Response of Soybean to Inoculation with *Bradyrhizobium spp.* in Saline Soils of Shinille Plains, Eastern Ethiopia

Anteneh Argaw*

School of Natural Resources Management and Environmental Sciences, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia

Abstract: Soybean [*Glycine max* (L.) Merrill] is an important crop in Ethiopia. However, its productivity is constrained by a number of factors among which soil salinity is one the major problems. Therefore, field and greenhouse experiments were conducted to examine the effectiveness of exotic and locally isolated *Bradyrhizobium* spp. nodulating soybean in a saline soil containing high soil N in Shinille area, Somali region, Ethiopia. The treatments of the glasshouse experiment consisted of effective isolates of bradyrhizobia nodulating soybean (TAL-379, UK isolate, and isolate) and an improved genotype of soybean. The treatments of the field experiment consisted of three bradyrhizobia isolates and control check. All treatments were replicated three times. The results of the experiments showed that inoculation significantly improved nodulation, growth, and productivity of soybean over the control treatment. Among the inoculation treatments, isolate and UK isolate inoculation significantly ($P < 0.05$) improved the nodulation and grain yield of soybean over the TAL-379 treatment. All investigated traits, except grain yield, did not display significant differences in response to the inoculation treatments. This indicates soil properties measured and evaluated at the late stages of growth of the crop especially native soil nitrogen content was high. The regression analysis indicated significant association of nodule number and nodule dry weight with highest R^2 scored in isolate inoculation. The multiple regression analysis revealed that nodulation and plant tissue nitrogen concentration had strong relationships with grain yield, indicating the importance of symbiotic nitrogen fixation. Hence, inoculation of elite isolate of *Bradyrhizobium* sp. improved the yield of the soybean in saline soils. Although *Bradyrhizobium* inoculation improved remakablely the productivity of soybean, the yield gap is still very wide as compared to the potential yield reported elsewhere. Therefore, further research is required to improve the yield of the crop by diagnosing othr soil constraints in the region.

Keywords: *Glycine max* (L.) Merrill*;* Grain Yield; Inoculation; Isolate; Nodule Number; Nodule Dry Weight; Saline Soils

1. Introduction

Soybean (*Glycine max* (L.) Merr.) plays an important role in the global agricultural nitrogen cycles by facilitating biological fixation of atmospheric N into plant-available N in symbiotic association with *Bradyrhizobium*. The N_2 fixation potential of soybean varies ranging from 0 to 185 kg N ha^{-1} with an average value of about 84 kg N ha^{-1} (Russelle and Birr, 2004). However, soil stresses such as salinity can adversely affect N_2 fixation by influencing both the host plant and the bacteria (Rai, 1987). Legumes have long been recognized to be either sensitive or only moderately tolerant to salinity (Lauchli, 1984; Subbarao and Johansen, 1993), particularly when the nitrogen needed for the growth of these plants is derived from symbiotic atmospheric nitrogen fixation. Unlike their host legumes, rhizobia can survive in the presence of extremely high levels of salt and show marked variations in salt tolerance (Singleton *et al*., 1982). The establishment of the *Rhizobium*-legume symbiosis has been also shown to be salt-sensitive (Rao *et al*., 2002). Reduction of rhizobial survival and growth, hindering the infection process, suppressing nodule function, and reducing plant ability to photosynthesize, grow, and take up N have been observed in saline soils (Singleton *et al*., 1982; Saxena and Rewari, 1992; Elsheikh and Wood, 1995). However, there is a better chance of improving symbiotic N_2 fixation through a simultaneous selection of both plant genotypes and *Rhizobium* spp. strains (Rai, 1983; Rai and Prasad, 1983; Rai *et al*., 1985). Research is needed to identify soybean host-*Rhizobium* strain combinations capable of forming root nodules, and fixing nitrogen symbiotically under salt stress.

Although identification of well adapted soybean and potential areas for producing the crop has been extensively done in Ethiopia (Asrat *et al*., 2001), little research has been conducted to address soil fertility problems affecting the production of the crop. Recently, a study has indicated that soils of Ethiopia have either nil or very few bradyrhizobia nodulating soybean (Anteneh, 2012), signifying the need to introduce competent symbiotic root nodule bacteria as inoculants. Several researchers reported that significant yield improvements by inoculating soybean with effective and competent bacteria (Joshi *et al*., 1986; Zhang *et al*., 2002; Egamberdiyeva *et al*., 2004b). Abbasi *et al*. (2008) also reported that *Bradyrhizobium*

*Corresponding author. E-mail: antenehargaw@gmail.com

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inoculation increased soybean seed yield by 85% over an uninoculated control. The presence of high native mineral N in the soil may inhibit symbiotic N2 fixation mediated by host plant and the endosymbiont (Mendes *et al*., 2003; Hungria *et al*., 2006). Differences in symbiotic response to mineral N have been reported and may arise from both variation in the host plant and *Rhizobium* spp. Herridge *et al*., (1990) and Wu and Harper (1991) indicated that a high soil mineral N tolerant species of soybean produced a lower yield compared to N intolerant genotypes of soybean. A study conducted by Evans (1982) indicated the inhibition effect of naïve mineral soil N on biological nitrogen fixation varied significantly in different strains of rhizobia nodulating soybean. The author also indicated strain dependence of tolerance of functioning nodules to exposure to mineral N.

It is evident that some recommended soybean cultivars formed ineffective symbiotic associations with elite isolates of *Bradyrhizobium* (Diatloff and Brockwell, 1976), which has been indicated to be a significant strain-host incompatibility that could be related to geographic and phenological barriers (Howieson *et al*., 2005). Inoculation with exotic rhizobia produced inconsistent results in the Guinea regions of Nigeria in spite of the low population of indigenous rhizobia in the soil (Pal *et al*., 1985). This incompatibility of introduced exotic strains can be solved by selecting elite isolates of rhizobia nodulating soybean originated from different locations. Therefore, the objective of this study was to evaluatethe symbiotic effectiveness of exotic and localy isolated *Bradyrhizobium* spp. nodulating soybean in a saline soil with a high total soil N content.

2. Materials and Methods

2.1. The Experimental Site

The field experiment was conducted in the irrigated agricultural field (Shinille Agricultural demonstration site, Somali region, Ethiopia) which is semi-arid in nature. The soil has no history of inoculation with Bradyrhizobia strains and was never used for soybean cultivation. The site has instead been used for maize (*Zea mays* L.) and tomato production in the previous years. There was also no history of fertilizer application at this site. The experimental field is located at 09°41' N latitude and 41°51' E longitude with an elevation of 1079 meters above sea level. The soil is dominated by a sandy clay texture and increased amounts of clay further down at the lower depth. The extent of the Rhizobial population was estimated with the most probable number (MPN) method (Vincent, 1970) within two weeks of sampling, using a base dilution of 10 and the soybean variety solitaire as the trap host. The physico-chemical properties of soil were determined following the procedures compiled by Sahlemedhin and Taye (2000). The soil physico-chemical properties and the rhizobia population nodulating soybean in the area are indicated in Table 1.

2.2. The plant Material

The soybean genotype used in this study was obtained from Pawe Agricultural Research Center, Pawe, Ethiopia, and was already tested under field conditions of Ethiopia.

2.3. Rhizobial Strains

Rhizobial strains, *Bradyrhizobium japonicum* (TAL-379), *Bradyrhizobium* sp. (UK isolate) and *Bradyrhizobium* sp. (isolate) were used as inoculants. These rhizobial strains were obtained from Holleta Agricultural Research Center (UK isolate) and National Soil Research Center, Addis Ababa (TAL-379 and isolate). The strain had been previously tested for infectivity under a controlled environment in National Soil Research Center, Addis Ababa, Ethiopia.

2.4. Preparation of Inocula

Sterile fine filter-mud was used as a carrier after adjusting the pH to 6.7. *Bradyrhizobium* spp. were separately incubated in the yeast-extract mannitol (YEM) broth at 30^0C for 7 days until the number of cells ml^{-1} reached 10^9 for inoculant preparation. The liquid medium containing 400 ml of *Bradyrhizobium sp.* culture was added to 1 kg of a carrier and mixed thoroughly and packed in plastic bags. The filter-mud-base inoculum was incubated at room temperature for 15 days.

2.5. Soils for Pot Experiment

The soil used for the pot experiment was a saline soil, collected to the depth of 20 cm from an area where the field experiment was conducted. A plant growth medium containing soil from Shinille agricultural experimental site was developed based on three requirements: absence of indigenous bradyrhizobia nodulating soybean, high native mineral soil N, and salinity of the soil. The soil was collected and dried under aseptic conditions and no rhizobia were detected by a plant infection technique (Brockwell, 1963) at sowing.

2.6. Treatments and Experiment Design for the Pot Experiment

A pot experiment was conducted in the semi-controlled greenhouse at Haramaya University, Eastern Ethiopia in 2012. The treatment consisted of (i) isolate, (ii) UK isolate, (iii) TAL-379, (iv) N-fertilized pot (pots fertilized with 20 kg Nha^{-1}), and (v) negative controls (unfertilized and uninoculated pots), with three replications. The experiment was laid out as a completely randomized design (CRD) and three replications.

2.7. Experimental Procedure

To make the seed free from rhizobial contamination, soybean seeds previously selected for saline soil were surface-sterilized with ethanol (1min) and sodium hypochlorite (5 min) and then washed several times with deionized water. The seeds were sown in each pot.

Five seeds were sown per pot. One week after emergence, the soybean seedlings were thinned to three plants per pot. Pots were regularly and daily watered to 70% water-holding capacity (WHC), avoiding waterlogging. Rhizobia were cultured to exponential phase in YEM broth, and then 1 ml of culture containing 1 x 10^8 rhizobia was applied to 7-day-old seedlings using glass pipette. Plants were harvested after eight weeks of growth at the late flowering and early pod setting stage. They were removed from the pots, and the roots were thoroughly rinsed with water, blotted dry using filter paper, and the nodules picked and counted. Total plant and nodule dry weights were recorded after drying at 70^0C for 48 h.

2.8. Field Experiment

Field experiments were conducted at the experimental farm of Shinille Agricultural Demonstration site using well-structured drip irrigation system in 2012. The treatments consisted of four inoculations (UK isolate, isolate, TAL-379 and uninoculated control) and two soybean genotypes (Giza and TGx-1332464). The experiment was laid out as a split plot in a randomized complete block design with three replications. The inoculants constituted the main plots whereas the soybean genotypes were the sub-plots.

The land was prepared by deep ploughing, harrowing and leveling. Then the area was ridged and divided into 3 m x 3 m plots. Each soybean genotype was planted in 10 cm spacing between plants, 60 cm between rows, 1.5 m between sub plots and 2.0 m between main plots. Before planting, a 20 g of the different bradyrhizobia inoculant was added into different polyethylene bags containing 200 g of soybean seeds. Sugar solution (48%) was added to each bag to enhance proper mixing and adhesion of the *Bradyrhizobium* carrier material to the soybean seeds. Two seeds were sown per hill. Plots were immediately irrigated after sowing to ensure uniform germination. Subsequently, plots were irrigated by a drip irrigation system at a 7-day interval.

Weeds were controlled over the growth period with hand hoeing. A set of five plants from each plot was randomly selected at the late flowering and early pod setting stage for nodulation potential (number of nodules, dry weight of nodules) and shoot characteristics (shoot height, shoot dry weight). Dried shoot parts were ground and analyzed for total N using Kjeldhal method.

2.9. Data Collection and Measurement

At physiological maturity, plants were harvested from a 3.0 m x 2.40 m net plot leaving two guard rows to avoid edge effects. The plant tops (stalks plus pods) were weighed to determine total dry matter yield before threshing and winnowing to separate the seed, which was then weighed to determine yield. Leaves were not included in total dry matter yield determinations, as they had already senesced and fallen to the ground. Seed moisture was adjusted to 10% when determining grain yield (Kenodulenumbereth and Hellevang, 1995).

2.10. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the SAS computer software package. The least significant difference (LSD) test was used to separate means at 5% level of significance. Microsoft excel was used for drawing bar graphs and regression analysis.

Table 1. Soil analysis of experimental sites before sowing.

Soil property	Shinille soil	Rating	Reference
pH in H_2O	7.74	Moderately alkaline	Murphy (1968)
EC(mS/cm)	4.12	Moderately saline	FAO (1988)
Organic carbon (%)	2.15	Medium	Tekalign Tadese(1991)
Total nitrogen (%)	0.29	High	Tekalign Tadese(1991)
Available P(mg kg^{-1})	25.85	High	Olsen *et al.* (1954)
Ca (cmol(+)kg^{-1})	31.10	Very high	FAO(2006)
Mg (cmol(+)kg^{-1})	3.22	High	FAO (2006)
Na (cmol(+)kg^{-1})	0.14	Low	FAO (2006)
K (cmol(+)kg^{-1})	2.22	High	Berhanu Debele (1980)
CEC (cmol(+)kg^{-1})	25.90	High	Hazelton and Murphy (2007)
Zn(mg kg^{-1})	1.19	High	Jones and Benton (2003)
B(mg kg^{-1})	0.86	Low	Jones and Benton (2003)
Fe (mg kg^{-1})	2.22	Low	Jones and Benton (2003)
NH_4-N(mg kg^{-1})	26.22		
NO_3-N (mg kg^{-1})	23.0		
Clay (g kg^{-1})	27		
Silt (g kg^{-1})	50		
Sand (g kg^{-1})	23		
Textural class	Loam		
Number of soybean nodulating rhizobia	None		

3. Results and Discussion

3.1. Physico-Chemical Properties of the Soil

The soil analysis indicated pH of 7.74 with higher electric conductivity of 4.21 mS cm^{-1}. The soil organic carbon (SOC) and total N contents in the experimental soil were 2.1 and 0.2%, respectively, with no native rhizobia nodulating soybean. Except Fe content, the soil had medium to high contents of total N, SOC, available P, CEC, exchangeable bases and other tested micronutrients, and could be classified as saline with mild alkalinity (Hazelton and Murphy, 2007). The textural class of the soil is loam with higher silt content (Table 1).

3.2. Effect of Inoculation on Nodulation

The results of the field experiment revealed that inoculation (I) was found to affect significantly ($P < 0.05$) all investigated growth attributes of soybean (Tables 2 and 3). There was no nodule formation in the control treatment throughout the experimental period, indicating that there was no rhizobia nodulating soybean in the area. Although the Shinille soil had high native mineral N (Table 1), nodulation was significantly improved by inoculation of *Bradyrhizobium* sp. A significantly higher nodule number was obtained from the isolate inoculation followed by UK isolatein oculation over the control treatment (Tables 2 and 4). In contrast to this result, however, several studies, earlier indicated the negative effect of higher native soil mineral N on nodulation induction (Gibson and Harper, 1985; Herridge and Brockwell, 1988). Inhibition of soil N has been substantially ameliorated by increased numbers of rhizobia (Herridge and Brockwell, 1988). Gibson and Harper (1985) noted the importance of selection of *Bradyrhizobium* and soybean genotype for better nodulation at high soil N. This strategy has been unsuccessful because the selected genotypes of soybean have low grain yields (Herridge and Rose, 1994).

The results of this study have also revealed that the isolate and the UK isolate inoculations resulted in significantly higher nodule dry weight over the control treatment in the greenhouse and field experiments (Tables 2 and 4). Similar findings were reported for pot experiments by Okereke and Onochie (1996) who found that exotic *Bradyrhizobium* sp. enhanced the nodulation of soybean. This indicates the importance of inoculation for enhancing nodulation. However, the nodule number obtained from the field and greenhouse experiments was generally lower than those obtained from experiments conducted in Nigeria (Okereke *et al*., 2001) but is comparable with nodules induced in calcareous soilin Uzbekistan (Egamberdiyeva *et al*., 2004a). The lower nodule number obtained in this study could be attributed to the negative effect of higher EC on root growth which in turn reduces the sites of nodulation (Rao and Sharma, 1995) and absence of rhizobia nodulating soybean in the experimental site.

Among the inoculation treatments, TAL-379 inoculation resulted in statistically lower nodule number and nodule dry weight compared to the other inoculation treatments. However, an experiment conducted in Congo found that inoculation of TAL-379 enhanced nodule dry weight up to 336-382% over the uninoculated control treatment (Mandimba and Mondiboye, 1996). The lower performance of TAL-379 in the present study might be ascribed to its high sensitivity to saline soil which could reduce growth and multiplication of rhizobia and formation of nodules in soybean roots (Tu, 1981; Sprent and Zahran, 1988; Talbi *et al*., 2013). El-Sheikh and Wood (1995) found that the salt-tolerant strain was more effective than the salt-sensitive strain under saline conditions. One of the main mechanisms of bacterial adaptation to hyperosmotic conditions is accumulation of compatible solutes, such as sugars, polyols, or amino acids (da Costa *et al*., 1998). Generally, the present experiment produced relatively higher nodule dry weight as compared to nodule dry weight produced in soils having high native mineral N (Herridge *et al*., 1984). Corroborating the results of this study, Okereke *et al*. (2001) reported nodule dry weights that were similar in amounts to the ones obtained in this experiment though the soil had native rhizobia nodulating soybean.

3.3. Shoot Dry Weight and Shoot Length

The shoot dry matter and shoot height at the late flowering and early pod setting stage produced by TAL-379 were in statistical parity with shoot dry matter and shoot height obtained from isolates that produced nodules well (UK isolate and isolate). Similar results were obtained in Nigeria (Okereke *et al*., 2001; Hungria *et al*., 2006). This implies that soybean plants that produced nodules poorly in response to inoculation with TAL-379 might draw mineral N in the soil during early growth, and perform equally well with the well nodulated soybean plants at the later stages of growth.

Under the greenhouse condition (pot experiment), UK isolate, isolate and N-fertilizer treatments induced significantly higher shoot dry matter when compared with TAL-379 inoculation and the control treatments (Table 4). This suggests the importance of symbiotic N_2-fixation even though the soil has a high content of native mineral N for growth and development of soybean at flowering stage. This result is consistent with a previous finding that confirmed that a symbiotic association between soybean and *Bradyrhizobium* fulfilled only 60% of N requirement of soybean (Schipanski *et al*., 2010).

Table 2. Growth performance of soybean at late flowering and early pod setting stage in response of exotic and *Bradyrhizobium* inoculation under field condition.

Inoculation	NN	NDW (g plant^{-1})	SDW (g plant^{-1})	SL (cm)	PH (cm)
TAL-379	31.9 ±1.98c	0.3286 ± 0.0158b	65.3 ± 2.70a	71.0 ± 1.74a	79.4 ± 2.05a
UK isolate	44.4 ± 1.81b	0.4122 ± 0.0098a	62.7 + 3.45ab	67.0 ± 2.40ab	81.2 ± 2.06a
isolate	52.6 ± 2.36a	0.4377 ± 0.0121a	54.0 ± 2.28bc	59.3 ± 1.71c	80.7 ± 3.64a
Control	0.0 ± 0.0d	0.0000 ± 0.0000c	47.8 ± 1.66b	62.6 ± 1.72bc	67.2 ± 2.20b
Mean	32.2	0.2946	57.5	65.0	77.1
F value					
Inoculation (I)	166.99***	330.92***	9.15***	7.05***	6.68***
LSD (0.05)	6.7	0.0413	9.71	7.14	9.59
CV (%)	23.5	16.0	19.2	12.5	14.2
SEM±	7.587	0.047	11.059	8.135	10.925

****significant at 0.001; Nodule number = NN; Nodule dry weight (gplant^{-1}) = NDW; Shoot dry weight at late flowing and early pod setting stage (gplant^{-1}) = SDW; Shoot length at late flowering and early pod setting stage (cm) = SL; Plant height at harvest (cm) = PH.*

Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Table 3. Growth performance of soybean at harvest in response of exotic and ly isolated *Bradyrhizobium* inoculation under field condition.

Inoculation	NPP	NSP	TBY (kg ha^{-1})	GY (kg ha^{-1})	TNC (%)
TAL-379	155.4 ± 6.36a	2.663 ± 0.0606a	7489.7 ± 262.4a	1881.51 ± 95.4c	4.0289 ± 0.0298a
UK isolate	149.6 ± 4.56a	2.643 ± 0.0098a	8060.7 ± 373.8a	2766.40 ± 70.5a	4.1578 ± 0.0490a
isolate	151.4 ± 2.56a	2.529 ± 0.0663a	7541.2 ± 413.8a	2398.25 ± 45.9b	4.0372 ± 0.0747a
Control	108.9 ± 3.70b	2.273 ± 0.0549b	5473.3 ± 191.3b	1520.72 ± 70.9d	3.7339 ±0.0810b
Mean	141.3	2.527	7141.2	2141.72	3.9890
F value					
Inoculation (I)	23.19***	9.43***	12.52***	57.18***	8.41***
LSD (0.05)	16.82	0.2173	1201.7	271.18	0.2315
CV (%)	13.6	9.8	19.2	14.4	6.6
SEM±	19.154	0.248	1368.8	308.9	0.264

****significant at 0.001; Number of pods per plant = NPP; Number of seeds per pod = NSP; Total biomass yield (kgha^{-1}) = TBY; Grain yield (kgha^{-1}) = GY; Tissue nitrogen concentration (%) = TNC.*

Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

3.4. Yield and Yield Traits of Common Bean

Even though nodule number and nodule dry weight showed significant differences for the inoculation treatments, the data indicated statistically insignificant plant height at harvest, number of pods per plant, number of seeds per pod, total N concentration and total biomass yield among the inoculations treatments (Table 2 and 3). Supporting the results of this study, Serraj and Drevon (1998) indicated that plant species dependent on symbiotic N_2-fixation for their N nutrition are more sensitive to saline conditions than plants which obtain N from native soil. This may have consequently resulted in lower N derived from symbiotic N_2 fixation.

3.4.1. Number of pods per plant and number of seeds per pod

Significantly higher number of pods per plant and number of seeds per pod were produced in response to the inoculation over the control treatment (Table 3). Similar results were reported for number of pods per plant (Patra *et al.*, 2012). The highest number of pods per plant (155.4) and number of seeds per pod (2.663) were scored for TAL-379 inoculation in spite of the fact that the isolate and the UK isolate scored the highest nodulation. This suggests that high native soil N could inhibit the symbiotic effectiveness of well nodulated isolates. In contrast, the results reported by Herridge and Betts (1988) indicated highly significant correlations among the indices of nodulation and N_2 fixation and poor correlation between those measurements and plant growth-seed yield. The average number of pods per plant produced by the inoculated soybean plants was 30% higher than number of pods per plant produced by soybean plants in the control treatment. Similarly, the average number of seeds per pod produced by inoculated soybean plants was 11% higher than the number of seeds per pod produced by soybean plants grown in the control treatment. Furthermore, number of pods per plant had no significant association with nodule number and nodule dry weight, except for UK isolate inoculation in which nodule number showed a significant association with number of pods per plant (Figures 1 and 2). This shows that nodule number may have determined variation in the number of pods per plant in the UK isolate treatment. The highest number of pods per plant and number of seeds per pod scored by TAL-379, however, could be due to higher native soil N. There were no any significant associations between number of pods per plant and nodule dry weight for inoculation treatments.

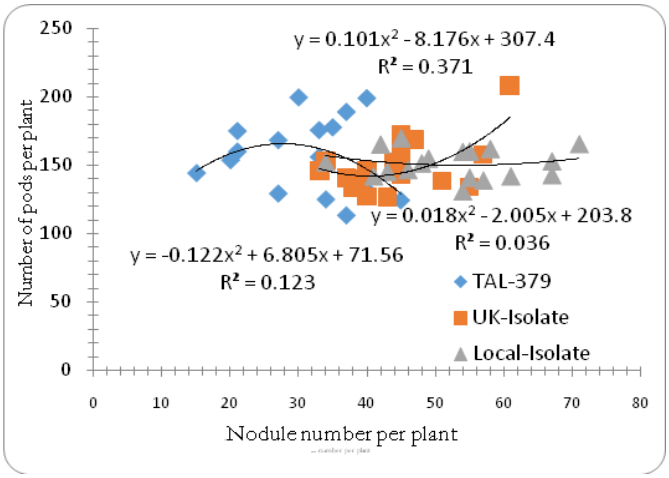

Figure 1. Regression analysis between number of pods per plantand nodule number among different *Bradyrhizobium* inoculation under field condition.

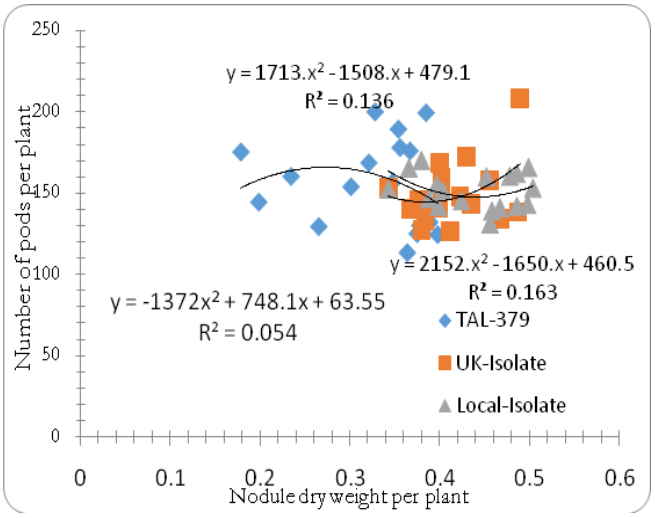

Figure 2. Regression analysis between number of pods per plant and nodule dry weight among *Bradyrhizobium* inoculation treatments under field condition.

3.4.2. Total biomass yield

All inoculation treatments resulted in significantly increased total biomass yields (kg/ha) over the control (un-inoculated) treatment (Table 3). Although the TAL-379 produced statistically lower nodulation, it performed statistically equally in terms of total biomass production with well nodulated isolates (isolate and UK isolate inoculations). Similarly, Simanungkalit *et al.* (1996) found non-significant differences in soybean productivity although the plants displayed significant differences in nodulation. There were no significant differences in total biomass yields among the inoculation treatments. However, soybean plants treated with the UK isolate inoculation produced the highest total biomass yield, exceeding the total biomass yield produced by plants in the control treatment by

about 47.3% (Table 3). Similar findings were reported by Douka and Xenoulis (1998) who found that inoculated plants produced 77% more dry matter yield over the uninoculated plants.

There was a significant association between nodule number and total biomass yield of soybean plants inoculated with isolate. However, there was a non-significant association between the nodule number and total biomass yield of plants inoculated with UK isolate and TAL-379 (Figure 3). Similarly, there was a significant association between nodule dry weight and total biomass yield of plants in isolate. However, there was no significant association between the same traits in UK isolate and TAL-379 isolate (Figure 4), indicating the importance of effectiveness of inoculated isolate and nodulation status for productivity of soybean.

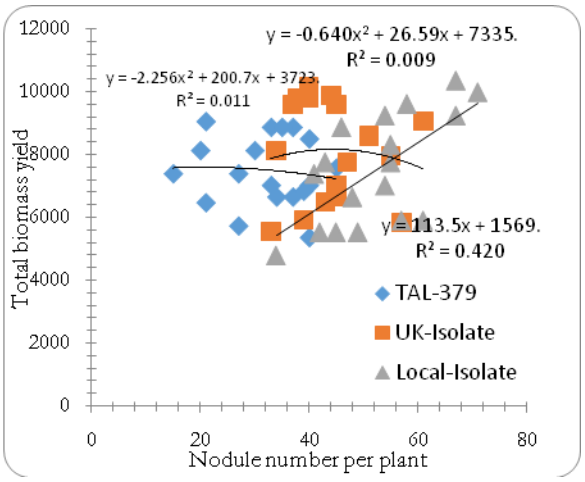

Figure 3. Regression analysis between total biomass yield and nodule number among different *Bradyrhizobium* inoculation treatments under field condition.

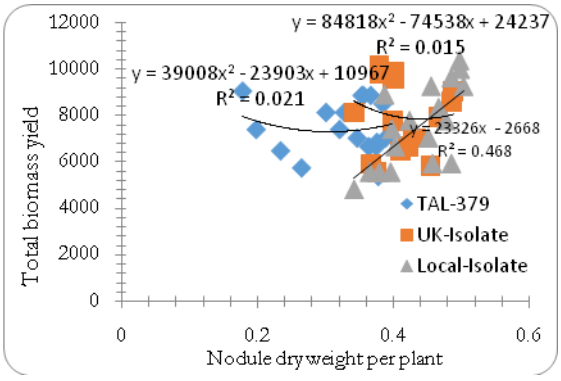

Figure 4. Regression analysis between total biomass yield andnodule dry weight among different *Bradyrhizobium* inoculation treatments under field condition.

3.4.3. Grain yield

Significantly higher grain yield was obtained from the UK isolate than the other treatments (Table 3). Similarly increased soybean grain yield using exotic *Bradyrhizobium* was previously reported in Nigeria by Okereke *et al.* (2001). UK isolate inoculation improved the grain yield by 82% over the control treatment. The increase of grain yield is higher than those reported in Congo (Mandimba and Mondiboye, 1996). The authors noted 35 - 55% increase in soybean grain yield in response to inoculation with *Bradyrhizobium*. Concurrent with the results of this study, Brutti *et al.* (2001) also found that inoculation with *Bradyrhizobium japonicum* increased the grain yield of soybean between 2 and 19% over the un-inoculated control treatment. The increase in grain yield in this study could be the consequence of higher nodule dry weight and total concentration N in response to inoculation with the UK isolate. Nodule number and nodule dry weight have accounted for 98% of the variation in grain yield in soybeans in Ontario, Canada (Hume and Blair, 1992). Anteneh (2012) reported that nodule dry weight and N_2 fixation had a strong correlation with the productivity of soybean. The lowest grain yield was obtained for the control treatment (1520.72 kg ha^{-1}). The highest grain yield (2766.40 kg ha^{-1}) was obtained in this study for the UK isolate inoculation is lower than previously reported grain yields of inoculated soybean in Greece by Douka and Xenoulis (1998), which amounted to 4455 kg ha^{-1}. This is attributable to soil salinity problem encountered in this study, which may have rendered the symbiosis less effective and productive. The control treatment produced lower grain yield than the inoculated treatments. The poor growth of soybean in the control treatment might be attributed to absence of specific rhizobia nodulating soybean (Vincent *et al.*, 1979) and the need of N either from N_2 fixation or mineral N. The inferior nodulation inducing isolate (TAL-379) resulted in the production of a lower grain yield (1881.51kg ha^{-1}). However, the grain yield produced by soybean plants subjected to this treatment exceeded the grain yield produced by plants grown in the control treatment by about 24%. This suggests that even though the soil had high native N, inoculation of effective isolate of *Bradyrhizobium* sp. is important to increase soybean grain yield (Herridge *et al.*, 1984). This result shows that soil N and nodulation are interactive and complementary in meeting the N requirements of a soybean crop grown.

3.5. Regression Analysis

Nodule number had significant quadratic concave upwards and downwards for UK isolate and isolate treatments, respectively, and linear (TAL-379 inoculation) with grain yield (Figure 5). This indicates highest response of soybean in terms of grain yield at higher nodule number in UK isolate than other treatments which consequently indicating the possibility of further increases in grain yield. In isolate

inoculation, the result indicated decrease the rate of grain yield increase with increasing nodule number. The coefficient of determination was higher in grain yield and nodule number association in isolate inoculation. This means variation in grain yield was highly determined by nodule numberin isolate inoculation than other treatments. Grain yield was also having significant quadratic (TAL-379 and UK isolate inoculations) and linear (isolate inoculation) with nodule dry weight (Figure 6). The R^2 value of the regression equation was also higher in isolate inoculation followed by UK isolate inoculation as has been indicated in nodule number. Similar result was indicated by Sogut (2005) who found that nodulation has been the important trait to improve the productivity of soybean when the experiment had been conducted in soils with no rhizobia nodulating soybean.

Significant variations were observed for tissue N concentration among the treatments. Significantly higher total N concentrations were obtained for soybean plants subjected to all inoculations over the control treatment (Table 3). However, the total N concentration of soybean plants subjected to all inoculation treatments were in statistical parity. This indicates that symbiotic N-fixation is very important to maximize the productivity of soybean by enhancing uptake of nitrogen in saline soils despite high native mineral N content in the soil. A similar result was obtained by Chen *et al.* (2002) who reported that inoculation improved the N accumulation of soybean in Paraguay soils. Furthermore, the enhanced concentration of nitrogen in the tissue of soybean plants could be related with high tolerance of soybean genotype for high soil N (Herridge and Betts, 1988).

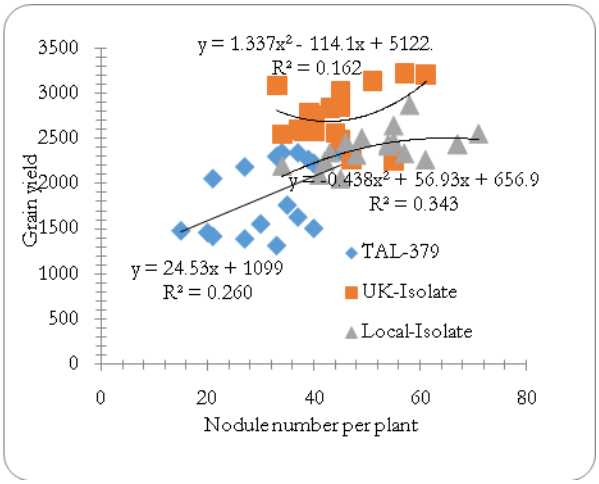

Figure 5. Regression analysis between grain yield and nodule number among *Bradyrhizobium* inoculation treatments under field condition.

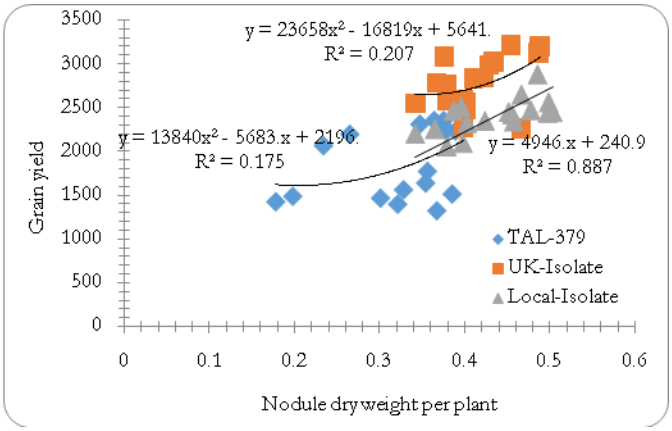

Figure 6. Regression analysis between grain yield and nodule dry weight among different *Bradyrhizobium* inoculation treatments under field condition.

3.6. Total N Concentration

Tissue nitrogen concentration exhibited significant quadratic and concave downward association with nodule number, with R^2 value of 0.196 (Figure 7) only in isolate treatment. This shows nodule number highly responsible for variation of total N concentration in isolate inoculation. Similarly in isolate inoculation resulted in a significant quadratic and concave downward association between nodule dry weight and total N concentration, with R^2 value of 0.338, indicating that 33.8% of the variation of total N concentration in isolate inoculation could be explained by nodule dry weight (Figure 8). The R^2 also suggested that nodule dry weight is more responsible for total N concentration variation than nodule number.

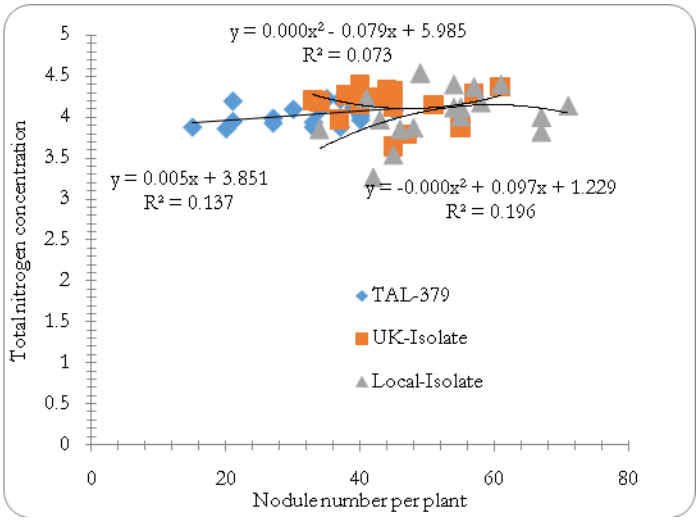

Figure 7. Regression between total n concentration and nodule number among different *Bradyrhizobium* inoculation treatments under field condition.

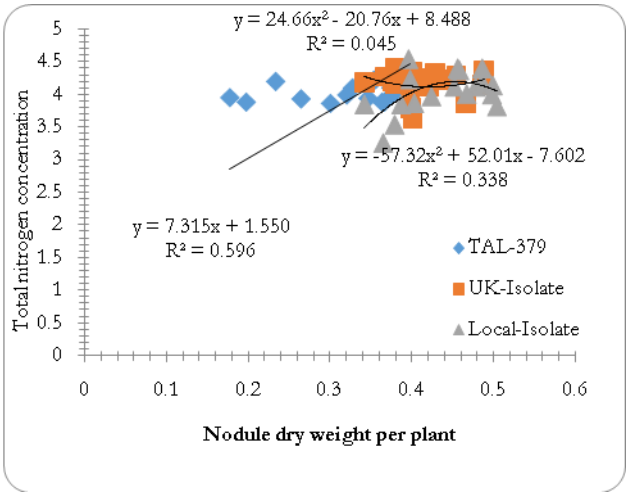

Figure 8. Regression between total n concentration and nodule dry weight among different *Bradyrhizobium* inoculation treatments under field condition.

The investigated parameters were further analyzed using multiple regressions to understand the relationship between grain yield and other measured traits (Table 5). These relationships were done for each inoculation treatments and overall mean of grain yield. The highest determinations of dependency were scored by TAL-379 (R^2=0.808) and UK isolate (R^2=0.692) followed by overall mean (R^2=0.654). Nodule number was the determinant trait for TAL-379 inoculation and nodule dry weight for UK isolate and overall mean. Total N concentration was also a parameter that determined grain yield in all inoculation treatments except isolate. Nodulation and total N concentration are the main parameters strongly related with grain yield when soybean inoculated highly effective isolate of *Bradyrhizobium* sp. as reported in various previous studies (Okogun *et al*., 2005; Thuita *et al*., 2012; Til'ba and Sinegovskaya, 2012).

Table 4. Growth performance of soybean at late flowering and early pod setting stage in exotic and *Bradyrhizobium* inoculation under greenhouse condition.

Treatment	NN	NDW	SDW
UK isolate	46.3a	0.3274a	8.550a
TAL-379	10.8b	0.1551b	7.094b
	41.9a	0.3207a	8.233a
+VE control	0.0c	0.0000c	8.222a
-VE control	0.0c	0.0000c	5.972c
Mean	19.8	0.1606	7.621
CV(%)	25.8	24.3	14.5
LSD	4.8	0.0365	1.031
F value	8.42***	7.29***	4.31***
SEM±	26.033	0.001519	1.2083

****significant at 0.001; Nodule number = NN; Nodule dry weight (g/plant) = NDW; Shoot dry weight at late flowing and early pod setting stage (g/plant) = SDW; +VE control -positive control (N treated pot); -VE control- N untreated and uninoculated pot.*
Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Table 5. Coefficients and statistics of multiple regression models of relating grain yield with other measured traits for each inoculation treatments.

Treatment	Model	Adjusted R^2 value
Overall data	GY=817.855 + 2758.782NDW+-7.010SDW + -6.863NPP+ 472.190TNC	0.654**
UK isolate	GY = -501.950 + 2243.800NDW+ -9.254SDW+ 703.202TNC	0.692**
isolate	GY= 1881.458 + 0.69TBY	0.343*
TAL-379	GY= -79.716 + 13.080NN+ -9.151NPP+ -109TBY+ 939.186TNC	0.808***
Control	GY= 952.382 + 19.091SDW+ 396.810TNC	0.440*

** Significant at 0.05; **significant at 0.01; ***significant at 0.001; Nodule number = NN; Nodule dry weight (gplant^{-1}) = NDW; Shoot dry weight at late flowing and early pod setting stage (gplant^{-1}) = SDW; Number of pods per plant = NPP; Total biomass yield (kgha^{-1}) = TBY; Tissue nitrogen concentration (%) = TNC.*

4. Conclusion

The results of this study have demonstrated that inoculating soybeans in the saline soil of Shinille with effective exotic and isolates of *Bradyrhizobium* significantly improved the productivity of the crop. High soil native N might cause the non-significant effect of inoculation on the yield of above ground part of soybean at early flowering stage. High soil N, however, did not have antagonistic effect on the effectiveness of inoculation on the grain yield of soybeans in this study. Even though inoculation remarkably improved the productivity of soybean, the grain yield obtained in this study was half of the potential yield of the crop (4 ton ha^{-1}). Therefore,

further research would be recommended to minimize the yield gap by diagnosing other constraints for soybean production in Ethiopia.

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Bottom Sediment Chemistry, Nutrient Balance, and Water Birds in Small High Altitude Tropical Reservoirs in the Rift Valley, Kenya

Francis Mwaura*

Department of Geography and Environmental Studies, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya

Abstract: Water bird characteristics, nutrient loadings, and the levels of bottom sediment silicon oxide (SiO_2), aluminium oxide (Al_2O_3), ferric oxide (Fe_2O_3), calcium oxide (CaO), copper (Cu), phosphorus (P) and organic carbon (C) was studied in eight high altitude (2040-2640m) small shallow (0.065-0.249 km^2; 0.9-3.1 m) reservoirs in the central rift valley of Kenya. The general aim was to assess the nature of the bottom sediments in relation to nutrient balance in the water bodies and their birdlife from a geographic perspective of spatial comparative analysis. The findings showed positive correlation between the levels of SiO_2, CaO and P with the levels of total-N and total-P. In addition, there was an inverse correlation between C, Al_2O_3, Cu and Fe_2O_3 in the bottom sediment and two nutrients. A total of six water bird counts across the eight sites recorded 49 species for all the reservoirs and an overall average of 60 individuals per reservoir. The counts of nine water bird species were established to increase significantly with increase in the levels of total-N and total-P. The results indicated a correlation with the levels of SiO_2, C, P, Fe_2O_3, and CaO in the bottom sediment for 12 water bird species, namely, African Fish Eagle, African Jacana, Black-headed Heron, Brack Crake, Common Teal, Great Egret, Great White Pelican, Grey Crowned Crane, Knob-billed Duck, Purple Gallinule, Ringed Plover, and Yellow-billed. The most sensitive species were the African Fish Eagle, Brack Crake, Common Teal, Great White Pelican, and Purple Gallinule. The actual impact of sediment chemistry on the utilization of reservoirs by water birds was not established and should, therefore, be an important subject for further investigation.

Keywords: Bottom Sediments; Total-N; Total-P; Tropical Reservoir; Waterbirds

1. Introduction

According to the International Commission on Large Dams (ICOLD, 1998), over 300 new dams were constructed in the world each year between the 1950s and 1980s. Although this number dropped to about 250 dams a year in the 1990s, the rate continued increasing particularly in developing countries. By the late nineties, the total area occupied by reservoirs worldwide was about 384 000 km^2 or roughly the size of Zimbabwe (Tundisi, 1993; WWF, 1999). Apart from the large reservoirs, there are an estimated 800000 small reservoirs worldwide (WWF, 1999). The construction of man-made reservoirs results in the establishment of artificial wetlands around the world. Presently, some of the world's most important wetlands including some Ramsar and World Heritage Sites are associated with the construction of dams and reservoirs. The rapid colonization of reservoirs by wetland birdlife provides good opportunities for recreation and ecotourism in an increasingly congested world. In the USA, constructed wetlands are managed to benefit waterfowl by mimicking the state of natural wetlands (Duffy &LaBar, 1994).

Water quality in reservoirs is an important aspect which determines the ecological character and spatio-temporal dynamics of aquatic life in water bodies including birdlife. The bottom sediments are known to have a significant influence on the state of water quality in reservoirs because they constitute an important internal storage for incoming materials and can provide an environmental chronological snapshot of what has been happening in a reservoir and its catchment. Large amounts of nutrients in lakes and reservoirs can be accumulated in the bottom sediments due to natural binding as explained by Keller *et al.* (1998). Widespread transfer of phosphorus to bottom sediments is, for example known to occur in water bodies through deposition of organic debris and particulate matter (Grobbelaar & House, 1995). In a study of the nitrogen cycle in a Brazilian floodplain lake, Howard-Williams *et al.* (1989) established that the bulk of the nitrogen storage for the lake existed in the sediments (87%) and only about 3% was in the water column. Nutrients in the bottom sediments can recirculate back into the water column depending on the prevailing environmental condition especially wind and the level of dissolved oxygen. In this way, bottom sediments can contribute to occasional internal loading of nutrients through fluxes at the sediment-water interface thereby affecting nutrient budgets, trophic systems and the entire ecology including birdlife. Melack (1995) identified and summarized the key environmental drivers of nutrient release from the bottom sediments to the water column in water bodies as water pH, temperature and dissolved oxygen.

Some previous studies have attempted to investigate the connection between bottom sediment and nutrient balance in aquatic environments. However, the majority of these have been undertaken in the temperate water bodies (e.g. Søndergaard *et al.*, 2003; Small *et al.*, 2013). A few studies have been done in Asia such as the study on Bukit Merah Reservoir in Malaysia by Ismail and Najib (2011) and the one on Jagadishpur Reservoir in

*Corresponding author. E-mail: mwauraf@uonbi.ac.ke

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Nepal by Gautam & Bhattarai (2008). These kinds of studies are quite rare in Africa with the study on the freshwater reservoirs in Mauritania as an exception (Segersten, 2010). Similarly, there has been very limited work on the linkages between reservoir bottom sediments, nutrient balance and birdlife which makes this a major gap in aquatic research both in Africa and beyond. Yet most water bodies in the region are currently experiencing accelerated sedimentation as a result of widespread environmental transformations in the catchments. A few studies such as Gwiazda *et al.* (2010) have attempted to investigate this linkage from a top-to-bottom perspective by considering the impact of waterbirds on water quality and nutrient balance through *bird* fecal *droppings*. There has been very limited effort to consider the bottom-up impacts by considering the indirect influence of bottom sediments on balance and birdlife.

The link between bottom sediments, nutrient balance and biodiversity is therefore an important subject for understanding reservoir ecosystem dynamics. The general aim of this study was to assess the nature of the bottom sediments in relation to nutrient balance in the small high altitude tropical reservoirs in Kenya and their birdlife. The key research question was whether there were statistically significant relationship between reservoir bottom sediment chemistry, nutrient levels and bird characteristics. Given that these are young water bodies (< 100 yrs) it is interesting to know whether sedimentation which commences immediately after dam construction can be used to predict the expected nutrient balance and birdlife characteristics of new reservoirs. The findings were expected to indicate whether there is need to pursue this line of focus in future research.

2. Materials and Methods

The eight reservoirs selected for investigation in the study are located at the distance of 100-200 km northwest of the city of Nairobi in the rift valley escarpment zones of Nyandarua County (3,500 km^2) and Nakuru County (7,200 km^2). Figures 1 & 2 shows the location of the study sites and Table 1 gives their general characteristics. All the reservoirs are located within the watersheds of three Ramsar Sites in the rift valley, namely Lake Naivasha (3,400 km^2), Lake Elementaita (600 km^2) and Lake Nakuru (1,800 km^2). The reservoirs are located within two key physiographic zones, namely, the flat rift plateaus (Muruaki, Kahuru, Murungaru and Kanguo) and the rift escarpments (Gathanje, Kiongo, Rutara and Gathambara).

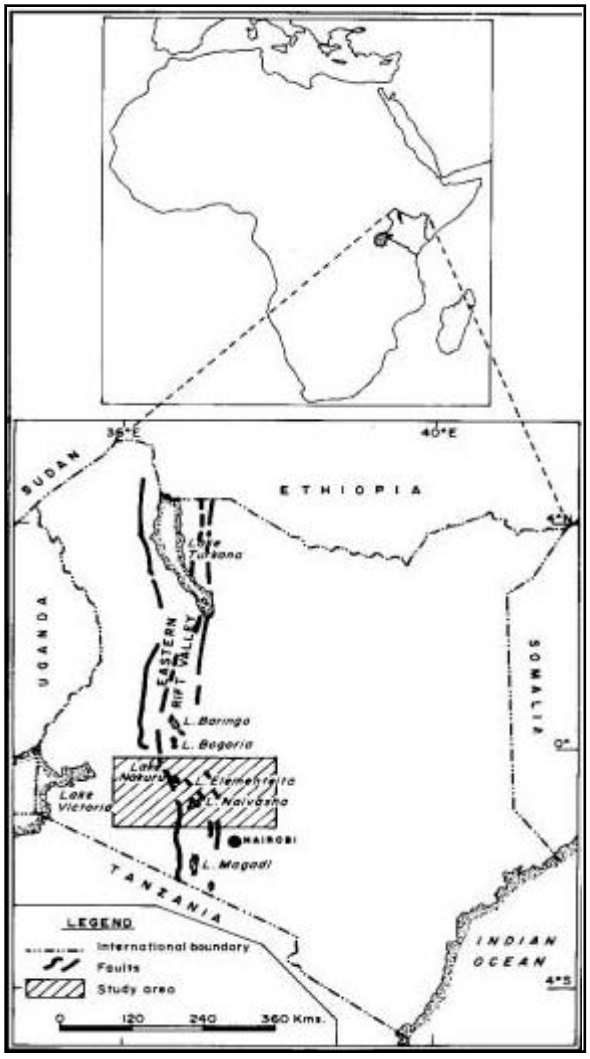


Figure 1. Map of the the study reservoirs.

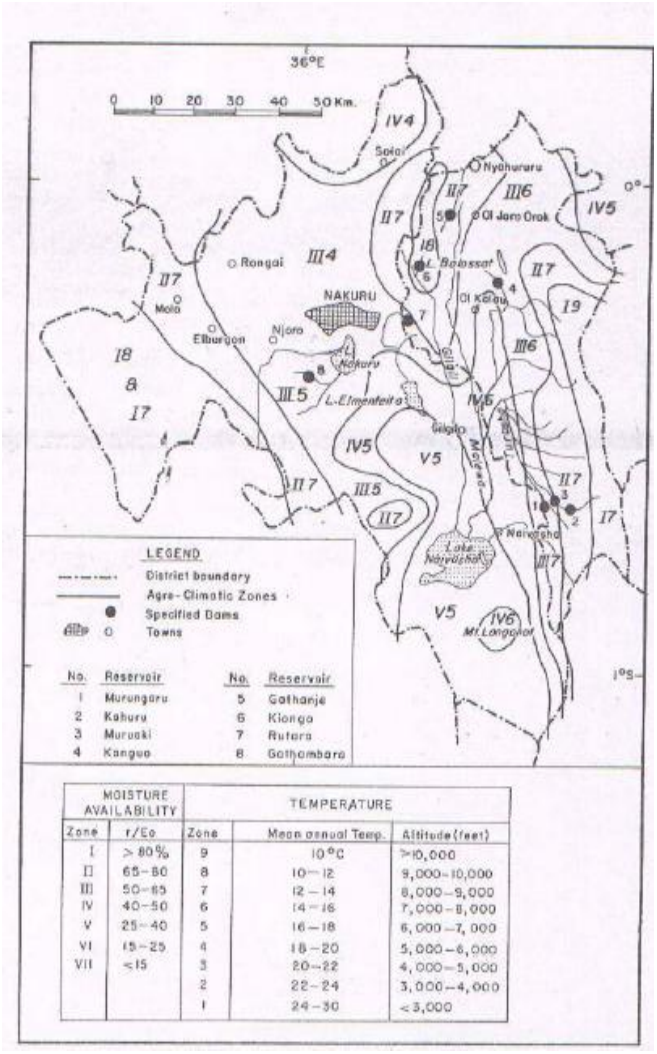


Figure 2. Map of the study area.

Table 1. The geographic and morphometric characteristics of the study reservoirs.

Reservoir	Location	Altitude (m)	Age (yrs)	Catchment area (km^2)	Estimated volume (10^3 m^3)	Water depth (Z_{max}, m)
Muruaki	0°38'S,36°33'E	2440	45	29.1	230	3.5
Kahuru	0°37'S,36°32'E	2420	46	31.4	240	4.5
Murungaru	0°36'S,36°30'E	2360	48	57.3	280	3.8
Kanguo	0°12'S,36°25'E	2340	45	14.1	240	2.2
Gathanje	0°03'S,36°19'E	2460	45	22.4	400	6.0
Kiongo	0°10'S,36°15'E	2640	48	0.05	580	3.3
Rutara	0°17'S,36°15'E	2400	46	1.50	230	3.6
Gathambara	0°27'S;36°02'E	2040	40	50.0	50	1.5

The plateaus reservoirs were characterized by pyroclastic rocks and soils dominated mainly by an assortment of clays especially humicplanosols, vertisols, andosols and phaeozems. The escarpment reservoirs were characterized by volcanic rocks with largely soft volcanic ashes and tuffs and loamy soils dominated by lithic leptosols with nitosols and luvisols. All the reservoirs were constructed in the 1940s by colonial European farmers in the former White Highlands as sources of year-round water supply. After independence in 1963 they became shared communal assets. The plateau reservoirs were mostly situated in open moorland environments consisting mainly of *Pennisetum-Eleusine* grasslands while the escarpment reservoirs were associated with natural forest and woodland zones. The littoral habitats of most of the

eight reservoirs had a wide range of emergent macrophytes such as *Kyllingaodorata* and *Cyperus immensus*. Some of the reservoirs like Kiongo, Kanguo and Kahuru were characterized by a substantial cover of submersed plants such as *Ceratophyllum demersum* (Planch*), Potamogeton richardii* and *Crassulagrainkii.* Both Gathanje and Gathambara had distinct stands of *Cyperus immensus* and *Jussiae repens* at the river mouth. The land use around all the reservoirs was small scale agriculture while Kiongo reservoir was located near the OlJororok town (Figure 2).

A total of twenty-one sampling sites with at least two in each of the eight reservoirs were considered and field measurements undertaken at different seasons between 1998 and 2001. Water depth was estimated by sending a weighted line to the bottom of every reservoir. Bottom sediment samples were collected thrice in 2001 in the months of March, June, October and December using an Ekman Grab. The samples were dried at 105°C and ground in a ball mill after which approximately 1g of sediment was shaken with 40 cubic centimetres of 0.5 M hydrochloric acid for 16 hours and the solution centrifuged and the supernatant filtered through 0.45 μm membranes. Sediment analysis was then conducted at the University of Nairobi using an atomic absorption spectrophotometre (AAS) to establish the levels of silicon oxide (SiO_2), aluminum oxide (Al_2O_3), ferric oxide (Fe_2O_3), calcium oxide (CaO), copper (Cu), phosphorus (P) and organic carbon (C).

Nutrient analysis for both nitrogen and phosphorus was determined from 500 cubic centimetres of integrated surface to bottom samples which were collected using a MacVuti water sampler (Litterick & Mavuti, 1985). The samples were collected in March (dry season), June (wet season) and December (intermediate season). Phosphorus as total-P was determined using the molybdenum blue-ascorbic acid technique after digestion of 25 or 50 cubic centimetres samples with 30% hydrogen peroxide and readings made from a Bausch and Lomb spectronic 88 spectrophotometer (Mackereth *et al.*, 1989). Nitrogen as total-N was determined using the Kjeldahl method after digestion of 25 cubic centimetres duplicate samples with 30% hydrogen peroxide (Kalff, 1983).

Seasonal observations of reservoir water birds were undertaken in 6 censuses usually between 10 am and 5pm through both point and transect counts using an inflatable rubber dinghy (Zodiac) according to Rumble and Flake (1982). Between 3-5 point counts lasting 15 minutes were undertaken in the inflow, middle and outflow zones of the reservoirs along a designated transect. At each point, the types and numbers of birds both in the open water and the riparian environment were recorded at different times using the sight and call method. Only positively identified birds were recorded and flying birds were not recorded unless they landed near the reservoir, or took flight from the reservoir. Species identification was done according to Williams and Arlott (1980). Data analysis for the study included computation of summary statistics, correlation and regression analysis.

3. Results

The results of reservoir bottom sediment analysis are shown in Table 2. They showed that the sediments were quite rich in both SiO_2 and organic C, moderately rich in Al_2O_3, Cu and Fe_2O_3, and quite poor in CaO. The plateau reservoirs especially those in Kinangop contained more sediment SiO_2 and less sediment C, Al_2O_3 and Fe_2O_3 when compared with the escarpment reservoirs. The highest content of organic carbon was found in the bottom sediments of Gathanje Reservoir (Table 2).

Table 2. The quality of reservoir bottom sediments.

Variable	R1	R2	R3	R4	R5	R6	R7	R8
Altitude (m)	2440	2420	2360	2340	2460	2640	2400	2040
Depth (Z_{max}m)	2.3	2.6	2.3	2.0	3.3	2.3	3.1	0.9
SiO_2 (%)	65.3	61.7	61.7	52.7	34.7	63.5	52.5	55.5
C (%)	10.82	14.22	14.18	16.9	36.03	26.09	22.16	17.23
Al_2O_3 (%)	13.3	13.7	13.3	16.5	18.3	16.9	14.6	14.5
Cu (ppm)	7.0	8.0	7.0	9.6	10.4	7.5	8.0	5.3
Fe_2O_3 (%)	4.6	5.4	5.0	7.3	7.4	7.6	7.9	7.8
CaO (%)	0.43	0.48	0.54	0.56	0.43	0.41	0.40	0.85
P (%)	0.8	0.2	0.13	0.29	0.22	0.42	0.20	0.19

Key: R18 for Muruaki, Kahuru, Murungaru, Kanguo, Gathanje, Kiongo, Rutara and Gathambara.

Figure 3 shows the total-N levels in the reservoirs with the nutrient loadings for the eight reservoirs in the three monthly measurements shown in different shades in the legend. The range of mean total-N concentration was 220-16 800 μg/l with an increase during the long rains and a maxima in the short rains towards the end of the year. There was no consistent spatio-temporal pattern for total-N concentration because the levels varied greatly from site to site and season to season (Figure 3). The highest concentration occurred in December and the lowest occurred in March. Some of the reservoirs with a high total-N loading included Kahuru in March, Kanguo and Gathanje in June and Gathambara in December.

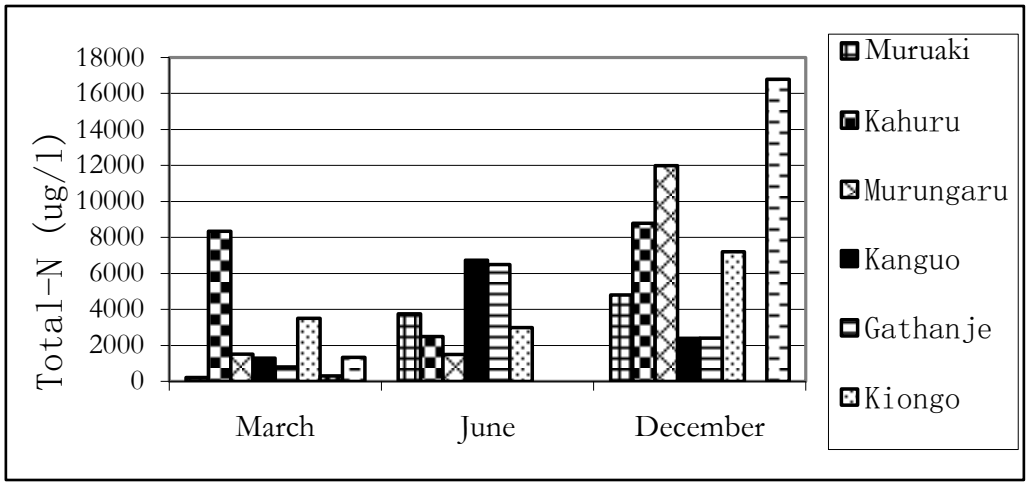


Figure 3. Seasonal total-N levels in the reservoirs.

Figure 4 shows the total-P levels in the reservoirs with the nutrient loadings for the 8 reservoirs in the three monthly measurements shown in different shades in the legend. The concentration of total-P ranged between 30-700 μg/l with the highest loading at the on-set of the long rains in March and the lowest after the short rains in December (Figure 4). There was a spatially consistent pattern whereby the rift plateau reservoirs especially Muruaki and Murungaru had the highest levels compared to the rift escarpment ones like Gathanje and Rutara which had the lowest levels throughout the study period. However, Gathambara reservoir was an exceptional escarpment site with high total-P.

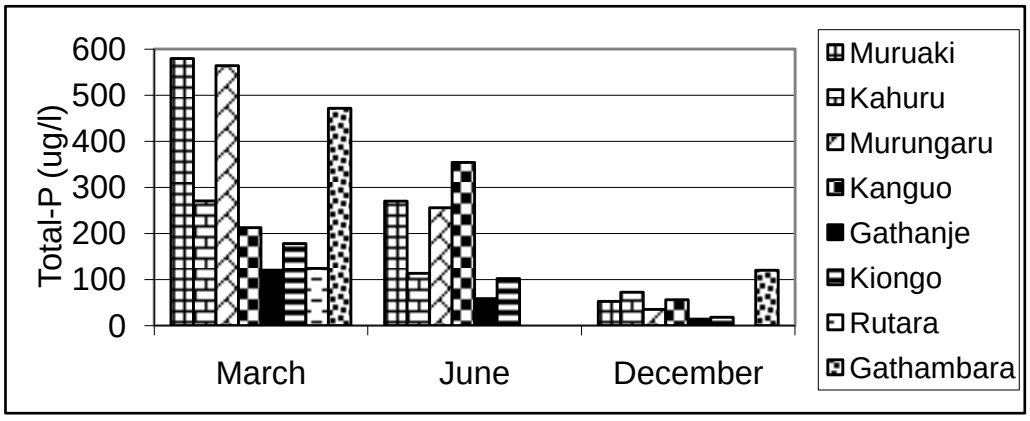
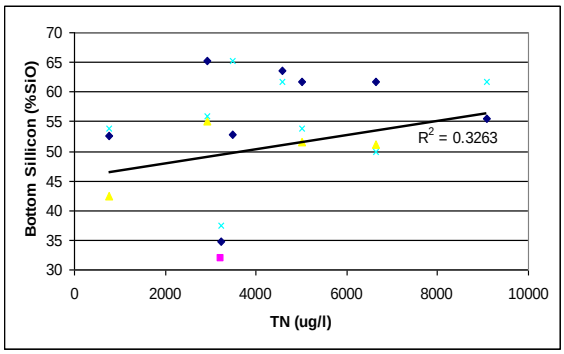
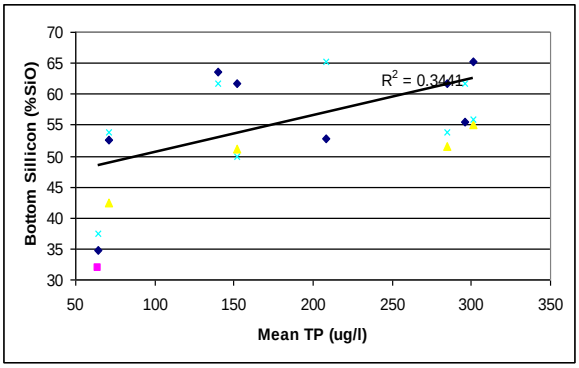


Figure 4. Seasonal total-P levels in the reservoirs.

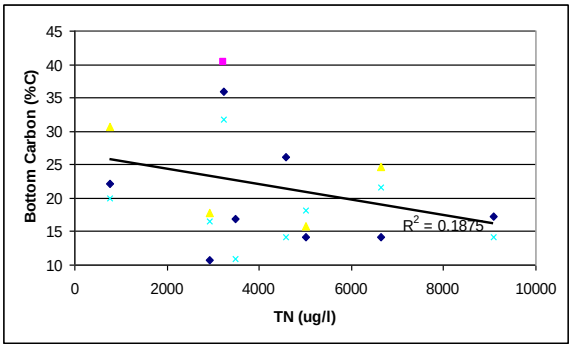
Correlation analysis between the reservoir bottom sediments chemistry and the levels of total-N and total-P in water indicated positive relationships between sediment SiO_2, CaO and P on one hand and total-N and total-P on the other as shown in Figure 5. It appeared that the higher the level of these parameters in the sediment, the more available the macronutrients. The correlation analysis in addition, established inverse relationships between sediment C, Al_2O_3, Cu and Fe_2O_3 on one hand andtotal-N and total-P on the other (Figure 5). However, the regression analysis between the levels of total-N and total-P and the concentration of bottom sediment parameters established that total-N was only significantly related with the level of CaO (r^2 0.606, df 1, 6, t 3.041, p 0.023) while total-P was only significantly related with the level of C (r^2 0.589, df 1, 6, t -2.933, p 0.026) in the bottom sediments. On the overall, the correlations were very weak between TN in the reservoir water column on one hand and Al_2O_3, Fe_2O_3, P, and C in the bottom sediments. In the case of TP, there was very weak correlation between nutrient loading in the water with Fe_2O_3, CAO and P in the bottom sediments as shown in Figure 5.

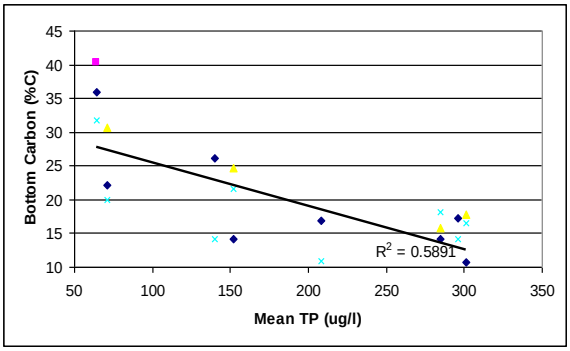
(a) Silicon (SiO_2)



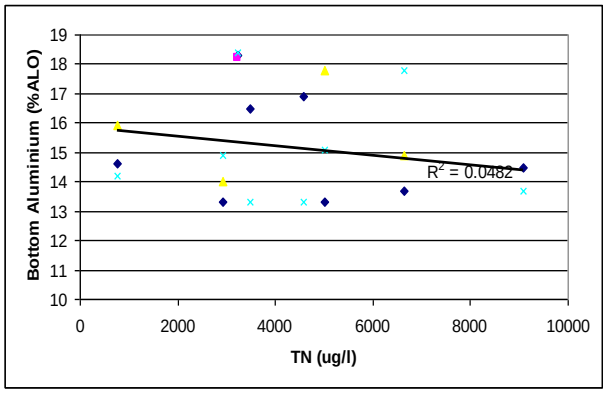


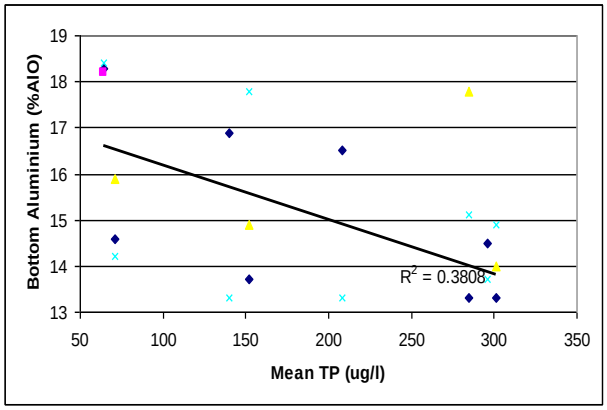
(b) Carbon (C)



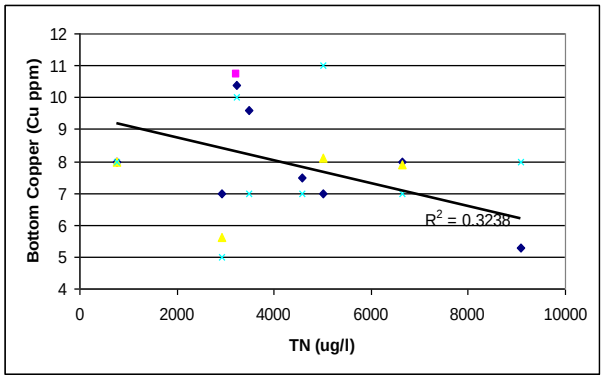


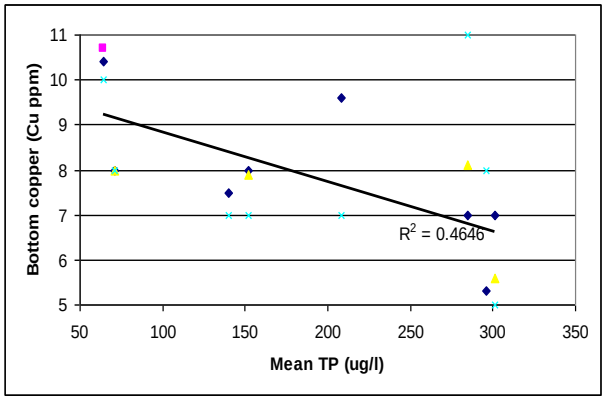
(c) Aluminium (AL_2O_3)



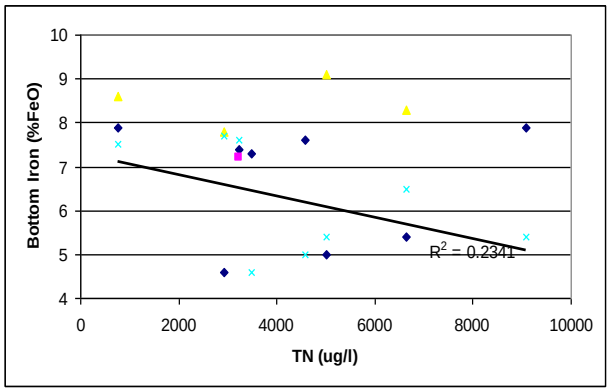


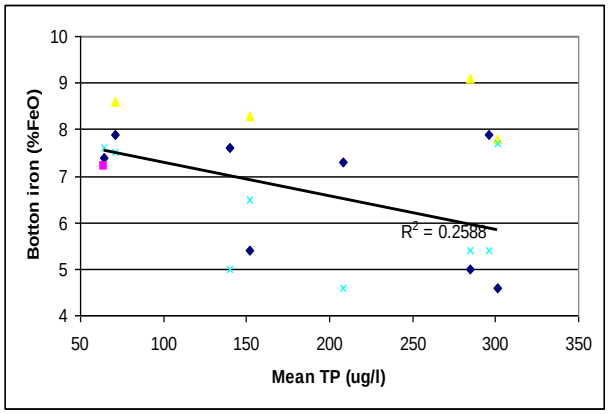
(d) Copper (Cu)



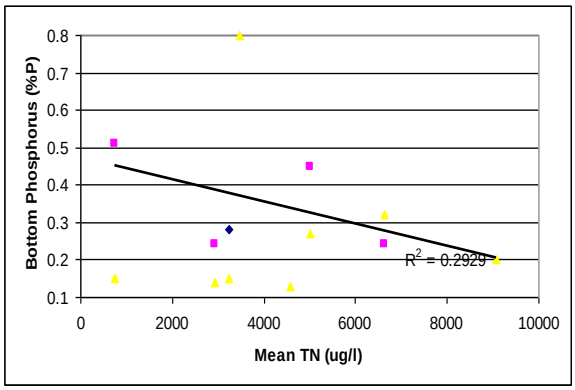


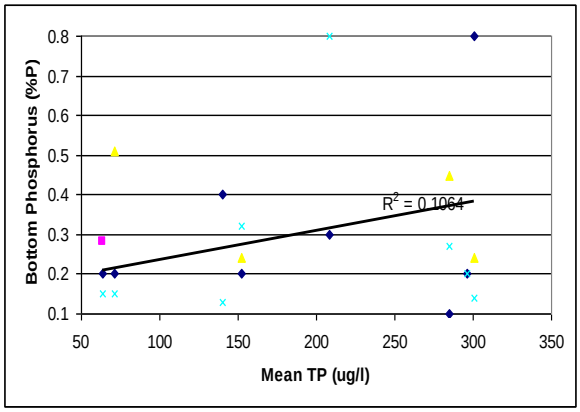
(e) Iron (Fe_2O_3)



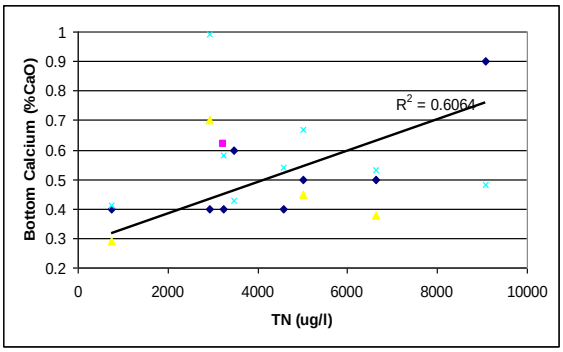


(g) Phosphorus (P)





(f) Calcium (CaO)



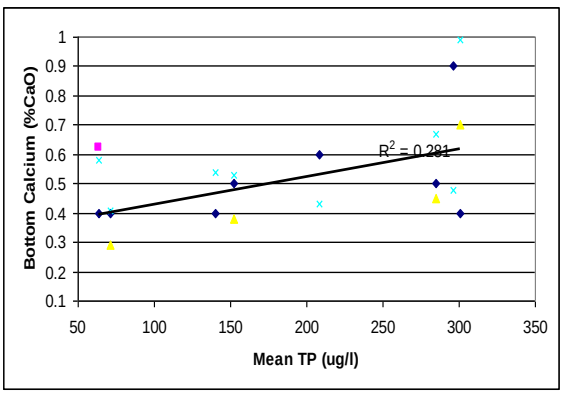


Figure 5. A summary of the relationship patterns between bottom sediment chemistry and reservoir nutrient levels (■ = March, ▲= June, ◊ = October, × = December).

A total of six water bird counts across the eight sites recorded 49 species for all the reservoirs and an overall average of 60 individuals per reservoir. The range of species number was 5-11 and that of the count was 60-80 birds per site while the density range was 0.3-1.2 birds/m². Table 3 gives a summary of the water bird species number, counts and density per square metre. The most dominant birds in terms of cumulative total counts included the Red-knobbed Coot (*Fulica cristata*), Black-headed Heron (*Ardea melanocephala*), Egyptian Goose (*Alopochen aegyptius*), Yellow-billed Duck (*Anas undulata*), Little Grebe (*Tachybaptus ruficollis*), White-necked Cormorant (*Phalacrocorax carbo*), Hadada Ibis (*Bostrychia hagedash*), Blacksmith Plover (*Vanellus armatus*) and Cattle Egret (*Bubulcus ibis*). From the counts, it was evident that the resident avifauna comprised of herons, coots, ducks, geese, grebes, ibises and egrets. The analysis of the monthly patterns for total counts and bird species showed that the bird populations were high in the dry season and onset of the long rains in February and March, decreased during the rains in June and July and peaked again towards the end of the year (Table 3).

The analysis of bird species showed that the reservoirs served as important hot and dry season refugia for the Red-knobbed Coots (*Fulica cristata*),

Pink-backed Pelicans (*Pelecanus rufescens*), Little Grebes (*Tachybaptus ruficollis*), Grey Crowned Cranes (*Balearica regulorum*), Egyptian Geese (*Alopochen aegyptius*) and Black-headed Herons (*Ardea melanocephala)* (Figure 3). Figure 3 also shows that in the cold and wet season (June) the dominating birds were Cattle Egrets (*Ardeola ibis*), Grey-headed Gulls (*Larus cirrocephalus*), White-necked Cormorant (*Phalacrocorax carbo*), Red-knobbed Coots (*Fulica cristata*), and Blacksmith Plovers (*Vanellus armatus*).

Table 3 shows that the highest density of water birds occurred in Gathambara and Muruaki which were also the smallest and shallowest water bodies (Table 2). Moderate density occurred in Kahuru and Murungaru which were of medium size and medium depth and the lowest density in Gathanje and Kiongo which were the largest and deepest (Table 2). Although there were no definite regional patterns in terms of the number of species per reservoir, general comparative assessment showed that the plateau reservoirs appeared to have slightly higher number of bird species per site than the escarpment reservoirs (Table 3). Similarly, the bird counts and species numbers were higher in the reservoirs located within the flat plateau rather than rugged escarpment terrain (Table 3).

Table 3. Monthly reservoir water bird counts, density, and species number in 1998-2000.

	Feb	Mar	Jun	Jul	Oct	Dec	Mean
(a) Mean number of species							
Muruaki (plateau, 2440m)	11	8	12	9	10	14	11.3
Kahuru(plateau, 2420m)	8	9	15	8	10	8	10.0
Murungaru(plateau, 2440m)	7	10	12	10	11	24	13.3
Kanguo(plateau, 2340m)	6	7	9	10	10	11	8.8
Gathanje (escarpment, 2460m)	4	6	5	5	10	9	6.5
Kiongo (escarpment, 2640m)	11	5	5	5	12	13	8.5
Rutara (escarpment, 2400m)	5	6	7	6	9	5	5.3
Gathambara (escarpment, 2040m)	5	5	6	7	10	6	6.5
Mean	7.6	7.0	9.0	6.7	9.6	11.8	
(b) Mean of total counts							
Muruaki (plateau, 2440m)	171	35	51	74	82	166	105.8
Kahuru(plateau, 2420m)	58	26	73	49	56	61	54.5
Murungaru(plateau, 2440m)	61	54	57	59	66	128	75.0
Kanguo(plateau, 2340m)	23	77	35	31	24	57	41.2
Gathanje (escarpment, 2460m)	20	97	15	13	19	24	31.3
Kiongo(escarpment, 2640m)	80	54	30	111	146	92	85.5
Rutara(escarpment, 2400m)	30	71	47	46	40	27	49.5
Gathambara (escarpment, 2040m)	6	102	63	16	60	144	83.6
Mean	71.1	64.5	43.5	42.8	57.3	87.4	
(c) Mean density (birds/m^2)							
Muruaki (plateau, 2440m)	1.7	0.3	0.5	0.7	0.8	1.6	1.0
Kahuru(plateau, 2420m)	0.7	0.3	0.8	0.4	0.6	0.7	0.6
Murungaru(plateau, 2440m)	0.4	0.5	0.5	0.4	0.5	1.1	0.6
Kanguo(plateau, 2340m)	0.2	0.7	0.3	0.3	0.2	0.5	0.4
Gathanje (escarpment, 2460m)	0.2	0.8	0.1	0.1	0.2	0.2	0.3
Kiongo (escarpment, 2640m)	0.3	0.2	0.1	0.4	0.6	0.4	0.3
Rutara (escarpment, 2400m)	0.1	1.0	0.5	0.5	0.6	0.4	0.7
Gathambara (escarpment, 2040m)	0.5	1.5	0.8	0.2	0.9	2.2	1.2
Mean	0.7	0.7	0.4	0.3	0.5	0.9	

Table 4 shows the results of curve linear regression between the levels of reservoir bottom sediment parameters and the bird counts. The results indicated that high bottom sediment C, P and CaO was associated with high water bird counts for 14 bird species namely, African Fish Eagle, Common Teal, Grey Crowned Crane, Purple Gallinule, Brack Crake, Great White Pelican, Ringed Plover, Yellow-billed Stork, African Jacana, Black-headed Heron, Knob-billed Duck and Great Egret. On the other hand, high bottom sediment SiO_2, and Fe_2O_3, was associated with low water bird counts for 7 bird species namely, African Fish Eagle, Common Teal, Grey Heron, Purple Gallinule, Brack Crake, Great White Pelican and Pink-backed Pelican. The levels of Cu and AL_2O_3 had no significant impact on the birds.

Table 4. Summary of curve linear regression results between levels of sediment parameters and reservoir birdlife population.

Sediment parameter	Impact on TN and TP	Bird species	Df	r^2	b1	F	∞
SiO_2	Positive	African Fish Eagle	1,6	0.556	-0.158	7.52	0.034
		Common Teal	1,6	0.642	-0.200	10.74	0.017
		Grey Heron	1,6	0.577	0.2131	8.20	0.029
		Purple Gullinule	1,6	0.754	-0.124	18.35	0.005
		Black Crake	1,6	0.754	-0.031	18.35	0.005
		Great White Pelican	1,6	0.754	-0.280	18.35	0.005
C	Negative	African Fish Eagle	1,6	0.603	0.200	9.11	0.02
		Common Teal	1,6	0.531	0.221	6.80	0.04
		Grey Crowned Crane	1,6	0.493	0.688	5.84	0.05
		Purple Gullinule	1,6	0.651	0.140	11.17	0.02
		Black Crake	1,6	0.651	0.035	11.17	0.02
		Great White Pelican	1,6	0.651	0.314	11.17	0.02
Fe_2O_3	Negative	Grey Heron	1,6	0.544	-1.473	7.15	0.04
		Pink-backed Pelican	1,6	0.625	-6.053	9.98	0.02
CaO	Positive	Ringed Plover	1,6	0.841	27.725	31.65	0.001
		Yellow-billed Stork	1,6	0.595	31.078	8.81	0.03
P	Positive	African Jacana	1,6	0.840	1.1471	31.58	0.001
		Black-headed Heron	1,6	0.554	327.941	7.45	0.003
		Common Snipe	1,6	0.721	4.118	15.47	0.01
		Knob-billed Duck	1,6	0.840	2.941	31.58	0.001
		Great Egret	1,6	0.615	2.647	9.58	0.02

Table 5 shows the relationships between reservoir water bird counts and nutrient content. The analysis indicated that the relationship between reservoir total-N and total-P and reservoir birdlife was only significant for 9 bird species. The number of birds was found to increase significantly with increase in total-N and total-P for species including the Cape Teal, Grey-headed Gull, Hottentot Teal, Long-tailed Cormorant, Pink-backed Pelican, Red-knobbed Coot, Ringed Plover, Squacco Heron and Yellow-billed Stork (Table 5).

Table 5. Regression results for number of water birds and reservoir nutrient content.

Nutrients	Water birds	df	r^2	b_1	SE	T	∞
TN	Ringed Plover	1,6	0.545	379.513	3.462	109.613	0.000
	Grey-headed Gull	2,5	0.306	128.125	2.633	48.656	0.000
	Squacco Heron	3,4	0.111	-596.467	21.484	-27.763	0.001
	Long-tailed Cormorant	4,3	0.062	365.983	11.335	32.289	0.001
	Red-knobbed Coot	5,2	0.005	-4.058	0.545	-7.440	0.018
TP	Yellow-billed Stork	1,6	0.661	11.607	0.425	27.280	0.000
	Pink-backed Pelican	2,5	0.253	3.723	0.340	10.964	0.002
	Cape Teal	3,4	0.064	15.956	2.487	6.416	0.008
	Hottentot Teal	4,3	0.002	-90.11	1.722	-5.232	0.014

Three bird species namely the Ringed Plover, Yellow-billed Stork and Pink-backed Pelican were found to have significant relationships with both the bottom sediment chemistry and total-N and total-P in water. It seems, therefore, that the reservoir bottom chemistry might have a wider implication on the characteristics of water birds in the water bodies.

4. Discussion

The findings showed that the tropical high altitude reservoirs were quite rich in bottom sediment SiO_2 and organic carbonand poor in CaO. Sediment SiO_2 is a common weathering product of acidic and intermediate igneous and pyroclastics rocks which are quite common in the study area. The rocks in the reservoir catchments are known to be quite rich in oxides especially Al_2O_3, Fe_2O_3 and CaO (Thompson, 1962). This condition is known to enhance the binding of phosphorus in the bottom sediments, which could amplify the process of internal loading as reported in other areas (e.g. Vikhristyuk & Varlamova, 1994). Several recent studies have closely linked the nutrient content in lakes and reservoirs to the physico-chemical profile of the bottom sediments, which can act as sinks, and sources of various elements (e.g. Barko *et al.*, 1991;

Harper, 1992; Daldorph & Price, 1994; Pacini, 1994; Vikhristyuk & Varlamova, 1994; Tiessen, 1995). Such studies have, for example, shown that the accumulation of iron in the sediments should bind the phosphorus and limit the rate of phosphorus release into the water column which can reduce the risk of eutrophication.

The main sources of C in the reservoirs was organic matter and this was more abundant in the escarpment reservoirs probably due to the steeper terrain and more woody vegetation in the riparian zone due to the presence of remnant forest in the catchment. This was a key source of dead litter along the river ways which feed into the reservoirs. Much lower sediment carbon content was recorded in the plateau reservoirs because of their location within a fairly flat meadow or moorland landscape where the release of organic debris was minimized by the gentle gradient and high trapping effect by moorland grass cover which greatly reduced surface transfer of detrital matter into the water bodies. The high level of organic carbon at Gathanje Reservoir was attributed to the raising of the dam which caused the submergence of a section of natural forest in the upper zone. The submergence generated a lot of debris, which accumulated within the reservoir. The burning of natural forests in the catchment also appeared to release a lot of carbon into the reservoir in the form of waste charcoal.

The results showed that the small reservoirs contained higher total-N and lower total-P levels at an average of 220-16 800 μg/l and 30-700 μg/l, respectively, than some large man-made lakes in Africa such as Kariba and Cabora Bassa where the concentration has been estimated at 790 and 1267 μg/l, respectively (Mhlanga, 2001). The three reservoirs in the Kinangop area which were within the Lake Naivasha basin had almost twice as much total-N than in terminal end of the largest river (River Malewa) and almost five times higher than in the open waters of Lake Naivasha (Harper *et al*., 1993). The water bodies were established to be bordering on the point of experiencing a frequent eutrophication which has already been reported by Mwaura, Koyo & Zech (2004). The lower total-N loading in some reservoirs like Gathanje indicated that the macrophyte-shored water bodies are probably better buffered against high nutrient loading than the open grass covered reservoirs. This has been found to occur in other areas. Sharpley *et al.* cited in Tiessen (1995), for example, have reported that forested areas can form suitable riparian buffers around streams or water bodies to reduce nutrient movement from agricultural land. Similarly, Hillbright-Ilkowska *et al.* cited in Tiessen (1995) have reported that zero-tillage along the water ways effectively reduces nutrient loss relative to conventional tillage by reducing soil erosion.

The high total-P content in the rift plateau reservoirs was attributed to the slow rate of overland flow within the reservoirs, which maintained longer contact of water with riparian soils. Most of the plateau landscape had higher clay content in the soil, which is known to increase P-binding in bottom sediments. The high content of total-P, which occurred in the reservoirs at the on-set of the long rains in March, indicated that the main route of phosphorus movement was the soil. High leakage of fertilizer from the land to the water was quite possible during the ploughing and planting season in March because of poor land cover, which could enhance rainfall erodibility. The low total-P in the rift escarpment reservoirs was attributed to a low clay content and greater presence of submersed macrophytes especially *Ceratophyllum demersum*. Such plants apart from accelerating nutrient uptake can also elevate the sediment redox potential thereby lowering the concentration of soluble phosphorus.

The high total-P in Kiongo Reservoir was largely attributed to anthopogenic factors because the reservoir is located 50-100 m away from Gwa-Kiongo, a rapidly growing market centre of approximately 5 000 people and whose runoff is flowing directly into the reservoir. With such a population, anthropogenic phosphorus loading can translate to an average export of about 55.1 kgP/person/year at about 2-4 gP/person/day through domestic sewage. The high total-P in Gathambara Reservoir was mainly as a result of heavy siltation. It is possible that re-suspension of phosphorus in the reservoir, which was also the shallowest, was returning large amount of phosphorus from the bottom sediments back into the water column through internal loading. The steeper gradient, high population density and rapid deforestation could explain the high loading of phosphorus in the Gathambara reservoir. A recent study in the area has shown that forest cover in the area has sharply decreased by as much as 19-24% in the 1986-2003 period (Baldyga *et al*., 2007). This is consistent with findings from other parts of the world. A survey of 928 catchments in the USA showed that phosphorus export increased proportionally with decrease in forest cover and increase in agricultural land (Sharpley *et al*., 1995). Rapid land cover change from natural vegetation to agro-ecosystem usually leads to loss of riparian buffer zones along the river ways thereby resulting in greater movement of phosphorus from the catchment into the water bodies especially in steep terrain.

The positive correlation between total-N and sediment calcium in the reservoirs was mostly linked with the movement from the catchment of phosphorus-rich particulate matter. This was attributed to the common use of Calcium Ammonium Nitrate (CAP) and Calcium Nitrate fertilizers for both agriculture and horticulture. This CAP fertilizer contains a mixture of calcium/magnesium carbonate and nitrogen that is often used to raise the soil acidity in the form of lime.

The range of total N: total P ratios in the reservoirs was 10-43. A high ratio above 30 is often associated with oligotrophy or mesotrophy while low values below 30 and in many cases even below 10 characterize the eutrophic and hypereutrophic waters. Based on this, only Muruaki, Murungaru, Kanguo and Gathambara were in the hypertrophic state. The others could to be

considered in the oligo-mesotrophic category. The results indicated that both phosphorus and nitrogen limitation are likely to occur in the reservoirs thereby affecting their biological productivy and biodiversity support capacity including birdlife. In June, half of the reservoirs where the total-N:total-P ratio was 10-17, namely Muruaki, Murungaru, Rutara and Gathambara could experience either nitrogen or phosphorus limitation or both as predicted by Forsberg *et al.* (1978) and Hillbricht-Ilkowska *et al.* (1995). In October, 37.5% of the reservoirs including Murungaru, Rutara and Gathambara, where the ratio was < 10 could experience nitrogen limitation. In the rest 50% including Kahuru, Kanguo, Gathanje and Kiongo where the ratio was >17 phosphorus limitation is expected. Finally, phosphorus limitation is likely to occur in all reservoirs during the month of December.

The inverse relationship between sediment carbon and total-P indicated that the former was acting as phosphorus sink. Activated carbon is known to have a strong affinity for many elements including phosphorus (Xie *et al.*, 2014, Newcombe *et al.*, 2010). On the overall, the results of the study indicated marginal influence by bottom sediment Al_2O_3, Fe_2O_3, P, C on TN and Fe_2O_3, CaO and P on TP in the water. The case for the TN is clearly explainable from the point of the nitrogen cycle whose key reservoir is largely atmospheric. But the findings seem to contradict other studies which have indicated positive relationship between iron oxide reduction and organic nitrogen mineralization in tropical wetlands (Sahrawat, 2004). The case of TP and Fe_2O_3 appeared to indicate that Fe(III) oxides can act as a barrier to diffusive P flux as previously established by (Vitousek *et al.*, 1997).

The results revealed that both high TN and TP loading were associated with more vibrant aquatic birdlife due to higher ecological productivity. This was particularly evident for waders, open water and diving birds which heavily depend on the water bodies for food. This pattern has been found elsewhere such in the Jagdishpur reservoir in Nepal where (Thapa & Bahadur, 2012) established that phosphate was positively correlated with bird species richness (r = 0.19) and bird number (r = 0.53). Studies have previously shown that the release of nitrogen and phosphorus from the bottom sediments of lakes and reservoirs to water column plays a key role in influencing the overall nutrient balance (Gautamand Bhattarai, 2008). This will eventually affect the biodiversity including birdlife.

This study established that bottom sediment SiO_2, C, P, Fe_2O_3, and CaO had the strongest positive and negative relationships with the reservoir birdlife community. The most sensitive waterbird species based on the results of a regression analysis included the African Fish Eagle, African Jacana, Black-headed Heron, Brack Crake, Common Teal, Great Egret, Great White Pelican, Grey Crowned Crane, Knob-billed Duck, Purple Gallinule, Ringed Plover, and Yellow-billed out of these, the most sensitive species were the African Fish Eagle, Brack Crake, Common Teal, Great White Pelican, and Purple Gallinule.

5. Conclusion

The findings showed that the chemical state of bottom sediments in reservoirs has a significant influence on the nutrient balance especially the levels of key nutrients such as nitrogen and phosphorus mainly through adsorption and binding of the nutrients at the bottom with occasional release into the ecosystem. The results showed statistically significant relationships between reservoir bottom sediment chemistry, nutrient levels, and bird characteristics. The actual impact of sediment chemistry on the utilization of reservoirs by water birds was not established and should, therefore, be an important subject for further investigation. However, the findings appeared to indicate indirect influence of sediment chemistry on nutrient dynamics, ecological productivity and food availability. The findings indicated that organic carbon litter from forests in the reservoir catchment as well as the riparian zones along the influent streams and rivers will usually influence the state of sediment carbon. This eventually affects the level of influential nutrients such as total-P which can determine the water quality through eutrophication and also affect biological productivity including the typology of species. The findings indicated that the state of reservoir bottom sediments and their influence on nutrient balance and birdlife cannot be de-linked from the landform, land cover, and human activities especially catchment management.

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Predicting Heterosis and F_1 Performance based on Combing Ability and Molecular Genetic Distance of Parental Lines in Ethiopian Mustard

Wassu Mohammed*, **D.P. Pant, Chander Mohan and Mohan Arif Khan**

*School of Plant Science,Haramaya University P.O. Box 219, Dire Dawa, Ethiopia

Department of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar-263 145 (US Nagar, Uttarakand), India

Abstract: Ethiopian mustard (*Brassica carinata* A. Braun) is one of the oldest oil crops cultivated and utilized by farmers for many purposes. However, it is one of the most neglected and least genetically studied crops. The improvement of the crop mainly depends on line breeding, but the crop is amenable to heterosis breeding. However, information regarding heterosis is scanty and the identification of parental lines from phenotypic observation is expensive and time-consuming. Therefore, this study was conducted to determine the association of genetic distances of seven *Brassica carinata* A. Braun lines measured by random amplified polymorphic DNA (RAPD) markers with heterosis, F_1 performance, and general combining ability (GCA). The study was aimed at comparing the effectiveness of parental GCA effects and genetic distance in predicting heterosis and F_1 performance. Seven *Brassica carinata* lines and their 21 F_1s generated in a half diallel fashion were evaluated in a replicated field trial for two years (2009/10 and 2010/11) at G.B. Pant University, India. Per se performances and GCA effects of the parents, heterosis and F_1 performance were calculated based on mean values from the two years for 13 traits. Correlations were computed among genetic distance, heterosis, GCA, and F_1 performances. Genetic distances among the parents were calculated from 95 random amplified polymorphic DNA (RAPD) markers and dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Means (UPGMA) method, which effectively grouped the parental lines in to three major clusters. The measured genetic distance was significantly correlated with parental GCA sum only for plant height ($r = 0.6$) and percent oil content ($r = 0.55$). However, the correlations with mid and better parent heterosis and F_1 performance were non-significant for all traits except a negative and significant correlation observed between genetic distance and better parent heterosis for length of main shoot. The correlation between GCA and F_1 performance was positive and significant for most of the traits. Mid and better parent heterosis had positive and significant correlations with GCA for days to 90% maturity, length of main shoot, and number of secondary branches. In addition, better parent heterosis of number of seeds per pod was positive and significantly correlated with the GCA of the parents. Correlation of GCA effects and parental performance was positive for all traits and significant in most cases. It could be concluded that molecular marker based distances is not a reliable predictor of heterosis, combining ability, and F_1 performance whereas GCA is better in predicting heterosis, parental line, and F_1 performances for the crop species.

Keywords: *Brassica carinata* A. Braun; General Combing Ability; Genetic Distance; Heterosis; Random Amplified Polymorphic DNA (RAPD)

1. Introduction

Brassica carinata A. Braun evolved as a natural cross between *Brassica nigra* (BB) (n=8) and *Brassica oleracea* (CC)" (n=9), in the highlands of the Ethiopian plateau, the adjoining portion of East Africa and the Mediterranean coast with underwent further chromosomal doubling (2n = 34) (U, 1935 cited by Gomez-Campo and Prakash, 1999). Ethiopian mustard is one of the oldest oil crops cultivated in Ethiopia (Simmonds, 1979). Farmers grow the crop as a leafy vegetable in their gardens at altitudes between 1500 and 2600 m.a.s.l. Traditional utilization of this crop embraces quite an array of purposes including ground seeds are used to grease a bread-baking clay pan, cure certain ailments or stomach upsets, the leaves of young plants are good source of vegetable relish and to prepare beverages. It also plays a role as a break crop for the cultivation of cereals with comparable ecological amplitude (Nigussie *et.al.,* 1997). The crop is also the third most important oil crop next to niger seed (*Guizotia abyssinica* Cass.) and linseed (*Linum usitatissimum* L) (CSA, 2003). It is higher yielding, more resistant to diseases, insect pests, and resistance to seed shattering than *Brassica napus* with the additional agronomic advantages of better tolerance for semi-arid conditions (Knowles *et al.,* 1981; Malik, 1990). Hence, the crop can serve as an important source of genes, which are rare in other oilseed *Brassicas*. Because of its

*Corresponding author. E-mail:wasmoha@yahoo.com

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drought and heat tolerance, the crop is now considered as an alternative to *Brassica napus* and *Brassica juncea* in dryer areas of Canada (Rakow, 1995), Spain (Velasco *et al.,* 1995), Australia (Fletche, 1997), India (Singh, 2003), USA and Italy (Cardone *et al.*, 2003). Besides, the gene source to improve other *Brassicas*, it become the interest of European countries, Canada and Australia for biodiesel production (De Rougement *et.al.*, 1989; Bozzini *et.al.,* 2007; University of Western Australia, 2007).

Line breeding to some extent and mass selection are the dominant breeding methods used to improve Ethiopian mustard. However, development of synthetic or hybrid cultivars have been successful in other oilseed *Brassica* ssp. **(**Becker *et al.*, 1999; Miller, 1999). Ethiopian mustard (BBCC) sharing one of its genome with *Brassica juncea* (AABB) and the other with *Brassica napus* (AACC) (U, 1935 cited by Gomez-Campo and Prakash, 1999) could be amenable for heterosis breeding as to its close relatives. But, information regarding heterosis is scanty where only one published report is available so far (Adefris and Becker, 2005).

Heterosis has been exploited extensively in crop production and has been a powerful force in the evolution of plants. But, one of the most expensive steps in heterosis utilization is the identification of parental combinations that produce superior F_1 hybrids. In maize, heterosis has been extensively exploited and several methods have been developed to predict hybrid performance using genetic markers (Frisch *et al.*, 2010; Maenhout *et al.*, 2010; Schrag *et al.*, 2010; Steinfath *et al.*, 2010). Considering the cost and time required to evaluate hybrid heterosis in the field, the use of genetic markers to predict the best heterotic combinations is the best alternative. Because, DNA molecular markers, i) identify great polymorphism, ii) not influenced by environment, and iii) can be evaluated at any development stages of the crop (Williams *et al.*, 1990).

The prediction of heterosis from parental genetic distance has been of great interest to breeders. This increases the efficiency of hybrid breeding programs since the superior crosses could be predicted before field evaluations through parental line screening. Genetic diversity can be investigated with data from pedigree, morphology, isozymes, storage proteins or DNA markers.Estimated genetic distances can be compared with heterosis from field experiments. However, limitations in traditional methods made the prediction of heterosis difficult (Hinze and Lamkey, 2003). More recently, molecular markers have been used to detect the variation in the DNA sequence underlying the analysis of the existing genetic dissimilarity of the parents. Examples of DNA markers presently used in *Brassica* are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphism DNA (RAPD), and simple sequence repeat (SSR) and single nucleotide polymorphisms (SNP). These markers have the advantage of simplifying the screening of parents, which can be done directly in the DNA evaluation (Liu *et al.*, 2002; Adefris and Becker 2005; Balestre *et al.*, 2008; Dandolini *et al.*, 2008; Silva *et al.*, 2009; Riaz *et al.* 2011).

There are reports indicating that random amplified polymorphic DNAs (RAPDs) have been successfully used to estimate genetic distance in *Brassica*. Many scientists reported that RAPD is effective in estimating genetic diversity in *Brassica* species (Divaret *et al.* 1999; Wang *et al.*, 2000; Adefris and Becker, 2005; Waqar *et al.*, 2007; Ghosh *et al.* 2009; Wisal *et al.*, 2011). It is believed that genetic differences between parents are the primary cause of heterosis. Therefore, it is important to estimate genetic distance of *Brassica carinata* lines using random amplified polymorphic DNAs and to test the correlation of parental distance with heterosis. To our knowledge, there is only one report on the association of genetic divergence and heterosis in *Brassica carinata* A. Braun (Adefris and Becker, 2005), which call for similar studies to establish genetic divergence as the predictor of heterosis in this crop. It is also necessary to test combining ability of parents as predictor of heterosis and F_1 performance as compared with genetic distance measured from RAPD molecular markers. Therefore, the objectives of this study were i) to asses genetic distances among seven *Brassica carinata* lines using random amplified polymorphic DNA (RAPD) markers, and ii)to determine associations among genetic distances, heterosis, F_1 performance and general combining ability (GCA) effects of parents in the crop species.

2. Materials and Methods

2.1. Estimates of General Combining Ability and Heterosis

Seven parental inbred lines namely; HCO-211, HCO-288, PBC-2005-1, Kiran (bold), Kiran (early), Jayanti and PBC-2006-4 were used in this study. Apart from the self-fertile nature of *Brassica carinata,* the parental lines were selfed before crossing, followed crossing with each other in a half-diallel fashion. A total of 21 F_1 crosses were generated at G. B. Pant University of Agriculture and Technology, Crop Research Center in 2008/09 and 2009/10 cropping seasons. The crossing was done by hand emasculation and bud pollination on 25 to 35 plants per line.

Then, an experiment consisting of the seven parental lines and 21 F_1 progenies was conducted for two consecutive cropping seasons (2009/10 and 2010/11) using a randomized complete block design with three replications. Each plot consisted of three rows of 5 m length and 30 cm inter-row and 10 cm intra-row spacing. All necessary crop management practices were applied as recommended for *Brassica* spp. in the study area.

In both seasons, except days to 50% flowering and 90% plant maturity that were recorded on plot basis, all other phenotypic traits were recorded from the same 10 randomly selected plants of the central row as follows: days to flowering (days from sowing until 50%

of the plants in a given plot produced flowers); days to maturity (days from sowing until about 90% of the pods matured); number of primary branches per plant (counted as the number of productive branches originating from the main stem); number of secondary branches per plant (productive branches developed from the primary branches); pod length (length of six randomly taken pods per plant; two each from bottom-middle-, and top-borne branches); plant height (the length of the main stem measured from the base to the tip of the main stem); length of main shoot (the length of the main shot measured from base of most top primary branch to the tip); number of seeds per pod (number of seeds obtained from the same pods used to estimate pod length were divided by the number of pods); seed yield per plant (the average weight of bulk of seeds obtained from all pods borne by a 10 sampled plant at the central row); 1000-seed weight (g); percentage of oil content (determined by nuclear magnetic resonance spectrometry at Center for National Oil Seed, India); biological yield (10 randomly selected plants harvested from the base, dried and weighted), harvest index (seed yield per plant/biological yield per plant x 100).

Absolute and relative mid and better parent heterosis as increase or decrease of F_1 hybrid over mid and better parent values were computed using Microsoft Excel program for each character with the formulae proposed by Gravois (1994), Fehr (1987), Falconer (1989) and others as follows: absolute mid parent heterosis (AMPH) = F_1–MP and relative mid parent heterosis $\text{MPH (\%)} = \frac{\text{F1}-MP}{\text{MP}}\ x\ 100$, where mid parent value is $\text{MP} = \frac{\text{P1+P2}}{2}$ and absolute better parent heterosis (ABPH) was calculated as F_1-BP and better parent heterosis (BPH%) or heterobeltiosis $= \text{BPH (\%)} = \frac{\text{F1}-\text{BP}}{\text{BP}}\ x\ 100$, where BP was the mean value of the higher performing parent of the hybrid. Significance of mid parent heterosis were tested as per the method proposed by Panse and Sukhatme (1961) where critical difference is calculated for mid parent heterosis as CD = $\left[\sqrt{3xEMS/2r}\right]$ x t value at error degree of freedom and CD for better parent heterosis= $\left[\sqrt{2xEMS/r}\right]$ x "t" value at error degree of freedom; r is number of replications; EMS is error mean square and t is table value of 't' at error degree of freedom at 5% and 1% probability level.

Combining ability analysis was performed according to Griffing's method II Model I (Griffing, 1956). Data analysis was conducted using MSTAT-C 1986 Michigan University statistical software. The data from the F_1 crosses and parents were subjected to analysis of variance for randomized complete block design. Analysis of variance was computed for each season and the error variance ratio of each trait was computed and the homogeneity of error variances was tested against table "F" value at 5% and 54 degree of freedom (Gomez and Gomez, 1984). All the error variance ratios computed for all traits were less than the F" value at 5% probability suggested the homogeneity of error variances. This allowed to calculate the mean values of the two seasons for each trait in each replication and it was used to compute analysis of variance and combining ability analysis on the basis of pooled mean. Further genetic analysis was performed for those parameters in which statistically significant differences existed among genotypes and GCA mean squares.

2.2. Diversity Study Based on RAPD Markers

DNA Extraction and PCR Amplification

Total genomic DNA was extracted from 0.2 g of young leaves. Leaves were taken from three weeks old seedlings from each line grown at G.B. Pant University of Agriculture and Technology Crop Research Center. Leaves were ground using a mortar and a pestle to fine powder and kept under liquid nitrogen in a 2 ml eppendorf tube. The powder was homogenized with 500 µL of DNA extraction buffer (4% SDS, 0.1 M Tris-Hcl, 10 mM EDTA, pH 8.0) and an equal volume of phenol: chloroform: isoamyl alcohol in the ratio of 25: 24: 1 respectively, was added to it. The whole mixture was vigorously shaken for 20-30 second and aqueous phase was recovered by centrifugation at 5000 rpm for 5 min. The supernatant was transferred to a fresh tube and the DNA was precipitated from it by adding $1/10^{th}$ volume of 3 M sodium acetate (pH 5.0) with an equal volume of isopropanol. The DNA was pelleted by centrifugation for 7 minutes, washed twice with ice cold 70% ethanol, dried at 37°C and dissolved in 40-45µg/ml of TE buffer (10 mMTris-Hcl, 1mM EDTA, pH 8.0) containing 40 µg/ml RNAse A. The concentration of DNA was estimated by comparing its intensity with that of the λDNA of known concentration on a 0.8% agarose gel ithTris borate EDTA (TBE) buffer. The DNA was diluted with double distilled, autoclaved and de-ionized water at the ratio of 1:5 concentrations for use in PCR.

Twelve random primers were used for PCR amplification (Table 2). The reactions were carried out in a 25 µl volume containing 1 x reaction buffer [200 mMTris–HCl, pH 8.55, 160 mM $(NH4)_2SO_4$ 0.1% (v/v)], 3.0 mM $MgCl_2$, 0.4 mM of dNTPs (dATP, dCTP, dGTP and dTTP), 0.16µM primer, 1.0 U of Taq DNA polymerase and 25 ng of genomic DNA template. DNA amplifications were performed in thermocycler programmed as indicated in the following table.

Table 1. DNA amplifications.

Cycle	Denaturation Temperature	Time	Annealing Temperature	Time	Extension Temperature	Time
First cycle	94°C		4 min.			
35 cycle	94°C	1 min.	36°C	2 min	72°C	2 min
Last cycle					72°C	10 min

Table 2. Detailed description and sequence information of primers.

Primer	Sequence	Size	TM^{0}C	GC content (%)	Mol.Wt (Da)
OPA-03	5'AGTCAGCCAC 3'	10bp	34.3	60	2997
OPA-04	5'AATCGGGCTG 3'	10bp	35.1	60	3068
OPA-07	5'GAAACGGGTG 3'	10bp	33.2	60	3117.1
OPA-10	5'GTGATCGCAG 3'	10bp	33.1	60	3068
OPA-11	5'CAATCGCCGT 3'	10bp	36.7	60	2988
OPA-18	5'AGGTGCCGTT 3'	10bp	38.1	60	3059
AC-11	5'CCTGGGTCAG 3'	10bp	35.1	70	3044
AC-20	5'ACGGAAGTGG 3'	10bp	34.3	60	3117.1
TIBMBB-17	5'ACGGAAGTGG 3'	10bp	41	60	2973
TIBMBB-02	5'TGCTCGGCTC 3'	10bp	40.1	60	2995
TIBMBB-13	5'CTTCGGTGTG 3'	10bp	32.7	60	3050
TIBMBB-16	5'CTGGTGCTCA 3'	10bp	34.3	60	3019

TM^{0}C = Melting temperature of primer, bp = base pair, Mol.Wt (Da) = Molecular weight of the primer.

Then samples were stored at 4^{0}C until the RAPD fragments were separated by electrophoresis using 1.8% agarose gel and visualized with ethidium bromide under UV light.

For data analysis, total number of bands and number of polymorphic bands generated by each primer were determined. For statistical analysis, all the scorable bands were considered as single locus/allele. The loci were scored as present (1) or absent (0). Bivariate 1-0 data matrix was generated. Jaccard's coefficient of similarity (JS) was calculated from polymorphic RAPD bands as JSjk = $\left[\frac{N11}{N11+N10+N01}\right]$ (Jaccard, 1908) as cited by Adefris and Becker (2005) and others where; JS_{jk} = is similarity between parents j and k;N11 is number of bands present in both parents; N10 is number of bands present only in parent j; N01 is number of bands present in parent k.

Similarities were computed using NTSYS-pc version 2.1 (Rohlf, 2001). The distance matrix from molecular markers was used to construct dendrograms based on the Unweighted Pair Group Method with Arithmetic Means (UPGMA) (Nei and Li,1979)using the same NTSYS software. Distances of lines were calculated from JS as Jaccard distance/genetic distance (JD/GD) = 1- JS, where Jaccard's coefficient of similarity was computed as indicated above.

2.3. Correlation of Heterosis, General Combining Ability, and Genetic Distance

Correlations of parental genetic distances with absolute mid parent heterosis (AMPH), absolute better parent heterosis (ABPH), GCA sum of parents and F_1 performance were computed. GCA sum is calculated as the sum of two parents' GCA effects involved in producing the hybrids under consideration. Correlation coefficients were also computed to detect associations of GCA sum of parents with mid parent heterosis and F_1 performance for each trait. In addition, correlation was calculated for parental performance and their GCA effects. Correlation was computed using STATISTICA 7 basic statistical analysis software (STATISTICA Software, 2002).

3. Results

3.1. Analysis of Variance and Mean Performance of Genotypes

Analysis of variance for data from each cropping season as well as for pooled means over the two years showed significant genotypic differences for all yield and yield related traits studied (Tables 3 and 4). GCA mean squares were highly significant for all traits except for number of pods per plant (Table3).

Table 3. Mean squares for yield and yield related traits from the pooled mean analysis of variance in a 7x7 diallel cross of Ethiopian mustard (*Brassica carinata* A. Braun), 2009/10 and 2010/11.

Trait	Genotype (27)	GCA (6)	Error (54)
Days to 50% flowering	606.01**	1465.10**	18.69
Days to 90% plants maturity	147.09**	468.53**	18.93
Plant height (cm)	367.98**	631.10**	150.19
Length of main shoot (cm)	373.14**	1151.45**	69.90
Number of primary branches	10.63**	23.99**	1.95
Number of secondary branches	151.51**	277.31**	34.67
Number of pods per plant	24837.14**	9006.99	6698.91
Pod length (cm)	0.11**	0.192**	0.03
Number of seeds per pod	2.76**	4.22*	1.51
Seed yield per plant (g)	40.01**	52.15*	21.55
Thousand seeds weight (g)	0.18*	0.433**	0.12
Biological yield (g)	1118.38**	1161.6*	466.75
Harvesting index (%)	9.85**	7.604*	3.08
Percent oil content (%)	5.52**	15.59**	0.96

** & **, significant $P < 0.05$ and $P < 0.01$, respectively. Numbers in parenthesis indicates degree of freedom.*

The mean values of F_1 hybrids were higher than the values for parents for all traits except for seed oil content, for which early flowering and early maturing are considered as desirable traits. Moreover, five best performing genotypes out of the 28 (21 hybrids & 7 parental lines) were identified of which all or four were hybrids. Among the parents, P1 for early flowering and maturing, P2 for number of seeds per pod and harvest index, P3 for seed yield per plant and harvest index, P5 for pod length and seed oil content, P6 for plant height and number of primary branches and P7 for harvest index were selected among the five best performing genotypes (Table 5).

Table 4. Mean squares from analysis of variance of separate years (2009/10 & 2010/11) and error ratio variance in a 7x7 diallel cross of Ethiopian mustard (*Brassica carinata* Braun).

	2009/10 cropping season			2010/11 cropping season			Error variance ratio
Trait	Replication (2)	Genotype (27)	Error (54)	Replication (2)	Genotype (27)	Error (54)	
Days to 50% flowering	83.61	606.01**	18.69	190.23	583.68**	25.29	1.35
Days to 90% plants maturity	44.3	147.09**	18.93	214.1	175.07**	23.75	1.25
Plant height (cm)	428.57	367.98**	150.19	4878.45	775.67**	206.35	1.37
Length of main shoot (cm)	57.07	373.14**	69.9	130.82	402.81**	61.83	1.13
Number of primary branches	9.08	10.63**	1.95	12.95	11.11**	2.35	1.21
Number of secondary branches	74.01	151.51**	34.67	359.52	99.15**	36.3	0.96
Number of pods per plant	37853.01	24837.14**	6698.91	122677.18	16062.77**	5698.02	1.18
Pod length (cm)	0.01	0.11**	0.03	0.01	0.19**	0.03	1.00
Number of seeds per pod	0.01	2.76**	1.51	0.87	2.56**	1.23	1.23
Seed yield per plant (g)	64.89	40.01**	21.55	243.29	50.93**	18.77	1.15
Thousand seeds weight (g)	0.43	0.18*	0.12	0.28	0.18**	0.11	1.09
Biological yield (g)	1653.77	1118.38**	466.75	6052.04	1245.15**	399.54	1.17
Harvesting index (%)	12.36	9.85**	3.08	53.31	5.87**	3.89	1.26
Percent oil content (%)	0.87	5.52**	0.96	0.004	4.40**	0.703	1.37

** & **, significant at P < 0.05 and P < 0.01, respectively. Numbers in parenthesis indicates degrees of freedom.*

Table 5. Summary of mean values of genotypes and five best performing genotypes for 14 yield and yield related traits in desired direction in a 7x7 diallel cross of Ethiopian mustard (*Brassica carinata* Braun).

DAF (50%)		DAM (90%)		PLH (cm)		LMS (cm)		NPB		NSB		NPP	
Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.
P2 x P3	67	P1 x P3	144	P5 x P7	236.9	P1 x P4	75.33	P5 x P7	16	P5 x P7	39	P4 x P6	484
P2 x P4	67	P1 x P4	144	P2 x P5	225.2	P1 x P7	74.93	P4 x P6	14	P4 x P6	39	P4 x P5	436
P2 x P6	67	P1 x P5	144	P6	224.8	P1 x P2	72.87	P6	13	P2 x P6	35	P5 x P7	431
P1	69	P1	146	P5 x P6	224.2	P2 x P6	72.7	P5 x P6	13	P2 x P5	35	P2 x P4	395
P1 x P4	69	P1 x P2	146	P3 x P6	222.4	P1 x P3	71.47	P3 x P6	13	P4 x P5	32	P2 x P5	377
Mean parents	92		155		203.7		51.34		10		18		182
Mean F_1s'	79		153		211.3		58.27		11		27		318
Grand mean	83		153		209.4		56.53		11		24		284
CD (5%)	8.65		8.7		24.51		16.72		2.8		12		164
CD (1%)	11.5		11.57		33.39		22.2		3.71		16		218
CV (%)	5.24		2.84		5.85		14.79		12.6		24.1		28.9

DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) =plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches per plant, NPP = number of pods per plant, Geno = genotype, Perf = performance, P1=HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4, CD (5%) = critical difference at 5% probability, CD (1%) = critical difference at 1% probability and CV (%) = coefficient of variation.

Table 5. Continued

POL (cm)		NSP		SYP (g)		TSW (g)		BIOY (g)		HI (%)		% Oil	
Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.
P1 x P5	4.15	P2 x P5	16	P4 x P6	24.05	P2 x P3	4.35	P2 x P4	135	P7	20.43	P5	43.15
P2 x P7	4.14	P2	16	P2 x P4	23.48	P2 x P7	4.28	P2 x P7	127.7	P4 x P6	19.44	P5 x P6	42.88
P5	4.13	P4 x P7	15	P5 x P7	22.42	P2 x P4	4.13	P5 x P7	125	P1 x P3	19.24	P5 x P7	42.34
P1 x P2	4.12	P3 x P4	15	P2 x P5	21.13	P3 x P5	4.11	P4 x P6	125	P3	19.14	P3 x P6	42.14
P2 x P6	4.11	P1 x P6	15	P3	20.68	P3 x P6	4.1	P2 x P6	115	P2	19.04	P3 x P5	41.81
Mean parents	3.8		14		16.64		3.69		83.79		18.68		41.01
Mean F_1s'	3.9		14		16.79		3.82		97.79		17.14		40.72
Grand mean	3.9		14		16.75		3.79		94.3		17.53		40.79
CD (5%)	0.3		2. 5		9.28		0.69		43.21		3.51		1.25
CD (1%)	0.4		3.3		12.35		0.92		57.47		5.06		1.70
CV (%)	4.22		8.79		27.87		9.11		22.89		10.01		2.41

POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvest index, % Oil = percent oil content, Geno = genotype, Perf = performance, P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4, CD (5%) = critical difference at 5% probability, CD (1%) = critical difference at 1% probability and CV (%) = coefficient of variation.

3.2. Estimates of General Combining Ability and Heterosis

Estimates of general combining ability (GCA) effects showed that parental lines had either positive or negative significant GCA effects for all traits except for seed yield per plant, biological yield, and harvest index (Table 6). Among the parents, P5 (Kiran early) and and P6 (Jayanti) had positive and significant general combining ability (GCA) effects for 7 and 6 out of 13 traits, respectively, including seed oil content. These parents could be considered as good combining parents whereas other five parents had positive GCA effects only for three and two traits and negative significant GCA effects at least for two and three traits. Particularly, P1 (HCO-211) showed negative and significant GCA effects for six traits that can be considered as poor combiner parent.

The magnitude of mid parent heterosis varied for the different traits and cross combinations (Table 7). Mid parent heterosis ranged from -42.18 for harvest index to 100% for number of secondary branches. Hybrid mean MPH ranged from -13.23% for days to 50 flowering to 48.13% for number of secondary branches. All hybrids showed negative mean MPH for four traits namely; days to 50% flowering, days to 90% maturity, harvest index and percent oil content. On the other hand, all hybrids displayed positive MPH (%) for number of secondary branches, which ranged from 11.76 to 100%. More than half of the hybrids (11 and above) exhibited MPH for 10 traits. Nine and seven hybrids displayed positive MPH for number of seeds per pod and percent oil content, respectively. Minimum number of hybrids displaying positive MPH was for harvest index. Three heterotic hybrids, P1 x P3, P2 x P4 and P2 x P5 recorded positive and significant MPH (%) for seven traits followed by P5 x P7 which registered positive and significant MPH (%) for six traits. Other seven hybrids (P1 x P2, P1 x P7, P2 x P7, P3 x P6, P4 x P5, P4 x P7 and P6 x P7) also displayed highest in magnitude and positive significant MPH (%) for five traits.

Varied number of F_1 hybrids displayed better parent heterosis (BPH%) in both direction ranging from -43.96 (harvest index) to 137.95% (number of pods per plant). Among the 21 F_1 hybrids, 15 for number of secondary branches, eight for pod length and thousand seeds weight, six for number of primary branches and seed yield per plant displayed positive and significant better parent heterosis. Among the five heterotic hybrids that registered the highest BPH (%) in the desired direction; P2 x P4 for seven traits, P5 x P6, P2 x P7, P2 x P5 and P2 x P7 for six and P2 x P6 for five traits displayed the highest and significant BPH (%). Three hybrids, P6 x P7, P1 x P3 and P4 x P5 for four traits exhibited the highest and significant BPH (%) (Table 8).

Table 6. Estimates of general combining ability (GCA) effects for yield and yield related traits of seven parental lines in a diallel cross of *Brassica carinata* A. Braun evaluated in 2009/10 and 2010/11.

Parent	DAF (50%)	DAM (90%)	PLH (cm)	LMS (cm)	NPB	NSB	POL (cm)	NSP	SYP (g)	TSW (g)	BIOY (g)	HI (%)	% Oil
P1	-9.33**	-6.95**	-5.54**	11.99**	-1.52**	-4.69**	0.07*	0.12	-1.82	-0.11	-11.6	0.35	-0.81**
P2	-11.1**	-4.95**	-1.38	1.74	-0.48	2.20*	0.08*	0.75**	0.23	0.19**	5.34	-0.4	-0.89**
P3	7.22**	2.09**	0.75	-2.79	-0.63*	-3.47**	0.03	-0.18	-0.55	0.15*	-7.03	0.50	-0.13
P4	3.63**	1.94*	-5.74**	-4.89*	-0.04	0.87	-0.1*	0.08	0.37	-0.14	2.27	-0.11	0.02
P5	5.70**	3.90**	5.63**	-8.25**	0.89**	2.20*	0.07*	0.04	0.79	0.02	3.83	0.01	1.26**
P6	4.22**	2.09**	6.50**	2.97*	1.00**	2.42*	-0.1*	-0.44	-0.10	-0.07	3.05	-0.61	0.64**
P7	-0.37	1.90*	-0.22	-0.77	0.79**	0.46	-0.02	-0.37	1.08	-0.05	4.12	0.27	-0.09
SE (gi)	0.77	0.78	2.18	1.49	0.25	1.05	0.03	0.22	0.83	0.06	3.85	0.31	0.18
SE (gi-gj)	1.18	1.18	3.34	2.28	0.38	1.60	0.05	0.33	1.26	0.09	5.88	0.48	0.27

** & **, significant P < 0.05 and P < 0.01, respectively. DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) = plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches, POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvesting index, % Oil = percent oil content. P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4.*

Table 7. Mid parent heterosis for yield and yield attributes in 7x7 diallel cross of *Brassica carinata* evaluated in 2009/10 and 2010/11.

Cross	DAF (50%)	DAM (90%)	PLH (cm)	LMS (cm)	NPB	NSB	POL (cm)	NSP	SYP (g)	TSW (g)	BIOY (g)	HI (%)	% Oil
P1 x P2	15.94**	-1.02	3.27	23.02**	25**	12.5**	7.15**	0.00	-3.37	1.08**	-1.22	-1.14	-2.59**
P1 x P3	-17.16**	-4.95	7.49	22.31**	12.5**	70.37**	1.4**	0.00	13.22**	4.69**	26.55	1.45	1.46**
P1 x P4	-17.37**	-4.95	5.2	39.08**	0.00	22.58**	-1.82**	-7.14**	3.73	4**	14.43	-7.6**	-0.83**
P1 x P5	-17.65**	-6.19*	0.14	5.47	11.11**	33.33**	3.23**	0.00	3.14	1.2**	17.39	-14.86**	-0.59**
P1 x P6	-12.94**	-2.97	3.38	14.21*	10.00**	31.43**	8.14**	15.38**	34.44**	3.55**	46.78**	-5.56**	0.82**
P1 x P7	-14.45**	-4.26	5.27	32.15**	5.26**	37.50**	3.05**	7.69**	3.83	-1.67**	16.81	-7.55**	-1.67**
P2 x P3	-20.71**	-3.27	6.38	2.89	22.22**	69.70**	0.52**	-13.3**	-13.6**	11.68**	7.77	-9.53**	0.38
P2 x P4	-19.76**	-3.27	10.62	19.33**	0.00	67.57**	2.51**	-6.67**	59.84**	12.84**	70.16**	-5.04**	-3.9**
P2 x P5	-15.29**	-4.52	11.82	0.41	30.00**	79.49**	0.63**	6.67**	30.92**	-5.01**	34.5*	-2.55*	-1.88**
P2 x P6	-21.18**	-3.27	1.54	33.55**	-9.09**	70.73**	15.45**	0.00	-23.39**	10.45**	36.9	-42.18**	-1.26**
P2 x P7	-20.23**	-3.9	7.12	30.91**	14.29**	63.16**	11.89**	0.00	16.44**	13.83**	48.17**	-17.46**	-1.71**
P3 x P4	2.02	0.00	-1.89	2.8	0.00	25**	-4.26**	7.14**	-31.34**	0.14	-19.54	-4.64**	-0.89**
P3 x P5	-1.49	1.26	1.25	-7.5	0.00	11.76**	0.87**	0.00	-22.75**	4.85**	-9.97	-4.31**	-0.64**
P3 x P6	-2.49	2.55	3.29	6.27	18.18**	38.89**	5.75**	0.00	-9.36**	10.51**	4.93	-1.00	4.01**
P3 x P7	-11.76**	1.9	4.65	17.66**	14.29**	27.27**	1.32**	0.00	-29.39**	-2.51**	-5.54	-17.46**	1.95**
P4 x P5	-3.52	1.89	2.96	8.1	9.09**	68.42**	-10.1**	-7.14**	24.73**	-3.93**	21.06	4.62**	-2.72**
P4 x P6	-10.55**	3.82	0.89	6.82	16.67**	95**	2.95**	7.69**	52.02**	-1.01**	44.78**	10.64**	-1.38**
P4 x P7	-26.73**	0.00	-3.13	11.92**	-4.35**	40.54**	2.83**	15.38**	-15.33**	4.79**	9.23	-18.14**	-3.91**
P5 x P6	-0.99	2.52	3.2	-17.25**	8.33**	33.33**	-0.67**	7.69**	-26.11**	2.96**	-10.27	-13.8**	1.16**
P5 x P7	-28.78**	1.88	14.46	-9.65	39.13**	100**	0.39**	7.69**	18.00**	-0.92**	32.04*	-7.4**	0.74**
P6 x P7	-32.68**	-5.06	-7.85	29.34**	-20**	12.2**	15.8**	16.67**	-32.82**	1.54**	-21.64	-7.52**	-23.67**
Mean	-13.23	-1.52	3.81	12.94	9.65	48.13	3.19	2.75	2.52	3.48	17.30	-8.14	-1.77
CD (5%)	6.11	6.15	17.33	11.82	1.97	8.33	0.25	1.74	6.57	0.49	30.55	2.48	0.38
CD (1%)	8.13	8.18	23.05	15.73	2.63	11.08	0.33	2.31	8.73	0.65	40.64	3.30	0.51

** & **, significant at P < 0.05 and P < 0.01, respectively. DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) = plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches, POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvesting index, % Oil = percent oil content. P1 = HC)-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4.*

Table 8. Better parent heterosis for yield and yield attributes in 7x7 diallel cross of *Brassica carinata* evaluated in 2009/10 & 2010/11.

Cross	DAF (50%)	DAM (90%)	PLH (cm)	LMS (cm)	NPB	NSB	POL (cm)	NSP	SYP (g)	TSW (g)	BIOY (g)	HI (%)	% Oil
P1 x P2	15.94**	-2.01	2.94	8.44	11.11**	-5.26	5.37**	-6.25**	-15.23**	-3.10**	-12.56	-1.79	-3.22**
P1 x P3	-30.00**	-7.01	3.85	6.35	0	64.29**	0.76**	0	-12.62**	-0.26	5.85	0.52	1.03
P1 x P4	-29.59**	-7.01	4.08	12.1	-18.18**	5.56	-3.32**	-7.14**	-7.87	2.54**	-1.22	-11.34**	-3.26**
P1 x P5	-30.69**	-9.32*	-4.11	-10.91	-9.09**	10.00*	0.48**	0	-15.07**	-3.57**	-4.26	-16.13**	-4.43**
P1 x P6	-26.73**	-7.01	-4.19	6.06	-15.38**	4.55	0.26	7.14**	11.25**	2.82**	21.24	-7.88**	-1.37
P1 x P7	-28.85**	-8.18*	2.06	11.5	-16.67**	15.79**	-0.77**	0	-19.79**	-3.01**	-5.24	-11.26**	-2.98**
P2 x P3	-33.00**	-5.1	3.11	1.29	22.22**	47.37**	-1.77**	-18.75**	-25.68**	10.97**	0.94	-9.77**	-0.79
P2 x P4	-31.63**	-5.1	9.79	7.53	-9.09**	63.16**	2.37**	-12.50**	57.58**	6.72**	65.3	-9.45**	-5.65**
P2 x P5	-28.71**	-7.45*	7.41	-4.43	18.18**	75.00**	-3.63**	0	21.58**	-5.61**	22.34	-4.62**	-5.08**
P2 x P6	-33.66**	-5.1	-5.62	26.22**	-23.08**	59.09**	8.73**	-12.50**	-28.44**	5.17**	26.37	-43.96**	-2.79**
P2 x P7	-33.65**	-6.29	4.18	24.44**	0	63.16*8	9.52**	-12.50**	0.29	10.59**	33.92	-20.26**	-2.54**
P3 x P4	1.00	0	-4.21	-6.04	-9.09**	11.11*	-6.31**	7.14**	-41.63**	-5.87**	-22.5	-9.30**	-3.81**
P3 x P5	-1.98	-2.48	0.31	-10.61	-9.09**	-5	-1.21**	0	-28.92**	4.85**	-12.77	-6.58**	-3.11**
P3 x P6	-2.97	0	-1.08	-1.04	0	13.64**	-2.53**	-7.14**	-17.07**	4.59**	3.3	-4.28**	1.23
P3 x P7	-13.46**	-1.26	4.28	13.55	0	10.53*	-3.03**	-7.14**	-29.50**	-5.87**	-9.08	-20.07**	0.07
P4 x P5	-4.95	-2.48	-0.38	2.03	9.09**	60.00**	-13.80**	-7.14**	14.33**	-9.69**	13.12	1.87	-4.36**
P4 x P6	-11.88**	0	-5.56	-8.45	7.69**	77.27**	-3.17**	0	40.15**	-1.71**	37.36*	8.79**	-1.65*
P4 x P7	-28.85**	-1.26	-5.09	5.78	-8.33**	36.84**	0.53**	7.14**	-27.93**	1.92**	1.41	-24.47**	-5.00**
P5 x P6	-0.99	0	-0.28	-25.35**	0	27.27**	-10.17**	0	-26.58**	-2.55**	-11.7	-14.65**	-0.63
P5 x P7	-29.81**	0	13	-9.78	33.33**	95.00**	-5.81**	0	8.73*	-4.34**	31.12	-12.38**	-1.88*
P6 x P7	-33.65**	-1.26	-12.05	16.55*	-23.08**	4.55	11.33**	16.67**	-38.46**	-0.55	-23.42	-13.31**	-1.47
Mean	-19.91	-0.04	0.59	3.11	-1.88	34.95	-0.77	-2.52	-8.61	0.19	7.6	-10.97	-2.46
CD (5%)	7.06	7.1	20.01	13.65	2.28	9.62	0.28	2.01	7.58	0.57	35.28	2.87	1.57
CD (1%)	9.39	9.45	26.62	18.15	3.03	12.79	0.38	2.67	10.08	0.75	46.92	3.81	2.05

** & **, significant at P < 0.05 and P < 0.01, respectively. DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) = plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches, POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvesting index, % Oil = percent oil content,. P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6= Jayanti, P7 = PBC-2006-4.*

3.3. RAPD Band Polymorphism and Parental Genetic Distance

Molecular sizes of amplified fragments (bands) ranged approximately between 150 to 2000 bp. In total, 95 RAPD bands were scored across the seven lines. Of these, 58 (61.05%) were polymorphic. Twelve primers generated between 5 and 12 bands with an average of 7.92 bands per primer. Primer OPA-04, OPA-07 and OPA-11, generated 12, 11 and 10 bands, respectively. The primers OPA-04 and OPA- 07 and TIBMBB-16 each with eight had also the highest number of bands. Two others namely; OPA-11 and TIBMBB-13 each with five exhibited relatively higher number of bands as compared to the other seven primers. The number of polymorphic bands ranged from one to ten with an average of 4.83 per primer. Primer OPA-04, OPA-07 and TIBMBB-16 had highest number of polymorphic bands of 10 (83%), 8 (73%) and 7 (88%), respectively (Table 9). Sample of PCR amplification profile of seven *Brassica carinata* lines from 12 RAPD primers are presented in Figures 1 to 2.

Table 9.Polymorphism exhibited by 12 RAPD primers in seven lines of *Brassica carinata* A. Braun.

Sr.No.	Primer Name	Sequence	Number of bands amplified	Number of polymorphic bands	% polymorphism
1	OPA-03	5'AGTCAGCCAC 3'	6	4	67
2	OPA-04	5'AATCGGGCTG 3'	12	10	83
3	OPA-07	5'GAAACGGGTG 3'	11	8	73
4	OPA-10	5'GTGATCGCAG 3'	9	4	44
5	OPA-11	5'CAATCGCCGT 3'	10	5	50
6	OPA-18	5'AGGTGCCGTT 3'	5	3	60
7	AC-11	5'CCTGGGTCAG 3'	6	3	50
8	AC-20	5'ACGGAAGTGG 3'	8	4	50
9	TIBMBB-17	5'ACGGAAGTGG 3'	7	4	57
10	TIBMBB-02	5'TGCTCGGCTC 3'	5	1	20
11	TIBMBB-13	5'CTTCGGTGTG 3'	8	5	63
12	TIBMBB-16	5'CTGGTGCTCA 3'	8	7	88
Average			7.92	4.83	58.75

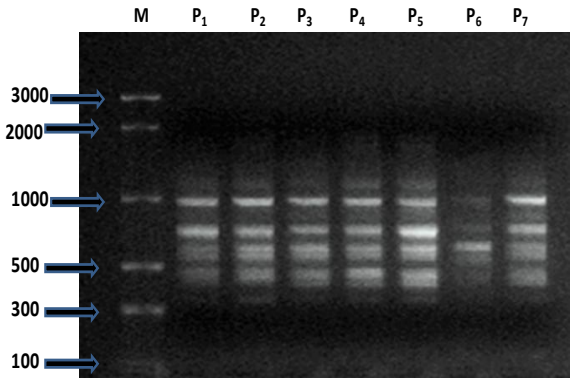

Figure 1. PCR amplification profile of seven lines of *Brassica carinata* using RAPD primer OPA-03.

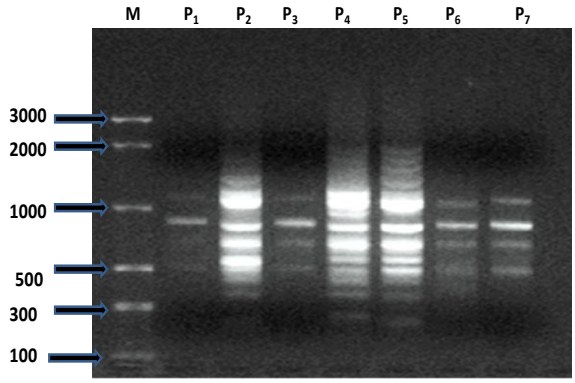

Figure 2. PCR amplification profile of seven lines of *Brassica carinata* using RAPD primer OPA-04P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7= PBC-2006-4.

On the basis of the data obtained from 12 RAPD primers, a dendrogram was constructed using Unweighted Pair Group of Arithmetic Means (UPGMA) (Nei and Li, 1979). Clustering based on JS (Jaccard's similarity) resulted in the formation of three clusters (Figure 3) of which one cluster consisted only one line i.e. Kiran (early) while the four *Brassica carinata* lines namely; HCO-288, Kiran (bold), PBC-2005-1 and PBC-2006-4 formed the second cluster and the other two lines, namely; HCO-211 and Jayanti formed the third cluster. Within the second cluster two sub groups were observed. The first sub-group comprised HCO-288 and Kiran (bold), while the other sub-group consisted of PBC-2005-1 and PBC-2006-4.

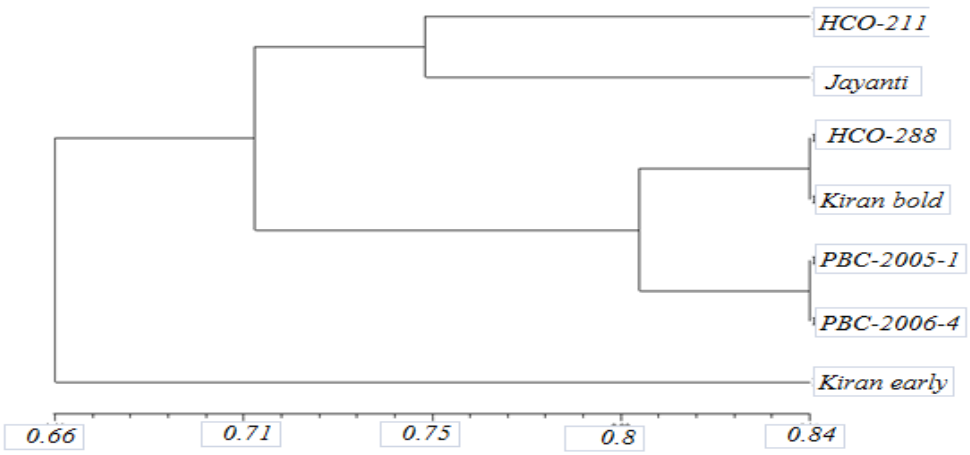

Figure. 3. Dendrogram constructed for seven lines of *Brassica carinata* A. Braun using 12 RAPD primers.

Jaccard's distance (JD) was calculated as 1-JS and JS (Jaccard's similarity) are presented in Table 7. Jaccard's distance (JD) ranged from 0.156 to 0.385 with the mean distance of 0.273 value. Among pairs of the seven lines, HCO-211 and Kiran (early) showed highest distance (0.385) followed by Kiran (bold)) and Jayanti with JD value of 0.375. Kiran (early) and PBC-2006-4, Kiran (early) and Jayanti, HCO-288 and Kiran (early) had JD values of 0.375, 0.365, 0.344 and 0.333, respectively. PBC-2005-1 and PBC-2006-4 and HCO-288 and Kiran (bold) had the lowest JD value (0.156). Other line combinations; PBC-2005-1 and Kiran (bold), Kiran (bold) and PBC-2006-4, HCO-211 and HCO-288 also had the lower distance values of 0.177, 0.187 and 0.198, respectively.

Table 10. Average estimate of Jaccard's distance (above diagonal) and Jaccard coefficients of similarity (below diagonal) among seven lines of *Brassica carinata* A. Braun using 12 RAPD primers.

	P_1	P_2	P_3	P_4	P_5	P_6	P_7
P_1		0.198	0.281	0.292	0.385	0.250	0.229
P_2	0.802		0.208	0.156	0.333	0.323	0.219
P_3	0.719	0.792		0.177	0.312	0.323	0.156
P_4	0.708	0.844	0.823		0.302	0.375	0.187
P_5	0.615	0.667	0.688	0.698		0.344	0.365
P_6	0.750	0.677	0.677	0.625	0.656		0.312
P_7	0.771	0.781	0.844	0.813	0.635	0.688	

P1 = *HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti and P7 = PBC-2006-4.*

3.4. Correlation among Heterosis, Genetic Distances, GCA of Parental Lines and F_1 Hybrids

Parental genetic distance was significantly correlated with parental GCA sum only for plant height (r=0.6) and percent oil content (r=0.55). However, the correlation coefficients between the parental genetic distances and GCA sums of parents were not significant for other traits. In addition, the correlation of parental genetic distance with absolute mid parent heterosis (AMPH) and F_1 performance was not significant for all traits. The correlation between parental genetic distance and absolute better parent heterosis (ABPH) was positive more than half of the traits (7 out of 13), but it was not strong and significant. On the other hand, the correlation between parental genetic distance and ABPH for length of main shoot was negative and significant (Table 11). The correlations between parental GCA sum and AMPH were significant for days to 90% maturity (r = 0.68), length of main shoot (r = 0.64), number of secondary branches (r = 0.41) and number of seeds per pod (r = -0.57). The correlation between GCA sum and ABPH was strong/significant for days to 90% plants maturity (r = 0.69), length of main

shoot (r = 0.46), number of secondary branches (r = 0.49) and biological yield (r = 0.43). Both AMPH and ABPH showed negative and significant correlations with GCA sum for number of seeds per pod. In general, both AMPH and ABPH showed positive correlations with GCA sum for 8 and 11 out of 13 traits, respectively.

Parental GCA sum was significantly correlated with F_1 performances for all traits except for number of seeds per pod (r = 0.26), seed yield per plant (r = 0.27) and harvest index (r = 0.35). This result was supported by the superiority of the hybrids obtained from the crossing of the two parents (Kiran early and Jayanti) that were identified as good combiner. Among the hybrids selected as best five performing genotypes for all traits, at least one hybrid at most all hybrids (plant height, percent seed oil content, number of primary and secondary branches) had one or both parents of these good combiners (Table 5).

Highly significant correlation was observed between parents' GCA effect with their *per se* performance for most of the traits. The correlation between parents' GCA effect with their *per se* performance was non significant but for pod length, seed yield per plant, biological yield, harvest index and percent oil content. The parents for which the trait that had higher performance also showed significant GCA effects. For instance, P1 had negative and significant GCA effects for days to 50% plants flowering and days to 90% plants maturity also identified as one of the five early flowering and maturing genotypes. The other parents, P6 for plant height and number of primary branches, P5 for seed oil content, P2 for number of seeds per pod and P7 for harvest index had positive and significant GCA effects that were also identified as one of the best performing genotypes for the same traits (Table 5 and Table 6).

The correlation between F_1 performance and mean and better parent mean values were positive for most of the traits. However, the correlation between F_1 performance and mean of the parental lines was positive and significant for days to flowering and maturity, length of main shoot, number of primary and secondary branches, thousand seeds weight and percent seed oil content. But better parent mean values showed positive and significant correlation with F_1 performance only for length of main shoot, thousand seeds weight and percent seed oil content. Both mid and better parent heterosis exhibited positive and significant correlations with F_1 performance for all traits except the correlation between better parent heterosis and F_1 performance for percent seed oil content was positive but significant correlation. The two heterosis estimates (mid and better parent heterosis) also showed positive and highly significant correlation for all traits (Table 11).

Table 11. Correlation coefficients of parental lines distances and GCA sum with heterosis and F_1 performance in 7 x 7 diallel crosses of *B. carinata* A. Braun for 13 traits.

	Jaccard's distance with			Parents' GCA sum with			F_1 performance with					
Trait	AMPH	ABPH	F_1 mean	AMPH	ABPH	F_1 mean	PM	BP	AMPH	ABPH	AMPH & ABPH	Parents' GCA & mean
Days to 50% flowering	-0.11	-0.05	0.08	0.08	0.33	0.92**	0.58*	-0.02	0.68**	0.87**	0.87**	0.92**
Days to 90% plants maturity	0.09	0.01	0.19	0.68**	0.69**	0.99**	0.84**	0.36	0.93**	0.93**	0.97**	0.99**
Plant height (cm)	-0.01	-0.18	0.37	-0.08	-0.2	0.89**	0.36	0.29	0.77**	0.60**	0.93**	0.89**
Length of main shoot (cm)	-0.34	-0.45*	-0.15	0.64**	0.46*	0.91**	0.77**	0.79**	0.91**	0.74**	0.90**	0.91**
Number of primary branches	0.16	0.13	0.39	-0.10	0.10	0.95**	0.58*	0.41	0.72**	0.72**	0.89**	0.95**
Number of secondary branches	0.28	0.23	0.39	0.41*	0.49*	0.96**	0.69**	0.34	0.96**	0.96**	0.98**	0.96**
Pod length (cm)	0.03	-0.18	0.05	-0.10	0.07	0.72	0.04	-0.15	0.71**	0.79**	0.91**	0.72
Number of seeds per pod	0.15	0.21	-0.06	-0.57*	-0.59*	0.95**	0.17	0.20	0.62**	0.49*	0.86**	0.95**
Seed yield per plant (g)	0.11	0.11	0.15	-0.03	0.13	0.57	-0.06	-0.09	0.89**	0.88**	0.96**	0.57
Thousand seeds weight (g)	-0.25	-0.21	-0.2	0.38	0.37	0.87**	0.57*	0.53*	0.87**	0.81**	0.93**	0.87**
Biological yield (g)	0.04	0.04	0.15	0.23	0.43*	0.64	0.15	0.18	0.93**	0.97**	0.96**	0.64
Harvest index (%)	0.11	0.14	0.01	0.18	0.17	0.49	-0.03	-0.1	0.95**	0.92**	0.98**	0.49
Percent oil content (%)	-0.01	-0.08	0.2	0.28	0.13	0.64	0.59*	0.68**	0.65**	0.36	0.83**	0.64

** & **, significant at P<0.05 and P<0.01, respectively. PM = parents of the hybrids mean value, BP = better parent mean value of F_1s', F_1 mean = F_1 mean performance, AMPH = absolute mid-parent heterosis, ABPH = absolute better parent heterosis, GCA sum = sum of general combining ability effects of the two parents' involved in producing hybrids.*

4. Discussions

The analysis of variance revealed significant differences among the genotypes (parental lines and F_1 crosses obtained from them) for all the traits. In addition, most or all of the high performing genotypes for all traits were F_1 hybrids. This suggestsa higher chance of creating variations through crossing of elite parental lines to improve the crop. The presence of variation is critical in any crop improvement which may be found in natural populations or induction created through crossing (Lewontiny and Birch, 1966; Stebbins, 1973; Eric *et al*., 2001).

Considerable number of hybrids exhibited positive and significant mid and better parent heterosis. The observed heterosis included the most economically important traits, namely, seed yield and seed oil content where 8 and 6 F_1s' displayed positive and significant MPH and BPH (%), respectively, though none of the hybrids exceeded their higher performing parents for seed oil content. This showed that hybrid production is important for the improvement of most of the traits including seed yield and early maturity in Ethiopian mustard. Negative or absence of heterosis for oil content is a common phenomenon in oil seed *Brassicas* (Banga and Labana, 1984; Brandle and McVetty, 1990;Schuler *et al*., 1992; Falk *et al*., 1994; Adefris and Becker**,** 2005). Heterosis for oil content could be much appealing, but the available experience in *Brassica napus* indicates that it is not an essential prerequisite for the success of hybrids as far as oil yield per plant could be maximized through higher yield. Early maturing might be considered as an advantage of hybrids for seed production in areas where growing seasons are short (short rainy seasons not supplemented with irrigation). But this might not be considered as a disadvantage in areas where the crop is used as a leafy vegetable where late (delayed) flowering is desired. The magnitude of heterosis observed in the present study was lesser than the magnitude of heterosis reported by Adefris and Becker (2005) for *Brassica carinata* which bMPH (%) for seed yield varied from 25.1 to 145.4 with a mean of 67%. However, the magnitude of MPH (%) observed in this study was higher than 15% (Leon, 1991) and 42% (Diers *et al*., 1996) in *Brassica napus* and 19% (Banga and Labana, 1984) in *Brassica juncea*. The magnitude of BPH (%) observed in this study across the traits was in the range of 50% (Pradhan *et al*., 1993) in *Brassica juncea*, and 69% (Brandle and McVetty, 1989) and 67% (Riazbb *et al.,* 2001) in *Brassica napus*.

In this study, RAPD was efficient in estimating the genetic distances of seven parental lines by grouping in three major clusters. The effectiveness of RAPD to estimate genetic distances was reported in *Brassica* species (Divaret *et al*., 1999; Wang *et al*., 2000; Adefris and Becker, 2005; Waqar *et al*., 2007; Ghosh *et al*., 2009 and Wisal *el al*., 2011). However, the estimated genetic distances of parents showed low correlation coefficients with heterosis and/or F_1 performance which could be attributed mainly to inadequate genome coverage, random dispersion of molecular markers and different levels of dominance. There are various reports on the extent of correlation between genetic distance and heterosis for various traits. Qian *et al*. (2007) observed a weak correlation between genetic distance and heterosis for interspecific crosses of European spring and Chinese semi winter lines. Kaur *et al*. (2007) observed a negative correlation between genetic diversity and hybrid performance in diverse morpho types of *Brassica rapa*.

For *Brassica carinata,* Adefris and Becker (2005) reported absence of correlation between the observed heterosis and genetic distance measured from RAPD markers. Riaz *et al*. (2011) also reported non-significant correlations between the genetic distance (GD) using SRAP molecular markers and oil content, plant height and maturity in *Brassica napus*. Similar to the results of other researchers, the results of this study confirmed that even though PCR based assays of RAPD estimate genetic distances of parents effectively, the estimated genetic distance could not precisely predict heterosis.

General combining ability effect of a population is an indicator of the relative value of the population in terms of frequency of favorable genes and of its divergence as compared to the other populations (Viana *et al*.,1999). The positive and significant correlations observed between GCA sum and F_1 performance for most traits and the relatively significant correlations of GCA sum with AMPH and ABPH for more traits, compared genetic distance measured from RAPD, indicates the importance of selecting parents on the basis of their GCA effect in producing hybrids with high performance. Similar to the current finding, Adefris and Becker (2005) reported positive and significant correlation of GCA sum with AMPH in *Brassica carinata*. This implies the importance of selecting parental lines on the basis of their combining ability to produce heterotic hybrids.

The observed strong correlations between F_1 performances and parents GCA effects for most of the traits and highly significant correlations of F_1 performances with both mid and better parent heterosis except in the case of better parent heterosis for seed oil content indicate the importance of additive gene action. However, the correlation coefficient did not attain unity (one) or drops to zero for any one of the traits. This indicates the involvement of epistasis other than dominance interactions in expression of F_1 performances and of heterosis. In a line crossing, the correlation of breeding values will be one if additive system is functioning, but if epistasis is functioning it drops below one and further falls to zero if dominance is involving in epistatic interactions (Pray and Goodnight, 1995; Goodnight, 1999). Poorer average performance of recombinant is explained by loss of favourable epistatic interaction present in the parents (Engquist and Becker, 1991). On the basis of the observed results and as suggested by Singh and Singh (1981), hybridization followed by selection could be suggested as a breeding procedure to develop pure-line

cultivars by taking the advantage of additive type epistasis (additive x additive) in all traits.

The observed positive and strong correlations of GCA sum of parents with parents and hybrid performances suggested that the parental lines included in this study performed well as a line as well as a parent in producing hybrids. This may be a good indicator for breeders to select their materials initially on the basis of parents' performance per se that can predict combining ability of parents and consequently the performance of crosses. Genetically, GCA is a consequence of additive gene action (Henderson, 1952; Welsh, 1981 and Falconer, 1989). If additive gene action is predominant in self-pollinated species, then the breeder can effectively select at various levels of inbreeding, because additive effects are readily transmissible from one generation to another (Gravois and Mc new, 1993).

5. Summary and Conclusion

The findings of this study indicated the possibility of predicting hybrid performances based on the general combining ability of parents. The strong and positive correlation between parents GCA effect and their performance also suggested that i) parental lines included in this study performed well as lines per se and also in hybrid combinations, ii) parents with desirable per se performances showed better combining ability and consequently hybrids performance, and iii) this may support the usual practice of breeders in selecting their materials on the basis of parental performance to include in crossing programs either to obtain high performing hybrids or to develop pure line cultivars through selection after hybridization followed by repeated self-pollination. This study also revealed that RAPD markers are effective in estimating genetic distances of few parental line of *Brassica carinata*, but parental distance computed from markers had no strong correlations with the observed heterosis, F_1 performance and parental GCA sum. This may be the consequence of using few number of primers that resulted inadequate genome coverage, random dispersion of molecular markers and different levels of dominance. Therefore, the future research should have to be directed towards the use primers as many as possible for adequate coverage of the genome. In addition, it is necessary to estimate distances of parental lines from phenotypic traits to predict heterosis in comparison to distances from molecular markers. The study generally suggested the importance of selecting materials initially on the basis of performance per se that can predict combining ability of parents and consequently the performance of crosses.

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Association of Stability Parameters and Yield Stability of Sesame (*Sesamum indicum* L.) Genotypes in Western Ethiopia

Chemeda Daba[1], Amsalu Ayana[2], Habtamu Zeleke[2] and Adugna Wakjira[3]

[1]Bako Agricultural Research Center, P. O. Box 03, Bako, West Shewa, Ethiopia
[2]Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia
[3]Ethiopian Institute of Agricultural Research, P. O. Box 2003 Addis Ababa, Ethiopia

Abstract:Information on phenotypic stability is useful for the selection of crop varieties as well as for designing appropriate breeding strategies. The present study was designed to determine the stability of sesame genotypes for seed yield and to elucidate interrelationships among the stability parameters and their associations with mean seed yield. Ten sesame genotypes were tested in four locations in 2011 and 2012 crop seasons using a randomized block design, with three replications. Nine statistical methods were used to determine seed yield stability of the sesame genotypes. The results of the various statistical analyses showed significant variations in seed yield due to genotype, location, and genotype x location interaction. Mean and cultivar superiority performance (Pi) showed high correlation with yield. Cultivar superiority measure (Pi) was significantly associated with S1 and S2. The positive correlation between Wricke and Shukla was perfect and the two procedures are equivalent for ranking purposes. Hence, either Wricke or Shukla can be used. Nassar & Hühn's absolute rank difference (S1) and variance of ranks (S2) were correlated positively and highly significantly ($r = 0.99^{**}$), hence either of them can be used. The correlations among the stability parameters S^2di, Wi, σ^2_i, ASV, S1 and S2 were positive and significant. Two genotypes, viz., EW002 and BG006, have been identified as stable with high mean seed yield and could be recommended for western Ethiopia. It could be concluded that both seed yield and stability should be considered simultaneously to exploit the useful effect of G x E interaction and using non-parametric stability measurements as an alternative to parametric stability measurements is important.

Keywords: Genotype; G x E Interaction; Sesame; Stability; Yield

1. Introduction

Sesame (*Sesamum indicums* L.) is an important oilseed crop grown for local consumption and export in Ethiopia, and it ranks first in area of production and as export crop among oilseed crops grown in the country (CSA, 2015). Ethiopia is among the world's top five producers of sesame and the third largest world exporter of the crop (Wijnands *et al*., 2011). Sesame production is increasing from year to year, which is mainly driven by increasingly high export market demand and availability of suitable agro-ecologies (Zerihun, 2012).

The national average seed yield of sesame in Ethiopia is 0.73 tons ha^{-1} (CSA, 2014), which is very low as compared to the productivity of sesame in such counties like China (1.3 tons ha^{-1}) (FAOSTAT, 2013). This low productivity of sesame is attributed to limited number of adaptable varieties with tolerance to biotic (e.g. bacterial blight) and abiotic factors (Dagnachew *et al*., 2011; Zerihun, 2012). Given the fact that western Ethiopia is one of the potential areas for sesame production (FAO, 2015), the demand for adaptable improved varieties of the crop is very high. All the nationally released varieties of sesame were evaluated for yield performance in western Ethiopia in 2004; however, all were out yielded by a local variety (Dagnachew *et al*., 2011).

For this reason, sesame breeding for western part of Ethiopia was started in 2005 at Bako Agricultural Research Center of the Oromia Agricultural Research Institute. To date, three varieties have been released and some elite breeding lines have been selected for the target agro-ecology. However, the genotypes have been selected based on their mean seed yield per hectare, with little or no reference to the stability of genotypes for seed yield across environments. Information on genotype by environmental interaction and stability is required as a basis for a sound breeding program to serve as a decision tool in releasing improved varieties and deciding the adaptation domain of such varieties (Yan, 2011). Past studies (Zenebe and Hussein, 2009; Hagos and Fetien, 2011; Fiseha *et al*., 2014; Mekonnen *et al.,* 2015) on genotype by environment interaction of sesame have not included western Ethiopia, where biotic factors such as bacterial blight exacerbates genotype by environment interaction and limit stability of varietal performance across different environments. Another issue is the presence of several parametric and non-parametric models for the statistical methods of stability analysis and absence of single method that can adequately explain stability of genotype performance across target environments (Kilic *et al*., 2010). The importance of comparing several models of stability analysis have been reported in Ethiopia for other

*Corresponding author. E-mail: chemeda2012@gmail.com

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oilseed crops like Ethiopian mustard (Kassa, 2002; Tsige, 2002), linseed (Adugna and Labuschagne, 2002; Adane, 2008), soybean (Fekadu *et al.*, 2009), linseed and niger seed varieties (Abeya *et al.*, 2014).

The present study, therefore, was designed with the objectives (1) to analyze the stability of sesame genotypes for seed yield across target environments in western Ethiopia, and (2) to study the interrelationships among the stability parameters and their associations with mean seed yield.

2. Materials and Methods

2.1. Plant Materials

The experimental materials for the present study comprised of two released varieties viz., Obsa and Dicho, seven elite breeding lines and a local check, Wama (Table 1). These genotypes were selected among the different landraces collected from Western Ethiopia based on their relative yield performance and disease resistance by Bako Agricultural Research Center. The local check was mostly grown by farmers in the Wama Valley. All the test genotypes are white seeded, having high market demand.

Table 1. List of genotypes used for the study and their silent feature.

No.	Genotype	Collection zone	Altitude (masl)	DM	PH	BP	CPP	YPP	BB
1	EW002	East Wellega	1470	124	140	9	143	17	R
2	BG006	Benshangul-Gumuz	1000	123	138	7	141	16	R
3	EW023- 2	East Wellega	1580	125	142	5	109	12	MR
4	EW003-1	Horo-GuduruWellega	1400	122	145	7	141	17	R
5	EW0011-4	East Wellega	1384	124	140	8	124	14	R
6	EW008-1	East Wellega	1402	121	137	7	138	16	MS
7	EW011-2	East Wellega	1342	124	139	7	144	16	R
8	Obsa	Horo-GuduruWellega	1395	119	135	7	125	14	R
9	Dicho	East Wellega	1460	120	140	8	130	16	MR
10	Wama	East Wellega	1430	121	137	6	131	15	MR

R = resistant, MR = moderately resistant, MS = moderately susceptible.

2.2. Experimental Sites and Experimental Procedures

The ten sesame genotypes were grown in four locations in 2011 and 2012 crop seasons (Table 2). The locations represent major sesame growing agro-ecologies for sesame production in western Ethiopia. The two locations namely Angar and Uke are found in the Angar and Didessa Valley, about 50 km apart from each other. Wama is found in the valley of Wama, while Bako is found in the Gibe basin. The four locations are also used as a testing site for sesame breeding by Bako Agricultural Research Center.

The genotypes were planted in the mid June each year at each location in randomized complete block design, with three replications. The seeds were drilled in each row at seeding rate of 5 kg ha^{-1} in plot consisting of 6 rows with spacing of 40 cm. A fertilizer rate of 46 kg N ha^{-1} was applied at planting. Twenty days after planting, thinning was done to 10 cm spacing between plants. Four times hand weeding was done at two weeks interval, starting fifteen days after planting. The genotypes were harvested on the second week of October each year. Seed yield per plot of the middle four rows were taken and used to estimate and report yield kg ha^{-1}.

Table 2.Description of experimental sites.

No	Location	Latitude	Longitude	Altitude m.a.s.l.
1	Angar	09^0 32' N	036^0 37' E	1355
2	Uke	09^0 22'N	036^0 31'E	1383
3	Wama	08^0 58'N	036^0 48' E	1436
4	Bako	09^0 04' N	037^0 02'E	1597

2.3. Statistical Analysis

Bartlett's test (Steel and Torrie, 1980) indicated heterogeneity of error variance for seed yield in each of four locations for two years and then the data was log transformed to proceed further for pooled analysis. Analysis of variance was conducted using Eberhart and Russell (1966) and Additive Main effects and Multiplicative Interaction (AMMI) (Zobel *et al.*, 1988) to test the presence of significant influence of genotype x environment interaction on seed yield of sesame genotypes. The AMMI analysis was performed using Genestat 15th Edition.

The statistical models used to estimate various stability parameters were Joint linear regression model (bi) and (S^2di) (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966), Wricke'secovalence (Wi) (Wricke, 1962), Shukla's stability variance (σ^2) (Shukla, 1972), Lin and Binns cultivar superiority measure (Pi), (Lin and Binns, 1988) and Nassar and Hühn's non-parametric measure of stability (Nassar and Huhn, 1987). For these stability measures statistical analyses were conducted using Agrobase Generation II (Agromix, 2008). In addition, the AMMI Stability Value

(ASV) was also used. It was computed as proposed by Purchase (1997) as follows:

AMMI Stability Value (ASV)

$$(\text{ASV}) = \sqrt{\left[\frac{\text{SSIPCA 1}}{\text{SSIPC 2}}\text{IPCA1score}\right]^2 + (\text{IPCA2 score})^2}$$

Where SS = sum of squares, IPCA1= Interaction principal component analysis axis one, IPCA2 = Interaction principal component analysis axis two. Spearman's coefficient of rank correlation was computed for each pair of the possible pair-wise comparison of the stability parameters by SAS 2002 statistical software.

3. Results

3.1. Analysis of Variance

The analysis of variance for the regression model for sesame seed is presented in Table 3. The results show that the variations among the genotypes and for G x E interaction (GEI) were significant. Further decomposition of the sum of squares due to environments and genotype x environment into environments (linear), genotype x environment (linear) and the pooled deviations from the regression model revealed that environment (linear) and pooled deviation were highly significant for seed yield; but G x E (linear) was non-significant.

Table 3. Analysis of variance from Eberhart and Russel's Model for seed yield of 10 sesame genotypes tested in eight environments in western Ethiopia.

Source of variation	DF	SS	MS
Genotypes	9	0.330	0.037**
Environment + (Geno x Env.)	70	1.701	0.024*
Environment (linear)	1	0.785	0.785**
Genotypes x Env. (linear)	9	0.180	0.020ns
Pooled deviation	60	0.736	0.012**
EW002	6	0.024	0.004
BG006	6	0.027	0.005*
EW023- 2	6	0.079	0.013**
EW003-1	6	0.132	0.022**
EW0011-4	6	0.028	0.005*
EW008-1	6	0.143	0.024**
EW011-2	6	0.140	0.023**
Obsa	6	0.055	0.009**
Dicho	6	0.058	0.010**
Wama	6	0.049	0.008**
Pooled error	160	0.247	0.002

, and ns, significant at P < 0.05, P < 0.01 and non significant, respectively. DF = degree of freedom, SS = sum of squares, MS = sum of squares, Geno x Env = genotype by environment interaction, Genotypes x Env. (Linear) = genotypes by environment interaction linear.*

The mean squares from AMMI analysis of variance indicated significant variations among the genotypes, the environments and their interaction for seed yield (Table 4). The GEI is highly significant (P<0.01), accounting for 47% of the sum of squares (nearly twice that of the genotypes). The GEI was partitioned into two interaction principal component axes (IPCA). The IPCA 1 score was highly significant, explaining 60.78% of the variability due to GEI. The IPCA 2 was significant, accounting for 26.16 % of the variability. As indicated by Sarwar*et al.* (2010), the highly significant differences in GEI under different models strongly justified the need for stability analysis.

Table 4. AMMI analysis of variance for seed yield of 10 sesame genotypes tested in eight environments in western Ethiopia in 2011 and 2012.

Sources of variation	DF	Sum of squares	Mean squares	Sum of square Explained % Total	% G x E
Treatment	39	3.525	0.090***		
Genotype	9	0.892	0.099***	25.00	
Environment	3	0.983	0.327***	28.00	
Rep within E	8	0.082	0.010ns		
G x E	27	1.655	0.061***	47.00	
IPCA 1	11	1.006	0.097***		60.78
IPCA 2	9	0.433	0.048***		26.16
Residuals	7	0.151	0.021		
Error	192	3.138	0.016		
Total	239	6.745	0.028		

****, significant at P = 0.001. DF = degree of freedom, Rep within E = replication within environments, G x E = genotype by environment interaction, IPCA 1 and IPCA 2 = interaction principal component axis one and two, respectively.*

3.2. Stability Analyses

The overall ranking and values of the ten sesame genotypes for stability are presented in Table 5. Based on cultivar superiority performance (Pi), S^2di, S1 and S2 genotype EW002 was found to be the most stable genotype with best mean seed yield. Genotype BG006 was ranked the 2nd stable genotype by bi, S^2di, Wi and σ^2i, although ranking third in terms of its mean seed yield. This same genotype ranked the first stable one by ASV. Genotype EW011-4 was ranked first according to bi, wi, $\boldsymbol{\sigma}^2i$ and ranked as the second stable genotypes by S1, S2 and ASV. However, this genotype was associated with low mean seed yield. Genotype EW023-2 was the most unstable genotype for its seed yield with low mean seed yield followed by genotype EW011-2.

Table 5. Mean yield (kg ha^{-1}), various stability measurements and their ranking order of 10 sesame genotypes evaluated in western Ethiopia in 2011 and 2012.

Genotype	Mean	R	Pi	R	Bi	R	S^2di	R	(Wi)	R	(σ^2_i)	R	S(1)	R	S(2)	R	ASV	R
EW002	881	1	0.010	1	1.57	8	0.003	1	0.045	3	0.021	3	2.107	1	3.109	1	0.27	3
BG006	750	3	0.011	3	0.90	2	0.003	2	0.029	2	0.010	2	3.179	4	6.109	4	0.09	1
EW023 -2	556	10	0.045	10	0.48	6	0.012	7	0.101	6	0.049	6	4.357	10	12.438	10	0.47	8
EW003-1	735	4	0.020	4	0.43	7	0.021	8	0.157	10	0.079	10	3.357	5	6.938	5	0.47	7
EW0011-4	608	9	0.032	7	0.91	1	0.003	3	0.028	1	0.010	1	2.893	2	5.234	2	0.17	2
EW008-1	625	8	0.038	8	0.71	3	0.022	10	o.150	8	0.075	8	4.071	8	10.000	7	0.46	6
EW011-2	710	5	0.030	6	1.36	4	0.022	9	0.150	9	0.075	9	4.214	9	11.000	9	0.65	10
Obsa	846	2	0.011	2	0.42	9	0.008	5	0.081	5	0.038	5	3.179	3	6.109	3	0.40	5
Dicho	704	6	0.028	5	1.38	5	0.008	6	0.070	4	0.032	4	4.036	6	9.984	6	0.33	4
Wama	645	7	0.038	9	1.82	10	0.007	4	0.102	7	0.049	7	4.036	7	10.109	8	0.52	9

Note: R = rank; Pi = Linn and Binn's (1988) cultivar superiority measures; bi = regression coefficient; S2di = Eberhart& Russell's (1966) deviation from regression parameter; Wi = Wricke's (1962) ecovalence; (σ2i) = Shukla's (1972) stability variance with no covariates; (S1) and (S2) Nassar and Hühn's (1987) absolute rank difference; ASV = AMMI absolute value.

3.3. Correlation of Stability Parameters

Spearman's coefficient of rank correlation between seed yield and the stability parameters as well as between each of the parameters is presented in Table 6. Mean seed yield and Pi were highly significantly correlated ($r = 0.94^{**}$). Mean seed yield was generally quite poorly correlated with the rest of the parameters. Cultivar superiority measure (Pi) was significantly associated with S1 ($r = 0.75^{**}$) and S2 ($r = 0.75^{**}$). This stability measure has also positive non significant association with S^2di, Wi, σ^2i and ASV. Regression coefficient (bi) showed positive non significant rank association with Wi, σ^2_i, S2 and ASV. Eberhart and Russell (1966) deviation from regression showed highly significant correlation with Wi ($r = 0.81^{**}$), σ^2i ($r = 0.81^{**}$) and S1 ($r = 0.75^{**}$) and significant with S2 ($r = 0.68^{*}$) and ASV ($r = 0.67^{*}$).

Wricke's procedure of stability statistic showed highly significant and positive association with (σ^2_i) ($r = 1.00^{*}$) and ASV ($r = 0.85^{**}$) as well as significant with S1 ($r = 0.65^{*}$) and S2 ($r = 0.64^{*}$) Shukla's (1972) highly positively and significantly correlated with ASV ($r = 0.85^{**}$) and significantly with S2 ($r = 0.64^{*}$) and S2 ($r = 0.64^{*}$). Nassar and Hühn's (1987) absolute rank difference (S1) and variance of ranks positive significantly associated with all the stability measures, except with mean seed yield and bi. The correlation of these two stability measures with mean seed yield is positive but not significant. The AMMI stability value was positively associated with all other stability parameters.

Table 6. Rank correlation between stability parameters for 10 sesame genotypes evaluated in western Ethiopia (2011 and 2012).

	Mean	Pi	Bi	S^2di	Wi	σ^2_i	S(1)	S(2)
Mean								
Pi	0.94^{**}							
bi	-0.36	-0.15						
S^2di	0.39	0.45	-0.10					
Wi	0.09	0.30	0.33	0.81^{**}				
σ^2_i	0.09	0.30	0.33	0.81^{**}	1.00^{**}			
S(1)	0.58	0.75^{**}	-0.03	0.75^{**}	0.65^{*}	0.64^{**}		
S(2)	0.58	0.75^{**}	0.05	0.68^{*}	0.64^{*}	0.64^{*}	0.99^{**}	
ASV	0.30	0.53	0.43	0.67^{*}	0.85^{**}	0.85^{**}	0.77^{**}	0.81^{**}

** Significant at 0.05 and ** significant 0.01 probability level; Pi = Lin &Binns's (1988) cultivar superiority performance, bi = regression coefficient, S^2di = Eberhart& Russell's (1966) deviation from regression parameter, Wi = Wricke's (1962) ecovalence, (σ^2_i) = Shukla's (1972) stability variance, S1 &2 = Nassar&Hühn's (1987) absolute rank difference and variance of ranks and ASV = AMMI stability value.*

4. Discussion

Data from multi-location trials help researchers to estimate yield stability more accurately and understand the interaction of yield with environments. In present study, seed yield was affected by GEI, accounting for 47% of the total, and it was far greater than that for genotype (25%) and environment (28%), indicating that there were substantial differences in genotypic responses across environments. The significant variation of genotypes and GEI suggests that genotypes exhibited different performance in the four testing locations, which can be due to their different genetic makeup, the variation due to the environments or both. Hagos and Fetien (2011) reported the highest share of sum of squares (73.1%) for environments. Thus, effective interpretation and utilization of a multi-environment trial data set remains a major challenge to researchers in making selection decisions in crop variety evaluation (Mortazavians and Azizi-nia, 2014).

The highly significant linearity for environment implies that the assumption for the differences among the linear response to environment is valid. The non-significance of G x E (linear) effect indicated that the behavior of the genotypes for seed yield is unpredictable over environments. The significance of pooled deviation from regression showed that the presence of non linearity for seed yield. As a result, the performance of different varieties fluctuated significantly from their respective linear path of response to environments.

The use of stability analysis other than the ANOVA and yield ranking would enhance prediction of cultivar choice for a target environment. The stability parameters that have been used in this study quantified the stability of genotypes with respect to mean seed yield, stability and the best combination of them. Both yield and stability of performance should be considered simultaneously to exploit the useful effect of GEI and to make selection of the genotypes more precise and refined (Farshadfar *et al.*, 2012). Most of the stability parameters used in this study were closely related in sorting out the relative stability of the evaluated genotypes. Though, some deviations were also observed.

Study on the association among stability statistics is essential to make any recommendations of a crop variety (Karimzadeh *et al.*, 2013). In the present study, rank correlation of mean seed yield was highly significantly with Pi. The highly significant correlation

of mean seed yield with Pi indicates that selection for yield would change yield stability by increasing Pi, leading to the for development of genotypes that are especially adapted to environments with optimal growing conditions. In agreement with this result, Pourdad (2011) also observed strong positive rank correlation of mean seed yield with Pi in safflower. A very serious concern in any breeding program is the possibility of rejecting a potentially useful cultivar whose mean may not be high but that shows good adaptability to a relatively narrow niche of environments, or accepting a cultivar whose mean may be high but that shows considerable variation over certain locations. Lin and Binns (1991) recommended the Pi measure to overcome this negative aspect of stability analysis.

Rank correlation of bi with mean seed yield was negative but no significant. On the other hand, Mekonnen *et al.* (2015) observed significant and positive correlation between mean seed yield and bi. The rank correlation between regression coefficient (bi) and deviation from regression (S^2di) was negative but not significant. This is in harmony with the reports by Mekonnen *et al.* (2015). However, Elfadl *et al.* (2012) for seed yield in sunflower and Mirza *et al.* (2013) for seed yield and branches per plant in sesame reported highly significant correlation of bi with S^2di.

Positive and significant rank correlation of S^2di with Wi, S1, S2 and ASV in the present study implies that S^2di and these parameters could be used independently. Similarly, Mekonnen *et al.* (2015) reported significant rank correlation of S^2di with ASV in sesame for seed yield. In the present study, the correlation between Wi and σ^2i was perfect ($r = 1.0^{**}$), indicating that the two procedures are equivalent for ranking purposes and either of them can be used. This is in agreement with the results of Schoeman (2003) and Mashayekh *et al.*, (2014), who reported significant and positive association of Wi with σ^2i in sunflower.

The present study showed that Nassar and Hühn's absolute rank difference S1 and variance of ranks S2 were positively correlated ($r = 0.99^{**}$) with each other, indicating that they were similar for classifying genotypes according to their stability under different environmental conditions. This suggests that the two statistics can be used alternatively to assess stability. Similarly, Balalić *et al.* (2011) reported in sunflower that these two non-parametric measures of stability parameters were nearly perfectly correlated. Mortazavians and Azizi-Nia (2014) also observed significant and positive association of S1 with S2 in canola. The two non-parametric stability measures S1 and S2 were positively significantly correlated with Pi, S^2di, Wi, σ^2i and ASV, suggesting that non-parametric stability measurements seem to be useful alternatives to parametric measurements. In other words, this non-parametric stability measures can complement the parametric stability measures used in this study.

In this study, AMMI stability value (ASV) was highly significantly correlated with Wi, σ^2i, S^2di, S1 and S2. In line with present result, Elfadl *et al.* (2012) also reported significant and positive rank association of ASV with S^2di for seed yield in sunflower. The association among S^2di, Wi, σ^2i, ASV, S1 and S2 was significant, which revealed that the parameters were similar in sorting sesame genotypes for stability. These results demonstrated that ranks of stability for genotype could be determined from any of these methods as they were in agreement and that these parameters can be used as alternatives to one another.

5. Conclusions

In conclusion, this study has emphasized that the effect of GEI was high, accounting to 47% of the total variation for sesame seed yield. The mean seed yield and cultivar superiority performance (Pi) showed significant rank correlation. In turn cultivar superiority measure has also showed significant rank association with S1 and S2. The Wricke and Shukla stability parameters showed perfect correlation, which indicates that either of the two procedures can be used for purposes ranking genotypes. Similarly, Nassar & Hühn's absolute rank difference (S1) and variance of ranks (S2) had near to perfect correlation suggesting that the two parameters can be used alternatively to assess stability. The two non-parametric stability measures (S1 and S2) were significantly and positively rank correlated with Pi, S^2di, Wi, $\sigma2i$ and ASV. This showed that non-parametric stability measures seem to be useful either to use as alternative or to complement the parametric stability measure. The rank correlation among S^2di, Wi, $\sigma2i$, ASV, S1 and S2 being positive and significant, indicated that the parameters were similar in assessing the stability of sesame genotypes. Two genotypes namely EW002 and BG006 were identified as stable genotype with high mean seed yield and recommended for western Ethiopia. The result of this study showed that considering more than one stability parameter is important to recommend stable genotype, with high mean seed yield to exploit the useful effect of GEI. It is also important to use the parametric and non-parametric stability measures jointly since results obtained from the two groups of stability measures can complement each other.

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Effect of Different Levels of Soybean /*Glycine Max*/ Meal Supplementation on Feed Intake, Digestibility, Live Weight Changes, and Carcass Characteristics of Black Head Ogaden Sheep

Kiflay Welday[1]*, Getachew Animut[2] and Mengistu Urge[2]

[1]Department of Animal and Range Sciences, Jigjiga University P. O. Box 1020 Jigjiga, Ethiopia,
[2]School of Animal and Range Sciences, Haramaya University P. O. Box 138 Dire Dawa, Ethiopia

Abstract: Sheep is an important animal kept as livestock in Ethiopia. However, productivity of the animal is constrained by scarcity of feed. Therefore, an experiment was carried out using twenty-four yearling male Black Head Ogaden sheep with an initial body weight of 12.95 ± 1.79 kg (mean ± SD) to evaluate the effect of different levels of soybean meal supplementation to natural pasture hay on feed intake, digestibility, average daily body weight gain, and carcass characteristics. The experimental sheep were blocked into six blocks of four animals based on initial body weight and randomly assigned to one of the four treatments. Treatments were *ad libitum* feeding of natural pasture hay and + 50 g/day wheat bran (T_1) supplemented with 125 g/day soybean meal (T_2), 250 g/day soybean meal (T_3), 375 g/day soybean meal (T_4). The experiment consisted of 90 days of feeding and 7 days of digestibility trials followed by evaluation of carcass. Supplementation of SBM increased dry matter and crude protein digestibility but neutral detergent fiber and acid detergent fiber digestibilities decreased with increasing levels of SBM supplementation. Average daily body weight gain and hot carcass was significantly increased as soybean supplementation increased. Dressing percentages both on pre-slaughter (37.3, 44.6, 46.4 and 48.6% (SEM = 0.94) and empty body weight (48.4, 51.9, 54.2 and 59.7% (SEM = 0.94)) basis for T_1, T_2, T_3 and T_4, respectively were highest for T_4, intermediate for T_3, and T_2, and the lowest for T_1. It could be concluded that feeding the Black Head Ogaden sheep on natural pasture hay supplemented with 375 g/day soybean meal resulted in superior biological as well as economic productivity.

Keywords: Body Weight Change; Digestibility Dressing Percentage; [Glycine max (L.) Merr.]

1. Introduction

Sheep in Ethiopia have many advantages over some other classes of livestock and are particularly well adapted to many agro-ecological zones of the country. However, sheep productivity very low and lag behind the growth of the population. Among the various factors constraining the productivity of sheep, feed scarcity is a core problem. Sheep productivity is also constrained by disease, inadequate utilization of indigenous sheep breeds, lack of infrastructure, lack of market information and lack of trained personnel (Markos, 2006).

Natural pasture and crop residues are the major feed resources for livestock in Ethiopia. However, such feed resources are characterized by high fiber, low protein, low minerals, and low vitamin content (Kayongo *et al.,* 1993). Thus, it is imperative to explore alternative, highly nutritious feed resources that could increase the total dietary value and eventually enhance animal performance (Yoseph, 2007).

One of the feasible methods of improving the nutritive value of natural pasture and crop residues is through strategic concentrate supplementation with energy and/or protein rich sources which can increase digestibility, nutrient supply and feed intake (Preston and Leng, 1987). Feed sources originated as by-products of various agro-industries are good sources of easily fermentable energy and protein (Ensminger *et al*., 1990).

Agro-industrial by-products such as oil seed cakes and cereal bran are potential supplements to animals on grazing (Shapiro *et al*., 2004) to alleviate qualitative deficiencies of feeds. Indeed the use of agro-industrial by-products that are locally available and complement each other is an advantage in utilization of nutrients, thereby improving animal performance. Soybean meal is one of the best plant protein sources for animals (McDonald *et al*., 2002). Griffiths (2004) found that soybean meal, in addition to being an excellent source of lysine, is also a rapidly degradable protein source. Soybean meal contains 46% CP, 5% CF, 18% fat and 84% TDN (McDonald *et al*., 2002). Soybean [Glycine max (L.) Merr.] was introduced in to Ethiopia in 1950. During that time its production and distribution was very low. But nowadays it is distributed to many regions of the country including Oromia, Tigray, Southern Nations, Nationalities and Peoples' Regional State (SSNNPRS), Amhara, and Benishangul Gumuz Regional States (MOA, 2009).

Soybean meal is commonly used as supplementary feed for small ruminants in many parts of the world.

*Corresponding author. E-mail: welday.kflom807@gmail.com

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But it is not commonly available in Ethiopia due to lack of modern soybean processing technologies. However nowadays huge modern oil extracting factories have been established at different parts of the country. Response of indigenous sheep breeds to improved feeding has not been widely investigated. As an example, the Black Head Ogaden sheep breed has not so far been exhaustively studied with regard to its response to different types of supplementation with agro-industrial by-products such as soybean meal that may enhance its productivity. This study, was therefore conducted with the objective of assess the response of Black Head Ogaden sheep to feeding on natural pasture hay and 50g/day wheat bran on dry matter basis, supplemented with different levels of soybean meals.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted at Haramaya University's sheep farm on the main campus. Haramaya University is located at the distance of about 515 km east of Addis Ababa, at 9° N latitude and 42° E longitude. The site is situated at 2050 meters above sea level has a mean annual rainfall of 790 mm and a mean annual temperature of 16 ºC (Mishra *et al.*, 2004).

2.2. Experimental Animals and Management

Twenty-four yearling male Black Head Ogaden sheep with initial body weights of 12.95 ± 1.79 kg (mean ± SD) were purchased from the local market in Babile district in eastern Ethiopia. The age of the animals was estimated by dentition. The sheep were quarantined for 21 days to adapt to experimental site (Haramaya University). During the period of adaptation all sheep were ear-tagged for identification, and drenched with 300 mg alebendazole against internal parasites and sprayed with acaricide against external parasites. The animals were vaccinated against anthrax and parasites based on the recommendation of the veterinarian. Then all sheep were transferred to individual pens and adapted to the experimental diets and pens for 15 days prior to the commencement of data collection.

2.3. Feeds and Feeding Management

The natural pasture hay (with majority of naturally cultivated grass hay) was collected from Haramaya campus. It was then it chopped to an approximate size of 4-5 cm, weighed and offered to the experimental animals *ad libitum*. Wheat bran was purchased from Dire Dawa food complex factory where as the soybean meal was purchased from Addis Ababa health care food manufactures private limited cooperation factory. The wheat bran and soybean meal were air dried to avoid moisture. Mineralized salt block and water were made available to the animals all the time, whereas different levels of soybean meal were offered in line with the treatments. The animals were offered the supplement feed in two equal halves at 8:00 and 16:00 hours daily.

2.4. Experimental Design and Treatments

The experimental design was completely randomized block design (RCBD). The experimental animals were grouped into six blocks of four animals based on their initial body weight. Initial body weight for blocking was taken after an overnight fasting by the animals at the end of the quarantine period. The four experimental treatment diets were randomly assigned to each animal in a block.

Table 1. Experimental treatment feeds.

Treatment	Natural	WB	SBM (g)
T1	*Ad libitum*	50	0
T2	*Ad libitum*	50	125
T3	*Ad libitum*	50	250
T4	*Ad libitum*	50	375

DM = Dry matter; SBM = Soybean meal; WB = Wheat bran.

2.5. Measurements and Observation

2.5.1. Feeding trial

The feeding trial lasted 90 days (from 1 January to the end of March 2011) following an acclimatization period of 15 days to the experimental conditions. The amount of feed offered and refused for each sheep was recorded daily throughout the experimental period. For an accurate estimation of nutrient digestibility, samples of feed offered were collected daily per batch and samples of feed refused were collected per animal and pooled over treatment, and sub sampled for chemical analysis. Daily feed intake of experimental animals was calculated on DM basis as the difference between the feed offered and refused.

Body weight of each animal was measured at the beginning of the feeding trial and every 10 days after overnight fasting. Body weight changes were estimated as a difference between the final and initial body weights. Average daily body weight gain (ADG) was calculated as the difference between final body weight and initial body weight divided by the number of feeding days. The feed conversion efficiency of the experimental animals was determined by the dividing ADG by the amount of daily feed consumed.

2.5.2. Digestibility

After the feeding trial, all sheep were harnessed with fecal bag to collect feces. After three days of adjustment period to fecal collection bags, feces were collected for seven days. In the morning total fecal output was weighed and recorded daily. Out of the total feces voided, 20% was sub-sampled and stored frozen at -20 ˚C to form a composite of fecal sample for each animal.

Samples of feed offered to and rejected by each animal were collected daily to form a composite of offered feed samples and refused or rejected feed samples. Refusal samples were bulked over treatments to form one refusal per treatment whereas a composite

sample from each feed was collected and ground to pass through a 1 mm sieve. Fecal samples were dried in an oven at 60 ˚C for 72 hours and ground to pass through a 1 mm sieve. The samples were stored in an airtight plastic pending chemical analysis. Then the apparent digestibility coefficient (DC) of nutrients was determined using the following equation**:**

$$\text{DC (\%)} = \frac{(\text{Total amount of nutrients in feed} - \text{Total amount of nutrients in feces})}{\text{Total amount of nutrients in feed}} x\ 100 \quad (1)$$

2.5.3. Carcass characteristics

At the end of the digestion trial, all the experimental sheep were made to fast for 12 hours and slaughtered for carcass analysis. Each sheep was weighed immediately before slaughtering. The sheep were slaughtered by severing the jagular vein and carotid arteries with a knife. The esophagus was tied close to the head to avoid leaking of gut contents. After dressing and evisceration, hot carcass weight was recorded to assess dressing percentage on slaughter and empty body weight basis. The hot carcass weight was estimated after removing the weight of the skin, head, thorax, abdominal, and pelvic cavity contents as well as legs below the hock and knee joints. Rib eye area was traced on transparency paper and measured by using plani-meters after cutting the vertebrae between the 12 and 13th ribs. Percentages of total edible offal components were taken as the sum of blood, tongue, heart, liver, bile, kidney, empty gut, omental fat, and kidney knob channel fat.

2.6. Chemical Analysis

Chemical analysis of the experimental feeds and feces was conducted at Haramaya University's animal nutrition lab. The chemical analysis of the experimental feeds, refusals, and feces was carried out after taking representative samples. The DM, ash, and CP (crude protein) contents of the feed was estimated as N*(6.25) after the analysis according to AOAC (1990) procedure. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed following the procedure described by Van Soest and Robertson (1985).

2.7. Statistical Analysis

Data on feed intake, body weight change, and digestibility, and carcass characteristics were subjected to analysis of variance (ANOVA) by using the general linear model procedure of SAS (2003), version 9. When treatment effect was significant, the Least Significant Difference (LSD) Test were used to locate differences between the treatment means. The model for data analysis was:

$$Y_{ij} = \mu + \alpha_i + b_j + e_{ij2} \quad (2)$$

Where:

Y_{ij}= Response variable

μ = Over all mean

α_i = Treatment effect

b_j= Block effect

e_{ij}= Random error

2.8. Partial Budget Analysis

The partial budget analysis was performed to evaluate the economic advantage of the different treatments by using the procedure of Upton (1979). The analysis involved the calculation of the variable costs of experimental sheep, feeds, and benefits gained from the result. The price range of the experimental sheep was between 200 and 300 Birr and the average purchase price of 250 Birr was used for the analysis of partial budget. At the end of the experiment, experienced sheep dealers from local market estimated the selling price of each experimental sheep. In the analysis, the total return (TR) wasdetermined by calculating the difference between selling and purchasing price of sheep in each treatment before and after the experiment. The cost of feeds was computed by multiplying the actual feed intake for the whole feeding period with the prevailing prices. The partial budget method measures profit or losses, which are the net benefits or differences between gains and losses for the proposed change and includes calculating net return (NR), i.e., the amount of money left when the total variable costs (TVC) are subtracted from the total returns (TR), mathematically estimated as:

$$\text{NR} = \text{TR} - \text{TVC}. \quad (3)$$

Total variable costs include the costs of all inputs that change due to the change in production technology. The change in net return (ΔNR) was calculated as the difference between the change in total return (ΔTR) and the change in total variable cost (ΔTVC), and this is to be used as a reference criterion for decision on the adoption of a new technology. The marginal rate of return (MRR) measures the increase in net income (ΔNI) associated with each additional units of expenditure (ΔTVC). This is expressed in percentage as:

$$\text{MRR\%} = (\Delta\text{NR} \div \Delta\text{TVC}) \times 100 \quad (4)$$

3. Results and Discussion

3.1. Chemical Composition

The chemical composition of treatment feeds is presented in Table 2. The Crude Protein (CP) content of the natural pasture hay used in this study was 4.67% which is below the 8% CP required to meet the maintenance need of the animals for protein (Van Soest, 1982). This low CP content of the natural pasture hay might be due to the maturity of hay at harvest. As a plant matures, the cell wall constituent or the structural carbohydrates such as cellulose and other components like lignin increases and the percentage of CP decreases (McDonald *et al.*, 2002).

The Neutral Detergent Fiber (NDF) content of the natural pasture hay used in this study was 80.33%. It is only partially digestible by any species of animals, but

can be used to a greater extent by ruminants, which depend on microbial digestion for utilization of most fibrous plant components (Pond *et al.*, 1995, McDonald *et al.*, 2002). The high content of Acid Detergent Fiber (ADF) in the natural pasture hay which was (50.97%) in the basal diet, might be an indication for low availability of nutrients in it since, ADF is negatively correlated with feed digestibility (McDonald *et al.*, 2002). In general, the chemical composition of natural pasture hay used in this study was characterized by high NDF, ADF, ADL (Acid Detergent Lignin) and low CP contents.

Table 2. Chemical composition of experimental feeds on DM basis.

Feed	Chemical composition				
	CP (% DM)	NDF (% DM)	ADF (% DM)	ADL (% DM)	Ash (% DM)
Hay	4.67	80.33	50.97	8.47	5.29
Wheat bran	15.20	50.68	15.05	4.76	5.12
SBM	39.76	19.03	10.10	3.31	5.67
Refusals					
Hay T_1	2.92	84.17	55.19	10.32	6.99
Hay T_2	2.94	85.16	57.93	10.14	6.30
Hay T_3	2.96	85.11	55.39	9.80	6.71
Hay T_4	2.99	86.83	57.09	8.88	6.60
SBM T_2	33.08	24.68	11.83	3.30	5.87
SBM T_3	33.69	20.97	8.85	1.82	5.81
SBM T_4	33.31	23.57	9.72	1.97	5.89

ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin; CP = Crude Protein; DM = Dry Matter; NDF = Neutral Detergent Fiber; OM = Organic Matter; SBM = Soybean meal; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

The NDF content of natural pasture hay used in this study was comparable to the value of 79.22% reported by Fentie (2007), but higher than the 70.7% and 71.8% reported by Asnakew (2005) and Getachew (2005), respectively. The CP, NDF, ADF and ADL of SBM used in the current study were slightly different from previous findings (Wilson *et al.*, 1995; McDonald *et al.*, 2002; Chumpawadee *et al.*, 2007). The variation in nutritional composition of SBM is dependent upon the amount of hull that is removed and processing method that is being used (Bedawy *et al.*, 2009). Furthermore, there are many factors that affect chemical composition and mineral content of feedstuffs such as oil extraction process (Mara *et al.*, 1999), stage of growth and maturity, species or variety (Keyserlingk *et al.*, 1996; Promkot and Wanapat, 2004), drying method, growth environment and soil types (Thu and Preston, 1999).

3.2. Dry Matter and Nutrient Intake

The mean daily dry matter and nutrient intakes of the experimental sheep during the feeding trial are presented in Table 3. The daily dry matter intake of natural pasture hay was significantly higher ($P < 0.05$) in non-supplemented sheep than in the supplemented ones, and decreased with increasing levels of SBM supplementation, indicating a positive substitution of SBM to the hay, and as such intake of pasture hay was not improved by supplementation. Thus, the higher intake of natural pasture hay for the control treatment as compared to the SBM supplemented ones might be an attempt to extract sufficient nutrients through relatively more natural pasture hay intake to satisfy nutrient requirements. When there is high CP inclusion in the animal feed, there could be substitution effect that could be satisfied with a limited amount of basal diet intake. However, when supplements were less in quantity, it might enhance the intake of basal diet to satisfy the nutrient requirement of animals (Nguyem *et al.*, 2008).

Total dry matter and organic matter intakes increased significantly ($P < 0.05$) with the increasing level of soybean meal (SBM) supplementation. Total dry matter intake increased by 5.25, 17.34 and 25.74% for T_2, T_3 and T_4 as compared to the non-supplemented group. The addition of supplementary SBM to wheat straw based diets increased the total dry matter intake since dietary protein supplementation increases intake by increasing the supply of nitrogen to rumen microbes that would consequently increase microbial population and efficiency (McDonald *et al.*, 1995; Willem, 2010).

Sheep in the control treatment had a lower ($P < 0.05$) CP intake than the supplemented ones. As supplementation increased, the CP intake also increased across the treatments. This increment in the CP intake with graded levels of supplementation might be due to the increased total dry matter intake and higher CP content of the supplement. Protein supplementation increases the supply of nitrogen to the rumen microbes, which have a positive effect on the rate of fermentation of the digesta (Van Soest, 1994). As the rate of degradation of digesta increases, feed intake is accordingly increased. According to NRC (1981), the average daily protein and energy requirements of a 19.1 kg body weight animal for

maintenance were 38 g CP and 4.0 MJ metabolic energy (ME), respectively. Based on this recommendation, the result of the current study showed that the average daily CP intake of 33.84 g for 13.28 kg body weight sheep in the control group was above the maintenance requirement and that is why sheep in the control treatment showed positive body weight gain. The estimated ME obtained by multiplying digestible organic matter intake with the coefficient of 0.0157 (McDonald *et al.*, 2002) was 6.3, 6.4, 7.5 and 8.3 MJ ME/head/day (SEM = 0.06) for T_1, T_2, T_3 and T_4, respectively and energy intake was above the maintenance requirement.

Table 3. Daily dry matter and nutrients intake of Black Head Ogaden sheep fed natural pasture hay supplemented with different levels of soybean meal.

Intake (g/day)	Treatment				P- Value	SEM
	T_1	T_2	T_3	T_4		
Hay DM	561.9[a]	471.1[b]	421.1[c]	367.2[d]	P < 0.0001	15.70
SBM DM	-	122.9[c]	246.9[b]	352.2[a]	P < 0.0001	22.77
Total DM	611.9[d]	644.0[c]	718.0[b]	769.4[a]	P < 0.0001	13.83
OM	562.6[d]	595.3[c]	666.4[b]	716.3[a]	P < 0.0001	13.36
CP	33.8[d]	78.5[c]	125.4[b]	164.8[a]	P < 0.0001	10.31
NDF	471.2[a]	422.9[b]	406.5[b]	383.7[c]	P < 0.0006	7.73
ADF	471.2[a]	422.9[b]	406.5[b]	383.7[c]	P < 0 .0007	7.73

[a-d] Means with in a row with different superscripts differ (P < 0.05); ADF = Acid detergent fiber; ADL = Acid detergent lignin; CP = Crude protein; DM = Dry matter; OM = Organic mater, NDF = Neutral Detergent Fiber; SBM; = Soybean meal; SEM = Standard Error of Mean; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

The total NDF and ADF intakes were greater (P < 0.05) for the control group than the supplemented groups. The basal diet used in this study, was 80% NDF and 51% ADF. This high content of fiber in the pasture hay used in this study coupled with the greater intake of hay for T_1 presumably resulted in more intakes of NDF and ADF for SBM non-supplemented group as compared to SBM supplemented ones.

3.3. Digestibility

The apparent digestibility of dry matter and nutrients are given in Table 4. The dry matter and organic matter digestibility was significantly impacted by treatment (P < 0.05), and was in the order of $T_4 = T_3 > T_2 > T_1$. The CP was also significantly affected by treatment and was increased as the level of SBM supplementation increased (P < 0.05); on the other hand NDF and ADF digestibility decreased (P < 0.05) with increasing level of SBM supplementation.

Digestion in the rumen is dependent on the activity of rumen microorganisms. Rumen microbes require energy, protein and other micro-nutrients to grow and multiply and in the due course efficiently perform the ruminal digestion of feeds (Ranjhan, 2001). Thus, the digestibility of dry matter and CP would expectedly increase when diets rich in CP are consumed (Broster, 1973), which presumably is the main reason for dry matter and CP digestibility differences among treatments observed in this study. Although soybean meal protein is degraded relatively rapidly in the rumen, much of such protein tends to bypass ruminal digestion, making it available for enzymatic digestion in the small intestine (Khorasani *et al.*, 1990). The digestibility of dry matter and CP was shown to increase in response to increase the amount of rumen undegradable protein in the diet of ruminants (Haddad *et al.*, 2005), which can be also another reason for the improvements observed in the dry matter and protein digestibility with SBM supplementation in this study. However, fiber digestibility in this study decreased in response to increasing the level of SBM supplementation in the diet despite the expected improvement in fiber digestibility with protein supplementation as noted before (Banamana *et al.,* 1990). However, if most of the SBM protein bypasses ruminal fermentation as noted above, the possible incremental effect of the additional dietary protein intake in ruminal fiber digestion could not have been achieved. On the other hand, the protein supplied by the 50 g wheat bran might have been sufficient to induce significant digestion of fiber especially in treatments with low intake of total dry matter and/or nutrients so that ruminal microbes extract sufficient nutrients from SBM and wheat bran that can satisfy their demand.

Table 4. Apparent digestibility of nutrients in Black Head Ogaden sheep fed on natural pasture hay supplemented with different levels of soybean meal.

Digestibility (%)	Treatment					SEM
	T_1	T_2	T_3	T_4	P-value	
DM	69.1[c]	71.0[b]	72.6[a]	73.4[a]	P < 0.0007	0.39
OM	70.8[c]	72.7[b]	74.3[a]	75.1[a]	P < 0.0017	0.39
CP	61.3[d]	83.9[c]	87.1[b]	89.3[a]	P < 0.0001	2.30
NDF	70.3[a]	66.9[b]	63.8[c]	59.3[d]	P < 0.0001	0.88
ADF	73.1[a]	70.1[b]	66.9[c]	62.4[d]	P < 0.0001	0.87

[a-d] Means with in a row with different superscripts differ (P < 0.05); ADF = Acid Detergent Fiber; CP = Crude Protein; DM = Dry Matter and OM = Organic Matter; NDF = Neutral Detergent Fiber; SEM = Standard Error of Mean; SL= Significance Level; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

3.4. Body Weight Change

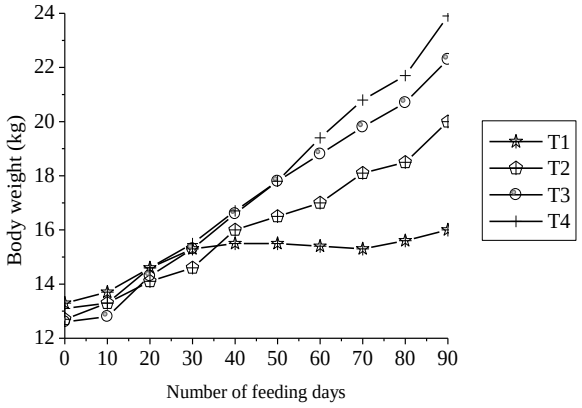
The body weight parameters of the experimental sheep are presented in Table 5. Initial body weights were similar (P > 0.05) among treatments. Final body weight and average daily body weight gain (ADG) increased (P < 0.05) in response to increasing the levels of SBM supplementation. Feed conversion efficiency (FCE) was lower for T_1 as compared to the SBM supplemented groups, and was significantly (P < 0.05) higher for T_3 and T_4 as compared to T_2. A positive ADG in SBM non-supplemented sheep in the present study indicated that the addition of 50 g wheat bran to natural pasture hay was more than the maintenance requirement of the animals. Improvements in growth rate and FCE with SBM supplementation in the current study was obviously associated with better dry matter and CP intake and digestibility that might have increased nutrient supply for growth in SBM supplemented sheep. According to Brown *et al. (*2001), animals that have high feed conversion efficiency are considered efficient users of feed. Therefore, among supplemented treatments, sheep in T_3 and T_4 were best feed converters. The improved FCE observed in the current study due to supplementation agreed with results from other similar studies (Solomon and Solomon 1995). The present experiment indicated that SBM supplementation promoted better ADG and FCE.

Table 5. Body weight change of Black Head Ogaden sheep fed natural pasture hay supplemented with different levels of soybean meal.

Parameter	Treatment					SEM
	T_1	T_2	T_3	T_4	P-value	
Initial BW (kg)	13.3	12.7	12.6	13.1	P < 0.4409	0.38
Final BW (kg)	16.0[d]	20.0[c]	22.3[b]	23.9[a]	P < 0.0001	0.69
ADG (g/d)	30.3[d]	80.9[c]	104.0[b]	116.2[a]	P < 0.0001	6.89
FCE (g ADG/g DMI)	0.05[c]	0.13[b]	0.14[a]	0.15[a]	P < 0.002	0.009

[a-d] Means with in a row with different superscripts differ (P < 0.05); ADG = Average Daily body weight Gain; BW = Body Weight; DMI = Dry Matter Intake; FCE = Feed Conversion Efficiency; SEM = Standard Error of Means; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

Body weight change of the experimental animals increased similarly until the animals became well adapted to SBM. However after the animals got well adapted to SBM. Supplemented animals significantly (P < 0.05) higher in body weight gain compare to non-supplemented animals. During the last 30 days of feeding regime, the control group showed little body weight change, after a pause between 30 and 70 days of the experiment, while the supplemented group showed increasing trend throughout the experimental period. This might be due to the insufficient amount of nutrients available for animals in T_1 as their requirement increase with increase in body weight.



T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

Figure1. The trends in body weight changes over the feeding days.

3.5. Carcass Characteristics

The pre-slaughter body weight (PSW), empty body weight (EBW), hot carcass weight (HCW), dressing percentage as a proportion of PSW and EBW and rib-eye muscle area were significantly ($P < 0.05$) higher for SBM supplemented group than the non-supplemented group (Table 6). Among the SBM supplemented treatments, PSW, HCW and rib eye muscle area increased with the increasing level of SBM supplementation. The dressing percentages as a proportion of EBW were eleven percent higher than the dressing percentages as a proportion of PSW due to effect of gut content. Black Head Ogaden sheep with pre-slaughter weight ranging from 20.1-23.9 kg had a dressing percentage of 38.8-44.9% and 53.1-57.3% on PSW and EBW basis, respectively (Embet, 2008), which are comparable with the results of the current study. Similarly, lamb slaughtered at 11.8-19.4 kg body weight had dressing percentages of 49.8-50% and 55.3-57.5% on PSW and EBW basis, respectively, (Manufredini *et al.,* 1988*),* which was also comparable with the results of current study.

Table 6. Carcass characteristics of Black Head Ogaden sheep fed on natural pasture hay supplemented with different levels of soybean meal.

	Treatment				
Carcass parameter	T_1	T_2	T_3	T_4	SEM
Pre-slaughter BW (kg)	15.8^{d}	20.0^{c}	22.3^{b}	23.9^{a}	0.72
Empty BW (kg)	12.2^{c}	17.2^{b}	19.1^{a}	19.5^{a}	0.67
Hot carcass weight (kg)	5.9^{d}	8.9^{c}	10.4^{b}	11.6^{a}	0.48
Dressing percentage (%)					
Pre-slaughter BW basis	37.3^{c}	44.6^{b}	46.4^{b}	48.6^{a}	0.94
Empty BW basis	48.4^{c}	51.9^{b}	54.2^{b}	59.7^{a}	0.94
Rib-eye muscle area (cm^2)	4.5^{d}	6.8^{c}	8.8^{b}	10.8^{a}	0.51

$^{a-d}$ Means with in a row with different superscripts differ; ($P < 0.05$); BW = Body Weight; SEM = Standard Error of Means; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

Generally, SBM supplementation improved dressing percentage, which is in line with the report of Ulfina *et al.* (1999), in which feeding aged Horro ewes on a concentrate improved carcass weight and dressing percentage.

Greater rib-eye muscle area with SBM supplementation in this study indicates that supplemented sheep were able to develop better muscling than the non-supplemented sheep. In agreement with the results of this study, Black Head Ogaden sheep fed on a basal diet of common bean haulms and supplemented with mixtures of wheat bran and brewers dried grain showed rib-eye muscle area of 8.2-10.4 cm^2 for supplemented treatments and 6.7 cm^2 for the control (Emebet, 2008). But rib eye muscle area in the control group of this study was slightly lower than results reported by this author. This could be attributed to the variation in slaughter body weight and feed type.

3.5.1. Main carcass components

The main carcass components of Black Head Ogaden sheep fed on a basal diet of natural pasture hay supplemented with different level of soybean meal are presented in Table 7. All main carcass components of the SBM supplemented treatments were significantly ($P < 0.05$) higher than the non-supplemented group, and values increased significantly ($P < 0.05$) with increasing level of SBM supplementation. Among the total main carcass components, the hind quarters made the highest share (35.94 -37.23%) of the hot carcass weight, followed by the forequarters (31.49-31.91%) and the third was the ribs that constituted 20.32-21.54% of the hot carcass weight.

Table 7. Main carcass components of Black Head Ogaden sheep fed on natural pasture hay and supplemented with different levels of soybean meal.

	Treatment				
Carcass parameter (kg)	T_1	T_2	T_3	T_4	SEM
Forequarter	1.2[d]	1.7[c]	2.0[b]	2.3[a]	0.09
Sternum (brisket)	0.4[d]	0.7[c]	0.8[b]	0.9[a]	0.04
Hind quarter	1.4[d]	2.1[c]	2.5[b]	2.7[a]	0.10
Ribs	0.8[d]	1.1[c]	1.4[b]	1.5[a]	0.06
TMCC	3.8[d]	5.6[c]	6.7[b]	7.4[a]	0.30

[a-d] Means with in a row with different superscripts differ; (P < 0.05); SEM = Standard Error of Means; TMCC = Total main carcass components; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

3.5.2. Edible offal components

Edible offal component of Black Head Ogaden sheep fed on the basal diet of natural pasture hay and supplemented with different levels of soybean meal are listed in Table 8. All edible offal components measured in this study showed significant (P < 0.05) difference among the treatments. Total edible offal components (TEOC) increased significantly (P < 0.05) with increasing level of SBM supplementation. Generally supplementation had a positive effect on the weight of most edible offal components which agrees with the report of Tesfaye (2007) in which supplemented Afar sheep had higher weight of visceral organs than the non-supplemented ones.

Table 8**.** Edible offal components of Black Head Ogaden sheep fed on natural pasture hay and supplemented with different levels of soybean meal.

	Treatment				
Edible offals	T_1	T_2	T_3	T_4	SEM
Blood (g)	707.2[c]	753.3[c]	892.3[b]	997.8[a]	26.59
Liver (g)	163.8[d]	306.7[c]	340.0[b]	408.7[a]	19.26
Kidney (g)	47.3[c]	59.3[b]	65.2[a]	69.3[a]	2.11
Heart (g)	71.7[c]	83.5[b]	84.7[b]	123.5[a]	4.34
Tongue (g)	50.8[b]	55.5[a]	56.2[a]	56.3[a]	0.71
Kidney fat (g)	17.7[d]	33.8[c]	40.2[b]	50.7[a]	2.54
Abdominal fat (g)	38.3[d]	75.0[c]	123.3[b]	170.7[a]	10.53
Reticulum (g)	57.2[c]	64.2[c]	73.0[b]	80.3[a]	2.17
Rumen (g)	344.8[d]	442.8[c]	481.2[b]	540.3[a]	15.38
Omasum (g)	59.7[c]	66.5[bc]	73.3[ab]	80.5[a]	2.04
Abomasums (g)	69.0[c]	77.8[b]	82.8[b]	93.8[a]	2.34
Small intestine (g)	281.0[c]	447.7[b]	476.5[ab]	512.3[a]	19.30
Large intestine (g)	236.7[d]	272.0[c]	292.3[b]	324.5[a]	7.15
TEOC (kg)	2.2[d]	2.7[c]	3.1[b]	3.5[a]	0.11

[a-d] Means with in a row with different superscripts differ; (P < 0.05);SEM = Standard Error of Means; TEOC = Total Edible Offal components; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

3.5.3. Non-edible offal components

Similar to that of edible offal components, all non-edible offal components also significantly (P < 0.05) differed among the treatments, and the total non-edible offal weight excluding the gut contents increased significantly (P < 0.05) in response to increasing the level of SBM supplementation (Table 9). Gut fill was 3.53 kg (22.41% of PBW) for T_1 and within the range of 2.78-4.42 kg (13.91-18.49% of PBW) for supplemented treatments. This might be due to the fact that animals on poor quality feed are compelled to fill their gut with less digestible roughage, and consequently, have proportionally bigger gut contents (Van Soest, 1994; Pond *et al.*, 1995). In the current study, it was observed that the proportion of gut fill to PBW of the control group is higher than the supplemented groups.

Table 9. Non-edible offal components of Black Head Ogaden sheep fed on natural pasture hay and supplemented with different levels of soybean meal.

	Treatment				
Non edible offals	T1	T2	T3	T4	SEM
Skin (g)	1256.3^{d}	1965.0^{c}	2401.5^{b}	2597.0^{a}	109.47
Feet (g)	367.5^{c}	415.2^{b}	424.4^{b}	466.8^{a}	8.15
Head without tongue (g)	1280.0^{b}	1303.2^{b}	1491.7^{a}	1527.2^{a}	25.13
Penis (g)	33.7^{d}	39.0^{c}	44.8^{b}	47.7^{a}	1.25
Testicles (g)	220.3^{c}	256.0^{b}	261.7^{b}	289.8^{a}	5.98
Lung and trachea (g)	181.0^{c}	205.2^{b}	216.5^{b}	240.7^{a}	5.48
Esophagus (g)	58.8^{b}	70.0^{a}	66.2^{a}	68.7^{a}	1.33
Spleen (g)	19.8^{d}	32.3^{c}	37.8^{b}	49.3^{a}	2.26
Gall-bladder with bile (g)	16.5^{c}	21.3^{b}	23.5^{ab}	25.8^{a}	0.85
Bladder (g)	13.8^{c}	14.2^{c}	17.8^{b}	20.8^{a}	0.68
TNEOC (g)	3447.7^{d}	4321.4^{c}	4985.9^{b}	5333.8^{a}	152.62
Gut fill (g)	3534.8^{b}	2782.8^{c}	3211.8^{b}	4421.2^{a}	143.55

$^{a-d}$ Means with in a row with different superscripts differ; ($p < 0.05$); = SEM = Standard Error of Means; TNEOC = Total None Edible offal Components; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

3.6. Partial Budget Analysis

As shown in Table 10, feed cost was the major cost of the feeding trial and selling price of sheep increased with increasing SBM supplementation. In this study, it was realized that the economic return of the feeding trial mainly depends on feed cost, purchasing and selling price of the experimental sheep. The most and important parameters of partial budget analysis are the change in net income (ΔNI) and MRR. These is due to the fact that MRR measures the net return increment associated with each additional units of expenditure (ΔTVC) and ΔNI shows the net return of the feeding regime by considering expense costs and return changes Upton (1979). Supplementation of different levels of SBM resulted in increments in NI and ΔNI. Animals that have high feed conversion efficiency are considered efficient users of feed (Brown *et al.*, 2001). Therefore, among the supplemented treatment groups, T_4 was the best recommended supplementation level based on biological performances and practical budget analysis. Therefore, natural pasture hay basal diet based feeding system for sheep should be supplemented with a good protein supplement like soybean meal to obtain good net income (profit) within a shorter duration.

Table 10. Partial budget analysis of Black Head Ogaden sheep fed on natural pasture hay and supplemented with different levels of soybean meal.

Parameter/Treatment	T_1	T_2	T_3	T_4
Purchase price of sheep (ETB/sheep)	250	250	250	250
Natural pasture hay consumed (kg/sheep)	50.6	42.4	37.9	33.0
Wheat bran consumed (kg/sheep)	4.5	4.5	4.5	4.5
SBM consumed (kg/sheep)	-	12.94	22.22	31.98
Cost of Natural pasture hay (ETB/sheep)	56.17	47.06	42.07	36.63
Cost of Wheat bran (ETB/sheep)	9	9	9	9
Cost of SBM (ETB/sheep)	-	80.54	138.2	198.26
Total feed cost (ETB/sheep) (TVC)	65.17	136.60	189.27	243.89
Selling price of sheep (ETB/sheep)	285	395	455	525
TR (ETB/sheep)	35	145	205	275
NR (ETB/sheep)	-30.17	8.40	15.73	31.11
ΔNI	-	38.57	45.90	61.28
ΔTVC	-	71.43	124.1	178.72
MRR	-	54.0	37.0	34.3

ETB = Ethiopian Birr; TR = Total Return; ΔTR = Change in total return; TVC = Total Variable Cost; Δ TVC = Change in Total Variable cost; NR = Net Return; ΔNI = Change Net Income; MRR = Marginal Rate of Return; SBM = Soybean meal; T_1 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran; T_2 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran +125 g DM/day Soybean meal; T_3 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 250 g DM/day Soybean meal; T_4 = Natural pasture hay ad libitum + 50 g DM/day Wheat bran + 375 g DM/day Soybean meal.

4. Conclusions

The result of this study have demonstrated that soybean meal (SBM) supplementation has a positive effect on feed intake, FCE, average body weight gain, carcass parameters and economic feasibility. The effects are more pronounced at the highest level of SBM. Thus supplementing natural pasture hay with 375 gm of dry matter soybean meal resulted in better biological and economic performances in the production of Black Head Ogaden sheep in the study area.

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Genetic Variability, Heritability and Correlation Coefficient Analysis for Yield and Yield Component Traits in Upland Rice (*Oryza sativa L.*)

Demewez Fentie[1*], Getachew Alemayehu[2], Mahesh Siddalingaiah[3] and TilahunTadesse[4]

[1]College of Natural and Computational Sciences, University of Gondar, P. O. Box 196, Gondar, Ethiopia
[2]College of Agriculture and Environmental Sciences, Bahir Dar University, P. O. Box 32, Bahir Dar, Ethiopia
[3]Indian Academy Group of Institutions, Bangalore: 560078, India
[4]Crop Research Directorate,Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia

Abstract:Rice (*Oryza sativa L.*) is one of the most important staple crops consumed by more than half of the world's population. To assess the range of genetic variability, heritability and association between yield and yield component traits of upland rice, a field experiment was conducted using twelve upland rice genotypes during 2013 main cropping season. The experiment was laid out in a randomized complete block design, with three replications under rain-fed condition. The analyses of variances (ANOVA) showed significant differences nearly for all traits tested, except panicle length and number of fertile tillers per plant. Grain yield ranged from 2340 kg/ha for genotype FOFIFA-3737 to 3400 kg/ha for genotype AD-48, with a mean value of 2868 kg/ha. High to medium phenotypic and genotypic coefficients of variability were observed for thousand grain weight, biomass yield and grain yield. High broad sense heritability estimates were observed for thousand seed weight, days to 75% maturity, days to 50% heading and biomass yield kg/ha. High to medium heritability and genetic advance were observed for plant height, thousand grain weight, biomass yield and grain yield. Days to 75% maturity, panicle length, plant height, number of fertile tillers per plant, number of spikelet per panicle, number of filled grains per panicle and biomass yield had a positive and significant correlation with grain yield. Generally from this study, plant height, number of spikelet per panicle, number of filled grains per panicle, and biomass yield were found to be important yield component traits.

Keywords*:* Broad Sense Heritability; Genetic Advance; Genotype; Grain Yield; GCV; PCV

1. Introduction

There is high potential for rice (*Oryza sativa L.*) production in Ethiopia. However, the crop has been introduced recently to the country. The finding of wild rice in the Fogera Plain in the early 1970s was the foundation for rice introduction in the area as well as in the Amhara region (Astewel, 2010). The number of farmers, who are growing rice, the area covered with rice and its production has been increasing from time to time (Tilahun *et al.,* 2013).

The development of rice cultivars which have high yielding potential is the most important aim in breeding programs (Jing and Jianchang, 2011). The genetic variation for the traits under selection process and high heritability and genetic advance are necessary factors to develop the high yielding cultivars in the breeding program (Ulloa, 2006). Heritability estimates provide information on the proportion of variation that is transmissible to the progenies in subsequent generations (Satheeshkumar and Saravanan, 2012). However, the estimates of heritability alone are not indicative of the genetic progress that would result from selecting the best plants (Chaghakaboodi *et al*., 2012). Genetic advance has also a considerable importance because it indicates the extent of the expected genetic gain from one cycle of selection (Hamdi *et al*., 2003). Grain yield is a complex character, quantitative in nature and anintegrated functionof a number of component traits. Therefore, selection for yield may not be much satisfying unless other yield attributing traits are taken into consideration (Akinwale *et al.,* 2011). There is no clear information regarding range of the genetic variability and yield attributing traits of upland rice in the study area. Hence, the objectives of this study were to estimate the genetic variability of upland rice genotypes using morphological traits and to assess the association between yield and yield related traits.

2. Materials and Method

2.1. Experimental Materials, Design, and Procedures

Twelve upland rice genotypes collected from Adet Agricultural Research Center: NERICA-3, NERICA-10, NERICA-12, NERICA-13, NERICA-14, Hidasie, Kokit, Superica-1, Getachew, Andasa, Tana and Kallafo-1 were used as experimental material. The experiment was laid out in a randomized complete block design (RCBD) with three replications and was conducted under rain fed situations in the 2013 main cropping season at Wereta rice research center. Each entry was sown with the seed rate of 60 kg per hectare by hand drilling in five row plots of 5 m length and 1 m width with 20 cm inter row space with three replications. There was 1m space between replications and 50 cm between varietal plots. DAP and Urea

*Corresponding author. E-mail: demewezfentie@yahoo.com

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fertilizers were applied at the rate of 100 kg per hectare. DAP was applied once at the time of planting, whereas Urea was applied in three splits equally at planting, tillering and panicle initiation. All other management practices were uniformly applied to all experimental plots as per recommendations.

2.2. Data Collection

Data were collected on plot and plant basis according to standard evaluation systems for rice (IRRI, 1988). Plant height (cm), panicle length (cm), number of effective tillers per plant, number of spikelet per panicle and number of filled grains per panicle were measured from ten randomly selected plants in the middle three rows of each plot. Data for days to 50% heading and days to 75% physiological maturity were collected from plot basis. However, thousand grain weight (g), biomass yield (kg/ha), and grain yield were collected on the three central rows.

2.3. Data Analysis

Analysis of variance (ANOVA) and correlation coefficient were conducted using the procedure for randomized complete block design in SAS (9.1 version). The phenotypic and genotypic coefficients of variation were estimated according to the method suggested by Burton and De Vane (1953). Broad sense heritability (H^2) expressed as the percentage of the ratio of the genotypic variance ($\sigma^2 g$) to the phenotypic variance ($\sigma^2 p$) as described by Allard (1960):

$$H^2 = \left[\frac{\sigma^2_g}{\sigma^2_p}\right] x100$$ Where,

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

Genetic advance under selection (GA) refers to the improvement of traits in genotypic value for the new population as compared with the base population under one cycle of selection at a given selection intensity (Singh, 2001). Genetic advance in absolute unit (GA) and Genetic advance in percent of the mean (GAM), was estimated in accordance with the methods illustrated by Johnson *et al.* (1955):

$GA = K\ \sigma_p H^2$

$GAM = (GA/\bar{x}) \times 100$ Where, k = the standardized selection differential at 5% selection intensity (K = 2.063).

σ_p = Phenotypic standard deviation

H^2 = Broad sense heritability

$\bar{x}$ = grand mean of a character

3. Results and Discussion

The analyses of variances revealed significant variations among the genotypes for all the characters examined with the exception of effective tiller per plant and panicle length (Table 1). The results show the presence of adequate variability which can be important for selection of the preferred genotype in rice breeding programme. Similar results were accounted by Surek and Beser (2003); Osman *et al.* (2012) and Mulugeta *et al.* (2012).

The maximum yield was obtained from the genotype AD-48 (3399.5 kg/ha) followed by AD-01 (3396 kg/ha) and AD-12 (3277.9 kg/ha) (Table 2). The highest grain yield performance of the genotypes could be attributed to plant height, number of fertile tillers per plant, spikelet per panicle, filled grain per panicle and biomass yield.

3.1. Phenotypic and Genotypic Coefficients of Variation

The phenotypic variance was separated into genotypic and environmental variances to estimate the contribution of each to the total variation. The minimum (3.34) and maximum (19.31) values of phenotypic coefficient of variation (PCV) were observed on biomass yield and days to maturity, respectively (Table.3).

Table 1. Analysis of variance (ANOVA).

Source of Variation	df	DH	DM	PL	PH	FTPP	SPP	FGPP	TSW	BY	GY
Replication	2	3.08	0.58	0.22	5.84	0.01	2.89	1.33	0.002	743361.83	84527.24
Genotype	11	44.09**	64.91**	2.41NS	95.51**	0.56NS	59.48*	33.76*	24.02**	3523820.64**	366650.02**
Error	22	5.63	0.79	1.73	22.31	0.49	24.58	12.58	0.23	535134.09	100109.56
CV		2.36	0.63	8.54	6.56	12.08	6.29	5.03	1.81	11.42	11.03

** = significant at P<0.05; ** = significant at P<0.01; NS = non significant. DH = Days to 50% heading, DM = Days to 75% maturity, PL = panicle length (cm), PH = Plant height (cm), FTPP = Number of fertile tiller per plant, SPP = Number of spikelet per panicle, FGPP = Number of filled grains per panicle, BY = Biomass yield (kg/ha), TGW = 1000 grain weight (g), GY = grain yield (kg/ha).*

Table 2. Agronomic performance of the tested upland rice genotypes.

Genotype	DH	D M	PL	PH	FTPP	SPP	FGPP	TSW	BY	GY
Kokit	94.33	138.33	14.97	65.37	5.67	72.07	67.03	30.33	5600.00	2895.82
Hedase	96.33	138.00	16.30	64.40	5.53	80.53	72.87	26.00	6000.00	2667.29
Superica-1	102.67	139.33	14.90	70.70	6.00	78.63	68.80	23.00	6511.11	2620.11
NERICA-12	104.00	140.00	14.50	73.73	5.77	76.60	68.33	28.67	5000.00	2447.46
Getachew (AD01)	106.67	147.67	16.73	78.00	6.50	91.80	80.53	25.00	8222.22	3396.15
NERICA-13	98.00d	137.67	16.00	77.87	5.37	77.83	70.80	31.00	6000.00c	2928.33
Andasa (AD012)	103.67	148.67	16.10	78.03	6.20	83.53	72.90	23.73	7666.67	3277.86
NERICA- 4	99.33	137.33	13.97	67.07	5.93	78.10b	69.23	25.00	6266.67	2691.98
FOFIFA-3737	101.00	139.33	14.57	69.90	5.63	77.30	69.60	30.00	5888.89	2339.67
NERICA-3	99.33	138.33	14.63	65.73	5.43	83.20	74.93	25.00	5555.55	2769.02
Tana (AD-048)	104.33	149.67	15.67	80.30	6.43	83.67	73.73	23.67	8333.33	3399.53
NERICA-10	96.33	138.67	16.27	71.57	5.07	76.17	67.87	27.67	5822.17	2977.62
P level	**	**	NS	**	NS	*	*	**	**	**
LSD value	4.0174	1.5102	2.226	7.9977	1.1855	8.3968	6.008	0.8146	1238.70	535.76
CV	2.36	0.63	8.54	6.53	12.08	6.29	5.03	1.81	11.42	11.03
SE±	0.69	0.76	0.22	1.11	0.11	0.97	0.71	0.46	203.19	71.29

** = significant at P<0.05; ** = significant at P<0.01; NS = non significant, CV = coefficient of variation, SE = standard error, P = probability level, LSD = least significance difference, DH = Days to 50% heading, DM = Days to maturity, PL = panicle length, PH = Plant height (cm), FTPP = Number of fertile tiller per plant, SPP = Number of spikelet per panicle, FGPP = Number of filled grains per panicle, BY = Biomass yield (kg/ha), TGW = 1000 grain weight (g), GY = grain yield (kg/ha).*

The PCV values for number of fertile tillers per plant, thousand grain weight, biomass yield and grain yield were medium. It indicated the phenotypic difference between the tested rice genotypes with the above traits is moderate. In agreement with the present results, Akinwale *et al.* (2011) also reported medium PCV for thousand grain weight and number of fertile tillers per plant.

Days to 50% heading, days to 75% maturity, panicle length, plant height, number of spikelet per panicle and number of filled grains per panicle had low PCV values. This result is in conformity with the finding of Akinwale *et al.* (2011), who observed low PCV for days to 50% heading, days to maturity, plant height and panicle length. Selvaraj *et al.* (2011) also observed low PCV for days to 75% maturity and panicle length.

Genotypic coefficient of variation measures the variability of any character. The extent of the environmental influence on any character is indicated by the magnitude of the differences between the genotypic and phenotypic coefficients of variation. Large differences reflect high environmental influence, while small differences reveal high genetic influence (Akinwale *et al.,* 2011)

Genotypic coefficient of variability (GCV) values were low for days to 50% heading, days to 75% maturity, panicle length, plant height, number of fertile tillers per plant, and number of spikelet per panicle. Similar results have been reported by Chakraborty and Chakraborty (2010) for days to 50% heading and panicle length. Likewise Selvaraj *et al.* (2011) for panicle length and days to maturity and Akinwale *et al.* (2011) for days to 50% heading, days to 75% maturity, panicle length, plant height and number of fertile tillers per plant. In contrast to the present study, Akinwale *et al.* (2011) reported low GCV for thousand grain weight and Selvaraj *et al.* (2011) observed high GCV for number of fertile tillers per plant. The difference between this finding and the previous findings may be related to environmental factors in which experiments conducted and genotypes used are quite different.

Medium GCV was observed for thousand grain weight, biomass yield and grain yield. It pointed out the possibility of yield improvement through selection of these traits. Similar results have been reported for thousand grain weight by Kumar and Saravanan (2012) and for grain yield by Akinwale *et al.* (2011). On the other hand Selvaraj *et al.* (2011) observed high GCV for grain yield.

The difference between PCV with the corresponding GCV values was relatively higher for number of fertile tillers per plant, panicle length, grain yield, biomass yield and number of spikelet per panicle, indicating the higher influence of the environment on the traits. However, this difference was comparatively low for days to 75% maturity, thousand grains weight, days to 50% heading, filled grains per panicle, and plant height. The small difference indicating that there is a minimal influence of environment on the expression of these traits. In addition, it indicates the presence of sufficient genetic variability for observed traits may facilitate the selection process (Yadav *et al.*, 2011). Therefore, selection based on phenotypic performance of the traits would be effective to bring considerable improvement in these traits.

3.2. Heritability and Genetic Advance

Broad sense heritability estimates ranged from 5.04 for number of fertile tillers per plant to 97.17% for thousand grain weight (Table 3). High broad sense heritability estimates observed for thousand seed weight, days to 75% maturity, days to 50% heading and biomass yield (kg/ha). Similar results have been reported by Yadav *et* al. (2011) for days to maturity; Akinwale *et al.* (2011) for days to 50% heading and days to maturity; Osman *et al.* (2012) for thousand grain weight. Whereas, Yadav *et al.* (2011) found low heritability for thousand grain weight, Akinwale *et al.* (2011) and Osman *et al.* (2012) found high heritability for plant height quite the opposite of the present result. High heritability values pointed out that the traits under study are less influenced by the environment in their expression (Babu *et al.,* 2012). Therefore, selection can be effective on the basis of phenotypic expression of these traits in the individual plant by implementing simple selection methods.

Medium broad sense heritability estimates were observed for plant height, grain yield (kg/ha), number of filled grains per panicle and number of spikelet per panicle, which indicates the possibility of using these traits in rice improvement programs, but the expressions could be influenced by the environment. A similar result has been observed by Mulugeta *et al.* (2012) for grain yield. The moderate heritability estimate for grain yield could be attributed to the fact that yield is a complex trait and is controlled by many genes (Osman *et al.*, 2012).

Table 3. Estimates of range, mean, phenotypic and genotypic variances, broad sense heritability, and genetic advance in 12 upland rice genotypes.

Trait	Range	Mean	σ2p	σ2g	σ2e	PCV (%)	GCV (%)	H² (%)	GA	GA (%)
DH	94.33_106.67	100.50	18.45	12.82	5.63	4.27	3.56	69.49	6.16	6.13
DM	137.33_149.67	141.08	22.17	21.37	0.80	3.34	3.28	96.41	9.36	6.64
PL	13.97_16.73	15.38	1.96	0.23	1.73	9.09	3.11	11.75	0.33	2.15
PH	64.40_80.30	71.89	46.71	24.40	22.31	9.51	6.87	52.24	7.37	10.25
FTPP	5.07_6.50	5.79	0.52	0.03	0.49	12.39	2.78	5.04	0.07	1.21
SPP	72.07_91.80	78.83	36.22	11.63	24.59	7.63	4.33	32.11	3.99	5.06
FGPP	67.03_80.53	70.48	19.65	7.06	12.59	6.29	3.77	35.93	3.29	4.66
TSW	23.00_31.00	26.59	8.16	7.93	0.23	10.74	10.59	97.17	5.73	21.53
BY	5000.00_8333.33	6405.55	1531363.00	996228.85	535134.09	19.31	15.58	65.06	1660.81	25.93
GY	2339.67_3399.53	2867.57	188956.42	88846.82	100109.56	15.16	10.39	47.02	424.65	14.81

DH = Days to 50% heading, DM = Days to maturity, PL = panicle length, PH = Plant height (cm), FTPP = Number of fertile tiller per plant, SPP = Number of spikelet per panicle, FGPP = Number of filled grains per panicle, BY = Biomass yield (kg/ha), TGW = 1000 grain weight (g), GY = grain yield (kg/ha), phenotypic (σ^2p) genotypic (σ^2g) and environmental (σ^2e) variances, phenotypic (PCV) and genotypic (GCV) coefficient of variability, broad sense heritability (H²), expected genetic advance (GA) and genetic advance as percent of the mean (GA%).

Low broad sense heritability was observed for number of fertile tillers per plant and panicle length, which indicates high influence of the environment on these traits. Similar results have been reported by Akhtar *et al.* (2011) and Akinwale *et al.* (2011) for number of fertile tillers per plant. The low heritability recorded for these traits indicates that direct selection for these traits will be ineffective.

Estimation of GA for grain yield was 424.68 kg/ha, indicating that when ever we select the best 5% high yielding genotypes as parents, mean grain yield of progenies could be improved by 424.68 kg/ha for the first cycle, that is, the mean genotypic value of the new population for grain yield will be improved from 2867.57 kg/ha to 3292.25 kg/ha.

The Genetic advance as percent of the mean (GAM) at 5% selection intensity was high for biomass yield per hectare followed by thousand grain weight. A similar result has been reported by Singh *et al.* (2011) for biomass yield. However, the authors observed high genetic advance for plant height and number of spikelet per panicle in contrast to the results of this study. Genetic advance as per cent of the mean (GAM) at 5% selection intensity was medium for plant height and grain yield. This result agrees with the finding of Akinwale *et al.* (2011); Babu *et al.* (2012) and Mulugeta *et al.* (2012).

Genetic advance as percent of the mean (GAM) at 5% selection intensity was low for number of fertile tillers per plant, panicle length, number of filled grains per panicle, number of spikelet per panicle, days to 50% heading and days to 75% maturity. Similar results have been reported by Akinwale *et al.* (2011) for number of fertile tillers per plant*;* Babu *et al.* (2012) forpanicle length and Mulugeta *et al.* (2012) for days to 75% maturity, panicle length, number of fertile tillers per plant and number of spikelet per panicle. However, this result is quite different comapare to the results of Nandan *et al.* (2010) for number of fertile tillers per plant and number of spikelet per panicle.

In view of the fact that, high heritability does not always indicate a high genetic gain, heritability should be used together with genetic advance in predicting the ultimate effect for selecting superior varieties (Ali *et al.*, 2002). In this study, high heritability and high genetic advance were recorded for thousand grain weight and biomass yield which could be considered as an essential traits for upland rice improvement by selection. Similar results have been reported by Shukla *et al.* (2005); Nandan *et al.* (2010); Sravan *et al.* (2011) and Toshimenla and Sapuchangkija (2013).

Moderate heritability with moderate genetic advance was recorded for the traits like plant height and grain yield. On the other hand, high heritability estimates with low genetic advance observed for days to heading and days to maturity. Similar results have been reported for days to maturity by Karim *et al.* (2007) and Akinwale *et al.* (2011).

Low heritability with low genetic advance was observed in the traits like panicle length and number of fertile tillers per panicle, this result agrees with the work of Chakraborty and Chakraborty (2010) for panicle length and Mulugeta *et al.* (2012) for number of fertile tillers per panicle.

3.3. Correlation

Among the tested parameters days to 75 % maturity (r = 0. 59**), plant height (r = 0.56**) and biomass yield (r = 0.76**) had a positive and highly significant correlation with grain yield. Panicle length (r = 0.39*), number of fertile tillers per plant (r = 0.34*), number of spikelet per panicle (r = 0.35*) and number of filled grains per panicle (r = 0. 39*) had a positive and significant correlation with grain yield. The positive and significant correlation indicates a strong association of these traits with grain yield. In addition to positive and significant correlation, high to moderate heritability is necessary for the indirect selection to enhance grain yield. Days to 75% maturity, plant height, number of spikelet per panicle number of filled grains per panicle and biomass yield reviled positive and significant correlation with high to moderate heritability. Therefore, a selection of these traits is very important in improving grain yield for upland rice. This result is in agreement with the result of Akhtar *et al.* (2011) and Akinwale *et al.* (2011). In contrast to present result, Hairmansis *et al.* (2010) reported a negative correlation between plant height and grain yield.

Table 3. Phenotypic correlation coefficients between pairs of traits studied in 12 upland rice genotypes.

TRAIT	DH	D M	PL	PH	FTPP	SPP	FGPP	TSW	BY	GY
DH	1.00									
DM	0.67**	1.00								
PL	0.043	0.29	1.00							
PH	0.39*	0.62**	0.16	1.00						
NFT	0.32	0.46**	0.28	0.27	1.00					
SPP	0.52**	0.55**	0.24	0.31	0.21	1.00				
FGPP	0.33*	0.41	0.12	0.17	0.11	0.88**	1.00			
TSW	-0.46**	-0.51**	-0.07	-0.14	-0.35*	-0.35*	-0.22	1.00		
BY	0.43**	0.74**	0.34*	0.63**	0.49**	0.47**	0.36*	-0.55**	1.00	
GY	0.18	0.59**	0.39*	0.56**	0.34*	0.35*	0.39*	-0.31	0.76**	1.00

** = significant at P<0.05 and ** = significant at P<0.01. DH = Days to 50% heading, DM = Days to maturity, PL = panicle length, PH = Plant height (cm), FTPP = Number of fertile tiller per plant, SPP = Number of spikelet per panicle, FGPP = Number of filled grains per panicle, BY = Biomass yield (kg/ha), TGW = 1000 grain weight (g), GY = Grain yield (kg/ha).*

4. Conclusion

In general, the study showed variation for almost all the traits studied among the tested rice genotypes, which is an indication of the presence of sufficient variability that can be exploited through selection. In the broad sense heritability, genetic advance and correlation analysis of the study revealed that plant height, number of spikelet per panicle, number of filled grains per panicle, and biomass yield were the most important yield component traits. From this result, genotypes AD-048, AD-01 and AD-012 can be suggested for commercial production in the upland ecosystem of Fogera district and other similar areas in the region.

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Influence of Mineral Nitrogen and Potassium Fertilizers on Ware and Seed Potato Production on Alluvial Soil in Eastern Ethiopia

Simret Burga*, Nigussie Dechassa, and Tekalign Tsegaw

*Haramaya University, Department of Plant Sciences, P. O. Box 138, Dire Dawa, Ethiopia

Abstract: Potato (*Solanum tuberosum* L.) is an important food security and cash crop in Ethiopia. However, the yield of the crop is low in the country due to a number of factors among which poor soil fertility management is a major one. Therefore, a field experiment was conducted on the main campus of Haramaya University in the 2009/10 cropping season to elucidate the effect of mineral nitrogen and potassium fertilizers on growth and tuber production of the crop. The treatments consisted of five rates of nitrogen (0, 50,100,150, and 200 kg N ha^{-1}) and three rates of potassium (0, 100, and 200 kg K_2O ha^{-1}). The experiment was laid out as a randomized complete block design in a factorial arrangement and replicated three times per treatment. The results of the experiment revealed that nitrogen had significant main effects on all parameters except tuber specific gravity whilst potassium did not influence any of the parameters studied. The maximum marketable ware potato tuber yield (21.4 t ha^{-1}) was obtained in response to the application of 100 kg N ha^{-1}. However, the highest yield (12.7 t ha^{-1}) and number (5.2 tubers $hill^{-1}$) of medium-sized tubers, which are appropriate for planting as seed, were attained at the rate of 200 kg N ha^{-1}. Thus, it could be concluded that the rate of nitrogen fertilizer required to enhance seed tuber production was found to be higher than that required to optimize ware potato production, and potassium application was not necessary to produce the crop.

Keywords: Soil properties; *Solanum Tuberosum* L.; Tuber number; Tuber Size Distribution; Tuber Yield

1. Introduction

Potato (*Solanum tuberosum* L.) is important for household food security and income generation for smallholder farmers in Ethiopia (Gildemacher *et al.*, 2009; Abebe *et al.*, 2010). However, the annual production of the crop in the country is low (about 525,657 t) (FAOSTAT, 2010). The national average yield of the crop in the country ranges only between 8 to 10 t ha^{-1} (Haverkort *et al.*, 2012). There are several causes for the low yield of the crop in the country among which depleting soil fertility, poor agronomic practices, and diseases and pests are the main ones (Gildemacher *et al.*, 2009).

A non-site-specific generic (blanket) recommendation of 111 kg N ha^{-1} and 90 kg P_2O_5 ha^{-1} [(165 kg urea and 195 kg diammonium phosphate (DAP) ha^{-1}] has been promoted for potato production in Ethiopia for a long time (EIAR, 2004). There are also no any site-specific mineral and organic fertilizer recommendations to optimize the yield of the crop. Consequently, like most smallholder farmers in other sub-Saharan African countries, farmers in Ethiopia use low rates of fertilizers for producing crops possibly due to prohibitively high prices (Bekunda *et al.*, 2010). They also apply low amounts of organic fertilizers owing to competing needs such as the use of cow dung and crop residues as a source of energy for cooking, construction, animal feed etc (Morris *et al.*, 2007; Wogi *et al.*, 2015). The application of low rates of fertilizers in sub-Saharan Africa may also be attributed to lack of knowledge as to which kinds and rates of fertilizers are recommended for the specific crops, soils, and agro-climatic conditions (Vlek, 1990).

Gildemacher *et al.* (2009) reported that the amounts of FYM, nitrogen, and phosphorus applied to the potato crop by smallholder potato farmers in the central highlands of Ethiopia averaged only 3.0 t ha^{-1}, 30.6 kg N ha^{-1}, and 33.4 kg P (76 kg P_2O_5) ha^{-1}, respectively. This indicates that potatoes are grown in the country under sub-optimal rates of nitrogen and phosphorus application. Therefore, nutrient deficiencies are very common in potato production in the country due to application of no or low rates of fertilizers including manure by potato farmers (Gildemacher *et al.*, 2009; Haverkort *et al.*, 2012).

Potato is a heavy feeder of potassium, nitrogen, and phosphorus. Potassium and nitrogen are found in the largest amounts in a potato plant, followed by Calcium (Ca) and Magnesium (Mg) (Westermann, 2005). On the other hand, the low root nutrient uptake efficiency of the crop (Perrenoud, 1983; Dechassa *et al.*, 2003) may exacerbate the problem of nutrient deficiencies in potato production. Therefore, ample application of fertilizers containing the major plant nutrients (N, P, and K) is required to obtain sufficient yield of the crop. Nitrogen and phosphorus are deficient in most areas of Ethiopia (Murphy, 1968). Research done on fertilizer requirements of potato on major soils of Eastern Hararghe Zone revealed significant effects of nitrogen and phosphorus on the yield of the crop. Beyene (1998) reported that application of 87 kg N ha^{-1} and 90 kg P_2O_5 ha^{-1} was required for optimum yield of the

*Corresponding author. E-mail: simretburga@yahoo.com

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potato crop on black soil in Haramaya district in eastern Ethiopia. Zelalem *et al.* (2009) reported that application of 138 kg N and 46 kg P_2O_5 ha^{-1} was necessary for obtaining optimum yield of potato on black soil of Debre Berhan in central Ethiopia. Balanced nutrient application is also important in crop production. For example, without application of phosphate and potassium, the yield response to increasing levels of nitrogen is smaller than when adequate amounts of P and K were applied (Mengel *et al.*, 2001).

The other major constraint to increased potato production is lack of good quality seed tubers. Agronomic practices of a seed potato crop are different from those of ware potatoes. Seed tuber production should be aimed at a high rate of multiplication, high yield of seed-sized tubers, and maintenance of healthy seed tubers that have optimum physiological quality (Lung'aho *et al.*, 2007).

Since potato is propagated vegetatively mostly using seed tubers, the quantity of planting material (tubers) required to plant a unit area of land is important. Therefore, it is not only the total tuber yield that matters in seed tuber production but also the size as well as the number of tubers produced. Small tubers have fewer eyes and produce only a few stems and larger tubers whilst large tubers have many eyes and produce a number of stems that would produce too many tubers, which may grow under stiffer competition for growth factors and become too small and unmarketable for use as either ware or seed potato. Besides, large tubers are too bulky and uneconomical to use as seed or to transport. Therefore, the best seed tubers are the ones that are medium-sized (39-75 g) (Lung'aho *et al.*, 2007). Fertilizer application may affect tuber size distribution of potato (Rosen and Bierman, 2008), and may influence ware as well as seed tuber production.

This study was, therefore, initiated with the objective of elucidating the effect of applying mineral nitrogen and potassium fertilizers on ware and seed potato production.

2. Materials and Methods

2.1. Description of the Experimental Site

The experiment was conducted at the crop research field on the main campus of Haramaya University. The site is located at 9°24'N latitude and 42°03'E longitude. The altitude of the site is 2006 meters above sea level. The site has a bimodal rainfall distribution and is representative of a sub-humid mid altitude agro-climatic zone. The short rainy season extends from March to April and constitutes about 25% of the annual rainfall whereas the long rainy season extends from June to October and accounts for about 45% of the total rainfall (Belay *et al.*, 1998). The mean annual rainfall and temperature are 760 mm and 17°C, respectively. However, during this cropping season, the mean monthly maximum and minimum temperatures and rainfall at the experimental area (July to November) were 23.72°C, 10.6°C, and 105.46 mm, respectively. Thus, the rainfall was particularly low during this period. During the previous cropping season, wheat was grown at the site. The trial was conducted under rain-fed condition. The soil of the experimental site is an alluvial deposit with sandy loam texture.

2.2. Materials Used in the Experiment

Plan Material

A potato variety named Badhasa was used as a planting material. The variety was released by Haramaya University in 2001, and is being grown as one of the improved potato varieties in eastern Ethiopia. It is a variety that requires about 90 to 100 days to mature.

Fertilizer Material

Urea (CO ($[NH_2]_2$) (46% N) and potassium sulphate (K_2SO_4) (52% K_2O) were used as sources of nitrogen and potassium, respectively. Tri-superphosphate $(CaH_2PO_4)_2$, which constitutes 46% P_2O_5, was used as a source of phosphate.

2.3. Treatments, Experimental Design, and Procedures

The treatments consisted of five rates of nitrogen (0, 50, 100, 150, and 200 kg N ha^{-1}) and three rates of potassium (0, 100, and 200 kg K_2O ha^{-1}). The experiment was laid out as randomized complete block design in a factorial arrangement and replicated three times per treatment. The size of a plot was 4.5 m x 3.6 m (16.2 m^2). A distance of 1 m between plots and 2 m between blocks was kept.

On 23 July 2010, uniform and medium-sized (39-75 g) tubers of the test variety with sprout lengths of 1.5 to 2.5 cm (Lung'aho *et al.,* 2007) were planted on ridges with inter-and intra-row spacing of 75 cm and 30 cm, respectively. Plants in the border row at each side of a plot and one plant at the end of each row were left out from data recording to avoid edge effects. The entire rates of potassium and 1/3rd of the rate of nitrogen fertilizer were applied at the time of planting in the form of potassium sulphate (52% K_2O) and urea (46%N), respectively, according to the specified treatments. The remaining N fertilizer was applied in two equal splits 38 days after planting and at the start of flowering of the plant. Phosphorus was applied uniformly to all plots as triple super phosphate at the rate of 46% kg P_2O_5 at the time of planting.

All the recommended cultural practices were followed to raise the crop. Harvesting was done at physiological maturity. However, before harvesting, the haulms of the potato plants were mowed 15 days earlier to toughen the tuber periderm so as to pre-empt the likelihood of bruising and skinning during harvesting. For determining certain yield components and growth parameters plant samples were taken randomly from the middle rows. To determine tuber yield, the entire plants were harvested from the middle rows on 23 December 2010.

2.4. Soil Sampling and Analysis

Soil samples were taken randomly in a W-shaped pattern from the entire experimental field. The samples were composited and replicated three times for determining physico-chemical properties.

The soil was air-dried and sieved through a 2 mm sieve. Soil pH was determined from the filtered suspension of 1:2.5 soils to water ratio using a glass electrode attached to a digital pH meter (Page, 1982). Texture of the soil was determined by the sedimentation method. Organic carbon was determined by the method of Walkley and Black (1934). Total nitrogen was determined using the Kjeldhal method (Jackson, 1975). Available phosphorus was determined by extraction with 0.5 M $NaHCO_3$ according to the methods of Olsen *et al.* (1954). Exchangeable potassium was extracted with 1 N ammonium acetate according to Hesse (1971) and determined using a flame photometer. Sulphur content of the extract was measured by the turbidmetric method as described by Okalebo *et al.* (2002) using a spectrophotometer.

2.5.Data Collection and Measurement

Data on total biomass were determined from 10 plants randomly sampled from each plot just at physiological maturity. Shoot dry mass was determined by oven-drying the fresh shoot biomass at 65 ^{0}C to a constant weight. Harvest index was determined as the ratio of fresh tuber mass to the fresh biomass yield at physiological maturity. Specific gravity of tubers (g cm^{-3}) was determined by the weight in air /weight in water method (Kleinkopf *et al.*, 1987). Seed tuber categories were identified as small (< 39 g); medium (39-75 g); and large (>75 g) (Lung'aho *et al.*, 2007). A healthy tuber weighing more than 20 g was considered marketable while rotten, diseased, insect-attacked, shrivelled, and deformed tubers and those having undersized tubers (less than 20 g) were categorized as unmarketable. Total tuber yields and numbers were recorded as the sum of marketable and unmarketable tuber yields and numbers, respectively. Medium-sized potato tubers (39-75 g) were categorized as seed tubers. To determine the tuber dry matter content (%), five potato tubers were randomly selected from each plot, chopped into small 1-2 cm cubes, mixed thoroughly, and two fresh sub-samples each weighing 200 g were weighed. Each sub-sample was placed in a paper bag and put in an oven until a constant dry weight was attained at 70^{o}C. Each sub-sample was immediately weighed and the mean recorded as dry weight. Percent dry matter content for each sub-sample was calculated based on the formula described by Bonierbale *et al.* (2006).

$$\text{Dry matter (\%)} = \frac{\text{Weight of sample after drying (g)}}{\text{Initial weight of sample (g)}} \text{ x } 100$$

2.6. Data Analysis

The data were subjected to analysis of variance using SAS statistical software (SAS, 2002) version 9.1. All significant pairs of treatment means were compared using the Least Significant Difference (LSD) test at 5% level of significance.

3. Results and Discussion

3.1. Physico-Chemical Properties of the Experimental Soil

The results of the soil analysis before planting are shown in Table 1. The textural analysis showed that the soil is sandy loam. The cation exchange capacity (CEC) of the soil is high according to the rating of Landon (1991). Therefore, there could be no limitation to the growth of the potato crop in terms of this soil chemical property. The pH of the experimental soil is moderately alkaline according to the rating of Murphy (1968) and that of Tekalign Tadese (1991). Potatoes can grow under a wide range of soil pH varying from neutral to alkaline reaction (Jadhav and Kadam, 1998; Fageria, 2011). However, the optimum soil pH for growing the crop ranges from 5.0 – 6.5 (Brown and McLean, 1984), which varies from very strongly acidic to slightly acidic reaction. This shows that the study site is stressful for potato growth due to the high soil pH. High soil pH has unfavourable effect on availability of nutrients such as phosphorus, which becomes precipitated in the form of calcium and magnesium phosphates (Holford, 1997). Alkaline soils also favour potato skin diseases such as common scab (*Streptomyces scabies*) (Jadhav and Kadam, 1998). Therefore, both potato yield and quality are likely to be negatively affected by the moderate alkalinity of the soil in the study area.

The organic carbon content of the soil is low according to the rating of Tekalign Tadese (1991), who categorized soil organic carbon contents of below 0.5, 0.5-1.5, 1.5-3.0, and >3.0% as very low, low, medium, and high, in the order mentioned here. Murphy (1968) categorized total soil nitrogen contents of below 0.10, 0.10-0.15, 0.15-0.25, and >0.25% as low, medium, high, and very high, respectively and Tekalign Tadese (1991) similarly categorized total soil nitrogen contents of below 0.05, 0.05-0.12, 0.12-0.25, and >0.25% as low, medium, high, and very high. Accordingly, the total nitrogen content of the experimental soil is medium. This shows that the soil is moderate in supplying nitrogen through mineralization during the cropping season for uptake by crops (Murage *et al.*, 2000). This signifies that external application of nitrogen and organic fertilizer is important for enhancing the fertility of the soil and yield of the crop. The available phosphorus content of the soil is high in accordance with the rating of Cottenie (1980) and Holford and Cullis (1985), who categorized available Olsen phosphorus contents of below 5, 5-10, 10-17, 17-25, and > 25 mg kg $soil^{-1}$ as very low, low, medium, high, and very high in the order listed here. This shows that application of phosphorus fertilizer is not required as a

regular fertilizer practice but just to replace offtake of the nutrient in the harvested potato crop (DEFRA, 2009). Landon (1991), FAO (2006), and Hazelton and Murphy (2007) categorized exchangeable soil potassium contents of 0 - 02, 0.2 - 0.3. 0.3 - 0.7, 0.7 - 2.0, and > 2.0 $cmol_c$ kg $soil^{-1}$ as very low, low, medium, high, and very high. In accordance to these categories, the exchangeable potassium content of the experimental soil is almost in the high category. This means that potassium fertilizer is not be required at least in the short-term. However, in the long term, mineral and/or organic fertilizers containing potassium may be required due to possible depletion as a result of continuous cropping (Sarkar, 2014). According to the rating of Bashour (2007), sulphate sulphur ranging from 0 -10, 10-20, 20-35, 35-45, and > 45 mg kg $soil^{-1}$ is very low, low, medium, high, and very high, respectively, for availability to plants. Therefore, sulphate sulphur content of the experimental soil is high. Thus, there seems to be no requirement for application of sulphur from an external source.

Table 1. Mean values of the physico-chemical properties of the experimental soil on the campus of Haramaya University, Ethiopia.

pH_{-water}	CEC	Total N	OC	SO_4-S	Exchangeable K	Available P	Soil texture		
	($cmol_c$ kg $soil^{-1}$)	(%)	(%)	(mg kg $soil^{-1}$)	($cmol_c$ kg $soil^{-1}$)	(mg kg $soil^{-1}$)	Clay (%)	Silt (%)	Sand (%)
8.0	27.0	0.11	1.15	35.2	0.65	18.2	17.44	19.64	69.92

CEC = Cation exchange capacity; OC = Organic carbon; K = Potassium; N = Nitrogen; P = Phosphorus.

3.2. Effect of Nitrogen and Potassium on Total Biomass

The main effect of nitrogen significantly influenced total dry biomass yield of the crop, but not its harvest index (Table 2). However, potassium application did not affect these parameters. The increase in total biomass yield continued up to the highest level of N (200 kg N ha^{-1}) which was higher than the biomass yield obtained from plants in the control treatment by about 31%. This result is in conformity with the findings of Millard and Marshall (1986) who reported a significant increment in canopy dry matter yield of potato in response to increased nitrogen application. On the other hand, neither the main effect of nitrogen nor that of potassium had a significant influence on harvest index. Consistent with this result, Zelalem *et al.* (2009) also reported that nitrogen had no significant influence on harvest index of potato.

Table 2. Main effects of nitrogen and potassium on total biomass and harvest index of potato at Haramaya University during the 2009/10 cropping season.

Treatment	Parameter	
	Total Biomass (g $hill^{-1}$)	Harvest index
Nitrogen (kg N ha^{-1})		
0	658.72^{c}	0.78
50	683.89^{bc}	0.77
100	745.33^{b}	0.79
150	701.44^{bc}	0.78
200	864.07^{a}	0.78
F-test	***	ns
Potassium (kg K_2O ha^{-1})		
0	719.71	0.78
100	724.29	0.79
200	748.07	0.77
F-test	ns	ns
CV(%)	10.90	4.43

*Means sharing the same letter within a column are not significantly different at 5% level of significance; *** = significant at 0.001 probability level; ns = non-significant at 5% probability level; CV= coefficient of variation.*

3.3. Effect of Nitrogen and Potassium on Tuber Yields

Both the total and marketable tuber yields increased significantly ($P < 0.01$) in response to the increase in the rate of nitrogen application. However, both yields did not increase in response to increasing the rate of nitrogen beyond 100 kg ha^{-1} (Table 3). The total and marketable tuber yields of plants treated with 100 kg N ha^{-1} exceeded the total and marketable tuber yields of plants not supplied with nitrogen by about 33 and 26%, respectively. These increments were 19 and 35%, respectively, over plants treated with 50 kg N ha^{-1}. Corroborating these results, Mulubrhan (2005) and Zelalem (2009) reported highly significant increases in

total tuber yield in response to increased levels of nitrogen application.

Both the total and marketable tuber numbers increased significantly ($P < 0.05$) with the increase in the rate of nitrogen application. However, similar to the tuber yields, the increase in the tuber number in response to the increase in the rate of nitrogen application occurred only up to 100 kg N ha^{-1}. Thus, increasing the rate of nitrogen application from nil and 50 kg N ha^{-1} to 100 kg N ha^{-1} increased total tuber number by about 26 and 12%, respectively. Similarly, increasing the rate of nitrogen application from nil and 50 kg N ha^{-1} to 100 kg N ha^{-1} increased marketable tuber number by about 92 and 30%, respectively. In agreement with this finding, Sharifi (2005) reported a significant increase in tuber numbers in response increased rates of nitrogen application. Similarly, Jenkins and Mahamood (2003) observed that the number of tubers varied considerably as a result of N fertilization, and doubled when the rate of nitrogen was increased to higher levels. Consistent with the results of this study, Zelalem *et al.* (2009) also reported that total tuber yield was strongly associated with average tuber weight and total tuber number signifying that the increase in both tuber number and size substantially contributed to increased tuber yields. Concordant with this result, Kanzikwera *et al.* (2001) also reported that the number of tubers per plant and mean fresh tuber weight increased as a result of nitrogen application. In contrast, however, there are reports demonstrating the absence of strong relationship between rates of nitrogen application and tuber number in potato (Sharma and Arora, 1987).

The lack of response of the potato crop to the increased application of nitrogen from nil to 150 and 200 kg N ha^{-1} in terms of tuber production implies that higher rates of nitrogen than required by the plant may lead to growth of more shoot at the expense of tubers (Sommerfeld and Knutson, 1965). This result indicates that increasing N fertilizer more than 100 kg N ha^{-1} would be wasteful for ware potato production

Table 3. Main effects of nitrogen and potassium on potato tuber yield and number at Haramaya University during the 2009/10 cropping season.

Treatment	Parameter					
	Total tuber Yield (t ha^{-1})	Total tuber (No. $plant^{-1}$)	Marketable tuber yield (t ha^{-1})	Marketable tuber (No .$plant^{-1}$)	Unmarketable tuber yield (t ha^{-1})	Unmarketable tuber (No. $plant^{-1}$)
Nitogen (kg N ha^{-1})						
0	17.60^{c}	9.75^{b}	14.08^{b}	3.96^{b}	3.52^{a}	5.79^{a}
50	19.80^{bc}	10.96^{b}	15.84^{b}	5.86^{b}	3.96^{a}	5.10^{ab}
100	23.48^{a}	12.25^{a}	21.35^{a}	7.61^{a}	2.13^{b}	4.64^{b}
150	22.64^{ab}	11.52^{a}	20.48^{a}	7.30^{a}	2.16^{b}	4.22^{b}
200	21.98^{ab}	11.98^{a}	19.78^{a}	7.02^{ab}	2.20^{b}	4.96^{b}
F-test	**	*	**	*	*	*
Potassium (kg K_2O ha^{-1})						
0	20.33	10.87	15.99	6.23	4.34	4.64
100	21.13	10.82	16.60	6.27	4.53	4.55
200	21.87	11.42	16.89	6.06	4.98	5.36
F-test	ns	ns	ns	ns	ns	ns
CV(%)	16.74	9.26	21.27	23.26	29.94	18.84

*Means sharing the same letter within a column are not significantly different at 5% level of significance; ** = significant at 0.01 probability level; * = significant at 0.05 probability level; ns = non-significant at 5% probability level; CV= coefficient of variation; No. = number.*

The increase in the marketable tuber weight and number in response to the increased application of nitrogen could be attributed to the effect of the nutrient on enhancing leaf growth and leaf surface area. This would enhance the interception of photosynthetically active radiation by the leaves for production of carbohydrate, which would ultimately be partitioned to tubers. In line with this argument, Wilcox and Hoff (1970) reported that the positive effect of N fertilizer on potato growth and yield was rooted in its impact on promoting the number of tubers produced per plant, the average weight of tubers, and the establishment of optimum leaf area index and leaf area duration. In the present study, increasing the rate of nitrogen beyond 100 kg N ha^{-1} did not result in significant increases in total and marketable tuber yields. Reduction in yield due to supra-optimal N application could be ascribed to the phenomenon that extra N application often stimulates shoot growth at the expense of tuber initiation and bulking (Sommerfeld and Knutson, 1965). In agreement with this suggestion, Krauss and Marschner

(1971) reported that a larger and continuous supply of nitrogen to potatoes delays or even prevents tuberization.

In contrast to the total and unmarketable tuber yields and numbers, however, increasing the rate of nitrogen application decreased unmarketable tuber yields and numbers significantly ($P < 0.01$) (Table 3). However, the significant decrease occurred only up to 100 kg N ha^{-1}. Thus, from 100 kg N ha^{-1} onwards, the unmarketable tuber yields and numbers were all lower and in statistical parity. The higher unmarketable tuber yields and numbers at nil and 50 kg N ha^{-1} may be attributed to nitrogen deficiency, which often leads to poor growth and photosynthesis for accumulation of sufficient starch for tuber bulking.

Although unmarketable tubers may be controlled more importantly through manipulating other factors such as disease and insect-pest incidence, in this study, harvesting practice, etc. rather than mineral nutrition (Berga *et al.* 1994), nitrogen deficiency may have contributed to the development of at least very small tubers due to scarcity of photoassimilates for tuber enlargement and bulking.Thus, N deficiency may have enhanced the unmarketable tuber numbers and yield at the lowest and marginal levels of N supply. Therefore, it could be suggested that marketability of potato tubers could be improved through enhanced nitrogen application as well as disease and pest control.

In the present study, applying 100 kg N ha^{-1} resulted in optimum total tuber yield, marketable tuber yield, total tuber number, and marketable tuber number. Conversely, the markedly low unmarketable tuber yield and number obtained at 100 kg N ha^{-1} signify the superiority of this level of nitrogen for the production of optimum fresh potato tuber yield. The vigorous response of potato yield to nitrogen application could be attributed to the low native soil N, which is associated with the very low content of organic carbon and total nitrogen (Landon, 1991; Murage *et al.*, 2000). Under low organic carbon content of the soil, there is little nitrogen that may become available to plants in the form of nitrate or ammonium through mineralization during the growing season. In addition, nitrogen is lost through leaching during wet seasons and its deficiency would become severe (Mengel *et al.* 2001).

Potassium had no significant effect on any of the above-mentioned tuber characteristics (Tables 2, 3, 4, 5). It had also no significant interaction effect with nitrogen. Lack of response of potato to potassium application in terms of the aforementioned tuber characteristics is consistent also with the results of Mulubrhan (2005) who found a non-significant influence of the nutrient on potato tuber yields and yield components on pellic Vertisols of Mekelle in northern Ethiopia.

The average value of exchangeable potassium of the experimental soil just before conducting the experiment was found to be 0.65 $cmol_c$ kg $soil^{-1}$, which is equivalent to about 255 mg kg soil-1. This level of exchangeable potassium in the soil is sufficiently available for uptake by plants (FAO, 2006; Hazelton and Murphy, 2007) and may have led to the lack of response from the potato crop to the external application of the nutrient. This may also confirm the earlier report of Murphy (1968) which indicates that most soils in eastern Ethiopia have sufficient levels of available potassium for crop production.

3.4. Effect of Nitrogen and Potassium on Tuber Size Distribution

Yield of small-sized tubers decreased in response to increasing the rate of nitrogen application (Table 4). The results revealed a significantly lower yield of small-sized tubers at 200 kg N ha^{-1} than at 0, 50, and 100 kg N ha^{-1}.For example, the yield of small-sized tubers obtained from plants in the control treatment exceeded the yield obtained at the highest rate of N by about 35%.

Increasing the rate of nitrogen application also affected the number of small-sized tubers although the trend of increase or decrease in response to application of the fertilizer was not consistent. The smallest numbers of small-sized tubers were obtained at nil and 200 kg N ha^{-1}. For example, the number of small-sized tubers obtained at 50 kg N ha^{-1} was significantly higher than the number obtained at 200 kg N ha^{-1} by about 34.85%. The significant reduction in the number of small-sized tubers at the highest level of N supply may have evidently occurred at the expense of production of increased number and yield of large-sized tubers.

The yield and number of medium-sized tubers were significantly higher at 200 kg N ha^{-1} than at the other nitrogen rates. The increments in yield and number of medium-sized tubers in response to increasing the fertilizer from nil to at 200 kg N ha^{-1} amounted to 58% and 76%, respectively.

In line with the present results, Mulubrhan (2005) reported that higher rates of nitrogen (165 kg ha^{-1}) produced a significantly higher number of medium-sized tubers than the other tuber size categories.

Thus, the results of this study have revealed that cultivating potato by applying 200 kg N ha^{-1} on the experimental soil would yield a quantity of seed potato tubers that could plant over half more area of land (53%) than the quantity obtained at 100 kg N ha^{-1}.

The number and yield of large-sized tubers increased highly significantly ($P < 0.01$) at 100 and 150 kg N ha^{-1}, but decreased at the highest rate of nitrogen supply (200 kg N ha^{-1}) (Table 4). The increments in the yield of large-sized tubers at 100 and 150 kg N ha^{-1} were 101% and 119%, respectively, compared to the yields of large-sized tubers attained in the control treatment. Concurrent with this result, Rosen and Bierman (2008) reported that increased rates of phosphorus application significantly decreased the proportion of large and medium-sized tubers whilst increasing the proportion of small-sized tubers.In contrast to this result, however, Reddy and Rao (1968) and Sharma and Arora (1987) reported that increased application of nitrogen and

potassium increased the proportion of medium and large-sized tubers. Similarly, Sharma and Arora (1987) observed decreased number of small (less than 25 g), increased number of medium (25-75 g) and large (above 75 g) grade tubers with increase in the rate of nitrogen application from 0 to 250 kg N ha^{-1}.

Emergence, seedling vigour, subsequent plant growth and final yield are affected by seed tuber size. Although all sizes of seed potatoes can grow into a crop, seed growers should only plant tubers ranging from 39 to 75 g. Such tuber sizes produce optimum stem number (25-30 m^{-2}) since they have optimum number of eyes and lead to high yield. Since basic seed tubers are sold on a weight basis, planting large tubers (> 75 g) is usually expensive as more of them are required to plant a unit area. In addition, large tubers are also bulky to transport and handle during planting. On the other hand, small tubers have small number of buds and give rise to small number of stems resulting in lower vigour and yields. Therefore, potato seed producers should aim at producing medium-sized tubers (Lung'aho *et al.*, 2007). In this experiment, the highest number of medium-sized tubers was obtained at 200 kg N ha^{-1}. Therefore, for the purpose of seed tuber production, the rate of N fertilizer application should be about twice as much as the rate required for ware potato production. It can, thus, be suggested that production of seed potato or ware potato could be optimized by manipulating the rate of nitrogen application.

Table 4. Main effects of nitrogen and potassium on tuber size distribution at Haramaya University during the 2009/10 cropping season.

Treatment	Tuber size categories					
	Small tuber yield (t ha^{-1})	Small tuber No. $hill^{-1}$	Medium tuber yield (t ha^{-1})	Medium tuber (No. $hill^{-1}$)	Large tuber yield (t ha^{-1})	Large tuber (No. $hill^{-1}$)
Nitrogen (kg N ha^{-1})						
0	6.24^{ab}	6.20^{bc}	8.03^{d}	2.96^{d}	3.33^{c}	0.59^{c}
50	7.56^{a}	7.39^{a}	8.64^{cd}	2.98^{cd}	3.60^{c}	0.59^{bc}
100	6.07^{ab}	6.56^{ab}	10.72^{b}	3.40^{bc}	6.69^{ab}	2.29^{a}
150	5.76^{bc}	6.54^{ab}	9.60^{bc}	3.55^{b}	7.28^{a}	1.43^{a}
200	4.62^{c}	5.48^{c}	12.69^{a}	5.20^{a}	4.67^{bc}	1.30^{b}
F-test	*	*	***	***	.**	**
Potassium (kg K_2O ha^{-1})						
0	6.32	6.49	9.73	3.40	4.28	0.98
100	5.97	6.23	10.21	3.69	4.95	0.90
200	6.54	6.62	9.90	3.78	5.43	1.02
F-test	ns	ns	ns	ns	ns	..ns
CV(%)	23.32	14.95	17.94	16.54	25.26	29.83

** = significant at 0.05 probability level;** = significant at 0.01 probability level; *** = significant at 0.001 probability level; ns = not significant at 5% probability level. Means sharing the same letter within a column are notsignificantly different at 5% level of significance; CV= coefficient of variation; No. = number.*

3.5. Tuber Dry Matter Content and Specific Gravity

Tuber dry matter content was significantly lower in plots treated with 100 kg N ha^{-1}than the plots receiving other N application rates. The decrease in tuber dry matter yield (Table 5) at this level of N supply is consistent with the high total tuber yield and numbers obtained at 100 kg N ha^{-1}(Table 2). This could be attributed to the higher tissue water content of tubers growing optimally when nitrogen is sufficiently available. In agreement with this finding, Maier *et al.* (1994), found reduction in dry matter content when nitrogen and potassium rates were increased.

Table 5. Main effects of nitrogen and potassium on dry matter yield and specific gravity of potato tubers at Haramaya University during the 2009/10 cropping season.

Treatment	Tuber quality parameter	
	Dry matter content (%)	Specific gravity (gcm^{-3})
Nitrogen (kg N ha^{-1})		
0	25.02[a]	1.088
50	25.18[a]	1.091
100	24.25[b]	1.087
150	25.62[a]	1.086
200	25.25[a]	1.088
F-test	*	ns
Potassium (kg K_2O ha^{-1})		
0	25.18	1.086
100	24.72	1.090
200	25.28	1.087
F-test	ns	ns
CV(%)	3.52	0.74

** = significant at 0.05 probability level; ns = not significant at 5% probability level. Means sharing the same letter within a column are not significantly different at 5% level of significance; CV = coefficient of variation.*

On the other hand, potassium had no significant effect on tuber dry matter production (Table 5). Similarly, Patricia and Bansal (1999), Kanzikwera *et al.* (2001), and Mulubrhan (2005) demonstrated that application of potassium had no significant effect on tuber dry matter yield. The absence of change or decrease in tuber dry matter production in response to increased supply of potassium, which might result in luxury consumption of the nutrient, could be ascribed to accumulation of high water in the cells, which may dilute the dry matter yield (Maier *et al.*, 1994).In this study, application of both nitrogen and potassium fertilizers had no effect on the specific gravity of potato tubers.

4. Conclusion

The results of this experiment have revealed that increasing the rate of nitrogen fertilizer application increased the yield and most yield components of the crop. However, potassium did not affect the yield and yield components of the crop. Moderate rate of nitrogen supply (100 kg N ha^{-1}) resulted in optimum total and marketable tuber yields. However, medium-sized tubers (39-75 g), which are the appropriate-sized tubers for use as planting material, were produced in a significantly larger numbers and quantity at the highest rate of nitrogen supply (200 kg N ha^{-1}). This implies that enhanced production of seed potato tubers requires higher rates of nitrogen fertilizer application than production of ware potato. The results of this study have also revealed that application of potassium fertilizer is not required for potato production in the study area since the soil contains sufficiently available quantities of the nutrient for uptake by the plant. Therefore, for potato seed tuber production, farmers in the study area should apply higher rates of nitrogen fertilizer than the rate required for ware potato production. Furthermore, application of mineral potassium fertilizer is not required for potato production in the study area at least on a short-term basis. However, to make a conclusive recommendation, the results of this study should be confirmed by conducting similar research over locations and growing seasons in the region.

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