	17.51	12.02-16.52	12.81	16.14	11.32-18.12	13.12	16.96
					Gamma ray							
10kR	5.66-18.61	12.66	28.80	4.78-19.86	12.78	26.41	6.18-18.66	12.76	26.74	4.96-19.99	13.20	25.37
20kR	4.41-19.98	13.02	30.89	4.53-20.13	11.82*	31.36	5.44-19.69	13.84*	29.14	4.86-20.06	12.07*	26.81
30kR	4.12-20.16	9.69*	35.85	2.98-21.62	14.23*	37.87	4.61-20.46	9.98*	32.97	3.66-21.14	14.59*	35.42
40kR	3.55-21.18	10.98*	43.44	2.36-22.37	10.37*	46.81	3.98-21.88	11.59*	41.15	2.99-22.81	10.92*	44.72
					EMS							
0.2%	6.13-17.99	11.95	29.13	4.36+19.61	12.08	28.57	5.84-18.74	12.16	28.95	5.91-20.40	12.65	25.33
					Gamma ray+ EMS							
10kR+0.2%	5.10-18.61	12.34	30.28	4.68-20.61	12.23	35.77	6.14-19.79	12.67	30.11	5.86-20.15	12.47	31.81
20kR+0.2%	4.99-19.80	14.13*	37.39	3.98-21.65	13.66	40.33	5.32-20.84	14.47*	35.29	4.03-21.33	13.90	35.56
30KR+0.2%	3.09-21.42	11.62*	42.49	3.64-22.14	11.14*	43.33	4.32-21.97	11.76*	40.97	4.86-22.01	11.64*	40.16
40kR+0.2%	2.17-22.14	8.82*	48.56	2.84-23.19	9.12*	48.69	3.92-32.12	9.14*	44.26	3.66-22.89	9.74*	45.89
S.Em±		0.49			0.496			0.481			0.476	
CD at 5%		1.03			1.04			1.01			1.00	

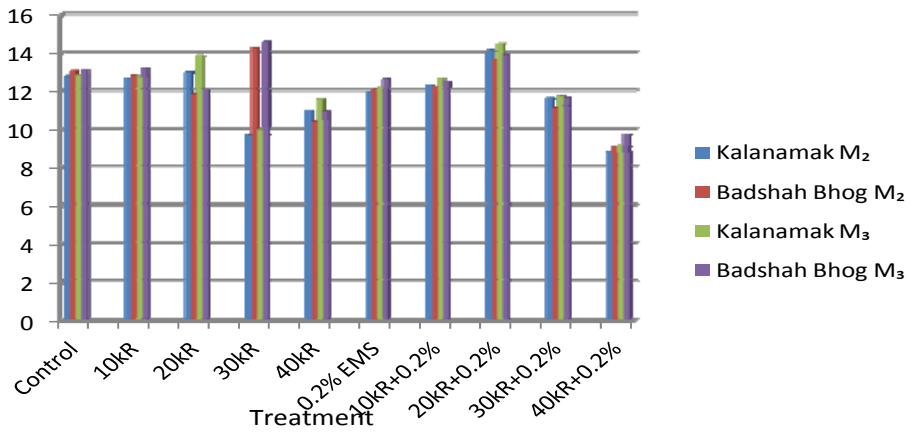

Figure 8. Grain yield per plant.

in the mutagen treated population. As such, sharp decline in grain yield per plant might be attributed to decrease in mean performance of these auxiliary traits affecting induced polygenic variability. The present investigation had clearly demonstrated the high potentials of combined treatments (gamma-ray + EMS) in releasing desirable variability for yield and component traits; treatments 40 kR gamma-ray +EMS followed by 40 kR gamma-ray and 30 kR gamma-ray +EMS were found to be most useful in releasing variability in desired directions in most of the characters in both the genotypes and generations. In few treatments, mean values higher than control were observed; 30 kR gamma-ray in *Badshah Bhog* and 20 kR gamma-ray + EMS in *Kalanamak* resulted increased mean values for most of the productive traits in both the generations. Similar results of increased mean than the control were also noted in rice (Gupta and Sharma, 1994; Siddiqui and Singh, 2010).

In general, there was reduction in variability, as judged from range and CV, in M_3 as compared to M_2 in all the treatments and traits in both the genotypes. Similar observations were also found by several workers (Singh, 2000; Siddiqui and Singh, 2010). This reduction in CV might be due to increase in the frequency of genetic death because of the homogygosity of harmful genes in M_3 generation. The differential genotypic response to different treatments was noted in present case; the genotype *Kalanamak* showed more variability than those recorded in *Badshah Bhog* for days to flowering, maturity and plant height, while more or less similar or low of variability was noted in both the genotypes for other traits (Singh, 2000; Siddiqui and Singh, 2010).

Conflict of Interests

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

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Full Length Research Paper

Effect of weather parameters on disease severity and rate of disease progress of yellow rust (*Puccinia striiformis*) of wheat in Jammu subtropics of India

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The effects of different abiotic factors such as average atmospheric temperature, relative humidity and rainfall on the development of yellow rust were studied under field conditions on two varieties of wheat *viz*., Agra local and PBW-343. Disease severity was recorded randomly in the plots following modified Cobb's scale at different dates. Epidemiological studies on wheat varieties *viz*., PBW-343 and Agra local revealed that there was significantly positive correlation between disease severity and minimum and maximum temperature, whereas, minimum and maximum humidity showed negative though significant correlation. The rainfall of 20.7 mm, minimum and maximum temperature of 9.8 and 19.4°C accompanied by minimum and maximum relative humidity of 84 and 87% were found conducive for disease development.

Key words: Yellow rust, *Puccinia striiformis*, severity, epidemiology.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's most extensively grown cereal crop and is staple food for over 10 billion people. It accounts for 30% in production amongst all cereal crops worldwide followed by rice (27%) and maize (25%). It is a major source of energy, protein and dietary fibre in human nutrition. Wheat is a staple food for nearly 40% of world population covering at least 43 countries (Rao et al., 1973). Yellow rust has traditionally been associated with wheat in the cool, temperate regions of the world, including Asia, Europe, North America, the middle-east and Africa (Line, 2002). In India, yellow rust is restricted in its distribution to cooler parts of North–West region as well as Nilgiri hills in South, where conditions are favourable for rust development (Kumar et al., 1989). Yellow rust is one of the most damaging disease of wheat and its distribution depends much upon climatic factors such as rainfall, humidity, temperature, etc (Emge and Johnson, 1972). The yellow rust is the most important of all the wheat rusts, favoured by mild winters and long, cool, wet springs. The development of yellow rust epidemics in addition to favourable weather depends upon the level of cultivar susceptibility, which affects the disease occurrence and its progress (Rapilly, 1979).

Growing resistant varieties and application of synthetic fungicides are the most effective means of controlling

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Table 1. Effect of abiotic factors on the severity of yellow rust in Jammu subtropics, during 2009 to 2010.

Standard week	Date of observation	Disease severity (%)		Infection rate		Rainfall (mm)	Temperature (°C)		Relative humidity (%)	
		Agra local	PBW-343	Agra local	PBW-343		Min	Max	Min	Max
3rd	18-01-2010	5.21	5.00	-	-	0.0	4.3	14.9	68	92
4th	25-01-2010	10.32	9.75	0.098	0.096	0.0	4.3	20.6	48	89
5th	01-02-2010	19.23	16.32	0.089	0.074	3.2	6.1	20.8	46	87
6th	08-02-2010	37.67	33.21	0.096	0.102	20.7	9.8	19.4	84	87
7th	15-02-2010	41.54	39.13	0.014	0.024	0.0	7.6	19.9	40	83
8th	22-02-2010	46.43	45.00	0.016	0.020	0.0	8.6	24.8	46	84
9th	01-03-2010	50.67	48.57	0.013	0.011	0.0	11.3	26.1	45	84
10th	08-03-2010	53.33	52.53	0.007	0.011	0.0	9.8	26.5	46	81
11th	15-03-2010	55.01	54.33	0.004	0.005	0.0	11.8	30.6	41	77
12th	22-03-2010	56.43	55.57	0.004	0.003	0.0	15.1	34.0	39	82
13th	29-03-2010	60.33	59.86	0.010	0.011	2.7	15.3	34.2	36	71
14th	05-04-2010	60.45	60.04	0.000	0.000	0.0	16.3	35.4	22	61
15th	12-04-2010	61.34	60.39	0.002	0.001	0.0	17.2	38.3	20	54
16th	19-04-2010	61.89	60.73	0.001	0.001	0.8	21.6	37.4	29	54

the disease (Kolmer, 1995).

MATERIALS AND METHODS

The effects of different abiotic factors such as temperature, relative humidity and rainfall on the development of yellow rust of wheat were studied under field conditions during *Rabi* 2009 to 2010 crop season. Two varieties of wheat *viz*., Agra local and PBW-343 were selected. Theurediospores of yellow rust (spore dust) were sprayed on the two test varieties (Agra local and PBW-343) at tillering stage. The field was irrigated after inoculation to maintain proper humidity. Disease observations were taken at weekly intervals using modified Cobb's scale (Peterson et al., 1948) and finally per cent disease severity was calculated from first week of February till the crop attained maturity final rust severity (FRS). Rate of disease progress was calculated using the formula given below (Vanderplank, 1963).

$$r = \frac{2.3}{t_2 - t_1} \log_{10} \frac{X_2}{X_1}$$

Where, r= rate of disease progress or infection rate, t_2-t_1=Time interval, X_1=Disease at time t_1, X_2=Disease at time t_2.

The disease rating was based on the leaf area covered with rust pustules. The disease severity (%) was correlated with meteorological data collected from the observatory of Agro-Meteorology, Section of the University and SKUAST-J, Chatha, Jammu. The effect of weather parameters on yellow rust was correlated using Minitab software. The regression and coefficient of multiple determination (R^2) values were also analyzed to find out the effect of single as well as combination of different meteorological factors on disease development.

Correlation analysis

All simple correlations were calculated between study variables Y and abiotic factors X_1, X_2, X_3…..X_5, where, disease severity (%), rainfall, relative humidity maximum and minimum, temperature maximum and minimum is represented by Y, X_1, X_2, X_3, X_4 and X_5, respectively. The test of significance for simple correlation was carried out using Student's t- test (Snedecor and Cochran, 1986). To study the cumulative effect of different variables (independent variables) in disease development (per cent disease severity) and multiple correlation coefficient were carried out and linear multiple regression model was developed for prediction of disease development. The coefficient of multiple determinations was used for the adequacy of regression equations.

Regression model

Linear multiple regression analysis were used to find out the effect of various environmental factors on disease progress by using prediction equation as:

$$Y = b_0 + b_1X_1 + b_2X_2 + \ldots\ldots\ldots\ldots + b_5X_5$$

RESULTS AND DISCUSSION

The data in Table 1 revealed that in the wheat varieties Agra local and PBW-343, under the field conditions, the disease initiation took place during the third week of January, when the average maximum and minimum temperature was 14.9 and 4.3°C and average maximum and minimum relative humidity was 92 and 68%, respectively.

Table 2. Correlation of different abiotic factors with disease severity.

Weather parameter	Disease severity (%)	
	PBW-343	Agra local
Rainfall (mm)	-0.161	-0.115
Min temperature (°C)	0.866*	0.862 *
Max temperature (°C)	0.875*	0.859*
Minimum relative humidity (%)	-0.645**	-0.621**
Maximum relative humidity (%)	-0.739*	-0.728*

*,** Significant at 1 and 5% probability levels, respectively.

Table 3. Multiple regression model of disease severity.

Variety	Multiple correlation coefficient	Coefficient of multiple determination	Regression model
PBW-343	0.901	0.811*	$\hat{Y}_1$ = - 60.2 + 0.46 X_1 + 2.68 X_2 + 1.10X_3 - 0.357 X_4 + 0.737 X_5
Agra local	0.896	0.802**	$\hat{Y}_2$ = - 56.8 + 0.74 X_1 + 2.96 X_2 + 0.84X_3 - 0.466 X_4 + 0.816 X_5

$\hat{Y}_1$, $\hat{Y}_2$ = Disease severity, X1 = Rainfall (mm), X_2 = Minimum temperature (°C), X_3 = Maximum temperature (°C), X_4 = Minimum relative humidity, X_5 = Maximum relative humidity; *,** Significant at 1 and 5% probability levels, respectively.

Subsequently, due to the rise in minimum relative humidity and rainfall in the month of February, a sharp rise in disease severity was recorded. The sharp rise in per cent disease severity on wheat varieties Agra local (19.23 and 37.67%) and PBW-343 (16.32 and 33.21%) was observed during 5th and 6th standard week when the weather variables *viz*., maximum temperatures (20.8 and 19.4°C, respectively), minimum temperature (6.1 and 9.8°C, respectively), maximum relative humidity (87 and 87%, respectively), and minimum relative humidity (46 and 84%, respectively) were favourable for the growth, establishment and spread of pathogen. From 11th to 13th standard week, the disease incidence and severity increased very slowly due to the rise in temperature and from 13th standard week onwards it remained more or less constant. NagarajanandJoshi (1978) reported positive correlation between number of rainy days and build up of brown and yellow rusts over N.W. India. Beest et al. (2008) have reported that the temperature was the most influential factor for disease severity and epidemics build up in wheat in United Kingdom. Similar observations were made by Papastamati and Vandan (2007) who have reported that the most important weather variable for the progress of yellow rust was temperature, followed by dew and light quantity. Dadrezaei and Torabi (2001) have also reported that the activity of *Puccinia striiformis*, including germination, penetration and development were limited due to rise in environmental temperature in late April which prevented further disease development as a result of heat and dryness. A correlation between abiotic factors and per cent disease severity on wheat varieties, Agra local and PBW-343, revealed that per cent disease severity was non-significant but negatively correlated with rainfall with a value of r = -0.161 and -0.115, respectively. The average maximum and minimum temperature had significant and positive correlation with disease development with the values of r = 0.859 and 0.862 in Agra local and 0.875 and 0.866 in PBW-343, respectively (Table 2).

Similarly, the average maximum and minimum relative humidity had significant and negative correlation with disease development, with the values of r = - 0.739, -0.728 and -0.645, while -0.621 for the varieties PBW-343 and Agra local, respectively. Christensen et al. (1993) found that the temperature in January and February is positively correlated with yellow rust severity. Disease severity did not show negative correlation with temperature in March because relative humidity seldom goes below 80%. Furthermore, yellow rust epidemic was sometimes halted by periods of hot weather (Beest et al., 2008).

Multiple correlation coefficients indicated strong relationship between disease and the weather variables taken understudy, thereby establishing that rainfall, maximum relative humidity,maximum temperature and minimum temperature during the course of disease development contributed more than 81.1 and 80.2% variations in yellow rust on varieties PBW-343 and Agra local respectively, during 2009 to 2010. Regression model developed for predicting unit increase/decrease in disease showed that different weather variables could influence disease severity to a given extent if the given weather conditions prevailed in an area over a specific period of time (Table 3).

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Full Length Research Paper

Trypsin (serine protease) inhibitors in peanut genotypes aiming for control of stored grain pests

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Peanut seeds from different genotypes were evaluated for activities of trypsin inhibitors (serine protease), based on *in vitro* and *in vivo* assays, aiming to further selection of genitors in breeding programs for tolerance of stored grain pests. The *in vitro* assays were based on inhibition of insect digestive enzymes and also on thermal and pH stabilities of seed protein, while the *in vivo* assays were performed with insects *Alphitobius diaperinus*, *Tribolium castaneum*, *Tenebrio molitor* and *Spodoptera frugiperda*. Seed inhibitors of all genotypes inhibited bovine trypsin at 70 to 94%. The seed extract of BRS Havana inhibited *T. castaneum* and *T. molitor* up to 80% while the extract of BRS 151 L7 inhibited *A. diapennu*s at nearly 20%. The seed inhibitors of both cultivars were stable at 80°C and also at different pH values. The two peanut genotypes are recommended as promising parents for breeding program aiming to selecting lines with tolerance to *Tenebrio* and *Alphitobius* insects.

Key words: Trypsin inhibitor, *Arachis hypogaea*, lepidoptera, coleoptera.

INTRODUCTION

Peanut is an important oleaginous known for its broad environmental adaptation. One of the major bottlenecks in the management is the post harvest phase mainly storage of grains. In this stage, the problems with storage pests are recurrent especially those caused by weevils: *Tribolium castaneum (Horbst, 1797) (Coleoptera: Tenebrionidae), Alphitobius diaperinus (Panzer, 1797) (Coleoptera: Tenebrionidae), Plodia interpunctella (Hübner, 1813) (Lepidoptera: Pyralidae), Tenebrio molitor (Linnaeus, 1758) (Coleoptera: Tenebrionidae), Corcyra cephalonica (Stainton, 1865) (Lepidoptera: Pyralidae) and Tenebroides mauritanicus (Linnaeus, 1758) (Coleoptera: Ostomidae)*. Depending on the level of infestation, the control of weevils can become unfeasible due to high costs with chemical treatment.

Genetic resistance to insect-pests is a desired goal by the most plant breeder. However, the acquisition of this trait is a big challenge due to heavy interactions between genetic and environmental factors. Thus, other natural strategies should be researched to detect tolerant

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Table 1. Main traits of peanut genotypes used in this study.

Genotype	G	SP	BT	GH	C	SS	O	CS	100S
BRS Perola Branca	C	H	Vi	R	110-115	L	50-52	White	55
176 AM	L	F	V	U	90-95	M	46-48	Red	43
173 AM	L	F	V	U	90-95	M	46-48	Tan	51
Florunner	C	H	Vi	R	120-130	L	49-51	Tan	50
IAC Caiapó	C	H	Vi	R	120-130	L	48-50	Tan	51
186 AM	L	F	V	U	90-95	L	46-48	Red	46
BRS Havana	C	F	V	U	88-90	M	43-45	Tan	50
L7 bege	L	F	V	U	88-90	L	45-47	Tan	61
175 AM	L	F	V	U	90-95	M	46-48	Tan	47
LGoPE-06	L	H	Vi	R	120-130	EL	50-52	Tan	72
BRS 151 L7	C	F	V	U	85-87	L	46-48	Red	67

G: Genealogy, C- Cultivar, L- Top line; SP- Subspecies: F- fastigiata, H- hypogaea; BT- Botanic type: V- Valencia, Vi- Virginia; GH- Growth habit: R- Runner. U- Upright; C- Cycle (days); SS- Seed size: M- Medium, L- Large, EL- Extra large; O- Oil content (%); SC- Seed colour and 100S- Average of 100 seed weight.

genotypes.

Focusing on stored grain pests, it is known that several plant species produce proteins with insecticidal property, such as inhibitor of proteases (IPs) which play a key role in plant defense against various orders of insects (Marinho et al., 2008; Pereira et al., 2007). The insecticidal activity of IPs is due to inhibition of proteolytic enzymes in the midgut of insects, leading to malnutrition, delay in larvae development and even death (Mosolov and Valueva, 2008). Among the groups of proteolytic enzymes affected by IPs, serine proteinases are the most investigated (Oliveira et al., 2005; Habib and Fazili, 2007).

Peanut seeds have different levels of IPs but information on using this trait for selecting genotypes tolerant to pests of stored grain is limited (Suzuki et al., 1987; Norioka et al., 1981). Considering the wide genetic base between intraspecific accessions of *A. hypogaea*, it is possible to identify promising materials for further use in hybridization works aiming subsequent selection of top lines with different level of tolerance to these pests.

The present research aimed to estimating the inhibitory activity of trypsin (serine protease) in seeds of different peanut genotypes for further recommendation of parents in breeding programs to tolerance to store grain pests.

MATERIALS AND METHODS

Extraction of proteins and determination of antitryptic activity

The peanut seeds used in this study were collected in January 2011 and January 2012 in Barbalha, CE (07 ° 18'18 "S, 39 ° 18'07" W, 414 m), semiarid region of northeastern Brazil. The study begin when the seeds were 8% moisture. The main traits of peanut genotypes are found in Table 1. Total crude protein of each genotype was extracted using methodology described in Bland and Lax (2000). The proteins were quantified by Bradford method (Bradford, 1976) at 595 nm using bovine serum albumin (BSA) asanalytical standard.

A previously described methodology was used to determine the antitryptic activity based on the following steps: a) bovine trypsin assay with seed total crude extract; b) bovine trypsin assay with partial purified seed trypsin inhibitor; c) insect digestive enzyme preparations assay with seed total crude extract; d) insect digestive enzyme preparations assay with partial purified seed trypsin inhibitor. A summary of the methodology is described below (Kakade et al., 1969).

In a microtube, 1.5 ml was performed following reaction: 5 µl of bovine trypsin (1 µg/µl) or 5 µl of insect digestive enzyme preparations (1 µg/µl), 20 µl of seed total crude extract or partial purified seed trypsin inhibitor (5 µg), 125 µl of 50 mM Tris-HCl buffer pH 8.5. The reaction was pre-incubated at 37°C for 20 min and 200 µl azocasein (1.5%, m/v) was added and again incubated at the same temperature and period. The reaction was discontinued with 300 µl of 20% trichloroacetic acid. After 5 min at room temperature, samples were centrifuged at 12.000 x g for 10 min. An aliquot of 250 µl of supernatant was collected and added to 250 µl of 2 mM NaOH; the reading was performed in a spectrophotometer (Femto, model 700S) at 440 nm. All assays were performed with three repetitions. Reagents from Sigma Aldrich (USA) were used in this assay.

Intestinal extract assays

Twenty third-instar larvae of *A. diaperinus*, *T. molitor*, *T. castaneum* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) were fed on artificial diet and then dissected for collecting the guts. *S. frugiperda* was included because it is an important crop pest and is susceptible to protease inhibitors of soybean (*Glycine max* L.) and bean (*Vigna radiata* L. Wilczek) seeds (Brioschi et al., 2007; Paulillo et al., 2000).

Dissected tissues were immediately immersed in 50 µl of 50 mM sodium phosphate buffer pH 7.5 and maintained at 4°C. After macerated, the homogenates were centrifuged at 12.000 x g for 20 min at 4°C. The supernatants were collected for further use in enzymatic assays for antitryptic activity assays with the seed total crude extract (Terra et al., 1977).

Hatching bioassay with *A. diaperinus*

Bioassays were carried out in order to estimate the hatching rate of

Table 2. Inhibitory activity of the seed total crude extract of peanut genotypes with bovine trypsin.

Genotype	Inhibition rate (%)
175 AM	94.2[a]
Florunner	92.6[a]
176 AM	92.2[a]
BRS Havana	90.3[ab]
186 AM	89.7[b]
173 AM	89.7[b]
IAC Caiapó	88.2[bc]
L7 Bege	79.3[bc]
BRS Pérola Branca	78.5[c]
LGoPE-06	78.0[c]
BRS 151 L7	70.7[d]

Means followed by the same letter do not differ significantly by the Tukey test (p≤0.05). Variance analysis to Inhibition rate: Mean square of treatment: 292.31, Standard error: 0.32, Freedom degree: 10, F test: 929.24**, Average: 85.60, Coefficient of variation: 0.66. **significant by the Friedman test (p≤0.01).

A. diaperinus fed on peanuts seeds (50 g). The seeds of each genotype were infested with 20 adults sexed and placed in plastic pots (8.0 cm of height x 11.0 cm in diameter). The pots were stored at room temperature for 90 days. Corn bran was used in control treatment. The bioassays were completely randomized with four replications.The number of larvae was registered at 53 and 90 days and hatching rates were estimated (Azevedo et al., 2010). The data were analyzed by the Friedman test (p ≤ 0.05) and the means were compared by Student Newman Keuls test (p ≤ 0.05).

Stability of seed trypsin inhibitors

This assay was performed in order to study the stability of seed-protease inhibitors at different temperature and pHs. The crude extracts were previously fractionated by sequential precipitation with ammonium sulfate at saturation ranges of 0 to 30, 30 to 60 and 60 to 90%. The 60 to 90% fraction showed the highest antitryptic activity (data not shown), and therefore was chosen as the peanut seed trypsin inhibitor fraction for stability assays. The procedures were based on that described by Gomes et al. (2005). To investigate thermal stability, the 60 to 90% fraction (1 ml, 1 µg/µl) was incubated for 30 min at 40, 60, 80 and 100°C, and thereafter cooled to 4°C. For characterization of the pH stability, the same volume of the 60 to 90% fraction was dialyzed for 16 h using the following buffers: 50 mM sodium phosphate, pHs of 5 to 8 and 50 mM Tris-HCl, pHs of 9 to 11. Then, the samples were incubated at 37°C and again dialyzed for 4 h in 50 mM Tris-HCl, pH 8.5. An aliquot of 5 µl of each sample was used for assessment of the remaining antitryptic activity with bovine trypsin. All assays were carried out in five replications. Data were analyzed by the Friedman test (p ≤ 0.05) and the means were compared by Tukey test (p ≤ 0.05).

RESULTS

Determination of antitryptic activity

Bovine trypsin was inhibited in all peanut samples analyzed, whose means ranged from 70 to 94% (Table 2). The genotypes 175 AM, Florunner, 176 AM and BRS Havana showed the same statistical classification with average of inhibition rate of 92%.

Based on the results in Table 2, all genotypes with inhibition rate at least 90% were selected for intestinal inhibition assays with *T. castaneum*, *T. molitor*, *S. frugiperda* and *A. diaperinus*. The genotype BRS 151 L7, with the lowest inhibition rate was also chosen considering that many have different response as to the types of protease inhibitors likely present in the seeds. As seen in Figure 1, the percentages of inhibitory activity of seed crude protein extract from different genotypes were almost uniform for *T. castaneum* (between 70 and 81%) and *S. frugiperda* (45 to 48%). However, different responses were obtained for *T. molitor* (35 and 83.6%) and *A. diaperinus* (8 to 19%).

The extract obtained from BRS Havana was very promising to inhibit *T. castaneum* and *T. molitor*, with inhibition rate up to 80%. For *S. frugiperda*, the extract of all genotypes showed average inhibition rate of 47% while for *A. diaperinus* BRS 151 L7 showed the highest inhibition rate close to 20% (Figure 1).

Feeding bioassay with *A. diaperinus*

In order to validate the information contained in Figure 1, a hatching bioassay was carried out using sexed adults of *A. diaperinus* fed on peanut seeds. This species was chosen due to high incidence in Brazilian grain storages. It was observed that larvae hatching were negatively affected by feed supplied and incubation period (Table 3). At 90 days, the number of larvae fed on peanut seeds was 33% less than at 53 days. As this average involved

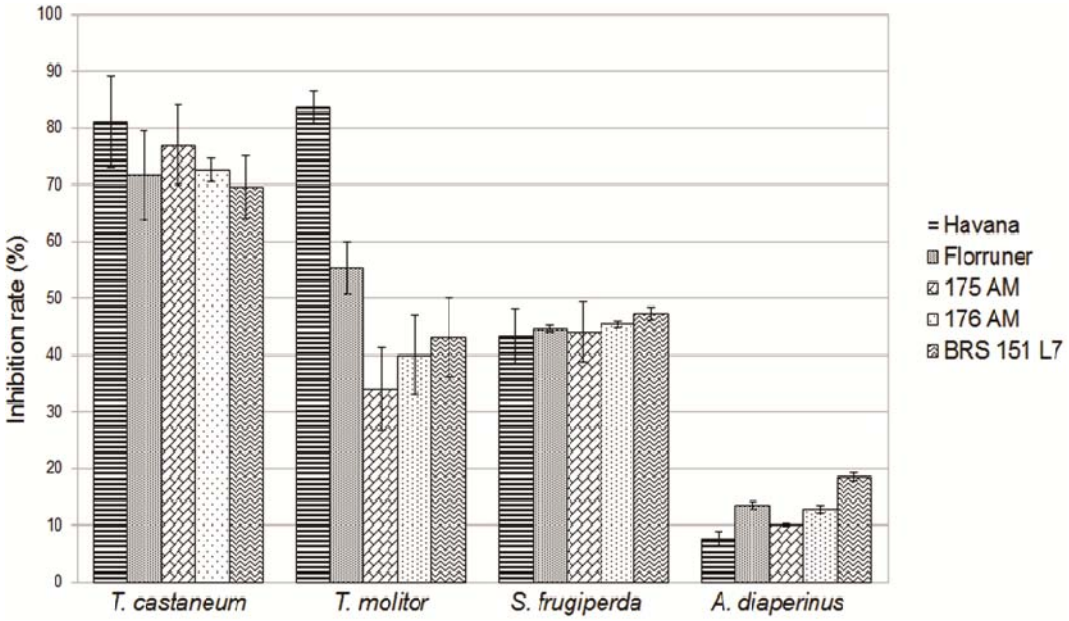

Figure 1. Inhibitory activity of seed total crude protein extracts from the peanut genotypes with insect digestive enzyme preparations of *T. castaneum*, *T. molitor, S. frugiperda* and *A. diaperinus.* Data are the mean values ± SD of three biological replicates.

Table 3. Number of hatched larvae of *A. diaperinus* fed on peanut seeds and corn bran.

Treatment	Number of larvae	DRC (%)
Period of incubation (days)		
53	9.00[a]	-
90	6.08[b]	33
Diet		
BRS 151 L7	5.52[bc]	51
Florunner	5.89[b]	47
175 AM	6.34[b]	43
176 AM	6.51[b]	42
BRS Havana	9.39[ab]	16
Corn bran (control)	11.17[a]	-

DRC- difference in hatching rate. Means with the same letter do not differ significantly by the Tukey test ($p \leq 0.05$). Means were transformed into $\sqrt{x}$ for statistical analysis. Variance analysis to period of incubation: Standard error: 91.16, Freedom degree: 1, F test: 24.41**, Average: 7.54; Variance analysis to Diet - Standard error: 40.91, Freedom degree: 5, F test: 10.77**; Average: 7.47. Coefficient of variation: 22.47%. ** significant by the Friedman test ($p \leq 0.01$).

most treatments with peanut seeds, it was suggested that this reduction may be associated with the greater period of insect feeding. This can be evident by the number of hatched larvae in different treatments. It was also verified that insects fed for 90 days on seeds from BRS 151 L7, Florunner, 175 and 176 AM had hatching rate reduced around 46% compared to control (corn bran). Among these genotypes, however, BRS 151 L7 revealed greater inhibitory rate (51%), which was in agreement with data recorded in Figure 1.

Stability assay of peanut seed trypsin inhibitors

The partial purified seed trypsin inhibitors from peanut genotypes showed thermostability in an interval of 40 to 100°C, with inhibition rate up to 60%, highlighting 175 AM who kept the inhibitory rate above 85% at all temperatures (Figure 2). An exception was verified to Florunner that had inhibitory activity substantially decreased at 100°C. As to pH stability of protease inhibitors, it was verified that the inhibitors from most

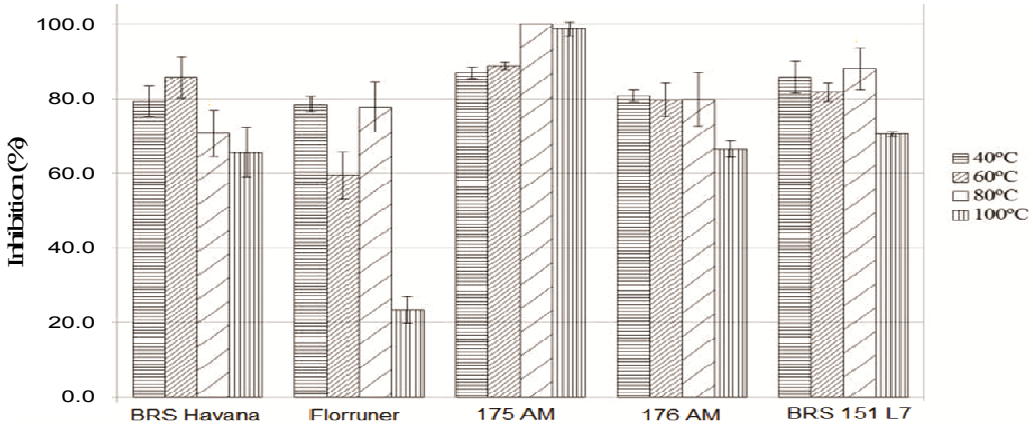

Figure 2. Thermal stability of peanut seed trypsin inhibitors from 60 to 90% fraction pre-incubated at different temperatures and tested with bovine trypsin. Data are the mean values ± SD of three biological replicates.

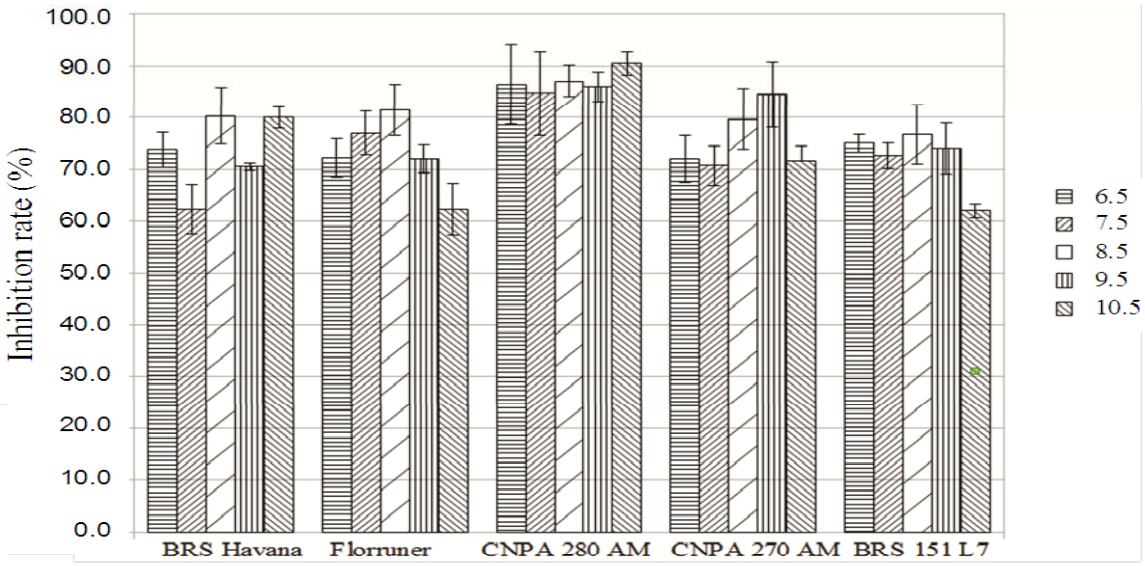

Figure 3. pH stability of peanut seed trypsin inhibitors from 60 to 90% fraction pre-incubated at different pHs and tested with bovine trypsin. Data are the mean values ± SD of three biological replicates.

genotypes maintained inhibition rate up to 70% at pH ranging between 6.5 and 9.5. Above this value, a small reduction in the rate was seen in extracts from Florunner and BRS 151 L7. Even so, it surpassed 60% which is still a reasonable rate of inhibition (Figure 3). The top line 175 AM showed large pH stability keeping the inhibition rate up to 80% at all pH range evaluated.

DISCUSSION

Based on high inhibition rates obtained in antitryptic activity assays using different peanut genotypes, we concluded that all materials showed great potential for further use in breeding programs aiming to tolerance to stored grain pests. Considering the rates obtained (70 to 94%, Table 2), it is suggested that proteases inhibitors are abundant in the seeds and this trait must be quantitative with high heritability. Such inference is based on studies developed by Dam and Baldwin (2003) and Kollipara et al. (1996), involving genetic inheritance of genes encoding to high expression of IPs in *Glycine tomentella* and *Nicotiana attenuata*. According to these authors, the inheritance is dominant and highly heritable. These findings are relevant when considering the perspective of transferring IP genes by conventional

hybridization.

Despite relevance of these data, *in vitro*-inhibition assays are not enough to define promising genotypes for a breeding program aiming to tolerance to stored grain pests. The bioassays with insects are essential to complement the information, mainly because a high inhibition rate to a given insect cannot be the same to another, due to particular differences in the families of intestinal proteases as well as to variations in pHs of insect guts. These are what really influence on binding and expressiveness of IPs to target proteins associated to insect digestion (Linser et al., 2009; Vinokurov et al., 2006; Dow, 1992).

The results shown in Tables 2 and 3 demonstrated the assertive in inclusion of cv. BRS 151 L7 in additional assays planned in this work. Although, this cultivar showed the lowest inhibition rate *in vitro* assay (70%, Table 2), it revealed expressive reduction in hatching rate (51%, Table 3), by using *A. diaperinus. T. castaneum* and *T. molitor*, the cv. BRS Havana was the most promising genotype due to its high inhibition rate (about 80%) in bioassays with intestinal homogenate (Figure 1). This is an expressive result comparing inhibition rate of others leguminous species. With *Crotalaria pallida*, Gomes et al. (2005) found 74% inhibition in intestinal homogenate of *Callosobruchus maculatus*. In further works, it would be interesting to test the extract of BRS Havana also with this coleoptera.

The inhibition rates obtained to *S. frugiperda* were reasonable but should be taken with caution in a breeding program since larvae are able to overcome the deleterious effects of IPs, possibly due to wide ability to activating new trypsin-like enzymes, which are less sensitive to inhibitors produced by plants (Paulillo et al., 2000; Xavier et al., 2005). This is a natural defense process also verified in several classes of insects.

In relation to thermal and pH stabilities of peanut seed trypsin inhibitors, these traits are quite important for further selection of genotypes because all seeds can be facing thermal variations in post-harvest processes. The instability of the trypsin inhibitors may be a negative factor for selection in a breeding program, even though genotype shows satisfactory results in inhibition assays. In this work, the results of pH and thermal stabilities of the partial purified trypsin inhibitors of the peanut genotypes were very expressive. The inhibitory potential of the seed extracts remained between pH 6.5 to 9.5 (Figure 3) indicating that peanut seed-IPs can affect a range of insect pests, especially Coleoptera and Lepidoptera, which intestinal lumen-pH varies from neutral to alkaline, acid to neutral or acid to alkaline, depending on the species (Linser et al., 2009; Vinokurov et al., 2006; Dow, 1992). The thermostability of the proteins were also satisfactory, since all extracts kept the inhibition above 60°C, excepting to Florunner that did not tolerate temperatures at 100°C. Plants rich in IPs and that hold the stability of inhibition in this range of pH and temperature are excellent sources genetic resources for plant defense by conventional hybridization or by transgenesis.

For food crops, such as peanuts, this information becomes more relevant due to several commercial products that are processed from the grains. Since many cereals are consumed boiled, losses in the activity of this compound may lead to the inactivation of its function. Serquiz (2012) tested the thermal and pH stabilities of trypsin inhibitor (TI) from peanut candy and verified that peanut-TI was fairly resistant, keeping the total inhibitory activity over trypsin when heated to 80°C and reducing only 8% when tested at 100°C. Gomes et al. (2005) evaluated the effect of trypsin inhibitor from *Crotalaria pallida* seeds on *Callosobruchus maculates*, *Ceratitis capitata* and obtained good results of inhibition from fresh seeds. However, when the extract was heated at 100°C for 30 min, the inhibition activity was reduced to 50%.

The results presented in this work can contribute greatly to the planning of a peanut breeding program aiming to tolerance to stored grain pests. The both earliness BRS Havana and BRS 151 L7, developed by Embrapa were promising candidates for this proposal. Additionally, the segregating arising from this crossing would also offer a range of variability for grain and oil, based on the characters presented in Table 1. The top line 175 AM was also a promising material based on the results obtained in inhibition assays against *T. castaneum* (Figure 1) and feeding assay with *A. diaperinus* (Table 3). Furthermore, the thermal and pH stabilities also contribute to explain its selection. The combination of this genotype, that is a Florunner-descendant (Gomes et al., 2007), with BRS Havana and BRS 151 L7 would generate large variability populations due to their broad genetic base which involves ancestors of fastigiata, hypogaea and vulgaris subspecies (Duarte et al., 2013; Gomes et al., 2007). The perspective in obtaining rich-IPs lines would naturally increase considering the dominant inheritance that controls the trait and also the percentage of inhibition shown in Table 2. Before beginning the breeding procedures, however, it is recommended to validate the information in natural storage conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Residual effect of transgenic soybean in soil microbiota

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Today, Brazil is the second major soybean (*Glycine max* (L.) producer of the world; however, few studies on the effects of transgenesis in the soil microbiota are completed. This study aims at assessing the residual effect of a transgenic soybean with tolerance to the imidazolinone herbicide group on the soil microbiota. The parameters examined were: the soil basal respiration, microbial biomass carbon (MBC) of the soil, metabolic quotient, spores of arbuscular mycorrhizal fungi and total soil fungal and bacterial biomass using epifluorescence technique in areas with eight years of no-tillage cultivated with non-transgenic (BRS133) and transgenic (BRS245) soybeans, in Londrina and Ponta Grossa - PR, Brazil, in the 2011/2012 growing season. The experiment was conducted in a randomized block design and each treatment with six replications. Results showed a significant increase on the MBC in Londrina soils with the BRS245 soybean, and also a significant increase in the total bacterial biomass in the both Londrina and the Ponta Grossa soil with BRS245 soybean, in contrast to those cultivated with BRS133. The BRS245 transgenic soybean crop changed the soil microbiota mainly by increasing the bacterial biomass, which was estimated by the epifluorescence microscopy method. The epifluorescence technique can be used to estimate soil biomass by fungi and bacteria with success.

Key words: Epifluorescence microscopy method, *Glycine max*, soil bacterial and fungal biomass.

INTRODUCTION

The soil is an extremely important component to the functioning of the terrestrial biosphere, not only for food production, but especially for the maintenance of the quality of the local, regional and global environments (Glanz, 1995).

The soil organic matter (SOM) is considered one of the main parameters in the evaluation of soil quality due to its influence on soil characteristics and responsiveness to management practices (Bayer and Bertol, 1999). One of the factors promptly responding to changes in soil use is the microbial biomass, which constitutes a means for processing all organic materials of the soil, acts as a reservoir of nutrients available to plants, and indicates the changes in the annual supply of organic materials by soils (Kaschuk et al., 2010, 2011).

Important parameters can be computed from the microbial biomass, as the metabolic quotient (qCO_2), which is the proportion of basal respiration (SBR) per microbial biomass carbon (MBC). The qCO_2 quotient indicates the influence of environmental conditions on the

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soil organic carbon. It is expected that stressed soils are presenting higher values of qCO_2 (Kaschuk et al., 2010). The SBR represents the amount of CO_2 released from respiration of soil samples and is a very responsive indicator of waste decomposition and disturbances in the ecosystem. This activity of soil organisms is considered a positive attribute for soil quality (Paul et al., 1999).

Agricultural management influences the size and activity of the soil microbiota (Bernardes and Santos, 2006). The ratio fungi/bacteria have been applied as good indicator of mineralization and immobilization of essential nutrients into soils, especially for N and P. Commonly there are more bacteria in conventional tillage and more fungi in non-tillage systems (Vries et al., 2006).

Microorganisms, especially fungi and bacteria, have been described as the principal degraders of organic matter in the soil and the water. Introducing chemical compounds into these environments means supplying microorganisms with nutrients, particularly C, N, and P (Kaschuk et al., 2010).

Currently, the soil capacity of crop production accounts for 40% of total degradation of the world agricultural lands. Among the sources of degradation is the extensive soil tillage. In Brazil, this extensive tillage increased by about 20% in recent years due to increasing demand for grains by the international market, especially for soybeans (*Glycine max* (L.) Merrill).

In the 2010-2011 cropping season, Brazil was the second soybean producer of the world, with 24.3 million ha of area cultivated with the crop and 75 million tons of grains produced, among which 90% from transgenic seeds (Embrapa, 2012). Transgenic soybean is no more than the result of the need for increased production and reduced costs in farming activities.

Genetic modified (GM) soybean was approved for trading firstly in USA in 1996. In 1997, a cultivar was developed by introducing the *ahas* transgene from *Arabidopsis thaliana*, resistant to herbicides of the imidazolinone group (Souza et al., 2013). It is known that the DNA of transgenic plants obtained from exudates, senescence, or degradation of plants or pollen are persistent in the soil (Lunch et al., 2004). Thence, it is important to investigate and understand the effects of transgenic crops on the soil microbiota (Kawalchuk et al., 2003; Dunfield and Germida, 2004; Widmer, 2007).

The BRS245 transgenic soybean, tolerant to herbicides of the imidazolinone group, received the *csr1-2* transgene in its constitution, which codes for the Acetohydroxyacid Synthase (AHAS) (EC 4.1.3.18), the first enzyme leading to the biosynthesis of valine, leucine, and isoleucine (first step of the synthesis of branched-chain amino acids) in plants and microorganisms (Souza et al., 2013). The inhibition of *ahas* activity leads to cell death by disabling cells to produce amino acids essential for protein synthesis and other key metabolic pathways (Souza et al., 2013).

Knowing the impact of transgenic crops and technologies associated with them on environment is an obligation of scientists, government and society. Most of studies on GM plants have assessed their potential only above soil; studies investigating their effects into soil are scarce (O'Callaghan and Glare, 2001; Bruinsma et al., 2003; Kowalchuk et al., 2003; Dunfield and Germida, 2004; Souza et al., 2013). GM plants can change soil microbiota by releasing several substances (exudates), However, the review by Bruinsma et al. (2003) does not address significant effects of transgenic plants on the bacterial and fungal population and microbial respiration of the soil.

On this way, it is important to understand the microbial ecology and soil quality aspects, since microorganisms act in the decomposition of organic matter, directly participating in biogeochemical cycles of nutrients and, consequently, contributing to the availability of nutrients in the soil (Balota et al., 2003; Kaschuk et al., 2010, 2011). Therefore, the soil microbial biomass is an important component of the SOM, responsible for regulating the microbiological transformations and nutrient storage.

Recently, both the soil microbial biomass and its biochemical processes are used as indicators of soil quality by responding promptly to changes in the soil environment. The contents of carbon (C), nitrogen (N) and phosphorus (P) on the soil microbial biomass as well as the activity of microorganisms are very important to understand the nutrient flow in natural ecosystems (Kaschuk et al., 2010, 2011). In addition, plant roots release exudates that can affect the soil microbiota (Koranda et al., 2011), and such changes may occur due to exudation of proteins by transgenic plants (Dunfield and Germida, 2004; Souza et al., 2013).

Many GM plants that are marketed can benefit from mutual associations with microorganisms. Soybean, for instance, form root symbioses with two different groups of mutualistic micro-organisms: bacteria of the genus *Bradyrhizobium*, which fix atmospheric N, and arbuscular mycorrhizal fungi (AMF), which can improve the absorption of nutrients and water by the roots of host plants (Powell et al., 2007).

In recent years, there has largely been cultivation of transgenic soybean in Brazil and in other countries of the southern hemisphere. However, little is known about the impact of GM crops on soil microbial populations and on related parameters, especially under cropping conditions (Souza et al., 2013).

This work aims at assessing the residual effect of the *ahas* transgene on soil microbiota by quantitative analysis, using non-transgenic (BRS133) and transgenic (BRS245 – transgenic version of the parent BR 133) soybeans in different areas, all with eight years of non-tillage (NT) system (Londrina and Ponta Grossa, Paraná, Brazil - 2011-2012 cropping season. Soil basal respiration (SBR), microbial biomass carbon (MBC) of the soil, metabolic quotient (qCO_2), spores density of

Table 1. Soil and local characteristics.

Place	Latitude (S)	Longitude (W)	Climate Koeppen's classification	U.S. soil classification	Previous crop
Ponta Grossa	25° 09´	50° 09´	Cfb	Rhodic Eutrudox	Soybean (summer) / wheat (winter)
Londrina	23° 18´	51° 09´	Cfa	Typic Haplustox	Soybean (summer) / wheat (winter)

arbuscular mycorrhizal fungi, and total fungal and bacterial biomass of the soil were the parameters examined.

MATERIALS AND METHODS

Description of sampling sites and soil sampling

Soil samples were collected in two areas (Londrina and Ponta Grossa, PR – Brazil) under eight years of NT system planted with non-transgenic (BRS133) and transgenic (BRS245 – transgenic version of the parent BRS133) soybeans, the transgenic seeds containing the *ahas* transgene, resistant to herbicides of the imidazolinone group. Soil samples were collected and provided by EMPBRAPA Soybean Division, Londrina - PR, which is a Brazilian experimental station certified to conduct experiments with transgenic material. Table 1 shows the main characteristics of soils and sites of each experimental treatment.

All farming practices as fertilization, irrigation, and pest and disease control were carefully standardized and addressed to each experimental site, following the recommendations for growing soybeans according to each region. The imidazolinone herbicide used was imazapyr at a rate of 70 g a.i. (active ingredient) ha^{-1} and the conventional herbicide consisted of a mixture of bentazon (400 g L^{-1}) + acifluorfen (170 g L^{-1}) (Volt) (570 g a.i. ha^{-1}), in addition to other herbicides such as tepraloxydim, according to the level of weed infestation in each area (ranging from 80 to 240 g a.i. ha^{-1}).

The experiments were conducted with four replications in a randomized block design. Plots were built as eight rows with five meters long and 0.5 m between rows, of which the four central lines were used as experimental area.

At R2 (50% of plants in full bloom) four soil subsamples were obtained at 0-10 cm depth and mixed to form a composite sample. This procedure was repeated six times to make the experimental replications, totaling 24 composite samples. Around 200 g soil per replicate were carefully homogenized and cleaned for plant and root debris and then sieved (4 mm sieve opening) and stored in plastic bags at ±4°C.

Determination of the microbial biomass carbon (MBC) in soil

Estimation of the MBC was made according to the fumigation-extraction method proposed by Vance et al. (1987) and Tate et al. (1988). For fumigation procedure, 10 g of soil sample was weighed and added to 1 mL of ethanol-free chloroform at flasks, which were closed and stored in location free from light for 24 h, with temperature ranging 25 to 28°C. After this period, the lids of the flasks were removed in laminar flow cabinet, leaving all the chloroform to evaporate, as proposed by Brookes et al. (1982) and Witt et al. (2000).

For non-fumigated samples, 10 g of soil sample was also weighed. Both the fumigated and the non-fumigated samples of each treatment were 2-time replicated and the means were obtained. Afterwards, C extraction was performed on fumigated and non-fumigated samples by adding them to 50 mL of 0.5 mol L^{-1} solution of potassium sulfate (K_2SO_4) and then, the samples was stirred for 30 min in an orbital shaker at 220 rpm. After decanting for 30 min, the supernatant was transferred to a filter paper to obtain an extract in a 50 ml tube. To determine MBC, 8 mL of extract was added to a 250 mL Erlenmeyer with 2 mL of 0.066 mol L^{-1} K_2Cr2O_7 (potassium dichromate), 10 mL of 95 to 98% H_2SO_4 (sulfuric acid), 5 mL of 85% H_3PO_4 (ortho-phosphoric acid); and after cooling the solution, 70 mL of deionized water was added. To the cold solution was added 4 drops of 1% (C_6H_5) 2NH (diphenylamine) to make up the titration under magnetic stirring using a solution of 0.033 mol L^{-1} (NH_2) 2Fe (SO_4) $26H_2O$ (ammonium ferrous sulfate). At the end of the titration, the color changed from purple to green.

MBC was estimated from extract by the formula MBC = (Cf – Cnf)/Kc, where Cf and Cnf are the C extracted from fumigated and non-fumigated soil samples and Kc is a constant value used for all samples, according to Hungria et al. (2009). The Kc value used in this study was 0.4, as suggested by Kaschuk et al. (2010).

Determination of basal respiration and metabolic quotient of the soil

The soil basal respiration (SBR) was determined according to Jenkinson and Powlson (1976). 30 g soil samples were weighed and stored in 100 mL flasks with each sample added to 10 mL of 1 mol L^{-1} NaOH (sodium hydroxide) within 30 mL flasks, which were transferred to a 500 mL glass jar tightly sealed. Three glass jars containing only 1 mol L^{-1} NaOH were used as a reagent blank (control).

The samples were incubated for 8 days in a free light area with 25 to 28°C temperature. After incubation period, the NaOH flasks were added to 2 mL of 10% $BaCl_2$ (Barium chloride) and 3 drops of phenolphthalein in alcohol solution at 3% for titration of NaOH with 0.5 ml HCl (hydrochloric acid). Titration was made under magnetic stirring, until the solution goes from white to pink color. Then, SBR was estimated according to Hungria et al. (2009). The metabolic quotient of soil (qCO_2) is the SBR and the MBC soil unit ratio Hungria et al. (2009).

Spore density of arbuscular mycorrhizal fungi (AMF)

Spores were extracted from 8 g soil subsamples by the wet sieving mesh methodology, using 0.710 and 0.053 mm sieves as described by Gerdemann and Nicolson (1963). After centrifugation with water (3000 rpm per 3 min) and with sucrose (2000 rpm per 2 min), the supernatant passed through the 0.053 mm wet sieving and was transferred to Petri dishes for spore counting and identification under a stereoscopic microscope (40X).

Determination of the total soil biomass by fungi and bacteria

The total soil biomass by fungi and bacteria was determined according to Bloem and Vos (2004). 6 g soil sample were weighed, ground, suspended in 190 mL of distilled water and stirred in a

Table 2. Chemical properties* (± standard error) of the soils where the field experiments were conducted.

Parameter[a]	Londrina		Ponta Grossa	
	Soybean varieties			
	BR133	BRS245	BR133	BRS245
pH ($CaCl_2$)	5.67±0.08	5.37±0.08	5.26±0.06	5.11±0.06
SOM (g dm^{-3})	24.85±0.79	25.26±1.04	32.17±1.97	31.57±0.39
P (mg dm^{-3})	27.93±1.45	26.96±2.44	18.51±2.56	19.35±2.44
K ($cmol_c$ dm^{-3})	0.56±0.04	0.48±0.05	0.31±0.02	0.30±0.02
Ca ($cmol_c$ dm^{-3})	4.44±0.14	4.18±0.21	3.45±017	3.28±0.11
Mg ($cmol_c$ dm^{-3})	2.72±0.28	2.03±0.18	1.62±0.09	1.50±0.08
S ($cmol_c$ dm^{-3})	0.89±0.17	1.89±0.34	3.06±0.30	3.13±0.13
CEC ($cmol_c$ dm^{-3})	11.73±0.30	11.28±0.24	10.17±0.23	10.10±0.11
BS (%)	65.55±2.14	59.07±2.59	53.26±1.84	50.15±1.73

[a] P = phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; S = Sulphur. * Methods: P, K, Ca and Mg; extracted by resin; S – extracted by resin $Ca(H_2PO_4)_2$ 0.01 mol L^{-1}; SOM (Soil Organic Matter) – Dichromate/colorimetric. Cation Exchange Capacity (CEC); Base Saturation (BS).

turbo-extractor at maximum speed (20,000 min^{-1}) for 1 min. A 9 mL aliquot of the suspension was transferred to a 15 mL centrifuge tube, gently homogenized in 1 mL solution of 37% aldehyde formaldehyde for 10 s, and allowed to stand for 2 min. After the rest period, an aliquot of 12 µL suspension was distributed uniformly over a microscope slide (9 mm diameter; 64 mm^2) previously sanitized and maintained at 50°C for 2 h to dry. Disinfestations of the slides consisted of washing with liquid detergent and 96% ethanol using absorbent paper. After drying, the slides were placed on damp paper towels.

For determination of fungi, 50 µL of freshly prepared solution of "fluorescent brightener 28 (Sigma F3397: Components C40, H42, N12, O10, S2 and Na2; and 1 mg mL^{-1} of 50% ethanol) was pipetted to each slide.

For the determination of bacteria, 50 µL of fluorescent dye dichlorotriazinyl aminofluorescein [5-(4,6-dichlorotriazin-2-yl)] or DTAF solution [2 mg of DTAF dissolved in 10 ml buffer solution of 0.05 M Na_2HPO_4 (7.8 g L^{-1}) and 0.85% NaCl (8.5 g L^{-1}), adjusted to pH 9] was pipetted to each slide.

The stained slides were stored at room temperature in a dark room for two hours. After this incubation period, the stained slides were immersed in distilled water for determination of fungus. For determination of bacteria, slides were dipped in buffer 3 times for 20 min to remove dye excess, and then dried in the dark at room temperature. Then, the slides received one drop of immersion oil, were covered with a slip and sealed with transparent glaze.

The length of fungal hyphae was estimated by epifluorescence microscopy (400x). The observation was made by the method of intersection of the grid, by randomly selecting one hundred sites and counting the presence or absence of fungal hyphae. The total length of hyphae (mg^{-1} of dry sample) and fungal biomass were calculated as described by Bloem and Vos (2004).

The amount of bacteria was estimated on these slides with epifluorescence microscopy (400x), by randomly selecting one hundred sites and counting for the presence of bacteria. The bacterial biomass was calculated according to equations described by Bloem and Vos (2004).

Statistical analysis

For local, Londrina and Ponta Grossa; and soybean varieties, BR133 and BRS245, T-test were done with independent bilateral averages. The interactions among the treatments with the combinations were submitted to an analysis of variance (ANOVA). Prior ANOVA analysis Levene's test were done for homogeneity. The averages were compared by Duncan's test ($p \leq 0.05$), utilizing the statistical program SPSS, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

From soil analysis, the pH values obtained were 5.52 in Londrina and 5.18 in Ponta Grossa (Table 2). The difference in pH values is probably due to soil constitution – in Londrina soil is classified as Eutroferric Red Latosol (Typic Haplustox), while in Ponta Grossa as Dystrophic Red Latosol (Rhodic Eutrudox). In Londrina, it was possible to verify pH values in soils cultivated with BRS245 soybeans lower than those of soils planted with BRS133 soybeans.

The nutrient contents and SOM are influenced by the soil management systems. In NT soils the SOM contents may be significantly higher (Kaschuk et al., 2010). The differences related to soil constitution can explain a variant influence on SOM between Londrina and Ponta Grossa soils, as the respective values 25.1 and 31.9 (mg g^{-1}) indicated (Table 2).

The SBR does not vary significantly between BRS133 and BRS245 varieties in both regions (Table 3), indicating that the transgene has no significant effect on the metabolic microbial activity of the soils. The same was verified by Bruinsma et al. (2003) based on their literature review. However, Bohm et al. (2010), who have studied the effect of herbicides recommended for transgenic soybean (glyphosate and imazethapyr) on MBC observed no significant change in this parameter but a significant increase on SBR with the application of these herbicides. The soybean transgene, however, did not result in significant changes in MBC, SBR and qCO_2 within this study.

Table 3. Values of the soil basal respiration (SBR µg C-CO_2 g^{-1} h^{-1}), microbial biomass carbon (MBC µg CO_2 g^{-1}), metabolic quotient (qCO_2 µg CO_2 $µg^{-1}$ C-microbial h^{-1}), arbuscular mycorrhizal fungi spores density (AMF nº g^{-1} of dry soil), total soil biomass by fungi (µg C g^{-1} of dry soil), total soil biomass by bacteria (µg C g^{-1} of dry soil), total soil biomass by fungi + bacteria (F+B) (µg C g^{-1} of dry soil) and the ratio of total biomass of fungi and soil bacteria (F/B) of soybean varieties (BRS133 – conventional and BRS245 – transgenic) grown in Londrina and Ponta Grossa, PR, in 8 years of non-tillage, in the 2011-12 growing season.

Parameter* / Treatments	SBR	MBC	qCO_2	AMF spores	Fungi	Bacteria	F+B	F/B
Local								
Londrina (LD)	0.75±0.03	139.88±4.40	5.43±0.37	2.79±0.25	23.32±1.60	158.64±12.44	181.96±12.94	0.13±0.01
Ponta Grossa (PG)	0.76±0.03	94.55±3.67	8.17±0.49	4.43±0.32	12.85±0.95	124.81±7.46	137.67±7.81	0.11±0.01
P value	0.841	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.029**	**0.008**	**0.009**
Soybean varieties								
BR133	0.78±0.03	110.18±7.25	7.47±0.58	3.26±0.39	17.55±1.98	117.11±7.13	134.66±7.99	0.15±0.02
BRS245	0.72±0.03	124.26±8.04	6.13±0.51	3.96±0.33	18.63±2.11	166.34±10.07	184.97±11.78	0.11±0.01
P value	0.195	0.207	0.097	0.182	0.712	**<0.001**	**0.002**	**0.032**
Soybean varieties X Local								
BR133 in LD	0.79±0.03	130.34±5.37^{b}	6.13±0.37^{bc}	2.42±0.22^{c}	22.74±2.11^{a}	125.87±11.59^{b}	148.62±12.08^{b}	0.19±0.02^{a}
BRS245 in LD	0.71±0.05	149.35±4.53^{a}	4.74±0.29^{c}	3.17±0.42^{bc}	23.89±2.59^{a}	191.41±10.82^{a}	215.30±12.10^{a}	0.13±0.01^{b}
BR133 in PG	0.78±0.06	89.93±6.21^{c}	8.81±0.79^{a}	4.10±0.58^{ab}	12.35±1.4^{b}	108.35±7.66^{c}	120.70±7.58^{c}	0.12±0.02^{b}
BRS245 in PG	0.74±0.05	99.18±3.47^{c}	7.53±0.52^{ab}	4.76±0.25^{a}	13.36±1.37^{b}	141.28±8.82^{b}	154.63±9.80^{b}	0.09±0.01^{b}
P value	0.582	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

*Means (± standard error). For LD and PG (n=12); BR133 and BRS245 (n=12) were done T-test with bilateral independent averages. In combinations, Duncan's test was used (n = 6). *P* values in bold are significant ($p \leq 0.05$).

Bohm et al. (2007, 2011) did not observe significant difference on MBC between transgenic (BRS244RR) and non-transgenic (BRS154) soybean when studied the effects of application and no application of herbicide. However, in present study, a significant increase on MBC was observed for BRS245 in comparison with BRS133 soybean in Londrina. In soils of Ponta Grossa this difference was not significant and the mean values were lower (Table 3).

The MBC increase is a positive aspect since it is an SOM component related to fertility and agricultural sustainability (Kaschuk et al., 2010, 2011). Similar results were found by Souza et al. (2013), who verified a reduced MBC in Ponta Grossa in comparison with Londrina. Low values of MBC can be explained by root exudates affecting soil microbiota (Widmer, 2007; Koranda et al., 2011) and by specific proteins released by transgenic plants (Dunfield and Germida, 2004). Souza et al. (2008) observed MBC changes in Londrina and Ponta Grossa soils during period of pre-sowing (R2 and R8) when studied transgenic and non-transgenic soybeans resistant to glyphosate. The authors found major changes in MBC in Londrina soil.

Zilli et al. (2007) reported a significant lower value of MBC in glyphosate + imazaquin treatment than in the control and the only glyphosate treatments. This result suggests that imazaquin is the responsible to reduce MBC since the glyphosate treatment had its MBC increased. However, some studies have reported no effect of glyphosate on diversity and soil microbiota and on functional activities as SBR and enzymatic action (Lupwayi et al., 2007; Weaver et al., 2007; Hart et al., 2009; Kremer and Means, 2009). But in other study, the application of glyphosate caused significant reduction on total microbial biomass at soybean

rhizosphere of a soil without previous exposition to glyphosate, by changing the microbial population structure (Lane et al., 2012).

The qCO_2 has been used as a biological indicator of soil equilibrium since more the microbial biomass becomes efficient less C is released as CO_2 by respiration and consequently more C is incorporated into microbial biomass (Anderson and Domsch, 1990). On this way, higher the qCO_2, more disturbed the soil is and less C can be incorporated to microbial biomass, causing reduced efficiency to sequester carbon and keep it as a part of SOM. According to Souza et al. (2006), high values of o qCO_2 are found under environmental stress conditions, where the microbial biomass demands more C for its maintenance. Therefore, the qCO_2 value of Londrina (5.43) indicates a less and significantly stressed soil than that of Ponta Grossa (8.17) (Table 3). The BRS245 soybean crop reduced the qCO_2 of Londrina soil (Table 3). This is a positive aspect since C is being kept within MBC, improving the SOM and contributing to the agricultural system sustainability.

The AMF are great bio-indicators of soil disturbance since they are very responsive to changes as soil fertility and plant species and also readily available (Bruinsma et al., 2003; Kowalchuk et al., 2003). In this study, significant effect of the *ahas* transgene was not observed resistant to imidazolinone herbicides of the BRS245 soybean on the AMF spore density. However, a significant higher spore density was observed in Ponta Grossa soil (4.43 no. g^{-1} dry soil), in contrast to a reduced spore density in Londrina (2.79 no. g^{-1} dry soil) (Table 3). Powell et al. (2007) analyzed nine varieties of transgenic soybeans resistant to glyphosate and did not observe significant effect of the transgene on AMF root colonization and on the number and volume of *Bradyrhizobium japonicum* nodules. Reis et al. (2010) also observed that glyphosate applied either single or in sequence did not affect AMF root colonization in soybeans with no seed treatment or treated with endosulfan + tebuconazole.

In Londrina, most of AMF spores were classified as *Glomus* sp., *Acaulospora* sp., and some as *Scutellospora* sp. (data not presented). In Ponta Grossa, most of AMF spores are *Glomus* sp., *Acaulospora* sp., and some are *Gigaspora* sp. These regional differences can be explained by geographical localization and their climate and soil conditions.

The total soil biomass by fungi and bacteria obtained by epifluorescence microscopy method was significantly higher in Londrina than in Ponta Grossa (Table 3). This confirms the results of high MBC obtained by traditional technique as shown in this study in Londrina. No significant differences were observed on fungal biomass in soils planted with transgenic soybeans. Similar results were reported in the literature review by Bruinsma et al. (2003).

A significant increased bacterial biomass was verified in soil planted with the transgenic soybean (BRS245) in both locals, but mainly in Londrina (Table 3). At both locations, studies were performed using the epifluorescence method for estimating the total biomass of bacteria of the soil, but it was not distinguished what soil bacterial groups were affected. Souza et al. (2013) concluded that the soil microbial population did not change significantly with the presence of *ahas* transgene in soybean, after 3 years of consecutive field analysis. From literature review by Bohm and Rombaldi (2010) it can be concluded that genetic transformation of soybean resistant to glyphosate did not affect soil microbiota and biological nitrogen fixation (BNF), but glyphosate applied in weed control can change the microbial soil population and affect BNF. The information survey by O'Callaghan and Glare (2001) shows that transgenic plants cause changes in soil microbiota and plant-microorganisms associations, including BNF (Souza et al., 2008).

The traditional technique for estimating the MBC with chloroform fumigation (Vance et al. 1987) and the epifluorescence microscopy method for estimating the total soil biomass by fungi and bacteria (Bloem and Vos, 2004) are correlated. The MBC correlates significantly with fungal ($r = 0.714$, $p < 0.001$) and bacterial ($r = 0.650$, $p < 0.001$) biomass. The same is observed by fungi + bacteria ($r = 0.707$, $p < 0.001$). These data indicates that the technique of epifluorescence microscopy is suitable to estimate soil biomass by fungi and bacteria and can be used in the further studies with success.

Kowalchuk et al. (2003), in a survey on the effects of all transgenic crops on soil microbes, states that effects have been generally slight and very small when compared to others sources of variation such as tillage, herbicide application and crop rotation. However, those slight changes in the total diversity of the soil microbiota caused by transgenic crops, such as the appearance and disappearance of certain microorganisms (soil bacteria), could affect the ecosystem (Dunfield and Germida, 2004).

Transgenic plants are likely to affect significantly soil populations of non-target bacteria and fungi, but more investigation in a case-by-case basis is needed to a deep assessment of the effects of transgenes on soil microbiota and ecosystems (Liu et al., 2005), as the proposition of this study.

Conclusion

The transgenic soybean crop (BRS245) significantly affected soil microbiota by causing an increased MBC and a reduced qCO_2 in Londrina. An increased total soil biomass by bacteria, obtained by epifluorescence technique, was also observed in soils planted with the transgenic soybean (BRS245) for Londrina and Ponta Grossa. Therefore, the cultivation of transgenic soybean (BRS245) modified the soil microbial population primarily by increasing the soil microbial biomass.

The traditional technique for estimating the MBC using

chloroform and the method for estimating the total biomass of bacteria and fungi are significantly correlated, indicating that the epifluorescence technique can be used to estimate soil biomass by fungi and bacteria with success.

Conflict of Interest

The authors have not declared any conflict of interest.

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