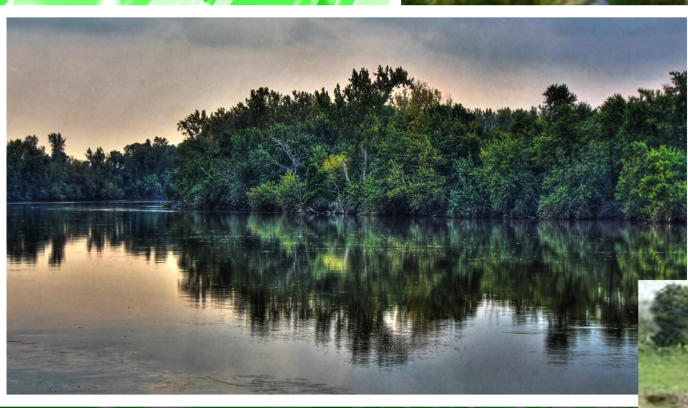
33rd Annual Research Proceedings

Theme I : Productivity and Environmental Sustainability for Food Security and Poverty Alleviation



Volume II

April 2016
Haramaya University

How to cite the proceedings

Wassu M, Jemal Y., Mengistu K., Nega A., Admikew H., Kidesena S., (eds.), 2015. "Proceedings of Annual Research Review Workshop", April, 2016, Haramaya University, Ethiopia

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1. Effect of *In-situ* Rainwater Harvesting Techniques on Soil Moisture Conservation and Grain Yield of Maize (*zea mays* L.) in Fedis District, Eastern Hararghe, Ethiopia

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Abstract: Inthe drier farming regions with arid and semi-arid environments, crop production is heavily dependent on rainfed agriculture and the main constraints of crop production are short growing period due to short term rainfall with high run-offand moisture deficit. In such areas, in-situ rainwater harvesting techniques are vital. This study was conducted under rain-fed conditions to investigate the effects of in-situ rainwater harvesting techniques (ridge furrow (RF), contour ridge (CR), and tied ridge (TR) on soil moisture conservation and grain yield of maize in Fedis district, which is one of the moisture stressed areas in Eastern Hararghe, Ethiopia. The experiment was conducted using spilt-plot design. Data collection on soil moisture content was conducted from three depths (0-20, 20-40 and 40-60cm) at three periods (viz. early, mid and late vegetative growth stages) during growing season. The yield and yield component data of maize were also collected. Moisture content means of 174.05mm/m, 170.9mm/m, 168.05mm/m and 115.65mm/m were observed for RF, CR, TR and FBF(control), respectively.The results showed that water harvesting techniques brought statistically significant effect on soil moisture conservation compared to control. In general, CR, TR and RF treatments resulted in 147.77 %, 145.31 % and 150.50 % more soil moisture conservation than FBP, respectively. The study also revealed that in-situ rainwater harvesting structures brought statistically significant effect on the yield of maize compared to the control. Tied ridge (TR) resulted in (130.55%), FR (126.23%) and CR (123.85%) higher grain yield compared to FBP on Melkasa maize variety, while TR resulted in 122.67%, FR (124.29%) and CR (138.31%) higher grain yield compared to FBP on BH maize variety. Therefore, in drier environment like Fedis in-situ rainwater harvesting techniques can be recommended for better moisture retention and subsequent crop production.

Keywords: In-situ rainwaterharvesting; soil moisture; contour ridge; tiedridge; ridgefurrow.

1. Introduction

Agriculture is the major economic activity for Sub-Saharan Africa countries, and it is strongly considered as the backbone of these countries' economic development and their people's wellbeing in the future (Giller *et al.*, 2009). Rapid population growth occurs in developing countries with a significant proportion still depending on a predominantly rainfed-based economy. Unfortunately, in several regions, including Africa in general and the Ethiopiain particular, rainfed agriculture as generally been associated to low yield levels, and high on-farm water losses. As result the majority of the people are not able to ensure their food security. Low crop productivity, food insecurity, hunger and malnutrition characterize poor rural smallholder agriculture based community (Bekele, 1998).

From 41% of semi-arid region of Sub-Saharan Africa farming land, only about 2% of the arable lands are irrigated, that is, rain fed agriculture is the dominant crop production system to meet the food demand (Zougmore *et al.,* 2002). However, the unreliability in rainfall and recurrent droughts lead to subsequent production failures and puts great pressure on the food self-sufficiency of the region. The low soil water retention capacity or the high potential evapotranspiration rate is the major problem.

Among the environmental problems people in eastern lowlands of Ethiopia are vulnerable to soil moisture stress problem and there have been notable droughts in this part of the country throughout human history (Tadesse *et al.*, 2008; UNEP, 2006; Gebre-Michael and Kifle, 2009). Some studies (Gebreyesus, 2012; McHugh *et al.,* 2007; Heluf, 2003; Aklilu and Mekiso, 2015). Except, Aklilu and Mekiso (2015) have been done on the effectiveness of micro-basin tillage to improve soil moisture in different parts of the semiarid areas in highlands of Ethiopia. However, the same problem (soil moisture stress) is happening in low lands of Eastern Hararghe. This indicates that there were less or no studies done to identify suitable in-situ rainwater harvesting techniques to solve crop production problem. Hence, this study was carried out in Fedis district, Eastern Hararghe zone of Ethiopia. The objective of this study was to determine the effect of in-situ rainwater harvesting structures on soil moisture conservations and grain yield of maize.

2. Materialsand Methods

2.1. Description of the Study Area

The field experiment was conducted during the main rainy season (May to December) of 2015 in eastern Ethiopia, at Fedis research sub- station of Haramaya University. Fedis is one of the woreda's of eastern Hararghe Zone in the semi-arid belt of the eastern low lands in the Oromiya regional state. The station is located west of Boko town in the semi-arid area of Fedis woreda. Climatically, the district is classified into Woinadega (15%) and kola (85%) agro climatic zones. The area is characterized by bimodal rainy seasons, "Belg" and "Meher". The "Belg" season is between March and May, and the second main rainy season is "Meher" which extends from July to October (Fedis Woreda Office of Agriculture). The site is situated at $9^0$07'N Latitude and $42^0$4'E

Longitude with an altitude of 1702 meters above sea level (GPS measurement). In the study area, the mean annual maximum and minimum temperature was 27.8^{0}C and 8.8^{0}C, respectively, and the area had annual rainfall of 714.3 mm (Fedis Agricultural Research Centre).

2.2. Treatments and experimental design

The experiment was conducted by using spilt-plot design with three replicates. Treatmentstestedon the main-plot were two maize varieties (Melkasa 4 and BH 661), and the sub plots consisted of contourridge (CR), tiedridge (TR), ridge furrow RF) and Fat bed planting (FBP), which is control. Recommended rates of N and P fertilizers were used. Buffer zones were left between plots and around the experiment area to facilitate crop management operations. Each plot consists of six (6) rows spaced 75cm between rows with row length of five meter. The spacing between plants within the row was 30 cm. Ridges with 20-30cm height; in CR, TR, and RF treatments of 0.75m spacing was constructed using a ridger implement. Cross earth ties in TR, 8-12cm in height, was manually constructed with hoes at 1.5m apart.So wing was done in the furrow.

2.3. Data Collection and Analysis

The soilmoisturecontent data was collected from three depths (0-20cm, 20-40cm and 40-60cm)at three periods(viz. early, mid and late vegetative growth stages) during growing season, and determined in the form of depth (mm) of water stored in the top 0.6 meters oil depth (assumed to be the depth of the root zone). The soil water stored (%) in each 0.2m incremental depth down to 0.6m was determined gravimetrically. It was then converted to water depth (mm) by multiplying by the specific bulk density values measured by the core sampler methods from the respective depths as described by Blake (1965). Grain yields and all other desirable data and samples were collected from the four central rows of each plot.

Statistical analysis was conducted for the collected data with the help of SAS software version 9.1. ANOVA was computed and mean differences were made by using least significant difference (LSD) at P=0.05. The results were presented by using tables, figures and text.

3. Result and Discussion

3.1. *In-Situ* Rain Water Harvesting Techniques and Soil Moisture Conservations

Amount of mean annual rainfall (mm) was measured duringthe experimentyear (2015) was 714.3mm. It waslessthanthe previous year (2014) mean annual rainfall, which was 926.5mm. Rainfall was measured at the Fedis meteorology station, which is 2Km far from the experiment site. Soil of the experimental site has sandy clay loam texture, moderate total nitrogen content(0.18%), low in organic matter(1.61 %), low organic carbon (0.93 %), low available phosphorus (1.78 mg Kg^{-1}), and moderately alkaline pH(7.76).

Soilmoisture content (SMC) of the soil profile (60cm) was measured at three periods, i.e. at early season, mid season and late season. The effects of the treatments on SMC are shown in Table 1.

Table 1. Treatments means for soil moisture conservations (mm/m) at three vegetative growth stages from three depths.

Treatments	Vegetative growth stages								
	Early			Mid			Late		
	0-20 cm	20-40 cm	40-60 Cm	0-20 cm	20-40 Cm	40-60 cm	0-20 Cm	20-40 cm	40-60 cm
TR	187.70[a]	197.75[a]	193.50[a]	185.00[a]	196.45[a]	172.05[a]	152.95[a]	157.80[a]	159.70[a]
FR	176.95[b]	177.45[b]	171.80[b]	166.55[b]	177.25[b]	163.30[a]	148.10[b]	155.95[a]	156.85[a]
CR	174.35[b]	176.30[b]	188.30[a]	175. 90[b]	190.10[a]	174.85[a]	144.35[b]	151.15[a]	154.65[a]
FBP	132.00[c]	134.10[c]	131.40[c]	123.35[c]	122.15[c]	111.95[b]	107.80[c]	115.95[b]	106.15[b]
LSD	8.10	14.90	12.25	10.70	9.30	14.25	4.70	10.85	18.50

LSD0.05 = least significant difference at 5% level and means followed by the same letter are not significantly different at P =0.05.

In all measurement depths at three vegetative growth stages, the results obtained showed significant ($P>0.05$) difference in SMC between *in-situ* water harvesting treatments and FBP. Where, *in-situ* water harvesting treatments (RF, TR and CR) recorded SMC values higher than FBP in all depths. This result is inagreement with the findings of Ibrahim (2008), Mohammed (2009), Li *et al.* (2000), Tian*et al.*(2003), and McHugh*et al.*(2007).

In this area, using of the conventional tillage method (FBP) may not help to conserve enough water for crop production, mainly due to the erratic rainfall that induces runoff. High intensity rain showers also enhance water losses through runoff. Crop growth conditions may further be hampered by a number of climatic factors suchas, low and erratic rainfall, low humidity levels, and high temperature during growing season (Botha*et al*,.2003).

The water harvested is retained and is far from the evaporative effects but within reach of plant roots. This is because of the presence of heavy textured soil at 40-60cm depth (sandy clay) than top 0-20cm (sandy clay loam). Lateral flow through which water harvested in the channels could benefit crops can only take place theoretically in the presence of a flow impeding layer at depth. This means water harvested in the channels feeds the soil until it reaches the impervious layer and starts flowing laterally or rising, thereby providing a reservoir of water to the crop at depth which on clays or heavy textured soils, rises by capillarity during dry spells and ensure the crop benefits.

3.2. Effect of *In-Situ* Rainwater Harvesting Techniques on Grain Yield

The effect of in-situ rainwater harvesting techniques on grain yield of maize was significantly higher compared to the control at $p<0.05$. The effect of in-situ rainwater harvesting structures on the grain yield as presented in Table 2, at $p<0.05$, the grain yield showed significant difference between treatments with rainwater harvesting structures and flat bed planting, which is control in Melkasa 4 variety. TR resulted in (130.55%), FR (126.23%) and CR (123.85%) higher grain yield compared to FBP. On BH 661 maize variety only CR treatment showed significant difference compared to control, while TR and FR were not shown significant difference but performed better than the control. TR resulted in (122.67%), FR (124.29%) and CR (138.31%) higher grain yield compared to FBP.

Table 2. The effect of in-situ rainwater harvesting techniques on grain yield of maize (quintal/ha).

Treatments	Grain yield of two varieties (Quintal/he)			
	Melkasa 4		BH 661	
	Mean	SD	Mean	SD
TR	43.89[a]	5.43	37.98[ab]	3.15
FR	42.44[a]	4.10	38.48[ab]	8.22
CR	41.64[a]	2.64	42.82[a]	6.55
FBP	33.62[b]	1.74	30.96[b]	1.41
CV (%)	9.28		14.73	
LSD 0.05	7.06		10.42	

LSD 0.05 = least significant difference at 5% level and means followed by the same letter are not significantly different at P =0.05.

All in-situ rainwater structure treatments performed much better than the controlled treatments in two varieties. This might be due to the fact that the harvesting structures store rainwater in-situ, enhancing infiltration, which provide a reservoir of water to the crop at depth which heavy textured soils (sandy clay), rises by capillarity during dry spells and ensure the crop benefits. This result is in agreement with the finding by Gebreyesus (2012) that tied-ridge and fertilizer, and its interaction significantly influenced the yield and yield components of sorghum and resulted in up to 48% increment. Tied ridges found to be very efficient in storing the rain water, which resulted in substantial grain yield increase in some of the major dryland crops such as sorghum, maize, wheat, and mung beans in Ethiopia (Georgis and Takele 2000). The average grain yield increase (under tied ridges) ranged from 50 to over 100 percent when compared with the traditional practice. This increase, however, will vary according to the soil type, slope, rainfall and the crop grown.

In the current result, the yield of maize was affected by all in-situ rainwater harvesting structures (Figure 2).The work of Heluf (2003) also supports this finding by the fact that the yield response to water conservation treatments was higher both under fertilized and unfertilized conditions than the control treatments.

4. Conclusion and Recommendations

In conclusion, flat bed planting produced the lowest soil moisture) and grain yields of maize in two varieties. Generally, furrow ridge, tied ridge and contour ridge planting produced higher grain yields of maize than flat bed planting in both maize varieties. Therefore, from the research results it is possible to make conclusion and recommendations as follows:

In-situ water harvesting techniques improved soil moisture stored within the root zone as compared to the flat bed planting and the improved varieties of maize responded significantly to *in-situ* water harvesting techniques, however, the magnitude of yield response to *in-situ* water harvesting techniques and the relative effectiveness of the different harvesting methods tend to vary with crop varieties. Finally, it could be recommended that *in-situ* water harvesting practices are indispensable agricultural operations for successful maize production in Fedis district and in other moisture stressed areas.

5. Acknowledgement

The authors would like to acknowledge the Haramaya University for funding this research

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2. Nodulation, Yield and Yield Traits of Faba bean (*Vicia faba* L.) Under Integrated Application of Vermicompost and *Rhizobium leguminosarum*bv. *Vicieae*Inoculation at Haramaya, Eastern Ethiopia

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Abstract: Low soil fertility and soil carbon depletion are the major constraints for crop production in Ethiopia and use of chemical input is very limited due to its unaffordable price by subsistent farmers. Therefore, this research was conducted to search locally available inputs for sustainable way of improving the soil fertility and crop production with specific objective of assessing the effect of integrated application of vermicompost and *Rhizobium* inoculation on nodulation, yield and yield related traits of faba bean at Haramaya, eastern Ethiopia. Ten treatments were made by factorial combination of five levels of vermicompost (0, 2, 4, 6 and 8 ton ha-1) and two levels of *Rhizobium* inoculation (inoculated and uninoculated). The treatments were laid out in randomized complete block design with three replications. Analysis of variance revealed that *Rhizobium* inoculation, vermicompost application, cropping season and their interactions significantly ($P<0.05$) affected all traits of faba bean. The highest nodule number (NN) (298.00) and nodule dry weight (NDW) (0.7598 g) were obtained at 4 ton ha^{-1} application of vermicompost but NN and NDW reduced by 41 and 48%, respectively, due to 8 ton ha^{-1}application as compared to 4 ton ha-1. Inoculation of *Rhizobium* increased the NN and NDW over uninoculated treatment by 6 and 11%, respectively. The remaining investigated traits remarkably increased by increasing rates of vermicompost application and *Rhizobium* inoculation, except number of seed per pod in 2012 cropping season. The highest grain yield (4822.1 kg ha^{-1}), which was 28.7% increased compared to unfertilized plants, was obtained from 8 ton ha^{-1} vermicompost application. Total P uptake also increased from 1.29% at unfertilized plant to 1.71% at 8 ton ha-1 vermicompost application. In contrast to this, total plant N concentration reduced from 3.81 to 3.37% when vermicompost application increased from 2 to 8 ton ha^{-1}. As compared uninoculated plants, inoculation of *Rhizobium* increased grain yield, total plant P uptake and plant total N concentration by 5.5, 8.6 and 1.1%, respectively. However, grain yield increased due to vermicompost application higher than that of *Rhizobium* inoculation which is 1076.7 and 224.8 kg ha^{-1} over the unfertilized and uninoculated plants, respectively. The effect of Rhizobium inoculation on grain yield production of faba bean was reduced when vermicompost application at the rates >6 ton ha^{-1}, probably due to inhibitory effect of inorganic N at high rates of vermicompost application on the effectiveness of *Rhizobium* inoculation. Hence, it is recommended the integrated application of *Rhizobium* and vermicompost application at 6 ton ha^{-1} to boost the productivity of faba bean without negatively affected the N2 fixation.

Keywords: Eastern Ethiopia; Faba bean (*Vicia faba* L.); Vermicompost, *Rhizobium*

1. Introduction

Vermicomposts are the product of the aerobic biodegradation of organic materials by integrated actions of various microorganisms and earthworms. Vermicomposts are major source of plant nutrients to reduce chemical fertilizer input thereby minimize cost of crop production (Adhikary, 2012; Lazcano *et al.*, 2013).The effect of vermicompost in improving plant growth can be broadly classified into four categories. These are increasing plant mineral nutrient content (Garcia-Ruiz *et al.*, 2008; Lim *et al.*, 2015), improving microbial activities and abundance(Zhang *et al.*, 2012), plant phytohormones production and solubilizing inorganic P by microbes(Hu *et al.*, 2009; Song *et al.*, 2015), and increasing humic/fulvic acid content (Atiyeh *et al.*, 2002; Aguiar *et al.*, 2013; Arancon *et al.*, 2003, 2006). Besides, organic input improves the soil physical properties by lowering bulk density, increasing water holding capacity, and improving infiltration rates (Werner, 1997; Petersen, *et al.*, 1999; Bulluck *et al.*, 2002; Gopinath *et al.*, 2008). Vermicompost contain large amounts of inorganic and organic form of N which is immediately available for plant uptake (Lazcano *et al.*, 2013). Plant available N in vermicompost is found in the forms of nitrate (NO3- and ammonium (NH4+). This input also contains higher mineral nutrients mainly available Mg, Ca, P, and K (Bedada *et al.*, 2014; Agegnehu *et al.*, 2016; Hernández *et al.*, 2016).

Low soil fertility is the major constraints for crop production in sub-Saharan Africa in general and Ethiopia in particular (Hurni, 1988; Smaling *et al.*, 1993). To improve the crop productivity in this region, adequate and balanced nutrients application is required especially in soils with poor nutrient content. Caliskan *et al.* (2008) also suggested that maintaining soil fertility and use of plant nutrient in sufficient and balanced amounts is one of the key factors in increasing crop yield in sub-Saharan countries. Recently, reports indicated that micronutrient deficiency which limits cell division, chloroplast development, enzyme activity, and reduced dry-matter yields is becoming the major yield limiting for crop production beside N and P in Ethiopia (Tulema *et al.*, 2007).

Inoculation of seeds with Rhizobium is known to increase nodulation, N uptake, growth, and yield parameters of legume crops (Adgo and Schulze, 2002; Rudresh *et al.*, 2005; Sogut, 2006; Stancheva *et al.*, 2006). Nitrogen fixation by different annual legumes has been reported to vary from 35-270 kg-1 ha-1 yr-1 (Nutman, 1969). However, the effectiveness of inoculationon nodulation and N derived from atmosphere by biological N fixation are reduced when Rhizobium inoculation integrated with high N-containing fertilizer (Rawsthorne *et al.*, 1985; Walley *et al.*, 2005; Ogutcu *et al.*, 2008). For instance, Clayton *et al.* (2004) examined response of field pea to N fertilization and reported that application rates greater than 40 kg N ha-1 had reduce nodulation and N fixation. However the nodulation and productivity of common bean is affected by the supply of other nutrients other than N regardless of indigenous rhizobial population (Amijee and Giller, 1998). They found poor nodulation less vigor growth of plants in soil with low extractable P. Major soil nutrients increase due to organic input amendment enhanced the nodulation and yield of peanut (Agegnehu *et al.*, 2015). The presence of high P attenuated the negative effect of available inorganic N on nodulation and N2 fixation (Hellsten and Huss-Danell, 2000). However, the information on the effect of vermicompost reached in inorganic N application in combination with Rhizobium inoculation on nodulation and growth of high N2 fixer faba bean is poorly studied. The hypothesis of this work has been formulated that use of vermicompost with high plant nutrients including N improve the plant production without affecting nodulation. Therefore, this study was conducted to determine the effect of vermicompost application and elite Rhizobium inoculation on nodulation, yield and yield traits of faba bean at Haramaya experimental site.

2. Materials and Methods

2.1. Description of Experimental Site

A field experiment was established in 2012 and 2013 cropping season on a sandy clay loam soil under rainfed conditions at the Research Farm of Haramaya University. The experimental site is situated at N09o24.954" and E042o02.037" at an altitude of 2020 m.a.s.l. The area placed in the semi-humid. During the experimental period, the mean monthly minimum and maximum temperatures were 23.4 and 8.25oC, respectively. The annual rainfall was 760 mm of which 75-85% occurred during the July-September period.

Soil samples taken from the surface 20 cm before treatment applications had sandy clay loam with 33, 18 and 49% of clay, silt and sand content, respectively, organic carbon (C) content of 1.96% determined as per Walkley and Black (1934), Kjeldahl nitrogen (N) of 0.12%, Olsen phosphorus (P) of 2.13 mg kg-1 soil , 1 N ammonium acetate extractable–potassium (K+1), Calcium (Ca+2), Sodium (Na+1) and Magnesium (+2) of 0.14, 31, 0.33 and 8.7 cmol(+) kg-1, respectively, and a pH (1:2.5 soil/water suspension) of 7.84. The cation exchange capacity and EC of the experimental soil were 25.98 cmol(+) kg-1 and 0.14 mS cm-1, respectively.

2.2. Experimental Materials Preparation

Vermicompost was produced on the Research Farm of Haramaya University, Ethiopia. Parthenium weeds and partially decomposed cattle dung were used in 2:1 ratio (w/w) for vermicomposting. These materials were thoroughly mixed and put into a pit 2 m long × 1.5 m wide× 0.75 m deep. Water was sprinkled to make the material sufficiently wet, and 4000 earthworms (*Eisenia foetida*) were introduced into the pit, which was covered overhead roof to prevent direct exposure to sunlight. The material in the pit was thoroughly mixed by hand twice, at an interval of 30 days. The vermicompost was removed from the pit after 90 days, and the earthworms were separated with a sieve. Matured vermicompost sample were collected for analysis of major chemical parameters. The result of the analysis is presented in Table 1. The vermicompost was applied by hand two weeks before sowing and was immediately incorporated after application with a spade.

Table 1. The chemical composition of vermicompost prepared from Parthenium weed and cow dung.

No.	Parameters	value
1.	$pH_{H2O(1:2.5)}$	7.86
2	EC (mS/cm)	9.35
3	Total N (%)	1.31
4	Organic Carbon (%)	35.22
5	NH_4^+N (mg/Kg)	37.60
6	NO_3^-N (mg/Kg)	8839.05
7	Available K (cmol(+)/Kg soil)	27.34
8	Available P (mg/Kg)	930.13
9	Zn (mg/kg)	39.57
10.	B (mg/kg)	7.75

Rhizobium leguminosarum biovar. viciae strain HUFBR-15 isolated from root nodules of faba bean grown in eastern Ethiopia soil (Minalku *et al.*, 2009) was used as inoculant preparation. This isolate was purified on yeast extract mannitol (YEM) agar medium by incubating at 28^{0}C for 5 days. Pure colony was further incubated in YEM liquid medium in shaker incubator at 120 rpm until the number of viable Rhizobium reached 108 ml-1 of culture broth. The viable number of isolate in the culture was monitored turbidimetrically at 620 nm with a spectrophotometer. The inoculum was produced by mixing culture liquid medium with well-decomposed filter mud. This production was undertaken at the Biofertilizer Research and Production Laboratory of Haramaya University, Ethiopia.

2.3. Treatments and Experimental

Improved cultivar Gachena of Faba bean, with favorable agronomic characteristics and high yielder in this region, was obtained from Haramaya University, highland pulses research project. The experimental design was randomized complete block design where10 treatments were randomized within a block and there were three blocks (10 treatments x 3 replications = 30 plots). Twelve treatments consisted of selected combinations of different rates of vermicompost (0, 2, 4, 6 and 8 ton ha-1) and two levels of Rhizobium inoculations (inoculated and uninoculated). Vermicompost applications were rated on a dry-weight basis. Each plot consisted of five rows, 3m long and 40cm apart. The area was moldboard-plowed and disked before planting. Seeds of inoculation treatments were inoculated with *Rhizobium leguminosarum*bv. viciae just before planting.

2.4. Data Collection and Analysis

At late flowering and early pod setting stage, five plants from three central rows were excavated to determine nodulation (nodule number and nodule dry weight) and shoot dry weight. The dried shoots were later ground to pass a 0.5 cm sieve. Total N determinations were done by the Kjeldahl method of Bremner (1965). The plants were harvested at physiological maturity on the second week of November, and yield components such as plant height, number of pods per plant, number of seeds per pod, and 100 seeds weight was recorded on 5 randomly selected plants from the central three rows. Grain and total biomass yields were determined by harvesting the middle three rows of each plot.

Analysis of variance (ANOVA) was done using the SAS computer software package. Differences between mean values were evaluated by a two-way analysis of variance (ANOVA) using least square difference (LSD) at the 0.05 probability level.

3. Results

Analysis of variances revealed that the main effect of vermicompost rates of application, Rhizobium inoculation and cropping season and their interaction significantly affected the nodule number enumerated at late flowering stage of faba bean, except the main effect of cropping season (Table 2). In 2011, the effect of vermicompost application did not significantly improve the nodule number of faba bean. The result indicated the reduction trends of nodule number when vermicompost application increased from 4 ton ha^{-1} to 6 and 8 ton ha^{-1}(Table 3). However, the result in 2012 cropping season revealed that 6 ton ha^{-1} vermicompost application significantly increased as compared to the remaining rates of vermicompost application. The effect of vermicompost on average nodule number over years was non-significant. The effect of Rhizobium inoculation on nodule number in the year 2011 was non-significant while this effect in the 2012 significantly improved when compared to uninoculated control.The effect of Rhizobium inoculation when compared to uninoculated control on NN along rates of vermicompost was significant only at the unfertilized and 4 ton ha^{-1} vermicompost applications (Figure 1a).

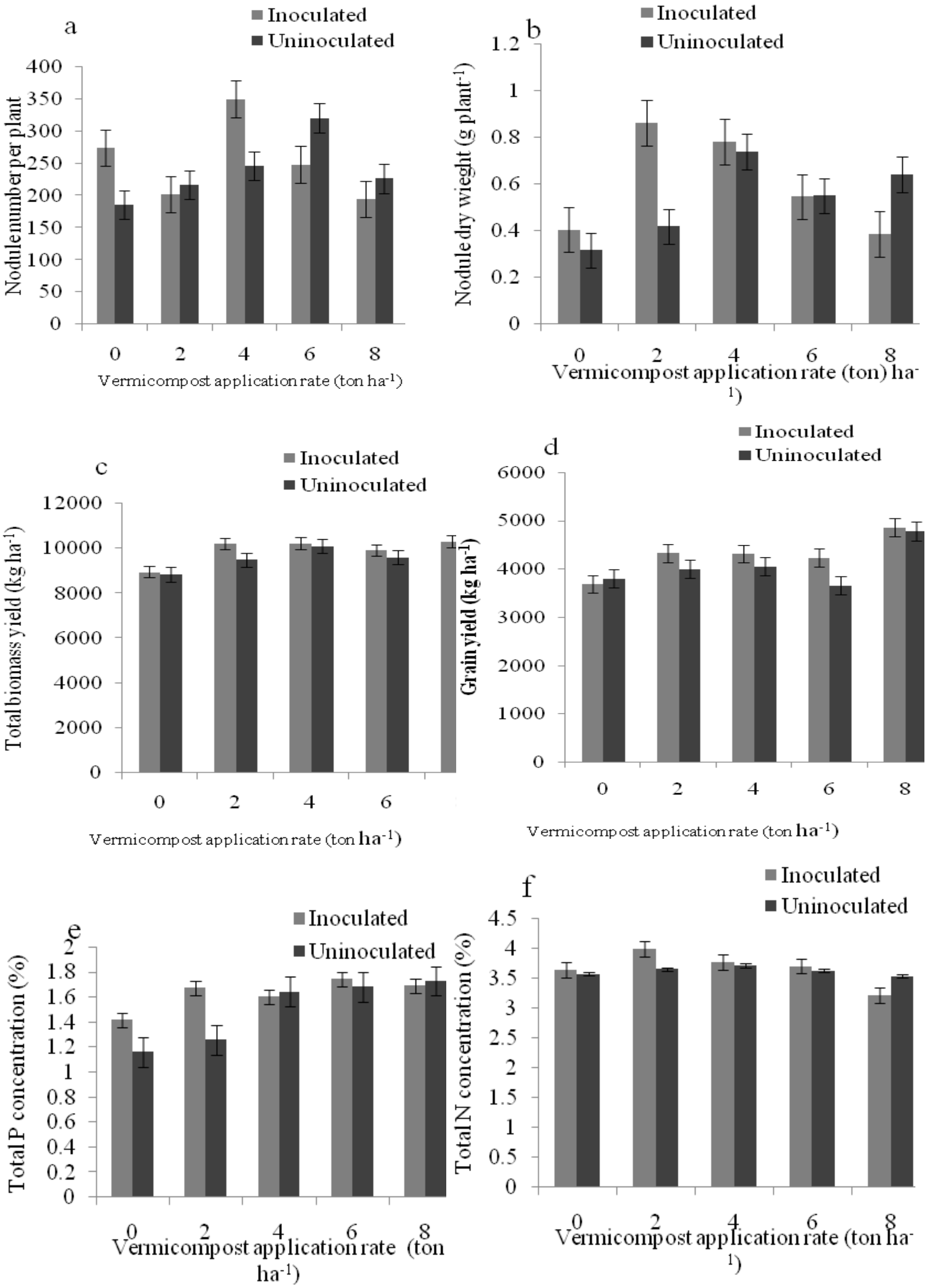

Figure 1. Effects of rates of compost application on (a) Nodule number, (b) nodule dry weight, (c) Total biomass yield, (d) Grain yield, (e) total P accumulation and (f) Total N accumulation

Table 2. Summary of ANOVA results for all investigated parameters affected by compost rates of application, *Rhizobium* inoculation and year of planting, in Haramaya, 2012-2013.

Sources of variation	df	F value											
		NN	NDW	SDW	SL	NT	NPP	NSP	100 wt	TBY	GY	Tot P	Tot N
Compost rates (C)	4	12.09***	54.22***	4.14**	10.83***	2.88*	16.07***	3.96**	4.26**	6.15***	24.12***	77.20***	25.74***
Inoculation (I)	1	9.17**	65.94***	0.64ns	5.01*	22.68***	31.94***	0.01ns	0.69ns	0.64ns	9.28**	48.76***	2.64ns
Year (Y)	1	0.13ns	432.51***	472.14***	124.57***	180.10***	304.25***	24.04***	64.06***	523.44***	61.57***	192.48***	1271.25***
C x I	4	11.79***	21.45***	8.52***	5.22**	0.45ns	5.19**	1.60ns	2.78*	0.73ns	2.47ns	22.94***	9.36***
Y x I	4	8.29***	47.14***	12.14***	7.34***	3.83**	7.62***	2.69*	3.60*	0.37ns	2.07ns	13.68***	4.42**
Y x I	1	15.67***	49.19***	43.94***	6.73*	3.89ns	31.92***	3.94ns	0.89ns	17.21***	24.05***	0.72ns	0.04ns
Y x C x I	4	17.29***	29.99***	3.97**	4.62**	9.15***	0.97ns	1.42ns	0.56ns	6.07***	5.32**	3.98**	8.71***

*NS= Non significant; *,**& ***, significant at P< 0.05, P<0.01 and P<0.001, respectively. NN= Nodule number; NDW= Nodule dry weight; SDW= Shoot dry weight; SL= Shoot length; NT= Number of tiller per plant; NPP= Number of pods per plant; NSP= Number of seeds per pod; GY= Grain yield; TBY= Total biomass yield; Tot N= total nitrogen and Tot P= Total phosphorus.*

Table 3. Nodule number, nodule dry weight and shoot dry weight of common bean as affected by rates of compost application and *Rhizobium* inoculation, Haramaya, 2012-2013.

Treatments (ton ha^{-1})	Nodule number			Nodule dry weight			Shoot dry weight		
	2012	2013	Average	2012	2013	Average	2012	2013	Average
0	239.83ab	220.00cd	229.92ab	0.5562b	0.1656c	0.3609b	121.07b	69.47ab	95.27a
2	245.33ab	173.33d	209.33b	1.0882a	0.1915c	0.6398ab	149.72a	52.05b	100.88a
4	294.33a	301.67b	298.00a	1.0122a	0.5073a	0.7598a	130.82b	85.15a	107.98a
6	235.83b	333.33a	284.58ab	0.6735b	0.4227ab	0.5481ab	133.25ab	86.40a	109.83a
8	173.33c	248.33bc	210.83b	0.6862b	0.3399b	0.5130ab	138.83ab	79.25a	109.04a
LSD	57.29	70.48	77.95	0.2167	0.1404	0.3709	18.54	18.59	43.72
Inoculated	219.13a	288.67a	253.90a	0.8468a	0.3447a	0.5957a	126.65b	84.77a	105.71a
Uninoculated	256.33a	222.00b	239.17a	0.7598b	0.3061a	0.5329a	142.82a	64.16b	103.49a
LSD	25.29	31.07	34.99	0.0955	0.0619	0.1665	8.17	8.19	19.63
CV (%)	13.97	15.98	27.37	15.61	24.97	56.89	7.96	14.45	36.18
Mean	237.73	255.33	246.53	0.8033	0.3254	0.5643	134.74	74.46	104.60

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

In 2011, 2 and 4 ton ha^{-1}application recorded significantly higher nodule dry weight (NDW) than those NDW from remaining rates of application including the control check while 4 ton ha^{-1} induced significantly higher NDW than the remaining rates of vermicompost except 6 ton ha^{-1}. Over years, 4 ton ha^{-1} resulted in significantly increased the average NDW over the control check. A significant effect of Rhizobium inoculation on NDW when compared to uninoculated treatment was observed in 2011cropping season.

The non-significant effect of Rhizobium inoculation across different rates of vermicompost application on NDW when compared to the corresponding rates of vermicompost without inoculationwas observed (Figure 1b).However, Rhizobium inoculation improved the NDW over the uninoculated treatment up to 4 ton ha-1vermicompost application, but the effect of inoculation gradually reduced when vermicompost application beyond 4 ton ha^{-1}. The Significant effect of Rhizobium inoculation, vermicompost application, cropping season and their interaction on shoot dry weight and shoot length measured at late flowering and early pod setting stage of fababean was observed (Table 2). Significantly higher shoot dry weight was recorded at 2 ton ha^{-1} in 2011than the control check (Table 3).However, this effect was non-significant in 2012.The data found that mean value of SDW over years exhibited increasing trend with increased rates of vermicompost application. In contrast, statistically higher shoot length was recorded at 8 ton ha^{-1} in 2011, but this effect was non-significant in 2012 (Table 4). Rhizobium inoculation resulted in significant improvement of SL and SDW in 2012 when compared to the uninoculated treatment but this effect was non-significant in 2011 cropping season.

The Rhizobium inoculation, vermicompost application, cropping season and their interaction significantly affected number of tiller per plant (Table 2).Significantly higher number of tiller per plant (2.62 and 4.05) was recorded at 2 and 8 ton ha^{-1} in 2011and 2012 cropping season, respectively (Table 4). In both cropping season Rhizobium inoculation significantly increased number of tiller per plant including average values over years. The mean number of tiller in 2012 was higher than that of 2011.

The number of pods per plant (NPP) was affected significantly by Rhizobium inoculation, vermicompost application, cropping season and their interaction (Table 2). Significantly higher NPP was recorded at 8 and 4 ton ha-1 in 2011cropping season while the rates at and beyond 4 ton ha-1 resulted in significantly increase NPP than the other treatments in 2012(Table 4). The effect of Rhizobium inoculation was non-significant in 2011 while significantly increased NPP in 2012. Though the effect of vermicompost application was non-significant on average NPP over years, the value was increased with increasing rates of vermicompost application.

Vermicompost application, cropping season and cropping season x Rhizobium inoculation had significant effect on number of seeds per pod (NSP) at $P < 0.05$ (Table 2). Applying 8 ton ha-1 resulted in significantly higher NSP and the average NSPover years in the 2011than unfertilized control (Table 4). In contrast, the non-significant effect of vermicompost on NSP was observed in 2012. The effect of Rhizobium inoculation on NSP was non-significant in both cropping season including its effect on the average NSP over years.

Vermicompost rates of application, cropping season, vermicompost application x inoculation and cropping season x inoculation had significant effect on 100 seeds weight of faba bean at $P < 0.05$ (Table 2). Hundred seed weight in 2011 was significantly increased at 4 and 6ton ha-1than the unfertilized plants (Table 4). In 2012, 8 ton ha-1application resulted in significantly higher NSP than the control check. However, this effect was non-significant on average value of 100 seeds weight over years. Beside, Rhizobium inoculation had no significant effect on 100 seeds weight of faba bean in both cropping season.

Total biomass yield (TBY) of faba bean was significantly influenced by main effect of vermicompost application and cropping season, and interaction effect of cropping season x inoculation and vermicompost application x cropping season x inoculation (Table 2). Significantly higher TBY as compared to the control check in both cropping season was recorded with8 ton ha-1 application (Table 5). However, this effect on the average value of TBY over years was non-significant. The effect of Rhizobium inoculation on TBY in 2011 and its average value over years was non-significant while significant in 2012 cropping season.The effect of Rhizobium inoculation on TBY when compared to uninoculated control with vermicompost application was non-significant (Figure 1c).

Table 4. Number of seeds per pod, 100 seeds weight and total biomass yield of common bean as affected by rates of compost application and *Rhizobium* inoculation, Haramaya, 2012-2013.

Treatments (ton ha^{-1})	Number of seeds per pod			100 seeds weight			Total biomass yield		
	2012	2013	Average	2012	2013	Average	2012	2013	Average
0	3.13c	3.50a	3.32b	57.17b	54.87b	56.02a	6208.3b	11564.8b	8887a
2	3.27bc	4.05a	3.66ab	58.82ab	55.25ab	57.03a	7477.8ab	12226.9ab	9852a
4	3.27bc	3.89a	3.58ab	61.77a	54.77b	58.27a	7555.6ab	12768.5a	10162a
6	3.53ab	3.55a	3.54ab	59.67a	55.73ab	57.70a	7169.4ab	12355.6ab	9763a
8	3.70a	3.94a	3.82a	59.78ab	57.80a	58.79a	8183.3a	12870.4a	10527a
LSD	0.36	0.70	0.48	3.34	2.93	3.27	1816.6	1021.0	3431.5
Inoculated	3.29b	3.86a	3.58a	59.41a	56.10a	57.76a	6950.0a	12901.9a	9925.9a
Uninoculated	3.47a	3.71a	3.58a	59.47a	55.27a	57.37a	7687.8a	11812.6b	9750.2a
LSD	0.18	0.31	0.22	1.47	1.29	1.47	800.9	450.14	1540.5
CV (%)	6.11	10.66	11.62	3.25	3.04	4.92	14.37	4.78	30.19
Mean	3.38	3.79	3.58	59.44	55.68	57.56	7318.89	12357.22	9838.06

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. Grain yield, total P accumulation and total N accumulation of common bean as affected by rates of compost application and *Rhizobium* inoculation, Haramaya, 2012-2013.

Treatments (ton ha^{-1})	Grain yield			Total P accumulation			Total N accumulation		
	2012	2013	Average	2012	2013	Average	2012	2013	Average
0	3418.7c	4072.1c	3745.4b	1.0815c	1.4973b	1.2894c	2.9706bc	4.2280ab	3.5993a
2	3692.1bc	4639.2ab	4165.6b	1.2923b	1.6365b	1.4644bc	3.2922a	4.3305a	3.8114a
4	4014.9b	4353.7bc	4184.3b	1.6061a	1.6380b	1.6220ab	3.1089ab	4.3660a	3.7374a
6	3679.7bc	4210.0bc	3944.9b	1.6256a	1.8008a	1.7132a	3.2000ab	4.1180bc	3.6590a
8	4609.7a	5034.6a	4822.1a	1.5521a	1.8698a	1.7110a	2.7476c	3.9863c	3.3669a
LSD	517.5	468.6	581.49	0.0969	0.1461	0.2043	0.2337	0.1795	0.7487
Inoculated	3814.5a	4755.22a	4284.9a	1.5040a	1.7453a	1.6247a	3.0927a	4.2182a	3.6554a
Uninoculated	3951.5a	4168.63b	4060.1a	1.3590b	1.6317b	1.4953b	3.0350a	4.1933a	3.6142a
LSD	228.16	206.6	261.04	0.0427	0.0644	0.0917	0.1030	0.0791	0.3361
CV (%)	7.71	6.08	12.06	3.92	5.01	11.34	4.41	2.47	17.83
Mean	3883.02	4461.93	4172.47	1.4315	1.6885	1.5600	3.0638	4.2058	3.6318

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

The main effect of vermicompost application, Rhizobium inoculation, cropping season and their interaction, excluding vermicompost x inoculation and cropping season x inoculation, had significant influence on grain yield (GY) at P <0.05 (Table 2). Applying 8 ton ha-1 application resulted insignificantly increase GY and the average GY over years when compared to the control check in both cropping season (Table 6).A significant increase in GY byRhizobium inoculation in 2012 was observed. This effect in 2011 and on average GY over years was non-significant. The highest GY obtained in 2011and 2012cropping season were 4609.7 and 5034.6 kg ha-1, respectively. The effect of Rhizobium inoculation when compared to uninoculate along increasing rate of vermicompost application on GY was non-significant (Figure 1d).

Vermicompost application, Rhizobium inoculation, cropping season and their interaction, except cropping season x inoculation, had significant effect on total plant P uptake at P <0.05 (Table 2). Significantly higher total plant P uptakes in 2011 and 2012 were recorded with 6 and 8 ton ha-1application, respectively. The average total P uptake over years was significantly increased at6 and 8 ton ha-1when compared with those recorded with the remaining rates of application. In both cropping seasons, Rhizobium inoculation resulted in significantly increase total plant P uptake than uninoculated control. Figure 1e indicated that inoculation significantly increased P uptake by the plant with unfertilized and 2 ton/ha vermicompost when compared to the uninoculated plants with respective rates of vermicompost.

Total plant N accumulation was significantly affected by vermicompost application, Rhizobium inoculation, cropping season and their interaction, except main effect of inoculation and the interaction effect of cropping season and inoculation (Table 2). Significantly higher total plant N accumulation than the control check in 2011 and 2012 were recorded with2 and 4 ton ha-1 application, respectively (Table 6). This effect was non-significant on average total N accumulation over years. The effect of Rhizobium inoculation on total N accumulation was non-significant in both cropping season, including its effect on average values over years. Like P uptake, a significant increase in plant N accumulation by inoculation was found with unfertilized and 2 ton/ha application of vermicompost.

4. Discussion

Due to soil nutrient depletion and other soil degradation, soil of sub-Saharan Africa including Ethiopia are deficient in essential plant nutrients. As a result, the crop productions in this region produced by subsistent farmers are very low in productivity. The cost effective and environmental friendly ways of improving the crop production is essential. Therefore, this study was initiated to evaluate the effect of Rhizobium inoculation integrated with vermicompost application in Haramaya experimental site, Eastern Ethiopia. Results of this study indicated a significant increase in NN and NDW up to 4 and 6 ton ha-1 rates of vermicompost application in 2011 and 2012 cropping season, respectively. This enhancement of nodulation could be due to the fact that vermicompost is the contained different essential nutrients including macronutrients and micronutrients necessitate for nodule initiation and development (Al-Chammaa *et al.*, 2014; Wu and Arima, 1992; Lawson *et al*., 1995).Organic input addition could have increase the volume of soil exploited by the roots (Jenkinson, 1985), leading to an improvement in the root system of faba bean, which in turn provided more area for Rhizobium proliferation and consequently improved the number of effective nodules per plant.

The present study found the significant reduction of NN and NDW at 8 ton ha-1 vermicompost application. This could be because of the fact that vermicompost contained relatively large amounts of N immediately available for plant uptake (Parmelee and Crossley, 1988; Ruz-Jerez *et al*., 1992; Datta *et al*., 2011) which may reduce the nodule initiation and development (Clayton *et al*., 2004). This negative effect is mainly due to the inhibition of Rhizobium infection to root hairs, nitrogenase activity, and transport of carbohydrates from shoots to nodules (Latimore *et al*., 1977; Streeter, 1988).

Though the soil had high indigenous rhizobia population nodulating faba bean, a slight increase in NN and NDW due to Rhizobium inoculation was also noted. This enhance of nodulation was previously reported by (Carter *et al*., 1994; Amanuel *et al*., 2000) who found that Rhizobium inoculation improved nodulation initiation and development. Mostasso *et al.* (2002) and Mrabet *et al.* (2005) found that significant increase in nodulation of common bean by using locally isolated Rhizobium inoculation though the soil had high rhizobial population.

The result of the current study revealed a significant increase in SDW, SL, number of tillers per plant, NPP and NSP of faba bean with increasing rates of vermicompost application with the highest value of these traits at 8 ton ha-1.The results obtained here are in agreement with those of Pandey *et al.* (2006) and Gopinath *et al.* (2011)who revealed a significant increase in growth and yield by organic input through deliveringgreater amounts of available C, Mg, Ca, P, and K for the plant (Lim *et al*., 2015; Zwieten *et al*., 2015). Besides, soils receiving greater amounts of vermicompost have been shown to contain larger amounts of microbial N than those receiving less vermicompost and this improves N-supplying potential(Arancon *et al*., 2004). In addition to mineral nutrition content of vermicompost, further stimulate plant growth through enhancing beneficial microorganisms and the microbially mediated release of phytohormones (Drinkwater *et al*., 1995; Frankenberger and Arshad, 1995).

Inoculating Rhizobium in 2012 cropping season was significant improved all investigated traits of faba bean when compared to the uninoculated treatment but this effect was non-significant in the 2011 cropping season. This difference could be related with the high competitiveness of the native rhizobia population in 2011 against the inoculated Rhizobium (Charman and Ballard, 2004). The results in 2012 agree with those of Ankomah *et al.* (1996) and Leffel (1989), who reported that QNdfa correlated well with grain yield and dry-matter production. We found that a significant increase in 100 seeds weight due to increasing rates of vermicompost application but this increase did not observe due to Rhizobium inoculation. Fageria and Santos (2008) suggested that this trait influenced by soil and plant management thought it is genetically controlled. Inoculation of seeds with effective bacteria strains is not alone sufficient to obtain higher quality and yield (Vincent, 1982; Tufenkci, 1995). In contrast to this, Elsheikh and Elzidany (1997) found a significant increase in 100 seed weight of faba bean due to Rhizobium inoculation. This difference could be due to the presence of high competitive rhizobia in indigenous soil in Haramaya soils.

In the present study, GY and TBY of faba bean increased significantly ($P < 0.05$)by increasing rates of vermicompost application in both cropping season. These results suggested that the growth of faba bean could be improved by the application of organic matter in low fertile soil (Zwieten *et al.*, 2015; Mete *et al.*, 2015). Inoculating Rhizobium significantly increased these traits only in 2012 cropping season, indicating the importance of Rhizobium inoculation beside vermicompost application. It has been reported that adequate and balanced application of nutrients supplied by organic input significantly increases the yield, especially in soils having poor nutrient content (Tufenkci *et al.*, 2006). Beside N2 fixation, rhizobia can produce different growth promoting substances and solubilizing insoluble plant nutrients from the soils (Werner and Newton 2005) and thus promoting seed germination, root elongation, and stimulate the leaf expansion. In addition, rhizobium enhances root development and thus enhances water and nutrient uptake (Chabot *et al.*, 1996).

The current work revealed that the higher mean TBY and GY were recorded in 2012 than in 2011 cropping season. This difference could be related with significant effect of inoculation in 2012 but not in 2011 in most of investigated traits of faba bean. It could have been because of increasing chlorophyll content (Elkoca *et al.*, 2010) by Rhizobium inoculation thereby improving the photosynthetic product and productivity of the host plant. Beside this, rainfall distribution in 2011 was not good as 2012 cropping season.

We found a significant increase in total plant P uptake and total plant N accumulation by increasing rates of vermicompost application. Similar findings were previously indicated by Parry, Kato, and De Carvalho (2008) and Al-Chammaa *et al.* (2014) who found that applying manure with had significantly increased P concentrations in cowpea and total N accumulation in soybean, respectively. This increase could be due to the fact that the application of organic matter to soil may increase P solubility (Gaur, 1984; Herencia *et al.*, 2007) and therefore the availability and the absorption of P by the plant. Their beneficial effects were mainly attributed to the enhancement of N2 fixation through root growth and soil property improvements besides being a source of P and other nutrients that are essential for N2-fixation process (Divitoa and Sadras, 2014).Inorganic P obtained from organic input could also play major role in ATP molecules synthesis required by nodules to enhance N-fixation capacity (Reddell *et al.*, 1988).In addition organic input can increase soil moisture capacity (Fahmy *et al.*, 2000), which could lead to better plant growth with high N2 -fixation capacity. The current study showed that the higher mean total plant P uptake and total plant N accumulation was recorded in 2012 than 2011 cropping season.

5. Conclusion

In general, the result indicates a remarkable improvement of faba bean production in the study site due to vermicompost application. Though the high rates of vermicompost application reduced the effectiveness of inoculated Rhizobium isolate and nodule formation, the result also revealed moderate increase in productivity of faba bean. We finally recommend the integrated application of vermicompost and elite isolate of Rhizobium inoculation to boost the faba bean production in country in general and in the study site in particular.

6. Acknowledgment

This research was funded by the Ethiopia Institute of agricultural Research, under Biofertilizer and organic fertilizer research project. The authors would like to express their sincere thanks to field and technical assistant for their field experiment management and data collection.

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3. Symbiotic Effectiveness of Rhizobium Leguminosarum bv. Viciae Isolated from Major Highland Pulses on Field pea (Pisum sativum L.) at Haramaya, Eastern Ethiopia

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Abstract: In Ethiopia, the productivity of field pea at farmer level is very low due to nitrogen deficiency, insufficient and ineffective native rhizobia as well as effectiveness of rhizobia isolated from homologous hosts on field pea production is not well known. Hence this research was conducted with the objective of evaluating the symbiotic effectiveness of indigenous rhizobia nodulating major cool season pulses grown in Ethiopia (faba bean, lentil and field pea) on nodulation, yield and yield related traits of field pea under control and field condition at Haramaya Agricultural Research Center. More than 72 isolates of rhizobia nodulating faba bean, field pea and lentil were identified from central part of Ethiopia and tested for their symbiotic effectiveness (%) using field pea variety Metti in greenhouse condition. Five best performing Rhizobia isolates were selected for evaluation on field condition with other three isolates from eastern Ethiopia. The greenhouse experiment revealed that the nodule number (NN/plant) and nodule dry weight (NDW/plant) were ranging from 24 to 240 and from 0.3440 to 0.3447 g, respectively. The number of Rhizobium isolates which produced higher shoot length (SL), root length and shoot dry weight were 9, 18 and 7, respectively, while 5 and 1 were for total N and P accumulation, respectively, over the N fertilizer treatment. Seven rhizobia isolates attained greater than 100% symbiotic efficiency. The field experiment conducted in 2011and 2012 cropping seasons revealed that some of Rhizobium inoculation treatments significantly increased the NN, NDW, SDW, grain and total biomass yield. Rhizobium inoculation did not significantly increase the shoot length and 100 seed weight in both cropping season. In addition, number of seed per plant, total N accumulation and P accumulation were not affected by Rhizobium inoculation in 2012 cropping season. Inoculating HUFPR-4 and NSLNR-5 increased the GY by 49.1 and 61.1% over the uninoculated treatment of the corresponding years in 2011 and 2012, respectively. In

2011 cropping season, the plant N concentration and P accumulation enhanced by 41.6 and 20.5% due to Rhizobium inoculations over uninoculated treatment, respectively. Although effects of Rhizobium inoculation on mean NN and NDW over seasons were not statistically significant, inoculating HUFPR-3 and HUFPR-4 significantly improved the mean TBY and GY over seasons, respectively. Although isolate NSLNR-5 scored the highest nodulation and symbiotic efficiency under greenhouse condition in sand culture, HUFPR-1 inoculation induced the highest increase in NN and NDW under field condition suggesting that NSLNR-5 was probably less competitive with indigenous rhizobia nodulating field pea. The highest increase in total biomass and grain yields were obtained from HUFPR-3 and HUFPR-4 isolates, respectively. In general, field pea was cross-infected by rhizobia trapped from lentil despite less effective. Field pea grain yield was increased only by isolates of rhizobia obtained from Field pea root substantiates host-specificity of rhizobia.

Keywords: Ethiopia;Field pea; Metti; Rhizobium.

1. Introduction

Field pea is the third most important food legume crop among the cool season pulses in Ethiopia (Telaye *et al.*, 1994). It serves as major source of protein and energy in many developing countries including Ethiopia. Field pea in Ethiopia is produced in mid altitudes range of 1800-3000 m asl and with annual rainfall of 600-700 mm (Telaye 1979; Ghizaw and Molla, 1994). It is one of legume crop serve as rotation crop with cereals (barley and bread wheat) to restore soil N content and minimize weeds, insect pests and disease in cereals (Taa 2001; Taa and Nefo 2006). The area allotted and total production of field pea in 2014/15 cropping season in Ethiopia has been 230,660.38 ha and 342,636.78 ton, respectively (CSA, 2015).

Despite its ecologic as well as economic importance, the productivity of the crop is only 1.485 ton ha^{-1}, which is far below the potential yield as recorded at research plot yield of 2.5-3.5 ton ha^{-1} (Fikreselassie 2012; CSA, 2015). The major yield limiting problems in the country are degraded soil fertility, recurrent droughts, and inadequate traditional agronomic practices (Kemal and Tibabu 1994; Ghizaw and Molla, 1994). Low soil fertility is the major constraint for field pea production in Ethiopia (Tsigie and Woldeab, 1994). Several research reports revealed an improvement of field pea yield up to 61% by N and P inorganic fertilizer application (Tsigie and Woldeab, 1994). However, N application alone had no significant effect on measured agronomic parameters of field pea in Bale and Arsi areas (SnARC, 2000). In addition, three years data from inorganic N response conducted in Ethiopia with different soil types had non-significant effect on field pea production (Tsigie and Woldeab, 1994). On the other hand, field peas have the inherent ability to obtain much of its nitrogen (N) requirement from the atmosphere by forming a symbiotic relationship with *Rhizobium* bacteria in the soil (Schatz and Endres, 2009).

Field pea is nodulated by *Rhizobiumleguminosarum* bv. *viciae* (Riah *et al*., 2014). This *Rhizobium* species can also form nodules with other legumes, including faba bean (*Viciafaba* L.), vetch (*Viciasativa* L.), lentil (*Lensculinaris* Medik.) and grass pea (*Lathyrussativus* L.) (Ruiz-Díez *et al*., 2012a). Hailmariam and Tsigie (2006) revealed that *Rhizobiumleguminosarum* bv. *viciae* inoculation increased the yield of major cools season pulses including field pea up to 38% as compared to the uninoculated treatment. Some studies, however, indicated the variable response of field pea to elite isolate of *Rhizobium* inoculation in different locations of the country (NSRC, 2002). This variable response could be due to the fact that *Rhizobium* bacteria have to compete with persistent and well-adapted indigenous microorganisms and survive variable environmental conditions (Rich *et al*., 1983; Sinclair and Serraj, 1995). Ruiz-Díez *et al.* (2012b) reported that rhizobial inoculant requires the selection of symbiotically efficient strains for every cropped legume in each specific area. Long time cultivation of legume increased the opportunity to select more highly effective and adapted strains to use as inoculants (Santos *et al*., 1999; Hungria *et al*., 2001; Ferreira and Hungria, 2002; Batista *et al*., 2007). Moreover, the effect of the locally isolated *Rhizobiumleguminosarum* bv. *vicieae* from different homologous hosts on nodulation, yield and yield traits of field pea is yet not known. Hence, the objective of this study were to evaluate the symbiotic effectiveness of *Rhizobiumleguminosarum* bv. *vicieae* isolates with field pea var. Metti under greenhouse condition and to determine the effect of elite isolates of *Rhizobium leguminosarum* bv. *vicieae* on nodulation, yield and yield traits of field pea in Haramaya experimental site, eastern Ethiopia.

2. Materials and Methods

2.1. Collection of Soil Samples for Nodule Trapping and Soil Analysis

Soil Samples from major field pea growing areas of Central Ethiopia were collected for nodules trapping experiment. The collection sites are known to be cultivated field pea and had no history of *Rhizobium* inoculation nodulating field pea. The corresponding GPS data including altitude and province name were also recorded. The map of soil samples collection is indicated in Figure 1.

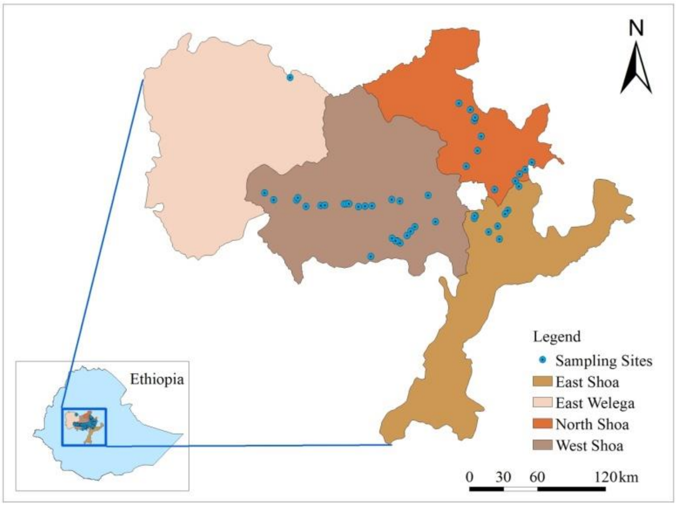

Figure 1. Map of soil samples collection from central part of Ethiopia.

Nodule trapping experiment was conducted in National Soil Testing center Greenhouse located in Addis Ababa, Ethiopia. Field pea seed (Metti variety), faba bean, lentil seeds were surface sterilized in 70% ethanol for 30 s and then in sodium hypochlorite solution (0.25% available chlorine) for 3 min and washed with sterile deionized water. Field pea var. Metti was planted as trap crops to trap the native rhizobia in the soil of each location. The seedlings were maintained for 6 weeks and harvested to obtain viable and effective nodules. The plants were cut at crown and soil was washed off from the root and nodules. Nodules already detached were picked and counted together with the intact ones. Ten fresh undamaged nodules were preserved in the refrigerator (4 ^{o}C) for isolation of the rhizobial isolates.

Isolation and purification of rhizobia was done following procedures of Somasegaran and Hoben (1994). After 4 weeks, nodules were randomly detached from the roots and sterilized in 70% ethanol for 3 min and sodium hypochlorite solution for 30 min. After washing in sterile deionized water, each nodule was crashed with pestle and mortar in sterile 0.9% NaCl solution under aseptic conditions. The extract was streaked on a yeast-extract–mannitol agar YMA (yeast extract-mannitol) agar medium, pH 7.0 containing yeast extract 0,4 g, D-mannitol 10.0 g, K_2HPO_4 0.5 g, $MgSO_4$-$7H_20$ 0.2 g, NaCl 0.1 g, and agar 15.0 g for 1,000 ml medium plate containing Congo red (Vincent 1970).

A total of 60 rhizobial isolates for all the locations were purified and characterized on YMA. Identification of fast and slow growers was carried out using Gram's staining method. The shape, texture and colour changes on YMA containing bromothymol blue were also observed. After incubation for another 3 or 7 days, a single colony was picked and transferred to YMA slant medium containing 3.0 g/liter $CaCO_3$ and incubated for 3 or 7 days, and then stored temporarily at 4 ^{o}C until further analysis. Beside this, rhizobia isolates were stored in YEM broth added with 20% of glycerol, at -20 ^{o}C.

To screen their infectivity and effectiveness, pot experiment was conducted under greenhouse condition in 12h of light at 28^{o}C and 12h of darkness at 20 ^{o}C. Sulphuric acid (98%) treated and three time autoclaved (at 121 ^{o}C for 2hr) river sand was used as growing medium. Among the indigenous strains isolated, 60 isolates were screened for nodulation field pea as test crops. Seeds were sterilized with 70% ethanol and sodium hypochlorite solution as described above. The sterilized field pea seed were then pre-germinated in moisten filter paper in petridish by incubating 25-30 ^{o}C for 48 hrs. Then after, three uniform seedlings were selected and planted semi-aseptically into a 3 kg capacity pot filled with sterilized river sand. Isolates cultured YEM broth at 28 ^{o}C for 6 days (Vincent 1970) were diluted with sterile deionized water to 10^6 cells ml^{-1}. One ml of the broth culture was supplied to each seedling.

Rhizobial isolates were assigned designations as follows: First and second letters indicat the name of research institute the rhizobia are isolated, plus a capital letter for pulse plant used as trap- plant (FP-field pea, FB-faba bean, LN-lentil) followed by a number (nodule sampled). The experiment was laid in completely randomised design and replicated four times.

Every other day, a 50ml of sterilized N-free nutrient solution (pH 6.8; K_2SO_4-0.28 g, KH_2PO_4-0.02 g, K_2HPO_4-0.145 g, $CaC1_2.2H_20$-0.014 g, $MgSO_4,.7H_20$-0.5 g, $CaSO_4$-$2H_20$-0.3 g, trace element solution 1.0 ml for 1,000 ml medium) was supplied to maintain balanced plant nutrients in the pot. Composition of the trace element solution was as follows: HBO_3-1.43 g, $MnSO_4$-$4H_20$-1.02 g, $ZnSO_4$. $7H_20$-0.22 g, $CuSO_4$-$5H_20$-0.08 g, $Na_2MoO_4.2H_20$-0.05 g, $COC1_2$-$6H_20$-0.10g, Fe-EDTA-1.0 g for 1,000 ml solution. The positive control treatment was supplied with KNO_3 (0.316 g/liter, 50 ppm N). All pots were regularly supplied with tap water during the experiment.

Harvesting was made at the beginning of the flowering period (six weeks after transplanting). The plants were uprooted and their roots were thoroughly rinsed with distilled water just before carefully detaching and rinsing with distilled water. Shoot, root, and nodule dry weights were recorded after drying for 48 h at 70 ^{o}C. Total N in plant samples was determined by the micro-Kjeldahl technique. Symbiotic effectiveness efficiency was also calculated following the formula indicated in Beck et al. (1993).

2.2. Soil Sampling and Analysis

Composite soil sample from the experimental site for the top 30 cm was collected before planting for determination of major physic-chemical characteristics. The soil at the experimental site has a textural class of sandy clay loam, with 1.965% organic carbon, 0.12% total soil N and 2.13 mg kg^{-1} soil available P. The amount of exchangeable bases (Ca^{+2}, Mg^{+2}, Na^{+1} and K^{+1}) were 31, 8.7, 0.33 and 0.14 cmol(+) kg^{-1}, respectively, with 25.98 cmol(+) kg^{-1} of CEC(cation exchangeable capacity). The pH(H_2O) and EC of the soil were 7.84 and 0.14 mS cm^{-1}, respectively. The zinc and boron content in the study site soil were 0.11 and 0.15 mg kg^{-1}, respectively.

2.3. *Rhizobial* Population Count

The indigenous rhizobial population of the soil on of 0–30 cm depth was estimated through most probable number (MPN) technique (Somasegaran and Hoben, 1994).

Field pea var. Metti was sterilized, pre-germinated and transplanted into sterilized growth pouches containing modified Jensen's N-free nutrient solution (Roughley 1984). A ten-fold soil dilution series having 6 levels and with four replications was used. Inoculations of the diluents were transferred to each pouch 1 week after planting. Nodule formation on plants was observed and recorded for 30 days. Accordingly, the most probable number of rhizobia nodulating field pea in the study site was $>10^3$ rhizobia g^{-1} soil.

2.4. Field Experiments

Field level symbiotic effectiveness evaluation experiment was conducted at Haramaya University for three consecutive years (2012-2013 cropping season) as a follow up of the green house screening work. Eight selected isolates of *Rhizobiumleguminosarum* bv. *viciae*: where four of which were obtained from the proceeding pot experiment while the other were obtained from previous work of Ketema, (2011). Beside this, one positive control (N fertilized at the rate of 20 kg N ha^{-1}) and one uninoculated control check were included. A total of ten treatments were arranged in Randomized Complete Block Design with three replications. Plot size was 3m length by 2m width. Each plot consisted of five rows and each row contained 30 plants, with 10 cm between plants and 40 cm between rows. All plots received a blanket application of fertilizer to supply 46 P_2O_5 kg ha^{-1} banded prior to seeding. The inoculants were prepared with sterile filter mud with final viable cells of rhizobia of 10^9 cells g^{-1} inocula. Inoculant was added at a rate of 500 g for 50 kg seeds with 300 ml 10% (w/v) sucrose solution to increase adherence. The plots were kept weed free throughout the experiment period.

2.5. Data Collection and Analysis

At late flowering and late pod setting stage, five plants from the central three rows were uprooted for the evaluation of nodulation and growth data collection. Nodule number was recorded after removing the soil from root system. For nodule dry weight and shoot dry weigh, the specimens were placed in over at 70 ^{O}C until constant weight was reached. At harvest, number of pod per plant, number of seeds per pod, total biomass yield, grain yield and 100 seeds weight were also recorded.

Grain yield was recorded at the final harvest, considering an area of 3.0 m x 1.2 m from the three central rows of each plot. Seeds were cleaned and records were adjusted to 13% moisture content, after determination of the humidity level in a grain moisture tester.

The collected data were subjected to test for normal distribution and variance homogeneity test using SAS version 9.1. The mean data were analyzed by analysis of variance (ANOVA), and the least significant difference (LSD) values were calculated at 5% level of significance to test significant differences among treatment means. Differences between mean values were evaluated by a one-way ANOVA.

3. Result and Discussion

3.1. Authentication and Evaluation of Symbiotic Effectiveness of Rhizobia Nodulating Field Pea

One of the major prerequisites for effective nodule formation is the selection of locally isolated bacteria strains (Bremer *et al*., 1990). The result of this experiment revealed that only thirty five isolates were nodulated field pea and authenticated as *Rhizobiumleguminosarum* bv. *vicieae* (Table 2). The remaining isolates, which were not induced nodules on the field pea root might be contaminants and rhizobia of other plants (De Lajudie *et al*., 1999; Mhamdi *et al*., 2002; Hameed *et al*., 2004). Mhamdi *et al.* (2005) showed that these non-nodulating agrobacteria were able to colonize root nodules of common bean. Due to the host-*Rhizobium* genotype specificity in nodule formation, those isolates from cross-inoculation groups may not form nodule with field pea (Slattery and Pearce, 2002). The highest nodules number per plant (266.67) was recorded with NSLNR2 followed by 240.00 with NSLNR5 inoculation. These records of nodules are higher than what has been reported from a similar study made by Keneni *et al.* (2013) where he obtained nodule number record of 19. Significantly higher nodule dry weight per plant (0.3447 g) was observed in NSLNR5 inoculated plant than those produced by other inoculated *Rhizobium*. None of the control and N fertilized treatments produced nodules, indicating that the experiment was conducted aseptically.

Shoot dry weight is often considered to indirectly measure symbiotic effectiveness when N is the only limiting factor for growth of the plants (Streeter 1988). Shoot and root length of field pea were significantly affected by inoculation treatments (Table 2). This result is in line with the findings of Amsalu (2005) and Argaw (2007). Inoculation of NSLNR2 and NSLNR5 isolates produced significantly higher shoot length (79.33 and 75.00 cm), respectively, than the check (uninoculated and unfertilized control), similar trend as nodulation performance. These isolates resulted in non-significant difference in shoot length with that of N-fertilized plant (positive control), suggesting that the inoculated plants obtained similar amount of N as of the N fertilized plant. Greater than 25.7% of tested *Rhizobium* isolates produced better shoot length than what is obtained in N fertilized treatment. Significantly higher root lengths; however, were produced by NSFPR13 followed by NSFBR7 inoculated plant but did not significantly differ from the control check. In general, more than 51% of tested isolates recorded higher root length than that of N fertilizer treatment.

Inoculation of different isolates of *Rhizobiumleguminosarum* bv. *vicieae* significantly affected shoot dry weight of field pea at $P < 0.05$ (Table 2). When compared to the check, inoculation of isolate NSLNR5 significantly increased shoot dry weight (7.03g). The difference in shoot dry weight between inoculated plants and N fertilized plant was non-significant. This result is in agreement with (Ballard *et al*., 2004) where he found non-significant difference in shoot dry weight between the inoculated and the N fertilized plants.

Symbiotic efficiency (SE) obtained up to inoculating seven *Rhizobium* inoculation isolates (NSFBR-1, NSFBR-2, NSFPR-1, NSFPR-4, NSLNR-2, NSLNR-5, and

NSFBR-8) was greater than 100%, indicating that the shoot dry weight produced by these isolates regardless of its host species was greater than that of N fertilized treatment. Fesenko *et al.* (1995) demonstrated that the proportion of strains effective on peas was equal among isolates from nodules of peas, horse bean and vetch. The result of the current study revealed that NSLNR-5 inoculation scored the greatest SE (114.7%). Similar finding was reported by Amsalu (2005) where isolates of central Ethiopia was higher in symbiotic effectiveness than those isolates obtained from other parts of Ethiopia. Ballard *et al.* (2004) found that least performed inoculation treatment produced nearly double the growth of the uninoculated treatment. Similarly, symbiotic effectiveness of rhizobia was recorded up to 138% in Otago soil (Gaur and Lowther, 1980).

Table 2. Nodule number, nodule dry weight, shoot length, root length, shoot dry weight, plant total N and P accumulation of field pea as affected by *Rhizobium* isolates inoculation and inorganic N application under greenhouse condition.

Treatment	NN	NDW	SL	RL	SDW	SE(%)	TN	TP
NSFBR1	91.00d-l	0.0590b-f	70.00abc	20.67ab	6.23ab	101.6	3.7940a-f	3046.7abc
NSFBR2	145.33b-g	0.1220bcd	58.67a-d	21.33ab	6.33ab	103.3	3.5863a-h	3980.0ab
NSFPR1	156.67b-f	0.0837b-f	69.00abc	20.33ab	6.47ab	105.4	3.7960a-f	4120.0ab
NSLNR1	94.33d-l	0.1123b-e	52.67a-d	23.33ab	5.17ab	84.2	3.8670a-f	3580.0abc
NSFBR3	147.33b-f	0.0453def	70.00abc	22.00ab	5.40ab	88.0	3.1970d-j	2820.0bc
NSFPR2	139.67c-g	0.1133b-e	75.33ab	21.33ab	5.53ab	90.2	3.6783a-g	3006.7abc
NSFPR3	130.67c-h	0.0673b-f	69.33abc	13.67b	5.43ab	88.6	3.4747b-i	3300.0abc
NSFPR4	123.33c-j	0.0850b-f	66.67abc	17.00ab	6.60ab	107.6	4.0110abc	3393.3abc
NSLNR2	266.67a	0.1350bc	79.33a	21.33ab	6.30ab	102.7	3.2247c-j	3280.0abc
NSLNR3	202.67abc	0.1423b	58.67a-d	21.67ab	6.13ab	100.0	2.6090j	3393.3abc
NSLNR4	40.67h-l	0.0647b-f	61.33abc	19.00ab	5.10ab	83.2	2.9703g-j	2966.7abc
NSFPR5	37.00h-l	0.0637b-f	65.33abc	23.00ab	5.77ab	94.0	4.0367ab	4146.7ab
NSFPR6	125.67c-i	0.0673b-f	64.33abc	20.33ab	5.80ab	94.6	3.4857b-i	2973.3abc
NSFBR4	108.00c-k	0.0537c-f	56.00a-d	22.00ab	4.90ab	79.9	3.6003a-h	3500.0abc
NSFPR7	203.00abc	0.0830b-f	70.67abc	20.00ab	5.37ab	87.5	3.9060a-f	2780.0bc
NSFBR5	178.00a-e	0.1003b-e	63.00abc	20.67ab	6.00ab	97.8	3.6703a-g	3246.7abc
NSFPR8	29.00i-l	0.0740b-f	50.67bcd	20.67ab	3.73ab	60.9	4.3203a	4813.3a
NSFBR6	48.00g-l	0.0733b-f	65.67abc	24.00ab	5.37ab	87.5	3.6443a-g	3913.3ab
NSLNR5	240.00ab	0.3447a	75.00ab	23.33ab	7.03a	114.7	3.3947b-j	1947.7c
NSFPR9	24.33kl	0.0573b-f	55.00a-d	21.67ab	4.70ab	76.6	3.1150f-i	3100.0abc
NSFPR10	187.67a-d	0.1013b-e	63.00abc	22.00ab	5.80ab	94.6	3.1383e-j	3686.7abc
NSLNR6	24.00kl	0.0407def	69.33abc	24.67a	4.60ab	75.0	2.7790ij	3826.7abc
NSFBR7	64.67f-l	0.0680b-f	64.00abc	26.33a	5.57ab	90.8	3.3823b-j	3346.7abc
NSFPR11	27.67j-l	0.0340ef	54.00a-d	22.33ab	3.77ab	61.4	3.9643a-d	2886.7bc
NSFPR12	159.00b-f	0.1063b-e	65.67abc	24.67a	6.10ab	99.5	3.5490a-i	2953.3abc
NSLNR7	130.00c-h	0.0547c-f	62.00abc	22.67ab	5.43ab	88.6	3.4673b-i	2560.0bc
NSFBR8	90.67d-l	0.1427b	54.00a-d	23.00ab	6.53ab	106.5	3.8307a-f	2834.7bc
NSLNR8	162.33b-e	0.0837b-f	62.23abc	25.00a	5.47ab	89.1	3.3853b-j	4340.0ab
NSFPR13	117.67c-k	0.0967b-e	62.00abc	26.67a	3.93ab	64.1	2.9937g-j	3193.3abc
NSLNR9	168.00bcde	0.997b-e	70.00abc	19.33ab	5.50ab	89.7	3.2713b-j	2953.3abc

NSLNR10	86.67e-l	0.1197b-e	46.00cd	22.00ab	4.13ab	67.4	3.6610a-g	3306.7abc
NSFPR14	100.67d-k	0.0693b-f	52.67a-d	19.67ab	4.97ab	81.0	4.0457ab	3786.7abc
NSFPR15	41.67h-l	0.0507c-f	59.33a-d	17.67ab	4.97ab	81.0	3.3227b-j	2993.3abc
NSFPR16	24.33kl	0.0400def	60.00a-d	19.67ab	5.83ab	95.1	2.8190h-j	3460.0abc
NSLNR11	93.33d-l	0.0820b-f	60.67a-d	22.00ab	4.83ab	78.8	3.9193a-e	3766.7abc
-VE	0.00	0	33.67d	17.67ab	3.13b	-	3.1777d-j	3086.7abc
+VE	0.00	0	66.33abc	21.67ab	6.13ab	-	3.9133a-e	4346.7ab
Mean	108.37	0.0876	62.21	21.47	5.41	-	3.5135	3368.53
LSD	97.65	0.0876	27.55	10.55	3.50	-	0.7944	1890.1
F value	15.67***	12.97***	3.26***	1.92**	2.05**	-	8.18***	2.91***
CV (%)	27.50	31.53	13.52	15.00	19.77	-	6.90	17.12

NN- Nodule number; NDW- Nodule dry weight; SL- Shoot length; RL- Root length; SDW- Shoot dry weight; SE(%)- symbiotic efficiency; TN- Total nitrogen; TP- Total phosphorus; -VE-negative control (no inoculation and N application), +VE -20 kg N ha^{-1}; NSFPR- National soil Field pea Rhizobium; HUFPR- Haramaya University Field pea Rhizobium.

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

The significant effect of inoculation treatments on total plant N concentration and plant P accumulation were observed at P <0.05 (Table 2). The highest total plant N was recorded with NSFPR8 inoculation followed by NSFPR14 though their performance on nodulation and biomass production was not good. Inoculating NSLNR3, NSLNR4, NSFPR9, NSLNR6, NSFPR13 and NSFPR16 which were induced good nodulation accumulated lower plant N than the control check. Previous report showed that the presence of great number of nodules not always guarantee effectiveness of an inoculum (Provorov *et al.,* 1994; He *et al*., 2011). The result of the current study revealed higher plant tissue N by some inoculated treatments than N fertilizer treatments. Similar results were reported by Fesenko *et al.* (1995) who found that *Rhizobium* inoculation increased N accumulation on average by 33.8%. The highest plant P accumulation (4813.3 ppm) was also recoded with NSFPR8 inoculated plant followed by NSLNR8 inoculations. Furthermore, NSFPR-8 inoculation resulted in significantly higher plant P accumulation than the N fertilized treatment. Research reports of Vance (2001) and Toro *et al.* (1998) revealed that increase in plant N concentration due to *Rhizobium* inoculation enhanced P accumulation. According to the greenhouse experiment result, NSLNR5, NSLNR2, NSFPR8 and NSFPR14 were selected for further evaluation under field experiment at Haramaya experimental site.

3.2. Effectiveness of Selected *Rhizobia* Isolates on Field Pea Production

3.2.1. Effect of *Rhizobium* inoculation on nodulation

In the field experiment the above 4 best isolates and other 4 isolates done by Ketema (2011) (HUFPR1, HUFPR2, HUFPR3 and HUFPR4) were used. The result indicated that inoculation treatments significantly affected all measured traits of field pea, except shoot length and 100 seeds weight (Table 3 and 4). Many studies reported that the benefit of *Rhizobium* inoculant on field pea production (Bremer et al. 1988; Rice et al. 1993; McKenzie *et al*., 2001). In 2011 cropping season, all isolates, excluding HUFPR3, significantly increased nodule number when compared to check. While in 2012, only isolates HUFPR3 and NSLNR5 inoculations recorded significantly higher nodule number/plant than other inoculation treatments including the N fertilized and control check (Table 3). This generally indicated the high competitiveness of inoculated isolates compared to the indigenous rhizobia nodulating field pea though the soil had $>10^3$ rhizobia population g^{-1} soil. This result is in line with the reported of Clayton *et al.* (2004) and Mishra *et al.* (2014) who found a significant increase in nodule number of field pea up on inoculation of *Rhizobium* over the uninoculated plants. In contrast to the current finding, Fettell *et al.* (1997) found the non-significant increase in nodules number/ plant through inoculation in soil with higher indigenous rhizobia. The same authors revealed that increasing rates of inoculation above 65, 000 rhizobia per seed did not further enhance nodulation. This result differed from the current work might be due to the use of indigenous rhizobia in Haramaya site thereby enhance the effectiveness of inoculated isolates in soil with high rhizobial population.

The highest nodule number in 2011 and 2012 cropping seasons were 256.3 and 73.67 with HUFPR-1 and HUFPR-3, respectively. In general the result indicated that the higher NN was recorded in the 2011 than the 2012 cropping season, though both experiments were conducted in the same experimental site with similar indigenous rhizobia population. The nodulation variation across the years was explained by the low rain fall recoded in the 2012. Climatic variability between growing seasons was often manifested through reduction of nodulation of legumes (Bidlack *et al.,* 2001). Similarly, reduction of nodule formation was reported by Yoshioka and Maruyama (1990) due to low shoot biomass production (Peoples *et al*., 2001). The lowest nodule number records in this work were 62.3 and 20.00 in 2011 and 2012, respectively, with N treatment. Field pea nodule development was highly sensitive to inorganic N than faba bean (Herdin and Silsbury, 1989).

The treatments effect over years on nodule number of field pea was non-significant (Figure 1a). However, the figure showed that HUFPR3 and NSLNR5 significantly increased nodule number as compared to N fertilized treatment. This implies that starter N was not necessary (Clayton *et al*., 2004). The highest amount of nodule number produced above the check was NSFPR14 followed by HUFPR1 inoculated treatments (Figure 2a).

Inoculating HUFPR1, HUFPR4, NSFPR2 and NSFPR8 resulted in significantly increased nodule dry weight than check in 2011 (Table 3). In 2012, all isolates, except NSLN2, NSFPR8 and NSFPR14, produced significantly higher nodule dry weight than the check. Rice *et al.* (2000) found a significant increase in nodules dry weight of field pea due to *Rhizobium* inoculation. The highest nodule dry weights (0.5520 and 0.1401 g) in the 2011 and 2012 were recorded with HUFPR1 and NSLNR5, respectively. Similar to the nodule number, *Rhizobium* inoculations over years on nodule dry weight was non-significant when we compared to check (Figure 1b). However, HUFPR3 and NSLNR5 inoculations resulted in significantly higher nodule dry weight than N fertilized treatment. This also suggests the reduction of nodule initiation and development due to inorganic N application. High levels of inorganic N were known to suppress root infection, nodule initiation and nodule development. In contrast to this, McKenzie *et al.* (2001) suggested that field pea nodulation and N_2 fixation may not be highly sensitive to residual soil inorganic N levels.

The highest amount nodule dry weight minus that of the control check was recorded with HUFPR1 followed by HUFPR4 treatments (Figure 2b). This justifies that rhizobia isolates from homologous host performs better than those isolated from cross inoculation group. Similarly, Hynes *et al.* (1990) found that strong preference of endosymbiont by field pea due to the presence of distinct plasmid profiles in *Rhizobium* strain isolated from homologous host plants. In addition, van Berkum *et al.* (1995) found that *Rhizobium* strains from *Vicia faba* nodules were distinguishable from those from *Pisum sativum*, *Vicia villosa*, and *Trifolium* spp.

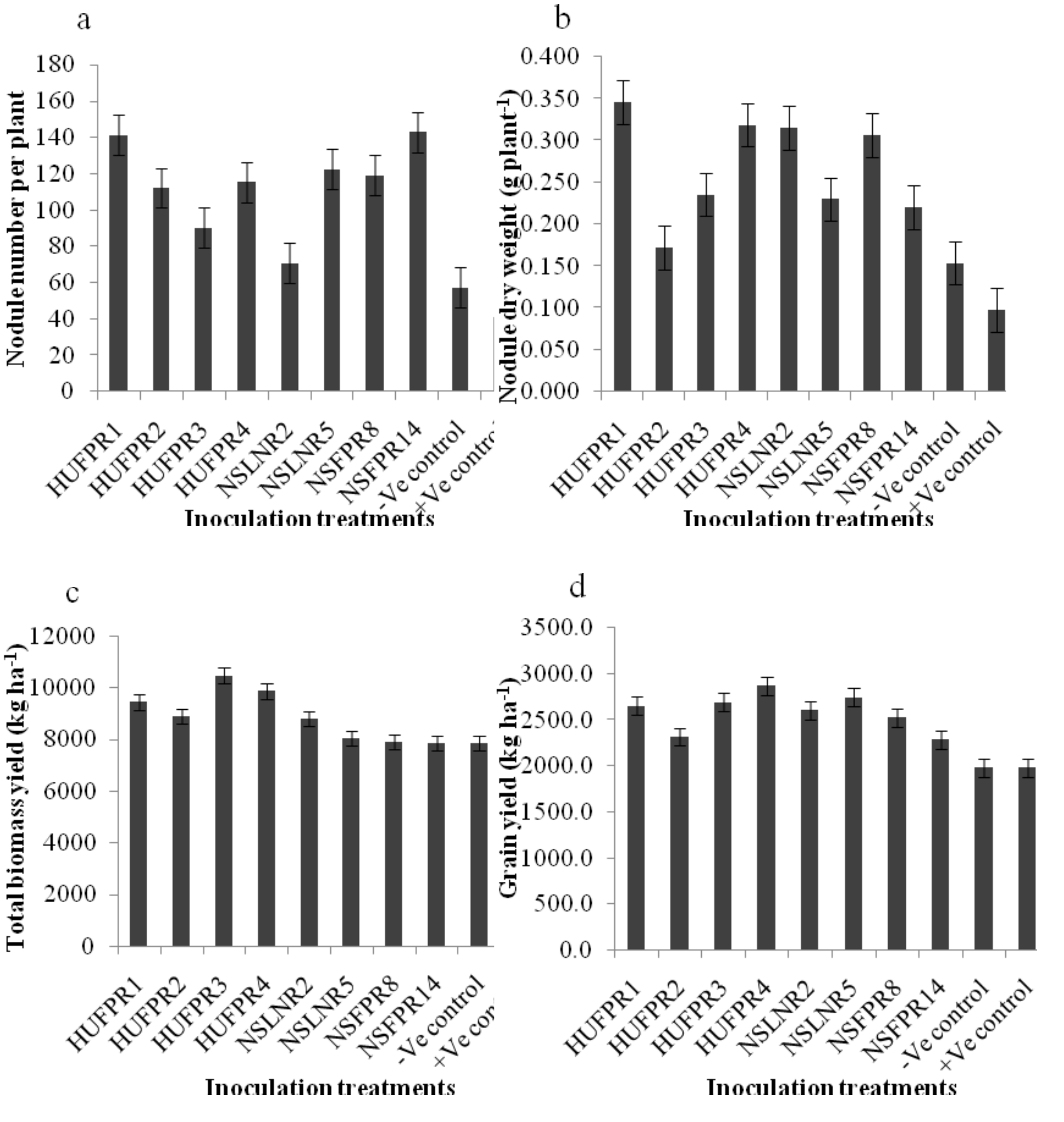

Figure 1. the effect of *Rhizobium* inoculation and inorganic N application on (a) Nodule number, (b) Nodule dry weight, (c) Total biomass yield and (d) Grain yield of field pea in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Table 3. Nodule number, nodule dry weight, shoot length, shoot dry weight and 100 seeds weight of field pea as affected by *Rhizobium* isolates inoculation and inorganic N application in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Treatments	Nodule number		Nodule dry weight		Shoot length		Shoot dry weight		100 seeds weight	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
HUFPR1	256.3a	26.33b	0.5520a	0.1401bc	159.97a	188.00a	29.33bc	64.73abc	19.0a	20.53a
HUFPR2	201.7a	22.67b	0.2830cde	0.0613e	159.30a	212.00a	35.12bc	63.78abc	18.7a	19.33a
HUFPR3	107.0bc	73.67a	0.2920cde	0.1791a	145.30a	183.33a	33.69bc	62.37bc	19.0a	20.53a
HUFPR4	197.0a	34.00b	0.4667abcd	0.1708ab	163.37a	191.00a	45.52ab	46.92c	19.3a	20.83a
NSLNR2	99.3bc	42.00b	0.5103ab	0.1205cd	176.53a	159.00a	31.76bc	59.21bc	18.7a	19.53a
NSLNR5	178.3ab	66.67a	0.2680de	0.1920a	138.75a	180.67a	20.87c	71.01ab	19.7a	21.17a
NSFPR8	214.3a	24.00b	0.4843abc	0.1287cd	174.30a	200.33a	57.69a	87.16a	19.0a	21.40a
NSFPR14	254.3a	31.33b	0.3223bcde	0.1178cd	157.03a	197.67a	32.69bc	58.43bc	19.5a	21.33a
-Ve control	83.7c	31.00b	0.2030e	0.1043d	143.97a	195.33a	18.56c	46.79c	19.9a	20.57a
+Ve control	62.3c	20.00b	0.1740e	0.0208f	177.07a	170.00a	35.47bc	70.09abc	19.0a	19.77a
Mean	165.43	37.17	0.3556	0.1235	159.56	187.73	34.07	63.05	19.17	20.5
LSD	86.80	24.22	0.2106	0.0324	43.62	67.16	17.83	23.58	2.43	2.99
F value	17.06	14.74	10.42	66.59	2.51	1.31	9.96	6.31	0.72	1.52
CV (%)	18.15	22.54	20.48	9.08	9.45	12.37	18.10	12.93	4.38	5.05

-VE-negative control (no inoculation and N application), +VE control -20 kg N ha^{-1}; NSFPR- National soil Field pea Rhizobium; HUFPR- Haramaya University Field pea Rhizobium.

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

3.2.2. Rhizobiuminoculation effect on shoot length and dry weight at late flowering stage

The non-significant effect of inoculation of selected isolates of *Rhizobiumleguminosarum* bv *vicieae* on shoot length measured at late flowing stage in both cropping seasons was recorded at $P < 0.05$. The effect of inoculations on shoot dry weight (SDW) of field pea measured at late flowering stage depicts significant difference (Table 3). This result is in synergy with the finding of Rice *et al.* (2000) which reported the significant improvement of SDW of faba bean due to *Rhizobium* inoculation. This may be attributed to increased nodulation and nitrogen fixation, more solubilization of inherent P, and production of secondary metabolites by the inoculated *Rhizobium* (Gaur 1984; Sattar and Gaur, 1987) and consequently improve the biomass production of field pea. Inoculating NSFPR8 in both cropping years produced significantly greater shoot dry weight than the check. The result, however, revealed statistically similar amount of shoot dry weight recorded between NSFPR8 and N fertilized treatments in the year 2012. The highest shoot dry weights produced in 2011 and 2012 were 57.69 and 87.16 g/plant, respectively, though nodule production was inferior in 2012. This could be due to low precipitation recorded at pod filling stage in 2011 cropping season. This shoot biomass in field condition is higher than what has been recorded in greenhouse condition.

3.2.3. Rhizobium inoculation effect on 100 seed weight and number of seeds per pod

The effect of *Rhizobium* inoculation on 100 seeds weight was non-significant at $P < 0.05$ (Table 4). This finding is in line with Jonah *et al.* (2012) who showed the non-significant influence of *Rhizobium* and N application up to 60 kg N ha^{-1} on 100 seed weight of garden pea. However, this trait significantly varied between two genotypes. In contrast to this, a study conducted by Argaw *et al.* (2015) showed a significant increase in seed weight due to *Rhizobium* inoculation in common bean. Significant effect of inoculation on number of seeds per pod was observed (Table 4) as has been found in common vetch (Albayrak *et al.*, 2006). Likewise, isolate HUFPR8 inoculation produced significantly higher number of seeds per pod than the check in year 2011. In 2012 cropping year, inoculation did not improve significantly number of seeds per pod as compared to the control check. The lowest number of seeds per pod in this cropping year was obtained from N fertilized treatment, which was probably due to nodule reduction when applied N (Herdin and Silsbury, 1989) which could consequently reduce N_2 fixation and then plant productivity (Pereira and Bliss, 1987; Peoples *et al.*, 2001). In contrary, Peoples *et al.* (2001) found suppression of N_2 fixation due to soil N without reduction of biomass production. The highest numbers of seeds per pod produced in the 2011 and 2012 were 5.73 and 6.73, respectively.

3.2.4. Effect of inoculation on total biomass and grain yield

The effect of *Rhizobium* inoculation on total biomass yield of field pea was significant at $P < 0.05$ (Table 4). In 2011, HUFPR4 inoculation resulted in significantly increase of the total biomass yield when compared to the control check. Inoculating HUFPR3 resulted in significantly greater amount of total biomass yield than the check in the 2012 cropping year, though many other *Rhizobium* treatments significantly increased nodulation over indigenous rhizobia. This suggests that N fixed and other benefit obtained from inoculation might not be sufficient to lead remarkable improvement in seed yield. Koutroubas *et al.* (1998) reported that accumulation nitrogen (N) at early growth stages and their later redistribution at seed filling stage were important factors in attaining high yield.

In both cropping years, the result also revealed the non-significant difference in total biomass between N fertilized and check treatments suggesting that the indigenous rhizobia might provide similar amount of N as inorganic N treated. The average total biomass yield over treatments produced in the 2011 was higher than that of the 2012. However, the highest total biomass yield (11,266.7 and 11228.4 kg ha^{-1}) in 2011 and 2012, respectively, were comparable to each other. The overall effect of *Rhizobium* inoculation over years when compared to the check was significant only in HUFPR3 treatment (Figure 1c). Beside this, HUFPR3 resulted in the highest change in total biomass yield (Δ TBY) over the uninoculated treatment followed by HUFPR4 treatment (Figure 2c). This indicates the need of homologous endosymbiot *Rhizobium* rather than those isolates from cross nodulation host plants species to improve the field pea. Report of several authors stated that field pea, lentil, and faba bean operated some rhizobial genotype selection (Hynes and O'Connell, 1990; Laguerre *et al.*, 1992; Laguerre *et al.*, 2003).

In contrary, when compared to the check, significantly inferior total biomass yield was produced from NSFPR14 inoculation. This suppression of field pea production by inoculation might be because competition for nutrients and other resources between inoculated and relatively effective indigenous rhizobia in rhizosphere of the plant. Kutcher *et al.* (2002) found a reduction in emergence of seedling in early stage due to inoculation.

Likewise, inoculation treatments significantly affected grain yield of field pea at $P < 0.05$ (Table 4). This study showed that indigenous rhizobia was incapable of supplying adequate levels of N_2 fixation though the rhizobial population in Haramaya soil had $>10^3$ g^{-1} soil. Several studies found to a significant increase in yield of field pea (Chemining'wa and Vessey, 2006) and common bean (Mulas *et al.*, 2015) by local *Rhizobium* inoculation in soil with abundant indigenous rhizobial population. Khurana and Dudeja (1997) found crop yield increase by 10-15% due to *Rhizobium* inoculation under field condition. In contrast to this study, McKenzie *et al.* (2001) found that greater response of field pea to *Rhizobium* inoculation in soil with low rhizobial population than soil having high rhizobial population. In addition, non-significant increase in grain yield of field pea was observed in soil with $>10^3$ rhizobial population g^{-1} soil (Evans *et al.*, 1993). Similarly, Yadav and Verma (2014) reported that a significant increase in chickpea grain yield was obtained by inoculating locally isolated *Rhizobium*. Inoculation of HUFPR4 recorded significantly greater grain yield than the check and N fertilized treatments in the 2011 cropping year. The non-significant effect of the remaining inoculation treatments on field pea could be related with the presence of field pea variety Metti variety-*Rhziobium* strain specificity. Under control condition, Hobbs and Mahon (1983) found specific interaction between field pea variety and single strain of *Rhizobium*.

Other isolates namely HUFPR1, HUFPR3, NSFP8 and NSDFPR14 resulted in significantly higher grain yield than the N fertilized treatment. This indicates that N fertilization and N_2 derived from nodules induced by indigenous rhizobia might not satisfy the N requirement of field pea. This finding is in line with McKenzie *et al.* (2001) who found that the average yield benefit due to N application was higher in trials with no history of legumes (no rhizobia detection) than in trials with a history of legumes (high *Rhizobium* population).

In 2012, NSLNR5 inoculation resulted in significantly increased grain yield as compared to the check and N fertilized treatments. Moreover, HUFPR1, HUFPR3 and NSLNR2 inoculation had significantly greater grain yield than the check. The highest grain yield produced in 2011 and 2012 cropping years were 2633.3 and 3655.6 kg ha^{-1}, respectively. However, the highest NN and NDW were recorded regardless of inoculation treatments in 2012 cropping season. This controversial result attributed to early termination of rainfall in 2012 and hence consequent reduction of the productivity of field pea. Significantly higher grain yield over years was produced due to HUFPR3 inoculation than the control check (Figure 1d). The highest grain yield minus the check (Δ GY) was recorded with HUFPR4 followed by NSLNR5 inoculation treatments. This indicates that more effectiveness of field pea self-inoculated isolates than cross-inoculated isolates obtained from homologous host species. The lowest grain yield was recorded in N fertilized treatment. This suggests that host plant could derive higher N from indigenous rhizobia nodulating field pea than N fertilization. McKenzie *et al.* (2001) found that less chance of effectiveness of inoculated *Rhizobium* in soil with high soil NO_3^- when compared to those fields with low soil NO_3^-. The report of Rice *et al.* (2004) also confirms the non-significant effect of N application up to 40 kg N ha^{-1} on field pea production when compared to the unfertilized treatment. In contrast to this 100 kg N ha^{-1} application was found to significantly improve the grain yield of field pea (Chemining'wa and Vessey, 2006).

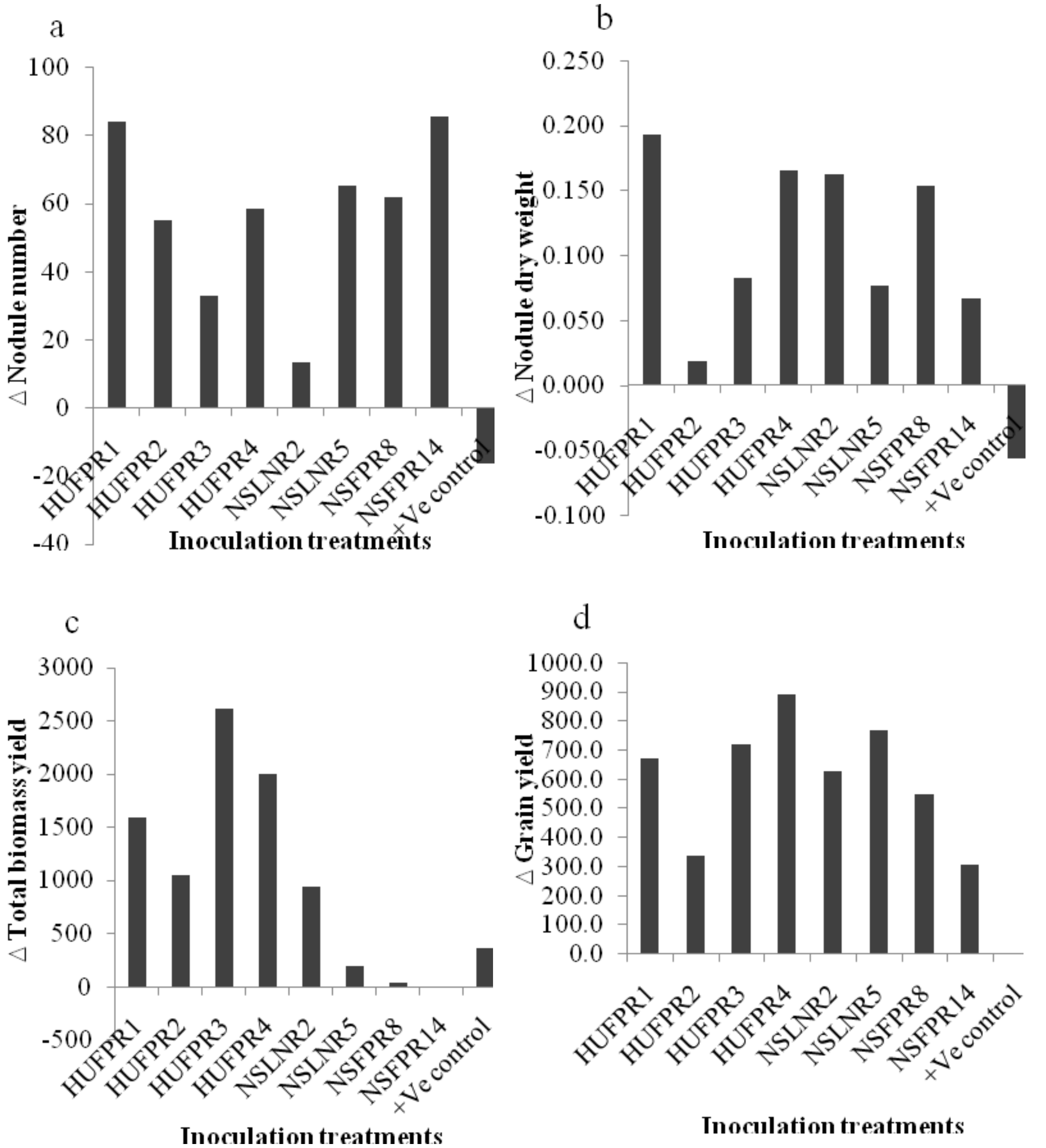

Figure 2. the effect of inoculation and inorganic n application over the control check on (a) Nodule number, (b) Nodule dry weight, (c) Total biomass yield and (d) Grain yield of field pea in Haramaya, eastern Ethiopia, 2012/13 cropping season.

3.2.5. Effect of rhizobium inoculation on total plant N and P accumulation

Significant effect of *Rhizobium* inoculation on the total plant N concentration and P accumulation of field pea was observed except, P accumulation in 2012 cropping year at P <0.05 (Table 4). In 2011, HUFPR4 and NSLNR2 inoculants resulted in significantly greater amount of total plant N than the check. Rennie and Dubetz (1986) found that 216 kg N ha^{-1} (85%) was derived from symbiotic N_2 fixation through inoculation of effective *Rhizobium*. Moreover, up to 90% of N accumulation was through inoculation of rhizobia on field pea when compared to uninoculated plant (Rice *et al.*, 2004). Mishra *et* *al.* (2014) also found remarkable increase in N derived from symbiosis due to inoculation when compared to uninoculated plants. However, the result of this study revealed non-significant difference of the trait among inoculated treatments with N fertilized plants. McKenzie *et al.* (2001) revealed significant reduction of protein concentration in field pea grain due to N application compared to *Rhizobium* inoculation. This finding is supported by Clayton *et al.* (2004) who found that N application did significantly improved the plant N accumulation as compared to inoculated plant. They found that total N accumulation was 36% higher in inoculated field pea than uninoculated. This increase could be because of achieving greater N_2 fixation by inoculated efficient rhizobia strains.

In 2012 cropping season, none of *Rhizobium* inoculation treatments improved significantly plant N concentration as compared to the check. However, all inoculation treatments, excluding HUFPR2, HUFPR4 and NSFPR14, accumulated significantly higher total plant N than N fertilized treatment. This finding is in line with the result of Bianco *et al.* (2013) who found that the N content of subterranean part of Adesmia bicolor was higher in *Rhizobium* inoculated plants than N fertilized plants. This could be due to reduction of N derived from symbiotic N_2 fixation as result of the N application (Bremer *et al.*, 1988).

As it was observed in nodulation, the total plant tissue N accumulated ranging from 5.0833% with HUFPR-4 to 3.5900 in uninoculated treatment in 2011 cropping season. While NSFPR-8 and N fertilizer treatments accumulated the greatest (1.9870%) and the lowest (1.3146%) plant total N, respectively, in 2012 cropping season. This indicates the need of rhizobial inoculation to increase the plant N content. The lower plant N accumulation in 2012 cropping season could be due to low and erratic precipitation that directly affects biomass production (Ronner *et al.*, 2016) and associated N accumulation reduction (Serraj *et al.*, 1999; Peoples *et al.*, 2001). Water stress at late flowering stage of field pea could be more severe in N_2 fixation than in other stages of growth. However, field pea is more tolerant to drought than faba bean and lentil in N_2 fixation (Bremer et al. 1988) and up to 50% increase in N_2 fixation due to effective *Rhizobium* inoculation (Mishra *et al.*, 2014). Under favorable condition without N application, field pea can derived 65-70% of accumulated N from biological N_2 fixation (Jensen, 1997).

Table 4. Number of seeds per pod, grain yield, total biomass, plant total N and P accumulation of field pea as affected by *Rhizobium* isolates inoculation and inorganic N application in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Treatments	Number of seeds per pod		Grain yield		Total biomass yield		Plant total N accumulation		Plant total P accumulation	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
HUFPR1	5.33ab	6.13ab	2000.0b	3299.9ab	9930.0ab	8944.4b	4.2767ab	1.9453a	4.2833a	3.2511a
HUFPR2	5.20ab	5.60ab	1833.0bc	2797.9abc	9230.0ab	8568.7c	4.1933ab	1.3529c	3.7857abc	3.7304a
HUFPR3	5.47ab	6.27ab	2067.0b	3321.4ab	9693.7ab	11228.4a	4.4900ab	1.9770a	3.9553abc	3.3911a
HUFPR4	5.33ab	6.13ab	2633.3a	3103.3abc	11266.7a	8441.5bc	5.0833a	1.3753bc	4.1890ab	3.5178a
NSLNR2	5.60ab	6.73a	1866.0bc	3346.3ab	9133.3ab	8455.6bc	5.0667a	1.9486a	4.1920ab	3.8022a
NSLNR5	5.47ab	6.00ab	1833.0bc	3655.6a	9300.0ab	6783.3bc	4.6333ab	1.8974a	3.8527abc	3.6311a
NSFPR8	5.73a	6.33ab	2066.3b	2980.2abc	8630.0ab	7144.4bc	4.1167ab	1.9870a	3.6227bc	3.2356a
NSFPR14	5.60ab	6.27ab	2033.0b	2533.1bc	9060.0ab	6638.9c	4.4300ab	1.4283bc	3.8423abc	3.1844a
-Ve control	5.07b	5.60ab	1766.0bc	2188.1c	8400.0b	7300.0bc	3.5900b	1.7828ab	3.5557c	3.3178a
+Ve control	5.20ab	5.20b	1533.0c	2431.3bc	8260.0b	8183.3bc	4.0533ab	1.3146c	3.9247abc	3.5756a
Mean	5.40	6.03	1963.1	2965.72	9290.67	8168.86	4.3933	1.7009	3.9203	3.4637
LSD	0.58	0.19	429.05	1001.2	2727.4	2261.5	1.2553	0.415	0.6169	0.6282
F value	3.33	3.53	11.26	5.51	2.57	8.91	3.34	12.54	3.88	3.04
CV (%)	3.70	6.8	7.56	11.68	10.15	9.57	9.88	8.43	5.44	6.27

-VE-negative control (no inoculation and N application), +VE control -20 kg N ha^{-1}; NSFPR- National soil Field pea Rhizobium; HUFPR- Haramaya University Field pea Rhizobium.

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

In the 2011, HUFPR1 inoculation resulted in significantly increase the plant P accumulation when (Table 4). This finding is supported by Zafar *et al.* (2012) who found that plant growth promoting rhizobia inoculation positively ($P < 0.05$) increased the P accumulation form the soil and plant N accumulation by improving N_2 fixation. Plant N accumulation due to increase in N2 fixation as a result of inoculation was reported also by Cong *et al.* (2010). The result also indicated the non-significant difference in P accumulationbetween *Rhizobium* inoculations and N fertilized treatment. The highest (4.2833 ppm) and lowest (3.5557 ppm) P accumulation were obtained from HUFPR1 and the control check, respectively. This shows that *Rhizobium* inoculation improved the P accumulation. However, the effect of *Rhizobium* inoculation and N application on P accumulation of field pea when compared to uninoculated treatment in 2012 was non-significant.

4. Conclusion

Nodulation and growth of field pea were highly improved by inoculation of rhizobia isolated from homologous host plants (Faba bean and lentil) and itself under greenhouse and field condition. Some of the tested rhizobia regardless of host species increased the growth of field pea when compared to N fertilized plants. Due to the environmental condition in two cropping seasons, the effectiveness of inoculated rhizobia was different. In general, low rainfall reduced the effectiveness of *Rhizobium* inoculation. Regardless of host species, nodulation field pea was increased by *Rhizobium* inoculation despite remarkable increase in grain yield of field pea was obtained through *Rhizobium* isolated from field pea root. Therefore, validation of the best isolate (HUFPR4) for one year over representative temperature regimes and soil types is suggested before getting recommended the *Rhizobium* of field pea as biofertilizer to improve its production in highland of eastern part of Ethiopia.

5. Acknowledgments

This research was funded by the Ethiopia Institute of agricultural Research, under Biofertilizer and organic fertilizer research project. The authors would like to express their sincere thanks to field and technical assistant for their field experiment management and data collection.

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4. Effectiveness of Native *Rhizobium* on Nodulation and Yield of Faba bean *(Vicia faba* L.) in Haramaya, Eastern Ethiopia

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Abstract: Ethiopia is the center of diversity for faba bean with the presence of high rhizobia diversity where the crop derive more than 80% of its nitrogen (N) from symbiotic association while N remains to be the major yield limiting nutrient in the country. Therefore, this study was initiated to evaluate the effect of locally isolated Rhizobium leguminosarum bv. *vicieae* on nodulation, yield and yield related traits of faba bean at Haramaya University experimental site, eastern Ethiopia. The experiment consisted of eight effective isolates of rhizobia, one uninoculated, and one N fertilized (20 kg N ha-1) treatments were laid out in randomized complete block design (RCBD) with three replications and conducted for three consecutive years (2011to 2013). All inoculation treatments increased nodule number (NN/plant)) and dry weight (NDW/plant) when compared to the control check in all cropping seasons. The highest NN of 413, 440.67 and 366.67 were caused by NSFBR-15, NSFBR-12 and NSFBR-1isolates in 2011, 2012 and 2013 cropping seasons, respectively, with 88.9, 67.8 and 31.7% better than the uninoculated treatments of the respective years. Rhizobium inoculation also increased NDW/plant by 185.7, 190.7 and 212.0% over uninoculated treatments in 2011, 2012, and 2013, respectively. Inorganic N application (20 kg N ha-1) improved the NN and NDW in all cropping seasons, except NN in 2011 and 2012. The result also showed the non-significant effect of Rhizobium inoculation on shoot length, number of tiller per plant and 100 seed weight in all cropping seasons. The non-significant difference between TAL-1035 and local isolates inoculations were recorded in all yields and yield traits of faba bean. However, inoculation of local isolates (NSFBR-15 and NSFBR-12) showed 64.8 and 80% relative NN/plant to TAL-1035 in 2011 and 2012, respectively. Similarly, NSFBR-30 inoculation showed 92.7 and 161.3% relative NDW/plant to TAL-1035 in 2011 and 2012, respectively. Inoculating HUFBR-15 in 2011 and NSFBR-30 in 2012 and 2013 induced 4330.0, 5267.0 and 4608.0 kg ha-1 grain yields with yield advantage of 75, 48 and 5% over the uninoculated treatment, respectively. Applying NSFBR-12 followed by NSFBR-30 gave the highest ΔTBY while NSFBR-30 proceeded by HUFBR-15 inoculations recorded the highest ΔGY. The result indicated that isolates from central Ethiopia were better than those isolated from eastern Ethiopia and TAL-1035 was better in enhancing faba bean production. Based on overall effect of inoculation, NSFBR-30 is recommended as a candidate isolate for faba bean biofertilizer production in eastern Ethiopia soils.

Keywords: Biofertilizer; Faba bean; Haramaya; Rhizobium leguminosarum bv. *Viciae*

1. Introduction

Faba bean might have been brought to Ethiopia from Middle East through Egypt around the 5th millennium B.C. (Telaye *et al*., 1994; Yohannes 2000). Ethiopia is considered as the secondary center of diversity and also one of the nine major producing countries of faba bean (Bond *et al*., 1985; Telaye *et al*., 1994) with annual production of 2,671,834.45 ton from 1,558,422.02 ha of land (CSA 2015). In Ethiopia, faba bean is grown in the highlands (1780-3000 m.a.s.l.) with 700-1000 mm annual rainfall (Yohannes, 2000). It is major source of protein beside N benefit for following crops is often high (Azaza *et al*., 2009; Nebiyu *et al*., 2014). Jensen *et al.* (2010) reported that the crop comes after faba bean benefits up to 100-200 kg N ha^{-1}.

Ethiopia is the world's second largest producer of faba bean next to china (Salunkhe and Kadam 1989). Its production share is only 13% of world production and 40.5% within Africa (Chopra *et al*., 1989; FAOSTAT 2008). This is because of the fact that the average yield of faba bean is about 1.89 t $ha^{-}1$ under small-holder farmers (CSA 2015). The world average grain yield of faba bean is around 1.8 t ha^{-1} (ICARDA 2008; FAOSTAT 2008).

Faba bean can form nodules with *Rhizobiumleguminosarum* symbiovars *viciae*, *trifolii* and *phaseoli*, *Rhizobiumetli* and *Rhizobiumfabae* (Van Berkum et al. 1995; Tian *et al*., 2007; Tian *et al.,* 2008). Faba bean and soybean fixes around the same amount of N up to 200 kg N ha^{-1} followed by pea and lentil (85 kg Nha^{-1}) and chickpea and common bean (50 kg N ha^{-1}) (Peoples *et al.,* 1995, Unkovich and Pate 2000; Hardarson and Atkins 2003; Walley *et al*., 2007; Yang *et al*., 2010). It have shown that faba bean can maintain a higher dependence on N_2 fixation for growth, and fix more N than field peas, lentils and lupins and green-manured pasture species under the same soil N supply (Rochester *et al*., 1998). To improve the N derived from nitrogen fixation and productivity of faba bean, the seed or soil must be inoculated with the appropriate strain of *Rhizobium* (van Kessel and Hartley 2000). Most cultivated soils contain large populations of indigenous rhizobia for faba bean and inoculation is usually not required; particularly if the land had previously been sown to faba bean (Murinda and Saxena 1985; Patriquin 1986; Jensen 1987). Under this condition, superior rhizobia that are competitive with the indigenous strains should be developed (Amarger 1986; Brockwell *et al*., 1995). Several authors found that inoculation of local or adapted varieties of host plant with effective native strains produced similar grain yield than the N fertilised control regardless of number of indigenous rhizobial population (Hungría *et al*., 2000, 2003; Mostasso *et al.,* 2002; Mrabet *et al*., 2005). In addition, native *Rhizobium* inoculation was more competitive and effective with clover than exotic *Rhizobium* in soil with previous history of inoculation (Batista *et al*., 2015). Due to faba bean genotypes-*Rhizobium* isolate specificity (Mytton *et al*., 1977) and selective in forming nodule with some *Rhizobiumleguminosarum* bv. *viciae* genotypes (Jorrin and Imperial, 2015), selection of locally isolated superior *Rhizobium* would be essential. The presence of high diversity of faba bean is expected to be accompanied by diverse symbiotically effective N_2 fixer indigenous rhizobia in Ethiopian soil. To our knowledge, prior to this study, there were no any studies on characterization of symbiotic effectiveness of selected native *Rhizobium* on faba bean in Ethiopia. Therefore, the objective of this study was to evaluate the effect of selected local isolates of *Rhizobiumleguminosarum* bv. *vicieae* inoculation on nodulation, yield and yield related traits of faba bean in eastern Ethiopia.

2. Materials and Methods

2.1. Description of the Study Site

A field experiment was established in 2012 and 2013 cropping season on a sandy clay loam soil under rainfed conditions at the Research Farm of Haramaya University. The experimental site is situated at N09°24.954" and E042°02.037" at an altitude of 2020 m.a.s.l. Climatically, the area placed in the semi-humid zone. During the experimental period, the mean monthly minimum and maximum temperatures were 23.4 and 8.25°C, respectively. The annual rainfall was 760 mm of which 75-85% occurred during the July-September period.

A composite soil sample (0–20 cm depth) was taken prior to planting for enumeration of indigenous *Rhizobiumleguminosarum* bv. *viciae* using the most probable number (MPN) plant grow out method (Vincent, 1970) and determination of selected soil physico-chemical properties. The physicochemical properties of the soil are: sand, 49%; silt, 18%; clay, 33%; EC, 0.14 mS cm^{-1}; pH, 7.3; 0.74% organic carbon; 0.08% total N; and 9.94 mg kg^{-1} available P. The exchangeable bases (Ca^{+2}, Mg^{+2}, Na^{+1} and K^{+1}) content in the study site soil were 31, 8.7, 0.33 and 0.14 cmol(+) kg^{-1}, respectively, with 25.98 cmol kg^{-1} CEC.

2.2. Treatments and Experimental Design

The faba bean variety was *Gachena* where as preexisting and indigenous effective *Rhizobiumleguminosarus* bv, *viciae*: NSFBR-11, NSFBR-30, NSFBR-12 and NSFBR15 developed by Anteneh Argaw (2012) and HUFBR-22 and HUFBR15 developed by Abere Mnalku (2009) were used. One exotic isolate (TAL-1035) obtained from Holleta Agricultural Research Centerwas also included.

Rhizobium was incubated in yeast extract mannitol (YEM) broth at 30°C for 5 days until the number viable cells were reached 10^8 ml culture broth. The liquid culture was added to the sterile filter mud and mixed thoroughly and packed in plastic bag. The filter mud based inoculant was incubated at room temperature for 15 days. Using pour plate technique, the number of viable *Rhizobium* in inoculant was tested and was 10^8 cells g^{-1} inoculant.

A total of ten treatments including eight *Rhizobiumleguminosarum* bv. *viciae* isolates, an uninoculated check and N treated (20 kg N ha^{-1}) were used throughout the three cropping seasons. The treatments were established in a randomized complete block design with three replicates. Individual plot size was 3 m x 2 m = 6 m^2. Spacing between rows was 40 cm whereas between plants was 10 cm. All plots received a blanket application of phosphatic fertilizer at a rate of 46 P_2O_5 kg ha^{-1} banded prior to seeding. The plots were kept weed free throughout the experiment period.

2.2. Data Collection and Analysis

Plants were sampled at 60 days after planting (DAP) which was at late flowing and early pod setting stage of faba bean. Five plants were randomly sampled from three central rows of each plot for measuring the following characteristics: nodule number (NN), nodule dry weight (NDW) and shoot dry weight (SDW). For NDW and SDW, the specimens were dried to constant weight at 70°C in an oven. Samples were milled and the nitrogen content was determined by the Kjehldahl procedure (Sahlemedhin and Taye, 1992).

At physiological maturity, the central three rows were harvested for yield assessment. Numbers of pod per plant, number of seeds per pod, number of tiller per plant, total biomass yield and plant height at harvest were recorded. 100 seed weights and grain yield were determined after air-drying to 13% moisture content.

The data were subjected to test for normal distribution and homogeneity of variance using SAS version 9.1. The mean data were analyzed by analysis of variance (ANOVA), and the least significant difference (LSD) values were used to compare treatment means ($P < 0.05$).

3. Result and Discussion

Analysis of variance revealed that the effect of *Rhizobium* inoculation treatments, cropping season and their interaction on nodule number and nodule dry weight were significant at $P < 0.05$, except the effect of cropping season on nodule number (Table 2). In the 2011 cropping season, NSFBR-15, NSFBR-30, NSFBR-12 and NSFBR-11 inoculation resulted in significantly higher nodule number (NN) and nodule dry weight (NDW) than the control check (Table 3). In 2012, NSFBR-12 inoculation increased NN while HUFBR-15, NSFBR-30 and NSFBR-12 inoculations increased NDW significantly as compared to the control check. Improving faba bean nodulation by inoculation of elite isolate of *Rhizobium*was previously confirmed by Ahmed and Elsheikh (1998). Carter *et al.* (1995) also found a significant increase in nodulation over the uninoculated check in previously inoculated soils. However, the current study in the 2013 indicated that none of *Rhizobium*isolates affected significantly the NN, though NDW was significantly increased by most of *Rhizobium* inoculated treatments. This non-response of nodulation to inoculation could be due to the presence of competent indigenous rhizobia nodulating faba bean in soil where this experiment was conducted. Carter *et al.* (1995) found that the alkaline and neutral soil in Australia possessed high populations of competitive native strains nodulating faba bean which reduced the infectiveness of inoculated isolate.

Though there was no significant difference in NN and NDW between the control check and N fertilized treatments, the lowest NN was recorded with the control check. In contrast to this, NDW was higher in the control check. This indicates the N application may inhibit effectiveness rather than infectiveness of indigenous rhizobia. Beside this, N fertilized application reduced the average NDW when compared to the control check. This finding is in line with Clayton et al. (2004) who found that N application reduced the nodule initiation and development. This could be through decreasing the genetic diversity among native *Rhizobium* strains (Cabarello-Mellado and Martinez- Romero 1999).

The result of the current study revealed a significant increase in average NN by all inoculated treatments excluding NSFBR-1 and HUFBR22 (Figure 1a). Inoculating NSFBR-30, NSFBR-12, TAL-1035 and NSFBR-11 isolates produced higher mean NDW than the control check treatment (Figure 1b). The highest relative NN increment over the control check was recorded due to NSFBR-12 followed by NSFBR-11 inoculation (Figure 2a). Isolate NSFBR-30 followed by NSFBR-12 inoculations produced the highest relative NDW increase over Control check (Figure 2b). This findings is in agreement with Habtemichial *et al.* (2007) who found that *Rhizobium* inoculation increased the nodule number and fresh weight of faba bean by 53% and 95% over the uninoculated in north Ethiopia.

The effect of *Rhizobium* inoculation, cropping season and their interaction was significant on the average shoot dry weight (SDW) at late flowering and early pod setting stage (Table 1). With respect to SDW, a significant increase due to *Rhizobium* inoculation was observed only in 2013 cropping season. Accordingly, the highest SDW was recorded in this season with NSFBR-12 and NSFBR-11, indicating that the inoculated isolates are better in N_2 fixation and improving plant growth than indigenous rhizobia in native soils. This finding agrees with the report of Badr El-Din and Moawad (1988) who came across in plant dry weight increase due to *Rhizobium* inoculation in faba bean.

Table 1. Nodule number, nodule dry weight and shoot dry weight of faba bean as influenced by *Rhizobium* inoculation and inorganic N application in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Treatment	Nodule number			Nodule dry weight			Shoot dry weight		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
TAL-1035	250.67bcd	244.67cde	290.00ab	1.2113bc	0.9917de	1.4499a	64.83b	152.30ab	94.37abcd
HUFBR15	335.67abc	384.67abc	296.67ab	0.9684bc	2.0177ab	0.5890de	73.63ab	196.63a	100.00abcd
NSFBR15	413.00a	191.33e	343.33ab	1.5870b	0.8809de	0.6323de	72.33ab	180.53ab	104.20abc
NSFBR-1	219.00cd	244.00cde	366.67a	0.8348c	1.4009cd	0.9901bc	75.03ab	162.40ab	69.37de
NSFBR-30	374.00ab	398.33ab	213.33b	2.3344a	2.5909a	0.8249cd	74.20ab	198.63a	81.53bcde
HUFBR-22	218.67cd	229.33de	250.00ab	1.4020bc	0.6144e	0.8500cd	95.90a	166.57ab	86.00abcd
NSFBR-12	400.00a	440.67a	363.33a	1.5246b	1.6274bc	1.3218ab	72.97ab	174.50ab	106.87ab
NSFBR-11	381.33a	345.00abcd	303.33ab	1.0248bc	1.0050de	0.8752cd	96.83a	155.47ab	115.43a
+VE	188.00d	185.00e	223.33a	0.8518c	1.2343cd	0.4673e	84.83ab	169.37ab	72.03cde
-VE	218.67cd	262.67bcde	278.33ab	0.8171c	0.8914de	0.4240e	61.23b	146.83b	56.00e
LSD	125.36	147.45	136.66	0.6394	0.6079	0.3319	28.60	48.94	33.45
F-value	12.57***	9.69***	3.92**	13.89***	15.86***	13.62***	4.32**	3.25*	8.03***
CV (%)	14.46	17.43	16.14	17.61	24.81	26.47	12.82	9.94	13.06
Mean	299.00	292.00	292.83	1.2556	1.3255	0.8425	77.18	170.32	88.58

, ** and *, significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. VE-negative control (no inoculation and N application), +VE control=20 kg N ha^{-1}; NSFBR= National soil Faba bean Rhizobium; HUFBR= Haramaya University Faba bean Rhizobium; TAL= Tropical Agricultural legume (International Rhizobium strain).*

Table 2. Shoot length, number of tillers per plant and number of pods per plant of faba bean as influenced by *Rhizobium* inoculation and inorganic N application in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Treatment	Shoot length			Number of tillers per plant			Number of pods per plant		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
TAL-1035	121.13a	143.00ab	155.00ab	1.67a	2.22a	3.99ab	41.33a	35.67ab	33.44ab
HUFBR15	113.43a	149.67ab	155.00ab	2.33a	1.78a	4.00ab	24.67bcd	24.22d	29.00ab
NSFBR15	118.63a	147.67ab	140.00b	2.67a	2.67a	3.44b	22.33bcd	25.22d	31.33ab
NSFBR-1	101.37a	158.00a	161.67ab	2.33a	2.33a	3.66b	26.67b	33.67bc	32.11ab
NSFBR-30	119.40a	148.00ab	165.00a	2.00a	2.78a	3.66ab	23.33bcd	43.00a	26.11b
HUFBR-22	99.90a	146.67ab	156.67ab	2.00a	2.89a	3.66ab	25.00bc	26.55cd	32.78ab
NSFBR-12	122.90a	147.67ab	151.67ab	2.00a	2.22a	4.66ab	27.00b	26.32cd	35.11a
NSFBR-11	105.66a	127.33b	161.67ab	1.67a	2.66a	3.44b	24.67bcd	27.67bcd	27.33ab
+VE	117.80a	148.00ab	153.33ab	2.33a	2.64a	5.44a	17.67cd	25.33d	31.78ab
-VE	99.00a	154.67a	165.00a	2.67a	2.10a	4.55ab	17.33d	22.78d	33.67ab
LSD	34.63	26.17	22.24	1.90	1.44	1.89	7.58	8.01	8.70
F-value	1.87ns	2.41*	4.92*	0.90ns	1.49ns	3.08*	19.23***	15.91***	2.79*
CV (%)	10.70	6.15	2.86	30.38	20.55	16.25	10.48	9.54	9.63
Mean	111.92	147.07	156.50	2.43	2.43	4.03	25.00	29.04	31.26

*NS= non significant; *, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. VE-negative control (no inoculation and N application), +VE control=20 kg N ha^{-1}; NSFBR= National soil Faba bean Rhizobium; HUFBR= Haramaya University.*

Faba bean Rhizobium; TAL= Tropical Agricultural legume (International Rhizobium strain).

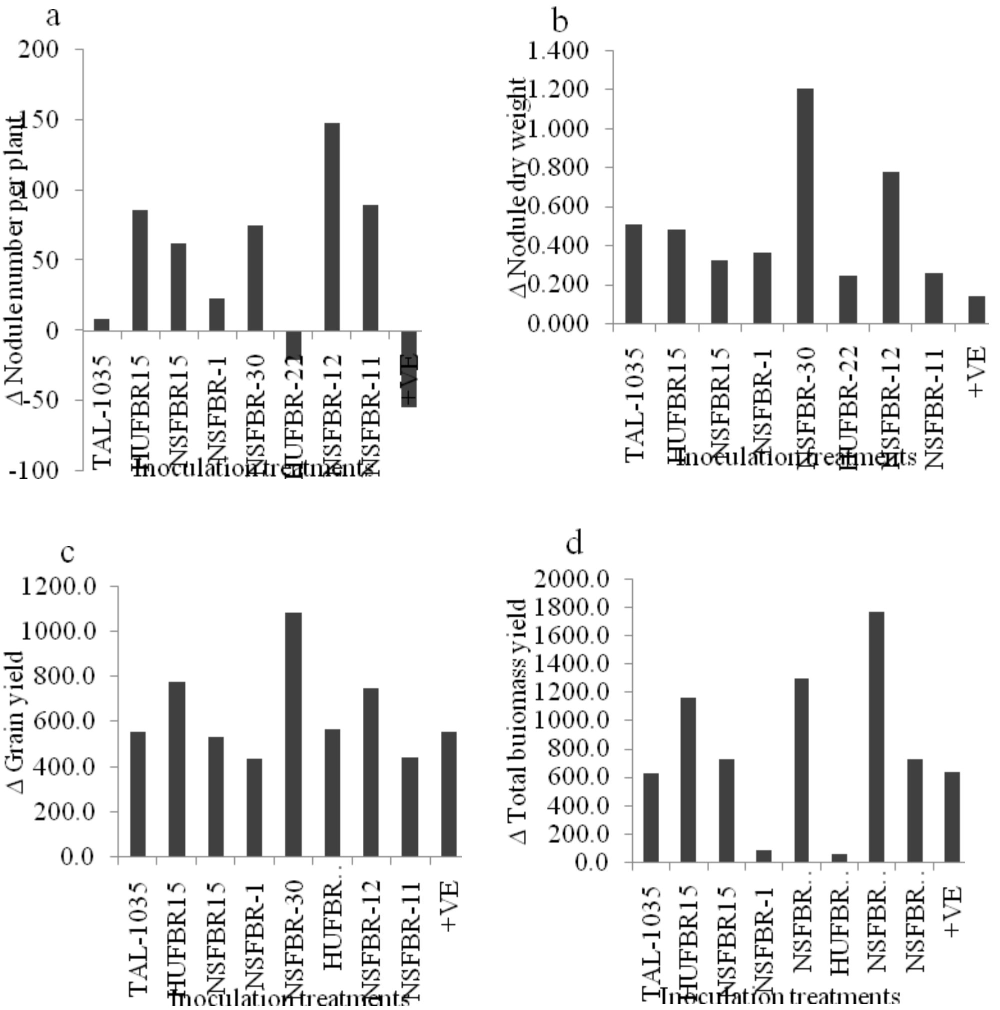


Figure 2. The effect of inoculation and inorganic N application over the control check on (a) nodule number, (b) Nodule dry weight, (c) total biomass yield, (d) Grain yield and (e) Total N accumulation in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Moreover, the result indicated the non-significant influence of inoculation on shoot length and number of tillers per plant in all cropping seasons (Table 2). This might have related that environmental and/or the genetic factor rather than the soil management dominate the performance of these traits. Beside this, N fertilized application did not improve the hoot length and number of tillers of faba bean in all cropping season. This result concurs with the finding of Tsigie and Woldeab (1994) who that the effect of N application on faba bean in soil containing >1000 rhizobial population was not satisfactory. It is also noticed that *Rhizobium* inoculation, cropping season and their interaction had significant effect on number of pods per plant (NPP) of faba bean at $P < 0.05$ (Table 2). Several studies found significant effect of *Rhizobium* inoculation on total number of pods of faba bean (Elsheikh and Osman 1995; Albayrak *et al.*, 2006), though it is an inherent property of the plant controlled by plant genetics makeup (Fageria and

Santos 2008; Meena *et al.,* 2015). In 2011, TAL-1035, NSFBR-1 and NSFBR-12 inoculations had significantly higher NPP than the control check (Table 4). Significantly higher NPP than the control check in 2012 season of cropping were produced with TAL-1035, NSFBR-1 and NSFBR-30 inoculation treatments. This could be due to better N_2 fixation, phytohormones production and solubilizing insoluble P (Vikram *et al.,* 2007; Zafar *et al.,* 2012). The effect of *Rhizobium* inoculation when compared to the control check was not significant on NPP in 2013. This result might be related with less competitiveness of the inoculated isolates against the background *Rhizobium* in soil (Blanco *et al*., 2010; Batista *et al*., 2015). On top of this, the effect of N fertilizer application on faba bean on NPP was non-significant in all cropping seasons, evidencing that N application may not satisfy the N requirement of the plant.

Table 3. Number of seeds per pod, 100 seeds weight and total biomass yield of faba bean as influenced by *Rhizobium* inoculation and inorganic N application in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Treatment	Number of seeds per pod			100 seeds weight			Total biomass yield		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
TAL-1035	3.90a	3.73a	3.89a	55.00a	56.50a	57.30a	10363.3a	8444.4bc	10833.3a
HUFBR15	3.27ab	3.53a	3.88a	54.00a	57.90a	55.37a	9530.0abc	10675.0ab	11037.0a
NSFBR15	3.03b	3.07a	3.89a	50.67a	55.77a	57.30a	9070.0abc	9202.8bc	11648.1a
NSFBR-1	3.00b	3.47a	3.76a	50.00a	56.03a	58.17a	7730.0c	9708.3abc	10555.6a
NSFBR-30	3.00b	3.33a	3.87a	56.00a	63.17a	56.30a	10070.0ab	9527.8abc	12037.0a
HUFBR-22	2.90b	3.27a	4.00a	50.33a	62.80a	55.43a	8330.0bc	8291.7c	11296.3a
NSFBR-12	3.10b	3.33a	4.22a	53.67a	60.70a	65.00a	9730.0ab	11847.2a	11481.5a
NSFBR-11	3.10b	3.43a	3.77a	52.33a	56.13a	67.37a	9360.0abc	9375.0bc	11203.7a
+VE	2.87b	3.13a	3.77a	49.67a	58.37a	61.13a	10360.0a	8955.7bc	10351.9a
-VE	2.87b	3.43a	4.33a	51.33a	58.83a	55.53a	8360.0bc	9270.0bc	10116.7a
LSD	064	0.75	0.86	6.84	8.32	12.43	1830.6	2322.3	2139.9
F-value	5.68***	1.67ns	1.26ns	2.70*	2.76*	2.91*	6.18***	5.14**	1.98ns
CV (%)	7.16	7.69	7.57	4.52	4.19	7.30	6.82	8.43	6.69
Mean	3.10	3.37	3.94	52.30	58.62	58.92	9290.33	9529.79	11056.11

*NS= non significant; *, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. VE-negative control (no inoculation and N application), +VE control=20 kg N ha*$^{-1}$*; NSFBR= National soil Faba bean Rhizobium; HUFBR= Haramaya University Faba bean Rhizobium; TAL= Tropical Agricultural legume (International Rhizobium strain).*

As we have found in NPP, number of seeds per pod (NSP) was significantly affected by *Rhizobium* inoculation treatments and cropping season (Table 2). A concurring finding of Argaw and Tsigie (2015) revealed that inoculation of *Rhizobium* remarkably increased the seed number of common bean. The significant effect of cropping season could also be related with moisture variability (Terefe *et al.,* 2015) and the presence of indigenous rhizobia with similar population and better competitiveness potential. This explains how biophysical environmental factors affect the performance of inoculated *Rhizobium* in to a certain soil.

Only cropping season affected 100 seed weight of faba bean significantly (P <0.05). This result is in agreement with previous studies on faba bean using different insitu water conservation technology (El-Sadek and Salem 2015). In contrast to this, Peoples *et al.* (2009) and Elsheikh and Elzidany (1997)found that inoculation has significantly affected 100-seed weight. This variability is might be attributed to the growth and development promoting potential of elite rhizobia that help facilitate the translocation of photosynthates to organs particularly to seeds.

Seed size has high heritability with a large additive genetic component and hence often displays a positive correlation with yield (Duc, 1997). On the other hand, the result indicated that *Rhizobium* inoculation did not significantly improve the 100 seeds weight of faba bean (Table 4). This is consistent with findings of Mulas *et al.* (2015) and Araujo *et al.* (2015) who reported that inoculation and N application did not significantly improve 100 seed weight of *Phaseolus vulgaris* and *Cajanus cajan*, respectively. Both findings demonstrated a significant variation of the trait across locations.

Analysis of variance reveled that *Rhizobium* inoculation, cropping season and their interactions had significant effect on total biomass yield (TBY) of faba bean at P <0.05 (Table 2). Unlike nodulation, in 2011 and 2013 cropping seasons, TBY was not significantly affected by *Rhizobium* inoculation when compared to control check (Table 4; Figure 1C). This implies that inoculated *Rhizobium* isolates did not show better performance in N_2 fixation over the indigenous rhizobia. Similar results were obtained in several locations and times. In cases where the number of the indigenous rhizobia >100 g^{-1} soil rhizobial inoculation often does not show significant for many traits (Ngeno *et al*., 2012). However, the result revealed that TAL-1035 inoculation was found to significantly increase TBY when compared to the N fertilized treatment. Biofertilization with native *Bradyrhizobium* strains was found to increase the yield of *Cajanuscajan* instead of chemical N fertilization (Araujo *et al*., 2015). Low performance of faba bean in N received treatment could be due to the fact that inorganic N is known to inhibit root infection, nodule initiation and nodule development (Chen and Phillips, 1977) thereby reducing N_2 fixation and plant biomass production. Similar studies conducted by by Tsigie and Woldeab (1994) in nitisols of Central highland of Ethiopia revealed non-significant effect of N rates of application at 23 kg N ha^{-1} in TBY of Faba bean. However, the same study reported significant increase in grain yield of faba bean at 46 kg N ha^{-1} with low indigenous rhizobial population. Faba bean fix as much as 80–90% by symbiotic association with indigenous rhizobia (Richards and Soper, 1979), ranged from 15 to 648 kg N ha^{-1} under field condition (Sprent *et al*., 1977; Schwenke *et al*., 1998). In addition to N_2 fixation, rhizobia can produce phytohormones, solubilizing organic and inorganic insoluble P (Gopalakrishnan *et al*., 2015) which could also increase faba bean production.

Table 4. Grain yield and total plant N accumulation of faba bean as influenced by *Rhizobium* inoculation and inorganic N application in Haramaya, eastern Ethiopia, 2012/13 cropping season.

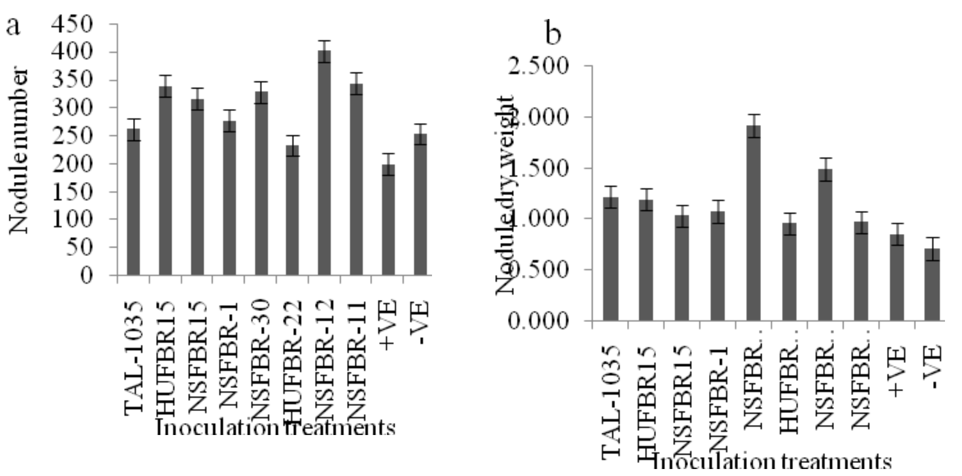
Treatment	Grain yield			Total plant N accumulation		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
TAL-1035	4260.0a	3698.3b	4072.3ab	3.8233a	3.4067a	4.7667a
HUFBR15	4330.0a	5158.2a	3219.4b	4.0900a	3.1733a	4.4767bc
NSFBR15	3430.0ab	4328.1ab	4219.1ab	4.2867a	3.2822a	4.6567ab
NSFBR-1	3266.7ab	4403.0ab	4019.5ab	4.0533a	3.2356a	4.5067abc
NSFBR-30	3740.0a	5267.0a	4608.0a	4.0400a	3.2044a	4.5067abc
HUFBR-22	3463.3ab	4721.5a	3889.9ab	4.4600a	3.3600a	4.3700cd
NSFBR-12	3700.0a	4959.1a	3962.8ab	4.2400a	3.0644a	4.2100d
NSFBR-11	3633.3a	4447.9ab	3620.2ab	4.2433a	3.2489a	4.4333bcd
+VE	3266.7ab	5054.9a	3711.8ab	4.5067a	3.1267a	4.5967abc
-VE	2466.7b	3549.7b	4358.2a	3.7733a	3.3289a	4.1967d
LSD	1103.8	986.64	1028	1.0360	1.0332	0.2614
F-value	5.80	9.00***	3.67**	1.38ns	0.26ns	12.04***
CV (%)	10.74	7.49	8.96	8.63	11.02	2.02
Mean	3555.67	4558.75	3968.11	4.1517	3.2431	4.4720

*NS= non significant; *, ** and ***, significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. VE-negative control (no inoculation and N application), +VE control=20 kg N ha^{-1}; NSFBR= National soil Faba bean Rhizobium; HUFBR= Haramaya University Faba bean Rhizobium; TAL= Tropical Agricultural legume (International Rhizobium strain).*

In 2012, inoculating NSFBR-12 resulted in significantly higher as compared to uninoculated control and N fertilized treatments. Moreover, the highest TBY improvements above the TBY control check (Δ TBY) were recorded with NSFBR-12 followed by NSFBR-30 treatment (Figure 2c). This result is in accord with results observed by Tumbure *et al.* (2013) who determined remarkable increase in biomass production of hairy vetch by *Rhizobium* inoculation. El-Ghandour *et al.* (1995) also found that *Rhizobium* inoculation alone and in combination with mycorrhizae significantly increased biomass production of faba bean over the uninoculated plants. This is probably because of higher rates of photosynthesis and delayed leaf senescence due to plants associated with rhizobia and result in increases biomass production of the legumes (Kaschuk, 2009). Beside this, overall effect of N fertilized on average TBY was decreased when compared to the control check treatment, indicates that N derived from indigenous rhizobia might be greater than N fertilized treatment.

Grain yield production of faba bean was significantly affected by inoculation treatments, cropping seasons and their interaction at P <0.05 (Table 2). This result is in accord with several studies showing that *Rhizobium* inoculation significantly improves the seed yield of faba bean (Carter *et al*., 1995; Habtemichial *et al*., 2007). A study in Saskatchewan showed that indigenous rhizobia were incapable of supporting adequate levels of nitrogen fixation by peas (Bremer *et al*., 1988). But the effect of *Rhizobium* inoculation in North showa on faba bean was more pronounced in depleted soil than fertile soil (Adamu *et al*., 2001) due to the number of rhizobia nodulating faba bean in fertile soil was $>10^2$ g^{-1} soil. Elsheikh and Elzidany (1997) also found that *Rhizobium* inoculation and 40 kg N ha^{-1} application of inorganic N gave comparable amount of grain yield increase. Grain yield increase by *Rhizobium* inoculation was found to be on average by 19% over the uninoculated treatments (McKenzie *et al*., 2001).

The result also indicated that the effectiveness of different isolates of rhizobia on GY of faba bean varied in different cropping season. In 2011 and 2012, none of inoculation treatments had significant improvement of GY when compared to the control check at $P < 0.05$ (Table 5). This indicates the presence of high effective and competitive rhizobia in Haramaya soils (Lopetinsky *et al*., 2014). Similar finding was previously observed in Arsi area of Ethiopia (Amanuel *et al*., 2000). However, NSFBR-30 and NSFBR-12 inoculations in 2011 resulted in significantly higher GY than N fertilized treatment. Beside this significant increase in GY over N fertilized treatment in 2012 was recorded with NSFBR-15 and NSFBR-30 inoculation treatments. Amager (1986) and Brockwell *et al.* (1995) found that significant increase in grain production in soil having higher indigenous rhizobia when used superior inoculant strains which are competitive with the indigenous strains. In 2013, *Rhizobium* inoculation did not significantly improve GY of faba bean as compared to the control check and N fertilized treatments. The reason for non-significant effect on grain yield might by early termination of rainfall at pod filling stage of faba bean in this cropping season. Peña-Cabriales and Castellanos (1993) determined that shortage of water at reproductive stage depressed the nodulation and reduced the grain yield of common bean. Water stress at late pod setting stage of soybean reduced the N_2 derived from symbioatic N_2 fixation and decreased the individuals seed mass (Mastrodomenico *et al.,* 2013).



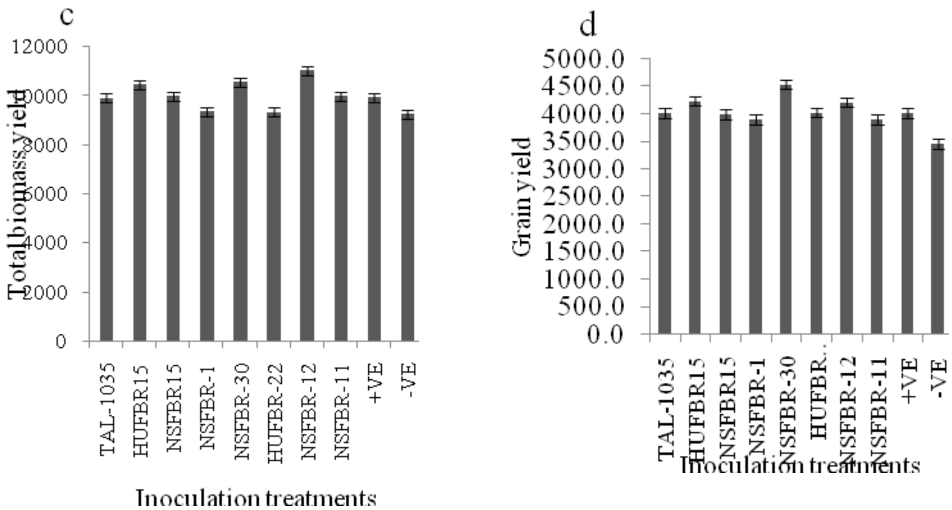


Figure 1. the effect of *Rhizobium* inoculation and inorganic N application on (a) Nodule number, (b) Nodule dry weight, 9c) Total biomass yield and, (d) Grain yield of faba bean in Haramaya, eastern Ethiopia, 2012/13 cropping season.

The overall effect of *Rhizobium* inoculation treatments across three years on average GY was non-significant (Figure 1d). However, the highest relative GY produced above the GY in control check was recorded at NSFBR-30 followed by NSFBR-15 and NSFBR-12 inoculation treatments (Figure 2d). This indicates the remarkable effect of cropping season on the competitiveness and effectiveness of inoculated *Rhizobium* against the indigenous rhizobia. Similar finding was obtained by Labidi *et al.* (2003). Similarly, the overall effect of inorganic N application on average GY was reduced when compared to the control check. In contrast, the effect of starter N together with P application enhances the effectiveness of inoculated *Rhizobium* in depleted soil of north Showa, Ethiopia (Adamu *et al.,* 2001).

Neither *Rhizobium* inoculation nor its interaction with cropping season had significant effect on total N accumulation of faba bean at $P < 0.05$ (Table 2). Only the effect of cropping season was significant on total N accumulation of faba bean. This was probably due to the presence of competitive and effective N_2 fixer indigenous rhizobia in the study site. However, Denton et al. (2013) found that highest rate of inoculation (100 times normal) provided increased N_2 fixed, even in a soil with an established population of rhizobia. The current result is attributed to the rainfall variation at late flowering stage prevailed in different cropping years and thus affects the photosynthetic products and N derived from symbiosis (Sprent *et al.*, 1977; Sprent and Bradford 1977; Siddique *et al.*, 2001). Similarly, grain yields, N_2 fixation and dry matter production of fababean were reduced in late-sown crops and those water-stressed during pod-filling (Rochester *et al.,* 1998). In the drier cropping season, the result could be attributed to the accumulation of free amino acids and amides in shoots, roots, and nodules, which may depress the nitrogenenase activity and N_2 fixation potential of legumes via a feedback system (Guerin *et al.*, 1990; Schubert, 1995). It is possible that the prolonged rainy season resulted in increased plant biomass, thereby increasing the capacity for N_2 fixation and the requirement for N.

The result in Table 5 displayed the non-significant effect of *Rhizobium* inoculation on total N accumulation in all cropping seasons as compared to the control check. This indicates that the inoculated isolates may be as effective as in N_2 fixation with indigenous rhizobia nodulating faba bean. This finding is in line with the previous work in Ethiopia by Amanuel *et al.* (2000) found the non-significant effect of inoculation on N_2 fixed from atmosphere by faba bean. However, TAL-1035, HUFBR-15, NSFBR-15, NSFBR-1 and NSFBR-30 inoculations were found to significantly improve total plant N accumulated compared to N fertilized treatment in 2013 suggesting that inoculated plants obtained greater N from symbiotic N_2 fixation without starter N than indigenous rhizobia with N application. This finding is supported by Voisin *et al.* (2002) who found that onset of symbiotic N_2 fixation was delayed due to receiving inorganic N at sowing. However, Rose*et al.* (2016) found that inorganic N application had significantly smaller rates of decrease in N2-fixing activity (acetylene reduction) in faba bean than in chickpea. Elsheikh and Elzidany (1997) determined a comparable increase in seed protein content due *Rhizobium* inoculation and 40 kg N ha^{-1}. The current result, in this regard, might be associated with the presence of sufficient amount N from the native soils.

4. Conclusion

In general, the *Rhziobium* inoculation improved the faba bean production in the study site. The effect of starter N application on faba bean production and nodulation is also depending on the cropping season. The effect of nodulation on the productivity of faba bean is also dependent on the cropping season. Good rainfall condition at late pod setting critically affects the performance of inoculation on nodulation of faba bean. Based on the result in NDW and GY production, isolate NSFBR-30 can be the candidate for biofertilizer production for faba bean production in the study site. The candidate isolate need to be tested further with different cultivars and in different soil conditions.

5. Acknowledgment

This research was funded by the Ethiopia Institute of Agricultural Research, under Biofertilizer and Organic Fertilizer Research Project. The authors would like to express their sincere thanks to field and technical assistants for their involvement in field experiment management and data collection.

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5. Semi-decomposed Bone Meal Amendment and *Rhizobium leguminosarum* bv. *Viciae* Inoculation: Effects on Nodulation, Yield and Yield Traits of Field pea (*Pisum sativum* L.) in Eastern Ethiopia

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Abstract: Field pea (Pisum sativum L.) productivity in Ethiopia is very low mainly due to the soils with low nitrogen (N) and phosphorus (P), therefore, improving field pea production using locally available N and P rich organic input such as bone meal is relevant but not studied. Therefore, the overall aim of this study was to evaluate the effect Rhizobium leguminosarum bv. *vicieae* inoculation and bone meal application on nodulation and production of field pea in eastern Ethiopia, Haramaya area. A total of ten treatments were established by factorial combination of five level of bone meal application (0, 100, 150, 200 and 250 kg ha-1) and two levels of Rhizobium inoculation (inoculated and uninoculated). The treatments were laid out in split plot design with three replications viz. Rhizobium inoculations and semi-decomposed bone meal rates were applied in the sub and main pots factors, respectively. Analysis of variance indicated that significant effect of Rhizobium inoculation, bone meal application, cropping season and their interaction on all measured traits, except number of seed per pod (NSP). It was observed a reduction of nodule number (NN), nodule dry weight (NDW), shoot dry weight and grain yield (GY) of field pea by 45.2, 12.9, 19.5 and 25.4% due to Rhizobium inoculation when compared to uninoculated plants in 2012 while total biomass production, grain yield, total plant N concentration and P uptake were increased by 9.3, 2.7, 2.6 and 3.4% due to inoculation over uninoculated plant in 2011. Bone meal application at 100 kg ha-1increased the NN by 5 and 22.7% and the nodule dry weight by 54.1 and 0.9% over the unfertilized plants in 2011 and 2012 cropping seasons, respectively. Rhizobium inoculation increased the NN by 37.7% over the uninoculated plant in 2011 cropping season. In both cropping seasons, all the remaining yield and yield traits, except NSP, showed increasing trend with bone meal rate of application. Grain yield increased from 2565.17 to 3040.17 and 915.63 to 1432.47 kg ha-1 1by application of 200 and 250 kg of bone meal ha-1 in 2011 and 2012, respectively, over unfertilized plant. Likewise, total plant N concentration increased from 3.17 and 3.43% at unfertilized plant to 3.51 and 3.72% due to 250 kg of bone meal ha-1 application in 2011 and 2012, respectively. When compared to unfertilized plant, 250 kg ha-1 bone meal application enhanced the plant P uptake by 8.4 and 15.8% in 2011 and 2012, respectively. The overall effect of Rhizobium inoculation in combination with bone meal rate of application on NN, NDW, GY and TBY were non-significant when compared to the corresponding rate without inoculation. However, slightly increase of GY and TBY were observed due to Rhizobium without and less than 150 kg ha-1 rate of bone meal application. Regression analysis revealed a significant and negative relationship between nodulation traits (NN and NDW) and GY of field pea, however, the relationship between GY and bone meal rates of application was positive. Therefore, it is recommend a combined application of Rhizobium inoculation with low rate of bone meal application (<150 kg ha-1) to improve the field pea in sustainable way.

Keywords: Bone meal; Eastern Ethiopia; Field pea (*Pisum sativum* L.), Rhizobium.

1. Introduction

More than 30-40% of the world's arable land limited the crop yield due to phosphorus (P) availability (Runge-Metzger, 1995; von Uexkiill and Mutert, 1995). Phosphorus has significant positive effects on the nodulation, the nitrogenase activity and the yield (Chmelíková and Hejcman, 2014; Medhi *et al.*, 2014). By some estimates, world resources of inexpensive P may be depleted by 2100 (Steen, 1998; Van Vuuren *et al.*, 2010). Hence, sustainable management of P via recycling P within the food systems is extremely important for crop production (Cordell, 2010). Use of locally available wastes, for instance, animal meals as substances rich phosphorus could help to maintain soils' fertility thereby improving crop productivity (Spychaj-Pychaj-Fabisiak *et al.*, 2007). Semi-decomposed bone meal is a better P source than the commonly used phosphate rock (Baker *et al.*, 1989; Kahiluoto and Vestberg, 1998). Bone meal application has been positively evaluated as fertilizer for improving crop production and soil fertility by many workers (Novelo *et al.*, 1998; Jeng *et al.*, 2004; Chaves *et al.*, 2005; Jeng *et al.*, 2006). Such organic input contains macronutrients comprising potassium (K), sulfur (S), Chloride (Cl), Sodium (Na) with large amount of Calcium (Ca) and phosphorus and other essential micronutrients (Jeng *et al.*, 2006; Gopinath *et al.*, 2008; Garcia and Rosentrater, 2008). These nutrients are released to soil via microbial decomposition and mineralization processes and become plant available in the first year of its application (Chaves *et al.*, 2005, Jeng *et al.*, 2004, 2006, Jeng and Vagstad, 2009; Nogalska *et al.*, 2012).

The relative efficiency of P in bone meal was about 50% of that of mineral P fertilizer for the first crop of barley and rye grass and that bone meal had residual P effects the following year (Jeng *et al.*, 2006). They found that the effect of bone meal application on cereal crops was larger in poor fertile soil with low soil organic matter and N content. Jeng *et al.* (2004 and 2006) believe that approximately 80% of the nitrogen in bone meal is released to plants in the first year, and the availability of this N to cereals was close to that of mineral fertilizers.

Field pea is one of the major cool season pulse crop grown in mid and high lands of Ethiopia. However, the productivity of this crop in Ethiopia is 1.294 ton ha^{-1} (CSA, 2015) which is very low when compared to the potential yield (2.5 to 3.5 ton ha^{-1}) reported under research field (Jarso *et al.*, 2006). Low soil fertility especially N and P deficiency is the major yield limiting plant nutrients for field pea production in Ethiopia (Beyene *et al.*, 1994). In order to resolve these constraints through environmentally and economically sustainable ways using locally available input needs to be considered. Due to low in plant nutrients and lack of effective *Rhizobium* strains in the soils, N_2 fixation is not sufficient to undertake good growth of the host plant (Erman, 1998). Several studies showed that inoculation of seeds with effective bacteria strains is not alone sufficient to obtain higher quality and yield (Vincent, 1982; Tufenkci, 1995) unless low soil fertility corrected through sustainable ways.

Inoculation of *Rhizobium* on field pea in Ethiopia and other part of the world indicated a remarkable increase in its production (Clayton *et al.*, 2004; Anteneh Argaw, 2013; Kumar *et al.*, 2014). Inoculation in combination with other essential plant nutrient including P improved the yield of different legume plant when compared to inoculation alone (Shah *et al.*, 2001; Cheng *et a*l., 2008; Dutta and Bandyopadhyay, 2009). On top of this, Hellsten and Huss-Danell (2000) found that nodule number, nodule dry matter and nitrogenase activity has been six times higher in plants grown with high N and high P than in plants with high N and low P. However, research work on the use of animal bone meal rich in inorganic N and water soluble P in integration with *Rhizobium* on pulse crops are not well studied. Hence, the aim of this research was to determine the effect of bone meal application and *Rhizobium* inoculation on nodulation, yield and yield traits of field pea at Raare, Haramaya University experimental site, eastern Ethiopia.

2. Materials and Methods

2. 1. Experimental Site

The research was conducted for two years (2011 and 2012)at Haramaya University research station located in eastern Hararghe, Ethiopia (09^{0}24.954' North and 042^{0}02.037' East), at 6628 ft. a. s. l.. The field was located in maize-sorghum-pulses cropping system where field pea had long been grown after maize and sorghum cultivation without inoculation. The annual rainfall is 760 mm which extending from June to September, most of which fall in a major cropping season which extends from May to September.

The selected soil physico-chemical properties of the experimental site were studied from the composite samples from the experimental sites before planting were taken using auger. The main soil properties in both experimental years were pH 7.84, electrical conductivity (EC) (0.14 mS cm^{-1}), organic carbon (1.96%), total N (0.12%), available P (2.13 mg kg^{-1} soil), and cation exchange capacity (CEC) (25.98 cmol (+) kg^{-1}). The exchangeable Calcium (Ca^{+2}), Magnesium (Mg^{+2}), Sodium (Na^{+2}) and Potassium (K^{+1}) were 31, 8.7, 0.33 and 0.14 cmol (+) kg^{-1} soil, respectively. The soil had sandy clay loam texture with 33, 18 and 49% of clay, silt and sand, respectively. The chemical properties of bone meal fertilizer were also studied and are indicated in Table 1.

Table 1. Chemical composition of semi decomposed bone meal.

Parameters	value
pH $_{H2O(1:2.5)}$	8.11
EC(mS/cm)	9.75
Total N (%)	2.77
Organic Carbon (%)	18.97
NH_4-N (mg/kg)	7615.58
NO_3-N (mg/kg)	3650.71
Available K (cmol(+)/kg soil)	3.02
Available P (mg/kg)	1218.14
Zn (mg/kg)	29.16
B (mg/kg)	5.28

2.2. Treatments and Experimental Design

Treatments consisted of two levels of inoculation (uninoculated and inoculated with HUIFPR-20) and five levels of semi-decomposed bone meal fertilizer rates (0, 100, 150, 200 and 250 kg ha^{-1}). Ten treatments were produced by factorially combining five levels of bone meal rates of application with two levels of inoculation. The actual plot was ploughed thoroughly twice with tractor and divided into sub-plots in accordance with the treatments. Experimental design was a split plot with three replications. Each plot consisted of six rows, 1.2 m long, 3m width, 5 cm between plants and 20 cm distance between rows, with a density of about 360 plants $plot^{-1}$. *Rhizobium* inoculations were applied in the sub pots factor and semi-decomposed bone meal rates in the main plots factor. Sowing was performed on first week of July. Manual weeding was practiced when required and all other cultural practices were accomplished throughout the growth period as per the recommendation for the crop.

The field pea cultivar Metti, which is well adapted and high yielding in the regionwas used. This cultivar was obtained from Highland Pulses Improvement Project, Haramaya University, Ethiopia. Superior isolate of *Rhizobium leguminosarum* bv. *viciae* (HUFPR-20), which was selected as effective isolate under greenhouse and field condition (Kassa Baye, 2012; Anteneh Argaw, 2013) was obtained from Soil Microbiology and Biofertilizer Research Project, Haramaya University, Ethiopia.

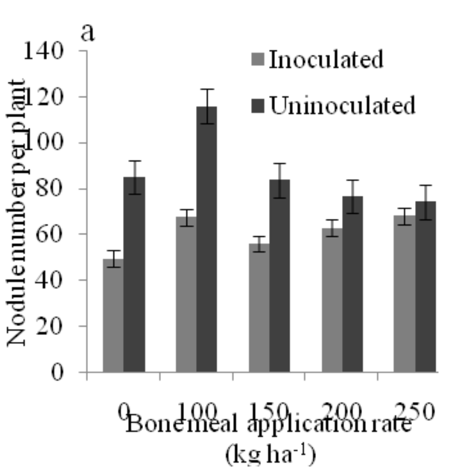
2.3. Data Collection and Analysis

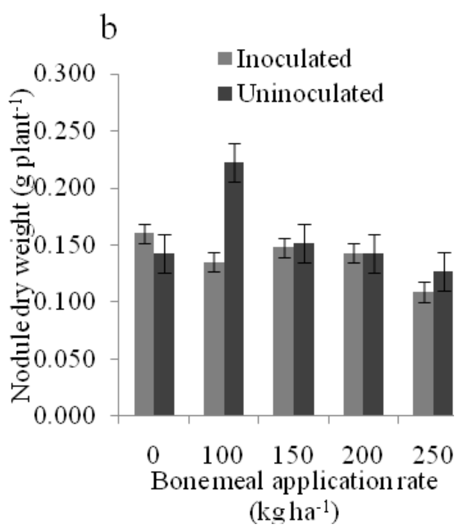
At late flowering and early pod setting stage, five plants were collected from central rows of each plot to evaluate the nodulation (nodule number and nodule dry weight per plant) and plant biomass production. Shoot dry weight was also measured after the samples were dried at 70°C in electrical oven until the weight of the samples became constant. The dried shoots were later ground to pass a 0.5 cm sieve. Shoot total nitrogen was determined by the Kjeldahl method. At physiological maturity, the grain yield, total biomass yield, number of pods per plant, number of seeds per pod and 100 seed weight recorded.

The influence of treatments on yield and yield related traits of field pea and differences among treatments were analyzed using analysis of variance procedures for split plot design in randomized blocks using SAS version 9.2 by and PROC GLM program. The least significant difference (Fisher's LSD) at P ≤0.05 was used to compare the means between treatments.

3. Results

Analysis of variance revealed that bone meal application, *Rhizobium* inoculation, cropping seasons and their combination significantly affected the nodule number (NN) and nodule dry weight (NDW) of field pea at $P<0.05$ (Table 2). In general, the result revealed that the NN was decreased with increasing bone meal rates of application with slight improvement at 100 kg ha^{-1}in both cropping season (Table 3). Applying 100 kg ha^{-1}produced the highest NN in both cropping seasons but this NN significantly differed from unfertilized plant in 2011.The lowest NN was produced at 200 and 250 kg ha^{-1} bone meal application in 2011and 2012, respectively. Nodule number in 2011 was significantly improved due to *Rhizobium* inoculation while significant reduced in 2012. However, the overall effect of *Rhizobium* inoculation and bone meal rates of application was non-significant when compared to the corresponding rates without inoculation (Figure 1a). Besides, the NN reduction in the figure was observed due to *Rhizobium* inoculation.





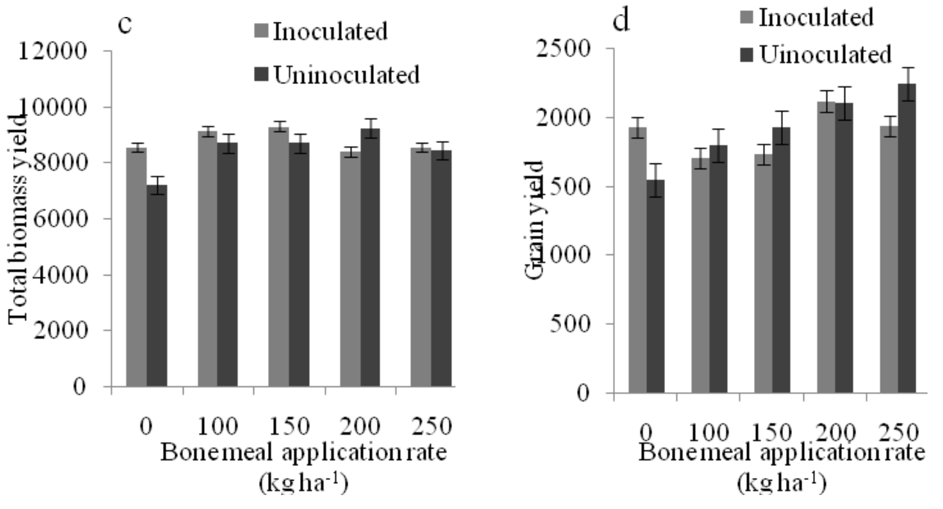


Figure 1. Effects of *Rhizobium* inoculation along different rates of bone meal application on (a) nodule number, (b) nodule dry weight, (c) total biomass yield and (d) grain yield.

The highest NDW was obtained with 100 kg ha^{-1} application in both cropping seasons. This effect was significant when compared to the unfertilized plant only in 2011. The result also revealed the NDW reduction with increasing rates of bone meal application. The lowest NDW was observed at 250 kg ha^{-1} bone meal application in both seasons. However, *Rhizobium* inoculation did not improve NDW of field pea in both cropping seasons; instead the significantly higher NDW was produced in uninoculated than inoculated in 2012. Among rates of bone meal application, a significant decrease in NDW was observed from application of 100 kg ha^{-1} bone meal (Figure 1b). This indicates that low rate of bone meal application enhances the competitiveness of indigenous rhizobia for nodule formation. This difference was non-significant with the remaining rates of bone meal application.

The effect of *Rhizobium* inoculation, cropping season, rates of bone meal application and their interactions was significant ($P < 0.05$) on shoot dry weight (SDW) at late flowering stage of field pea (Table 2). Similar to the nodulation result, 100 kg ha^{-1} application results insignificantly increased SDW at late flowering stage of field pea in 2011(Table 3). In 2012, the SDW showed increasing trends with increased bone meal rates application where 200 kg ha^{-1} resulted significantly higher SDW than those produced at <200 kg ha^{-1} bone meal application. In contrast, *Rhizobium* inoculation did not increase the SDW in both cropping season rather it reduced significantly.

The interaction effect of cropping season and rates of bone meal application on shoot length at late flowering stage (SL) was significant ($P < 0.05$) (Table 2). In 2011, 100 kg ha^{-1} bone meal application significantly increased the SL when compared to the unfertilized check while significantly higher SL at 250 kg ha^{-1} in 2012 than the unfertilized check. Though the main factor *Rhizobium* inoculation had no significant effect on SL of field pea, but it was observed a significant reduction in SL in 2012 by *Rhizobium* inoculation.

The number of seeds per pod (NSP) was significantly (P <0.05) affected by cropping season (Table 2). Besides, Table 3 shows the non-significant effects of bone meal rates of application and *Rhizobium* inoculation on the NSP in both cropping seasons (Table 3). However, data revealed slight increase in NSP by bone meal application. Analysis of variance revealed that *Rhizobium* inoculation, bone meal rates of application, cropping season and their interaction, excluding main effect of cropping season, was significantly (P <0.05) affected the number of pods per plant (NPP) (Table 2). The highest NPP was recorded with 250 kg ha^{-1} followed by 200 kg ha^{-1} of bone meal application and they were significant differed from the unfertilized check in the 2011cropping season (Table 4). In 2012, 200 kg ha^{-1} application had significantly increases the NPP when compared to the remaining rates of bone meal. In this season, *Rhizobium* inoculation significantly increased the NPP over uninoculated control.

Table 2. Mean squares from analysis of variance (ANOVA) for traits of field pea treated with different rats of bone meals and *Rhizobium* inoculation at Haramaya, eastern Ethiopia during 2011 and 2012.

Sources of variation	df	Field pea traits NN	NDW	SDW	NPP	NSP	100 seeds weight	GY	TBY	PH	Tot N	Tot P
Inoculation (I)	1	26.11***	16.48***	20.99***	4.90*	0.01ns	0.09ns	0.94ns	2.38ns	1.60ns	12.05**	63.09***
Bone meal rates (B)	4	10.69***	3.96**	8.26***	20.29***	0.47ns	7.57***	17.72***	8.93***	2.93*	4.42**	7.62***
Year (Y)	1	300.14***	27.52***	227.16***	3.60ns	26.10***	75.47***	1620.43***	268.77***	1.47ns	186.34***	81.13***
B x I	4	6.51***	16.10***	6.49***	9.59***	0.30ns	3.15*	3.54*	4.88**	2.51ns	1345***	7.38***
Y x I	1	67.93***	4.75*	16.86***	6.40*	0.70ns	0.18ns	48.56***	0.45ns	0.57ns	41.48***	9.84**
Y x B	4	15.53***	16.71***	14.50***	5.66**	0.59ns	3.54*	12.58***	1.85ns	3.91**	6.82***	0.76ns
Y x B x I	4	10.96***	6.52***	14.14***	6.96***	0.95ns	1.99ns	4.93**	8.40***	1.27ns	5.12**	0.82ns

*NS= non significant; *, ** and ***, significant at P<0.05, <0.01 and P< 0.001, respectively. NN= Nodule number; NDW= Nodule dry weight; SDW=Shoot dry weight; NPP= Number of pods per plant; NSP= Number of seeds per pod; GY= Grain yield; TBY= Total biomass yield; PH= Plant height; Tot N= total nitrogen and Tot P= Total phosphorus.*

Table 3. Nodule number, nodule dry weight, shoot dry weight and number of seeds per pod of common bean as influenced by different rates of bone meal application and *Rhizobium* inoculation in Haramaya, 2011 and 2012.

Treatments (kg ha^{-1})	Nodule number		Nodule dry weight		Shoot dry weight		Number of seed per pod	
	2012	2013	2012	2013	2012	2013	2012	2013
0	53.33a	99.83b	0.0982b	0.2041a	92.42b	191.38bc	6.13a	5.47a
100	56.17a	122.50ab	0.1513a	0.2060a	158.47a	190.78bc	6.43a	5.53a
150	39.33ab	100.33ab	0.1506a	0.1489bc	142.12a	167.28c	6.27a	5.47a
200	13.67c	130.50a	0.0947b	0.1913ab	128.98ab	233.50a	6.33a	5.77a
250	25.33bc	98.33b	0.01335ab	0.1021c	101.48b	213.10ab	6.07a	5.73a
LSD	17.97	3023	0.0407	0.0477	38.23	26.97	0.87	0.84
Inoculated	43.53a	78.13b	0.1193a	0.1587b	123.52a	177.73b	6.31a	5.55a
Uninoculated	31.60b	142.47a	0.1320a	0.1823a	125.87a	220.69a	6.19a	5.64a
LSD	7.92	13.33	0.018	0.0210	16.86	00.89	0.38	0.37
CV (%)	27.69	15.86	18.76	16.81	17.75	7.84	8.04	8.73
Mean	37.57	110.30	0.1257	0.1705	124.69	199.21	6.25	5.59

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

The *Rhizobium* inoculation, bone meal rates of application, cropping season and their interaction had significant ($P < 0.05$) effect on 100 seeds weight of field pea (Table 4). Bone meal application at 200 and 250 kg ha^{-1}had significantly increased the 100 seed weight when compared to the unfertilized check in 2012 while 200 kg ha^{-1}in 2012. In both cropping seasons, the lowest 100 seeds weight was obtained in the unfertilized control. However, the effect of *Rhizobium* inoculation on 100 seed weight was non-significant in both seasons.

The total plant N accumulation (TPNA) was significantly influenced by *Rhizobium* inoculation, bone meal application, cropping season and their interaction (Table 2). In both cropping seasons, 250 kg ha^{-1}application significantly increased TPNA when compared to the unfertilized check. Inoculating *Rhizobium* in 2011 resulted in significantly increase plant accumulated N but non-significant in 2012. Rather inoculation of *Rhizobium* significantly decreased the plant accumulated N when compared to the uninoculated treatment.

The *Rhizobium* inoculation, bone meal application, cropping season and their interaction, excluding cropping season x bone meal application and cropping season x bone meal application x *Rhizobium* inoculation, had significant ($P < 0.05$) effect on total P uptake of field pea (Table 2). Significant increase in plant P uptake in 2011 cropping season was recorded with 200 kg ha^{-1}while 250 kg ha^{-1} application in 2012 cropping season. In both cropping seasons, *Rhizobium* inoculation did not affect the plant P uptake when compared to the uninoculated treatment rather a significant decrease was recorded by inoculation in 2012.

Total biomass yield (TBY) was significantly affected by the main effect bone meal application and cropping season, bone meal x inoculation and inoculation x bone meal application x cropping season (Table 2). All bone meal rates of application, excluding 250 kg ha^{-1} produced significantly higher TBY than the unfertilized in 2011 and the highest TBY (8078.5 kg ha^{-1}) was obtained from 200 kg ha^{-1} (Table 4). In 2012, all rates of application except 150 kg ha^{-1} produced significantly higher TBY than unfertilized check. The highest TBY (11375.0 kg ha^{-1}) in this season was 20.3% over the unfertilized control. The effect of *Rhizobium* inoculation on TBY in both cropping season was non-significant.

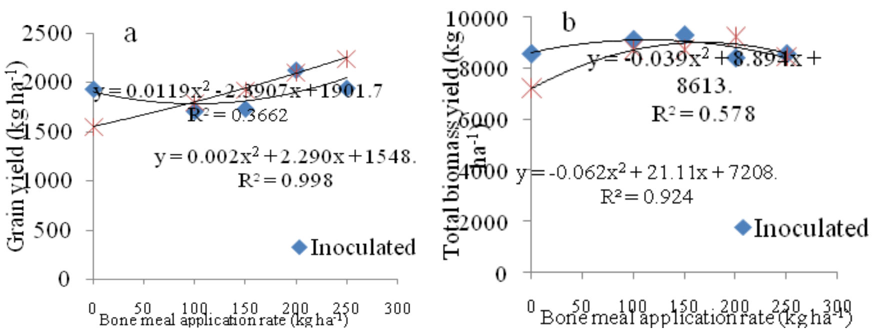


Figure 2. Regression analysis between bone meal application rate with (a) grain yield and (b) total biomass yield.

The effect of *Rhizobium* inoculation when compared to uninoculated treatment along increasing trends of bone meal application was non-significant (Figure 1c). However, the figure revealed the higher TBY with inoculation as compared to uninoculated with <200 kg ha^{-1} bone meal application. In figure 2a indicated the polynomial and significant relationship between the rates of bone meal application and TBY with both inoculation treatments. The higher y intercept was recorded in inoculated treatment than the uninoculated treatment.

Table 4. Number of pods per plant, 100 seeds weight, grain yield and total biomass yield of common bean as influenced by different rates of bone meal application and *Rhizobium* inoculation in Haramaya, 2011 and 2012.

Treatments (kg ha^{-1})	Number of pods per plant		100 seeds weight		Grain yield		Total biomass yield	
	2012	2013	2012	2013	2012	2013	2012	2013
0	11.00b	10.83b	19.25b	20.13b	2565.17b	915.63c	5947.9c	9454.2b
100	12.33ab	11.33b	19.42b	23.17a	2655.64b	853.47c	7618.1ab	11375.0a
150	11.00b	11.33b	19.83ab	22.95a	2567.55b	1091.81bc	6117.0bc	10458.3ab
200	13.17a	14.00a	20.52a	23.63a	3040.17a	1189.21ab	8078.5a	11263.9a
250	13.33a	11.333b	20.57a	22.13ab	2753.86ab	1432.47a	6694.2abc	10840.3a
LSD	1.37	1.45	0.94	2.54	291.12	245.48	1503.6	1175.7
Inoculated	12.13a	12.27a	19.93a	22.30a	2837.14a	936.75b	6984.2a	10867.8a
Uninoculated	12.20a	11.27b	19.90a	22.51a	2595.81b	1256.28a	6798.1a	10488.9a
LSD	0.61	0.64	0.42	1.12	128.35	108.23	662.9	518.36
CV (%)	6.54	7.11	2.74	6.56	6.20	12.96	12.63	6.37
Mean	12.17	11.77	19.92	22.40	2716.48	1096.52	6891.13	10678.33

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. Shoot length, total N accumulation and total P uptake for common bean as influenced by different rates of bone meal application and *Rhizobium* inoculation in Haramaya, 2011 and 2012.

Treatments (kg ha^{-1})	Shoot length at 50% flowering		Total N accumulation		Total P accumulation	
	2012	2013	2012	2013	2012	2013
0	1.66b	1.72b	3.1700c	3.4250b	1.4986bc	1.5758b
100	1.85a	1.83ab	3.1122c	3.6067ab	1.3877c	1.6918ab
150	1.72ab	1.84ab	3.2178bc	3.6050ab	1.5074bc	1.6888ab
200	1.72ab	1.86ab	3.3844ab	3.5502ab	1.7235a	1.6955ab
250	1.68b	2.01a	3.5133a	3.7213a	1.6368ab	1.8250a
LSD	0.14	0.24	0.1823	0.2500	0.1784	0.1543
Inoculated	1.72a	1.80b	3.3213a	3.3966b	1.5766a	1.5376b
Uninoculated	1.74a	1.91a	3.2378b	3.7667a	1.5250a	1.8532a
LSD	0.06	0.11	0.0804	0.1102	0.0786	0.0680
CV (%)	4.84	7.49	3.22	4.04	6.69	5.27
Mean	1.73	1.85	3.2796	3.5816	1.5508	1.6954

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

The effect of *Rhizobium* inoculation, rates of bone meal application, cropping season and their interactions, excluding the main effect of inoculation, were significantly ($P <0.05$) affected the grain yield (GY) of field pea (Table 2). The highest GY (3040.17 and 1432.47 kg ha^{-1}) which were 18.5 and 56.4% over the unfertilized control of respective years of were produced at 200 in 2011and 250 kg ha^{-1}in 2012 application, respectively. However, the effect of 250 kg ha^{-1} application in 2011 was non-significant when compared with unfertilized check. With both inoculation treatments, a positive and significant association between GY and different rates of bone meal application was observed (Figure 2b). This association was linear and quadratic in uninoculated and inoculated treatments with higher value of y intercept in inoculated treatment. The significant effect of inoculation over the uninoculated treatment was observed in the 2011 while a significant reduction of GY by inoculation in 2012.

The effect of inoculation with bone meal application on GY was non-significant when compared to respective rates of bone meal without inoculation (Figure 1d). However, the better GY in inoculated than uninoculated was observed in the unfertilized check. The regression analysis revealed that regardless of *Rhizobium* inoculation treatment, the association between NN and GY was negative and quadratic at $P <0.05$ (Figure 3a). Figure 3b indicates that association between NDW and GY in both inoculation treatments was negative and significant. This association was linear in uninoculated treatments and quadratic in inoculated treatment.

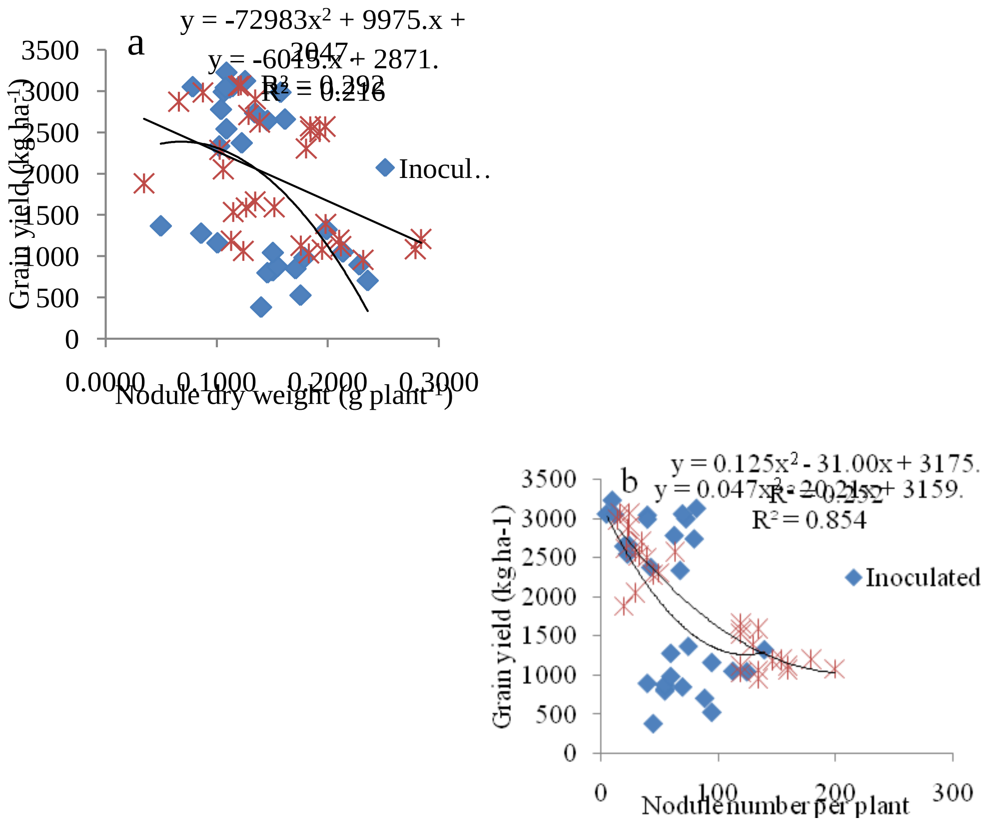


Figure 3. Regression analysis between grain yield with (a) nodule number and (b) nodule dry weight.

4. Discussion

The research result revealed slight improvement of NN and NDW of field pea by lowest rate (100 kg ha^{-1}) of bone meal application. This could be related to the presence of available P and other nutrients essential for normal nodule development and plant growth (Gopinath *et al*., 2011; Mete *et al*., 2015), thereby enhancing root growth (Kumari *et al*., 2012) and the site for nodule formation. The results obtained here are in agreement with that of Wu and Arima (1992) who found that nodulation enhanced by *Rhizobium* inoculation together with NPK application. However, the current result indicated continuous reduction of NN and NDW when applied the bone meal beyond 100 kg ha^{-1}. This reduction in nodulation could be due to the presence of inorganic N beside other essential plant nutrients derived from the decomposition of the organic input (Clayton *et al*., 2004; Sato *et al*., 2011).These authors also reported that the reduction in nodule formation might be mainly due to the inhibition of *Rhizobium* infection to root hairs. However, Hellsten and Huss-Danell (2000) and Chmelíková and Hejcman (2014) found that negative effect of inorganic N on nodulation was attenuated by high P availability.

The effect of *Rhizobium* inoculation in 2011 cropping season improved the NN but a significant reduction in NN and NDW was observed in 2012. The overall effect of inoculation with all rates of bone meal application on NN and NDW was non-significant. The reason might be due to the less competitiveness of inoculated isolates against the indigenous rhizobia nodulating field pea (Thies *et al*., 1991).Several result revealed the non-significant effect of inoculation on nodulation when the number of indigenous rhizobia was greater than 100 g^{-1} soil (Thies *et al*., 1991; Denton *et al*., 2007; Denton *et al*., 2009). On top of this, inoculated *Rhizobium* might be more sensitive for N application than the indigenous rhizobia.

In general, increasing trend of bone meal application significantly increased the SDW, SL, NPP and 100 seeds weight of field pea in both cropping seasons. Such enhancement could not be only due to increase in nodulation and N_2 fixation but also it provides several essential nutrients to the plants. Phosphorus obtained from bone meal application is playing major role in ATP molecules synthesis required by nodules to enhance N-fixation capacity (Ribet and Drevon, 1996), thereby enhancing photosynthesis and plant growth (Zhang *et al*., 2013; Bloomfield *et al*., 2014). This promotive effect of organic input would also be attributed to the improvement of the soil moisture status of the soils and nutrient uptake (Lawson *et al*., 1995). The result of current work indicated that the higher mean values of all investigated traits including NN and NDW excluding GY and TBY was recorded in 2012 than that obtained in 2011 cropping seasons. This indicates the importance of N_2 fixation derived from symbiosis beside essential nutrients obtained from bone meal application.

Many studies have documented the benefit of *Rhizobium* inoculant to field pea production (Kucey and Hynes, 1989; Hynes *et al*., 1995; Clayton *et al*., 2004). These reports support the finding of present study that indicate application of *Rhizobium* inoculants, with many positive effects, and a few negative effects on the field pea parameters assessed, were generally beneficial. Inoculating *Rhizobium* resulted in significantly lower in SDW than the uninoculated. This is in agreement with the study conducted by Xu *et al.* (2015) who found that reduction of shoot biomass yield was observed due to SCAUf32 isolate of *Rhizobium* inoculation. This reduction of productivity is probably due to reduction of indigenous beneficial microbes in field pea rhizosphere by the inoculated rhizobia via space and nutrient competition. Zhang *et al.* (2010) found reduction of microbial diversity by inoculation of microbe. In 2011 cropping season, the effect of *Rhizobium* inoculation on SDW, SL, NPP and 100 seed weight was non-significant. This could be due to the low competitiveness of inoculated *Rhizobium*when compared to the indigenous rhizobia nodulating field pea in Haramaya soils (Zhang *et al*., 2010). The result of the present study revealed that effect of bone meal application on NSP was non-significant in both cropping seasons.

The current study showed significant increase in TBY and GY with increasing in rates of bone meal application suggesting the importance of supply of essential nutrients at planting. This finding is in line with previously reported by Jeng *et al.* (2006) who found that bone meal application increase the yields of spring barley and rye grass. On top of delivering nutrients to the plants, organic input application improve water holding capacity (Bulluck *et al*., 2002) and biological activity in soil (Garcia-Ruiz *et al*., 2008; Pan *et al.*, 2009).This positive effect on soil could also increase in N_2 fixation as result of supply of P and thus increasing grain and biomass production (Zwieten *et al.*, 2015). Ankomah *et al.* (1996) reported that N derived from atmosphere correlated well with grain yield and dry-matter production.

In contrast to the previous highlighted traits of field pea, the higher GY and TBY were recorded in 2011 than 2012 cropping season with all rates of bone meal application. These differences may have arisen from environmental conditions such as precipitation recorded during the late stage of growth in the experiment. Precipitation values in the pod setting growth period of our study were lower in 2012, resulting in lower yields than 2011 cropping season. The current work also revealed that as light increase in average TBY by *Rhizobium* inoculation when compared to uninoculated treatment. This indicates the relevance of *Rhizobium* inoculation to boost field pea biomass production. Beside delivering N by symbiotic N_2fixation, rhizobia can synthesized phytohormones like auxin as secondary metabolites and promote seed germination, root elongation, and stimulation of leaf expansion (Wani *et al.*, 2007; Ahemad and Khan, 2012)).A positive effect of inoculation on TBY was recorded with<150 kg ha^{-1} bone meal application when compared to corresponding rates without inoculation. This suggests that the negative effect of inorganic N on the N derived from the symbiosis at high rate of bone meal application. In contrast to this Chmelíková and Hejcman, (2014) found that the negative effect of high inorganic N was attenuated by combined application P nutrient. In addition, Namvar *et al.* (2011) found that the yield increasein chickpea, with increasing rates of inorganic N application with *Rhizobium* inoculation.

Inoculating *Rhizobium* significantly increased GY in 2011 but a reduction was recorded in 2012 cropping season. Besides, slight increase in GY by inoculation was observed with unfertilized control when compared to control check without bone mean and inoculation. The regression analysis also revealed that an inverse and significant relationship between nodulation traits (NN and NDW) and GY of field pea. This indicates that increase in seed yield is due to improving inorganic N availability beside other essential nutrients rather than improving N_2 fixation by *Rhizobium* inoculation. Previous reports have shown that P application enhanced the microbial biomass and the microbial community composition (Liu *et al.*, 2012) which could beneficial microbes for plant growth thereby increasing crop yield. Inoculating *Rhizobium* alone did not increase field pea production in Ethiopia (Tsigie and Woldeab, 1997).The authors, however, found that combined application of inorganic N and P enhanced the yield of field pea at different parts of Ethiopia.

Increasing bone meal rates of application had significantly improved the total plant N accumulation and P uptake. This indicates the need of organic fertilizer which is rich in different essential plant nutrient to improve the N accumulation and P uptake by plants. Ismand (1989) and Mondinia *et al.* (2008) showed that organic input application increase release of appreciable amounts of N and P to the soil as result of high microbial activity. Significant increase in plant N accumulation due to manure and P application was observed in soybean (Al-Chammaa *et al.*, 2014). This finding is in line with Parry *et al.* (2008) and applying manure with large amount significantly increased P concentration in cowpea. Al-Chammaa *et al.* (2014) also found as significant increase plant N accumulation due to sheep manure application in soybean. Due to high P and N as starter in a form of bone meal application improves the N derived from symbiosis (Oghoghorie and Pate, 1971; Islam *et al.,* 2013) and efficient use of inorganic N from soil (Tufenkci *et al.,* 2006). Under favorable condition, field pea can derive as much as 80–90% from symbiotic association with rhizobia (Sim *et al.,* 1983). However the present study indicated an improvement of plant N accumulation and P uptake by *Rhizobium*inoculation in 2011 as has been reported by Elkoca *et al.* (2010) but a significant reduction in 2012 cropping season. This difference could be because of good weather condition in 2011 and thus enhancing in the amounts of N derived from field pea-*Rhizobium* symbiosis.

5. Conclusion

This research indicates the need of applying high quality organic fertilizer to boost the productivity of field pea in the study site in particular and in Ethiopia in general, though higher rates reduced the nodulation. However, the effect of *Rhizobium* inoculation was not satisfactory and highly depending on the weather condition of the cropping season. The effectiveness of *Rhizobium* inoculation was also dependent on the rates of bone meal application. Therefore, we recommend the application of bone meal alone and/or combined application of low rate of bone meal <150 kg ha^{-1} with *Rhizobium* inoculation for sustainable production of field pea when the native rhizobia is high enough and effective in N_2 fixation.

6. Acknowledgment

This research was funded by the Ethiopia Institute of Agricultural Research, under Biofertilizer and Organic Fertilizer Research Project. The authors would like to express their sincere thanks to Field and technical assistants for their involvement in field experiment management and data collection.

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6. *Rhizobium* Strain × Common bean Genotypes × Environment Interaction Effect on Nodulation and Productivity of Common bean

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Abstract: The interaction effect of Rhizobium strains, common bean genotypes and environment on nodulation and common bean production is not so far properly studied. Therefore, this research was conducted to assess the interaction effect of three common bean varieties (Dursitu, Gofta and Kufanzik), Rhizobium strains and four environments/locations (Haramaya, Hirna, Babillae and Fedis) on nodulation, yield and yield related traits in eastern Ethiopia. The treatments were laid out in randomized complete block design with three replications. The results revealed that *Rhizobium* inoculation, the genotype, environment and their interaction significantly ($P<0.05$) affected all traits of common bean. The effect of *Rhizobium* inoculation on number of nodules (NN) and nodule dry weight (NDW) was varied with different common bean varieties and locations. At Haramaya site, most of the tested isolates produced significantly higher NN than the control check in all varieties while less number in other locations. The effect of *Rhizobium* inoculation on NDW with Kufanzik and Gofta at Babillae was non-significant, while some of inoculated treatments significantly increased the NDW in all varieties at Haramaya, Hirna and Fedis sites. Regardless of the inoculation treatments, the highest NN and NDW above the uninoculated control across locations were observed in Dursitu variety though the highest NN and NDW of varieties were observed when inoculated with different isolates and grown at different locations. Inoculation of common bean varieties with some isolates significantly increased TBY except the inoculation effect was none significant on Kufanzik and Gofta varieties at Hirna and Haramaya sites, respectively. Dursitu produced the lowest mean total biomass (TBY) and grain yield (GY) over locations while the highest mean TBY of 6453.15 kg ha-1 by all the treatments was produced by Kufanzik at Haramaya while the highest mean TBY of 6525.93, 2589.44 and 5036.48 kg ha-1 was obtained from Gofta variety at Hirna, Babillae and Fedis, respectively. Likewise, the highest mean GY of 3358.89, 3257.82, 1499.25 and 2204.82 kg ha-1 over treatments were obtained from Gofta variety at

Haramaya, Hirna, Babillae and Fedis sites, respectively. The highest mean total plant N accumulation of 3.6257, 3.9950, 2.8543 and 3.5637% across the treatments were produced by Dursitu at Haramaya, Hirna, Babillae and Fedis, respectively. The results indicated that none of the tested isolates in all locations produced statistically better NN, NDW, TBY, GY and total plant N accumulation and none specificity between common bean variety × locations in eastern Ethiopia suggesting the need to develop specific strain of Rhizobium for different locations.

Keywords: Common bean; Ethiopia; locations; specificity; variety

1. Introduction

Symbiotic N_2 fixation (SNF),a biological process of transforming the atmospheric $N_2$by mutual interaction of the host plant and soil bacteria is an essential environmentally and economically sustainable sources of N to the soil (Silva and Uchida, 2000), thereby reduce the use of chemical N fetilizer. Different rhizobial species belonging to the genera *Rhizobium*, *Agrobacterium*, *Ensifer*, *Bradyrhizobium* and *Ochrobactrum* have been produced nodules with common bean plants (Wang et al. 2016). Inoculation is a key biological input to improve crop productivity and soil fertility through increasing the rhizobia in the plant rhizosphere (Keyser and Li, 1992; Remans *et al.*, 2008), thereby improve nodulation and N_2-fixation (Peoples *et al.*, 1995) and it can also fix exceed 200 kg N ha year^{-1}(Giller, 2001).The symbiotic N_2 relationship between common bean and *Rhizobium* contributed up to 90 kg N ha^{-1} which was 40 to 50% of the total N near physiological maturity (Westerman *et al.*, 1981).Several studies (Asadi Rahmani *et al.*, 2005; Garcia *et al.*, 2004; Remans *et al.*, 2008) indicated that it's promising potential of common bean to fix N_2 derived from the atmosphere.

The efficacy of rhizobial strains in nodulating and fixing atmospheric N with common bean is varied by both the host genotypes and the *Bacterium* strains (Aguilar *et al.*, 1998; Caballero-Mellado and Martinez-Romero, 1999; Faris and Navabi, 2015; Michiels *et al.*, 1998; Moawad *et al.*, 1998).The prevailed environmental condition significantly shaped the diversity and distribution of indigenous rhizobia nodulating common bean (Wang *et al.*, 2016).Deficiency of different essential nutrients have also been reported as environmental legume-*Rhizobium* symbiosis limiting factors, which may limit the nodulation and N_2 derived from the atmosphere (Divitoa and Sadras, 2014). Soil water availability which is one of major environmental factors also influenced the N_2 fixation derived from atmosphere by common bean (Devi *et al.*, 2013) and soybean (Collino *et al.*, 2015). This variability often limits the nitrogen-fixing performance of soil native rhizobia or use of commercially available inocula. Strains of rhizobia widely differed in their ability to survive nodulate and fix Nin soil environments (Slattery *et al.*, 2001).Considering the high level of adaptation by native rhizobia to local soil conditions, it is important to characterize the indigenous rhizobial collection for use in inoculant production.

Many research reports indicated that host genotypic factors affect nodulation and nodule activity in *Phaseolus vulgaris* (Graham and Temple, 1984; Rennie and Kemp, 1983). Nleya *et al.* (2001) also illustrated the different response of common bean genotypes to the application of *Rhizobium* inoculant. Hardarson *et al.* (1993) also found that N derived from atmosphere (% Ndfa) was varied from 35 to 70% among different common bean genotypes. Usually bushy growth habit of common bean has the lowest N fixation efficiency among all legume crops (Bliss 1993a; Hardarson *et al.*, 1993; Isoi and Yoshida, 1991; Martinez-Romero, 2003). Indeterminate varieties generally will fix more nitrogen than determinate varieties due to the greater "sink" in the indeterminate variety (Ofori and Stern, 1987). Bliss (1993) identified common bean varieties capable of fixing enough atmospheric N_2 to support the grain yield of 1000-2000 kg ha^{-1}. Therefore, improvement of bean BNF requires a multi-disciplinary approach that will increase the host capacity to fix N (Giller, 2001), and selection of effective *Rhizobium* strains that can compete for nodulation with native populations of bacteria present in most soils. So far, the effect of environmental condition on *Rhizobium*-common bean genotypes is not well known. Almost no attempt has been also made on effective bushy type common bean genotypes (with variable maturity time)-*Rhizobium* symbiosis which can give higher response in different environment condition. Hence, the objective of this research was to assess the interaction effect of bushy type common bean varieties, *Rhizobium* strains and environment on nodulation, yield and yield related traits of common bean in eastern Ethiopia.

2. Materials and Methods

2.1. Description of Experimental Sites

Field experiments were conducted on four locations, which are Hirna (N09^{0}13.157' and E041^{0}06.488'at an altitude of 5932ft .a.s.l.), Fedis (N09^{0}06.941' and E042^{0}04.835' at an altitude of 5476ft a.s.l.), Babillae (N09^{0}13.234' and E042^{0}19.407' 5478ft above sea level) and Haramaya (N09^{0}24.954' and E042^{0}02.037' at an altitude of 6628ft a.s.l.) agricultural research centers in 2012, representing the major common bean producing areas of Ethiopia. The fields were located in the eastern part of Ethiopia where common bean had long been grown intercropped with sorghum and maize without inoculation.

The initial soil samples for soil physic-chemical were collected from the top 0-20 cm. A composite soil comprising 20 auguring sampling point from each experimental site was taken and transported back to the laboratory within a day. Representative subsamples of 1 kg each were prepared for most probable number (MPN) assay and stored in a refrigerator at 4^{o}C until used enumerating indigenous rhizobial population. The soil physic-chemical properties were analyzed using standard procedure (Sahlemedhin and Taye, 1991).

The study sites had clay, sandy loam, sandy clay loam and silty clay loam in Hirna, Babillae, Haramaya and Fedis sites, respectively. The pH (H_2O) of the study sites were ranging from 6.66 to 7.84 which is the suitable pH ranges for *Rhizobium* species. All experiment sites had the electric conductivity less than 0.14 ms cm^{-1}. The soil organic carbon and total N content were 1.65 and 0.06%, 0.56 and 0.06%, 1.96 and 0.12%; and

1.32 and 0.12% in Hirna, Babillae, Haramaya and Fedis sites, respectively. The soil had the CEC ranging from 6.59 cmol(+) kg^{-1} in Babillae to 39.88 cmol(+) kg^{-1} in Hirna site. The soil of the study sites had exchangeable Ca^{+2}, Mg^{+2}, Na^{+1} and K^{+1} with ranges of 39.88-4.18 cmol(+) kg^{-1}, 12.87-3.5 cmol(+) kg^{-1}, 0.33-0.12 cmol(+) kg^{-1} and 1.09-0.14 cmol(+) kg^{-1}, respectively.

2.2. Treatments and Experimental Design

Eight isolates of *Rhizobium* spp. were obtained from Biofertilizer Research and Production Project, Haramaya University (Haramaya, Ethiopia). The isolates designated as HUCBR-1, HUCBR-2, HUCBR-3, HUCBR-4, HUCBR-5, HUCBR-6, HUCBR-7, and HUCBR-8. All isolates used in this study were isolated from Ethiopia soils. All isolates were characterized as superior isolates in nodule formation and shoot biomass production of common beann under greenhouse condition (Anteneh Argaw, 2007).

Seeds of *Phaseolus vulgaris* genotypes used in this study were obtained from Lowland Pulse Research Program, Haramaya University, Haramaya, Ethiopia. Varieties were selected characterized as highly productive varieties in this study sites. Beside this, maturity time was also considered for selection of varieties for this experiment. Accordingly, Gofta, Kufanzik and Dursitu varieties had early, medium and late maturing, respectively.

The pure culture of *Rhizobium* isolates were obtained from the laboratory in slant culture. The bacteria were purified by culturing in YEMA (Yeast extract mannitol agar) medium and then single pure colony was transferred into YEM broth medium and kept at 30°C for 7 days on a rotary shaker at 120 rpm. 400 ml of culture liquid medium containing appropriate *Rhizobium* sp. were added to 1 kg of carrier (sterile fine filer mud) and mixed thoroughly and then packed in plastic bags. Filermud-base inoculum was incubated at 26–28°C for 15 days. At the time of inoculation, the number of rhizobia in the inoculum was estimated using plate count method. One ml samples of serially diluted inoculum from 10^{-6} dilution were plated in YEMA medium. Colonies that developed after incubation at 28°C for 5–7 days were recorded. This test indicated that the number of rhizobia was more than 1 $x10^{9}$ gm^{-1}.

The experiments were designed as two-factor experiments in a randomized complete block design (RCBD). There were three replications of each treatment. Ten treatments containing eight *Rhizobium* isolates (HUCBR-1, HUCBR-2, HUCBR-3, HUCBR-4, HUCBR-5, HUCBR-6, HUCBR-7, and HUCBR-8) with uninoculated and N fertilized (20 kg N ha^{-1}) treatments were assigned as the main plot factor while three common bean varieties were the sub-plot factor. Before sowing, 20 kg P ha^{-1} as tri-superphosphate for all experimental plots were applied in furrows. A total of 30 treatments were used in the experiment. Identical field experiments were mounted in four locations. The experimental fields were ploughed thoroughly twice with tractor and divided into sub-plots in accordance with the treatments. The net size of each experimental sub-plot was 3 x 2 m^2. There were five rows per sub-plot and the spacing was 1.5 m between main plots, 1m between sub-plots, 40cm between rows and 10 cm between.

Common bean seeds were sterilized using 70% ethanol for 1 min and NaClO solution (0.25% as available Cl) for 3 min. The seeds were then washed carefully in sterilized de-inonized water five times before sowing. Then, 20 g of the different rhizobia inoculants was added to different polyethylene bags containing 200 g of common bean seeds. A 10% (w/v) sucrose solution to increase adherence was added to each bag to enhance proper mixing and adhesion of the rhizobia carrier material to the common bean seeds. After mixing, seeds were allowed to air-dry in the shade for 15 min and sown field layout. Two seeds were planted by hand per hole and later thinned down to one per hole 1 week after germination All standard local cultural practices were accomplished throughout the growth period. Manual weeding was done when required.

2.3. Data Collection and Analysis

At late flowering and early pod setting stage, 5 plants were randomly chosen from central three rows for the evaluation of nodulation and plant growth. Adhered soil on the sampled plants were loosen by placing into plastic buckets with full of water. Thereafter, nodules from roots were picked and following data were recorded: (1) Nodules number plant^{-1}, and (2) Nodule dry weight plant^{-1}. Shoot dry weight was also measured after dried the samples at 70 ^{0}C in electrical oven until the weight of the samples became constant. Shoots of the plants were later ground to pass a 0.5 cm sieve. Total N determinations were done by the Kjeldahl method of Bremner (1965).At full maturity stage, numbers of pods plant^{-1}, number of seed pod^{-1}, plant height at harvest and total biomass were evaluated. Grain yield was corrected for 13% moisture content after determining humidity level with a grain moisture tester.

Data were subjected to analysis of variance (SAS Institute, 1999). Statistically significant differences between means were also determined by the LSD test (SAS Institute, 1999).

3. Results

Analysis of variance (ANOVA) showed that *Rhizobium* inoculation, experimental location, the genotypes and their interaction significantly affected the nodule number (NN) at P <0.05 (Table 1). The effect of *Rhizobium* inoculation treatments on NN varied due to different genotypes and experimental locations. At Haramaya site, most of the inoculated isolates, except NSCBR-59 and NSCBR-31 inoculations, resulted in significantly increase NN with Dursitu genotype (Table 2). With Gofta genotype, in exception of isolate NSCBR-59, all tested isolates significantly increased the NN. With exception of NSCBR-31, all isolates resulted significantly increase NN with Kufanzik genotype.

At Hirna sites, NSCBR-59, NSCBR-31 and NSCBR-57 inoculations with Dursitu variety had significantly higher NN than the control check. However, most of inoculated treatments, except NSCBR-59, NSCBR-18 and NSCBR-57 inoculation with Gofta variety significantly increased the NN. With Kufanzik variety, statistically increase in NN was recorded in NSCBR-14, NSCBR-16 and NSCBR-18 inoculation treatments.

At Babillae site, NSCBR-$(25)_2$ and NSCBR-18 with Dursitu resulted in significantly higher NN than the uninoculated control. Significantly increase in NN of Gofta was recorded with NSCBR-14, NSCBR-$(25)_2$, NSCBR-59 and NSCBR-31 treatments. However, NSCBR-$(25)_2$ significantly increased the NN with Kufanzik. At Fedis site, significantly higher NN with Dursitu was produced in association with all inoculated treatments with exception of NSCBR-31 and NSCBR-18 while NSCBR-14, NSCBR-$(25)_2$, NSCBR-59 and NSCBR-31 isolates with Gofta.

The highest NN over the control check was recorded with Gofta genotype. Beside this the highest mean NN (216.17, 221.93, 106.27 and 152.37) over treatments were induced with Dursitu at Haramaya, Hirna, Babillae and Fedis sites, respectively. Similar NN in uninoculated control check at Haramaya, Babillae and Fedis sites with all tested common bean genotypes were recorded. At Hirna site, the higher NN (140 and 150) in uninoculated treatment were recorded with Durstu and Kufanzik, respectively than Gofta genotype.

The effect of *Rhizobium* inoculation, the genotypes, experimental locations and their interaction was significant on nodule dry weight (NDW) (Table 1).The effect of inoculated isolates on NDW was varied with different genotypes and in different experimental sites as the result displayed in NN (Table 3). At Haramaya site, Dursitu symbiosis with all*Rhizobium* inoculation treatments, except NSCBR-$(25)_2$, NSCBR-59 and NSCBR-18, recorded significantly higher NDW than the control check. Inoculating NSCBR-14 and NSCBR-$(25)_2$ and NSCBR-16 significantly increased the NDW of Gofta genotype. However, only NSCBR-59 inoculation with Kufanzik had significantly higher NDW than the control check.

At Hirna site, NSCBR-$(25)_2$, NSCBR-59, NSCBR-31 and NSCBR-57 inoculations significantly increased the NDW of Dursitu genotype. All except NSCBR-$(25)_2$ and NSCBR-18 inoculations had significantly higher NDW with Gofta than uninoculated control. Inoculating NSCBR-16, NSCBR-18 and NSCBR-57 with Kufanzik recorded significantly higher NDW than uninoculated control. At Babillae site, none of the tested isolates with Dursitu and Kufanzik recorded significant effect on NDW when compared to the control check. However, only NSCBR-$(25)_2$ significantly increased NDW of Gofta genotype. At Fedis site, most of the tested isolate excluding NSCBR-31 and NSCBR-18 had significantly higher NDW with Gofta than uninoculated control. A significant increase in NDW of Gofta was observed withNSCBR-14, NSCBR-59, NSCBR-31 and NSCBR-16 treatments. InoculatingNSCBR-14, NSCBR-59 and NSCBR-16 was significantly increased the NDW of Kufanzik genotype at $P < 0.05$.

Analysis of variance indicated that the main effect of *Rhizobium* inoculation, the genotypes, experimental locations and their interaction was significant ontotal biomass yield (TBY) at $P < 0.05$ (Table 1).At Haramaya site, TBY of Dursitu was significantly increased due to NSCBR-16, NSCBR-57 and NSCBR-25 inoculations (Table 4). Only NSCBR-14 isolate with Gofta and none of the tested isolates with Kufanzik resulted in significantly increase the TBY. At Hirna site, NSCBR-14, NSCBR-59 and NSCBR-31 with Dursitu and NSCBR-59 and NSCBR-18 with Kufanzik produced significantly higher TBY than the uninoculated control. However, the data presented the non-significant effect of inoculation on TBY of Gofta genotype.

At Babillae site, NSCBR-14 inoculation significantly improved the TBY of Dursitu over the control check. With Gofta, NSCBR-$(25)_2$ and NSCBR-16) inoculations significantly increased the TBY when compared to the control check. Inoculating NSCBR-$(25)_2$ with Kufanzik produced significantly higher TBY than the control check. Inorganic N application with all genotypes at Babillae site produced the highest TBY as compared to the other treatments.

At Fedis site, NSCBR-59, NSCBR-16 and NSCBR-57 isolates with Dursitu produced significantly higher TBY than the uninoculated control. Gofta in association with NSCBR-$(25)_2$, NSCBR-31 and NSCBR-16 inoculation had significantly higher TBY than the uninoculated control. Inoculating NSCBR-$(25)_2$resulted in significantly increase the TBY with Kufanzik. In contrast to nodulation, the highest TBY (2589.44 and 5036.48 kg ha^{-1}) across the treatments were produced with Gofta at Babillae and Fedis sites. At Haramaya and Hirna sites, all genotypes produced almost similar amount of TBY. With no inoculation, Gofta recorded the highest TBY (5954.6, 5835.2, 2294.4 and 4627.8 kg ha^{-1}) in Haramaya, Hirna, Babile and Fedis sites, respectively.

The grain yield (GY) of common bean significantly affected by *Rhizobium* inoculation, the genotypes, experimental sites and their interaction at $P < 0.05$ (Table 1).The effects of tested isolates on GY were found to be differed significantly due to different in genotypes and experimental locations. At Haramaya site, Dursitu inoculated with NSCBR-14, NSCBR-16 and NSCBR-57 presented significantly higher GY than the uninoculated control (Table 5). With Gofta, applying NSCBR-14 resulted in significantly increase in GY comparing with the uninoculated control. The response to NSCBR-14, NSCBR-59, NSCBR-16 and NSCBR-18 inoculations in GY of Kufanzik was significant.

At Hirna site, all inoculated treatments, except NSCBR-18, NSCBR-57 and NSCBR-25, resulted in significantly increase in GY with Dursitu genotype while none of the inoculated isolates significantly affected the GY of Gofta genotype. Applying NSCBR-14, NSCBR-16 and NSCBR-18 significantly ($P < 0.05$) increased the GY with Kufanzik genotype. At Babillae site, NSCBR-14 inoculation with Dursitu variety gave significantly higher GY than the uninoculated control. Significantly higher GY of Gofta over uninoculated control was recorded with NSCBR-$(25)_2$ and NSCBR-16 treatments. However, the data revealed the non-significant effect of inoculation on the GY of Kufanzik genotype.

Table 1. Summary of ANOVA results for all investigated traits of common bean affected by *Rhizobium* inoculation, locations and common bean varieties and their interaction, in Haramaya, eastern Ethiopia, 2012/13 cropping season.

Sources of variation	df	Mean of squares								
		NN	NDW	SDW	NPP	NSP	100 seeds weight	GY	TBY	Tot N
Inoculation (I)	9	57820.21***	1.3474***	390.95***	36.19***	0.8309***	5.97***	689219.2***	1901478.7***	0.3843***
Error a	18	6.93	0.0272	1.419	0.436	0.1307	0.2717	36.32	73.8	0.0438
Location (L)	3	162712.69***	12.0138***	18507.05***	24000.08***	8.8561***	222.18***	50871117.8***	303516204.1***	14.8800***
Variety (V)	2	99133.02***	4.9526***	1336.97***	192.52***	13.0149**	7713.64*	27841004.6***	13380537.9***	13.9593***
Error b	40	3.45	0.0118	0.903	0.354	0.0669	0.1485	27.32	43.6	0.0195
L x I	27	7791.62***	0.6423***	214.30***	23.12***	0.5278**	3.01***	224350.6***	1314309.9***	0.3052***
V x I	18	6338.46***	0.3683***	369.16***	11.66**	0.5495**	3.00**	1366719.0***	795260.8***	0.2897***
L x V	6	21688.09***	0.2510***	1273.77***	29.85***	1.7977***	74.17***	1234231.0***	2163372.5***	1.8200***
L x V x I	54	9120.29***	0.4940***	309.04***	13.97***	0.4452***	2.90***	208208.5***	639905.5***	0.2491***
Total	359									

*** and***,significant at P<0.01and P<0.001, respectively. NN= Nodule number; NDW= Nodule dry weight; SDW=Shoot dry weight; NPP= Number of pods per plant; NSP= Number of seeds per pod; GY= Grain yield; TBY= Total biomass yield; PH= Plant height; Tot N= total nitrogen.*

At Fedis site, a significant improvement of GY for Dursitu was observed with NSCBR-59 inoculation while *Rhizobium* inoculations did not affect the GY of Gofta and Kufanzik genotypes. The highest mean GY (2932.3, 2739.4, 1490.0 and 2065.6 kg ha^{-1}) across the treatments were recorded with Gofta in Haramaya, Hirna, Babillae and Fedis sites, respectively. In all experimental sites, Gofta produced the highest GY (3498.4, 3257.82, 1499.25 and 2204.82 kg ha^{-1}) with uninoculated control

Analysis of variance showed that the effect of *Rhizobium* inoculation, the genotype, experimental locations and their interaction on total plant N accumulation (TPNA) was significant at $P < 0.05$ (Table 1). The effect of *Rhizobium* inoculation was non-significant on TPNA of Dursitu genotype in Haramaya site (Table 6). In the same site, inoculating NSCBR-16, NSCBR-57 and NSCBR-25 resulted in significantly improve the TPNA with Gofta genotype while applying NSCBR-59, NSCBR-31 and NSCBR-16 produced significantly higher GY than the uninoculated control with Kufanzik.

At Hirna site, significant increase in TPNA due to NSCBR-$(25)_2$, NSCBR-59, NSCBR-57 and NSCBR-25 inoculations was recorded with Dursitu genotype. None of the *Rhizobium* inoculations significantly affected the TPNA with Gofta and Kufanzik genotypes. At Babillae site, all *Rhizobium* inoculations did not improve the TPNA of all the tested genotypes. At Babillae site, all *Rhizobium* inoculations, excluding NSCBR-14 and NSCBR-59with Dursitu produced significantly higher TPNA than the uninoculated control. However, there was no significant of inoculation on TPNA with Gofta and Kufanzik genotypes. The highest mean total plant N accumulation (3.6257, 3.9950, 2.8543 and 3.5637%) were recorded with Dursitu in Haramaya, Hirna, Babillae and Fedis sites, respectively. Beside this, Dursitu in uninoculated treatment accumulated the highest total plant tissue N (3.7400 and 3.7033%) at Haramaya and Hirna sites, respectively, while the highest plant N accumulation (3.7167 and 2.9933%) in Babillae and Fedis sites were recorded with Kufanzik genotype, respectively.

4. Discussion

Utilizing *Rhizobium* inoculation for pulses production is common practice in different part of the world including some countries in sub-Saharan Africa. However, the success of this inoculant technology with common bean is variable from location to location beside depending on common bean genotypes. Due to different rhizobia population size and its competitiveness in different locations and presence of specificity between *Rhizobium* strain-common bean genotypes (Aouani *et al.*, 1997), we need to develop genotype and location specific *Rhizobium* isolates to improve the effectiveness of inoculant. Hence, this study was initiated to evaluate the effect of locations and common bean genotypes on the effectiveness of selected *Rhizobium* isolates in major common bean growing areas of eastern Ethiopia.

In general, the *Rhizobium* inoculation, the locations, the genotypes of common bean and their interaction significantly affected the nodulation, yield and yield traits of common bean at $P < 0.05$ (Table 1). This indicates the need of specific *Rhizobium* isolate development for each of common bean genotype when cultivated different locations. Similar result was previously reported on common bean (Handley *et al.*, 1998; Mostasso *et al*., 2002; Popescu, 1998; Remans *et al*., 2008). This specificity could be the fact that the exchanges of chemical signals between the two partners are present. The legume roots exude organic compounds (flavonoids) (Hungria *et al*., 1997; Long, 2001), which differ between plant species and genotypes, and rhizobial bacteria respond with lipo-chitin oligosaccharides, known as Nod factors, which act as specific morphogenetic signal molecules to induce the roots nodule formation (Oldroyd and Downie, 2008). Besides, locational specific *Rhizobium* development also required.

The present study revealed that isolate performed good in improving NN, NDW, TBY, GY and TPNA with one of the tested genotype was not consistently exhibited with other genotypes, indicating the presence of specificity between the inoculated *Rhizobium* isolate and tested common bean genotypes. Similarly, Bouhmouch *et al.* (2005) reported the common bean genotypes-*Rhizobium* specificity. This also indicates the presence varied infectivity potential of inoculated *Rhizobium* isolates with different genotypes of common bean (Neila *et al.,* 2014).

We found that relatively more number of inoculated *Rhizobium* performed better in NN than the background rhizobia in Haramaya site than the other study sites. This indicates the presence of less competitive background rhizobia in infectiveness at Haramaya site when compared to the other study sites. The current study also showed that those isolates performed better in improving NN were not performed in NDW enhancement in all study sites. At Babillae site, the effectiveness of the inoculated *Rhizobium* on NDW was very less those performed in other study sites and produced the lowest mean NN and NDW across inoculation treatments with all tested genotypes. This is probably due to low rhizobial population in this site (Ojo *et al*., 2015) and consequently reducing the nodule formation. Elias and Herridge (2015) found that rhizobial population was positively correlated with soil moisture. Low nodulation formation might be also attributed to the prevailed adverse environmental condition at Babillae site (Hungria *et al*., 2003). Besides, the soil textural class of Babillae soil is sand and had low SOM (Table 1) which could reduce the survival of inoculated *Rhizobium* in the soil (Hagedorn, 1978; Mahler and Wollum, 1981). However, Bliss (1993) suggested that the limitation of N_2 fixation imposed by environmental factors could be resolved through the selection and breeding of improved common bean cultivars.

The highest NN and NDW in the control check without inoculation were produced with Dursitu at Haramaya and Hirna sites and Kufanzik at Babillae and Gofta at Fedis site. This suggests the presence of appropriate indigenous rhizobia population in Haramaya and Hirna soils for Dursitu, at Babillaae for Kufanzik and Fedis soils for Gofta genotype. Rodiño *et al.* (2011) determined a significant main common bean genotype effect and genotype × environment interaction for all nodulation parameters. Similar finding was previously observed in common bean genotypes in Canadian Prairie by Nleya *et al.* (2009) who found that common bean genotypes differed in nodulation formation. In addition, Ikeda (1999) found that the number of nodules was directly controlled by host genotype. This preference could have a major significance in resolving strain competition problem in *Phaseolus vulgaris* (Raposeiras *et al*., 2006).

The result of the present work indicated that the isolate induced the highest nodulation with one genotype was not consistently performed with the other genotypes. Similarly, Bonish and MacFarlane (1987) demonstrated that isolates mean effectiveness of 12% with 'Tamar' genotype were recorded 87% mean effectiveness with Huia genotype. Similarly, differences in host genotype among clover lines influence the effectiveness of the symbiosis (Hagedorn and Caldwell, 1981; Sherwood and Masterson, 1974).

The highest mean NN and NDW across locations and with all treatments including uninoculated control were produced by Dursitu. On the other hands, Dursitu at Haramaya, Babillae and Fedis sites and Kufanzik at Hirna site induced the highest mean NDW across the treatments. This indicates the presence of more infectiveness by inoculated *Rhizobium* and background rhizobia with Dursitu rather than other tested genotypes. This might also be attributed to the high promiscuity of Dursitu with several rhizobial species (Cardoso *et al.,* 2012) apparently resulting from the capacity of the host plant to perceive a variety of rhizobial molecular signals (Michiels *et al*., 1998).Besides, the current work found the presence of *Rhizobium* isolate-Genotype specificity in nodule production in different location. Significant environment by inoculant interactions for nodule dry weight was reported by Nleya *et al.* (2009).

The result of the present study indicated the highest mean total plant N accumulation across treatments and at uninoculated control was recorded with Dursitu as nodulation traits recorded. This implies that improving nodulation is important traits to enhance the total N in plant tissue. Variation for plant N accumulation among genotypes could be due to the presence of variability in SNF among different common bean genotypes (Hardarson *et al*., 1993; Nleya *et al*., 2002). Yadegari *et al.* (2010) found that Cultivar 'Akhtar' demonstrated highest potential for nodulation, nitrogen fixation and seed yield production compared to cultivars 'Sayyad' and 'Goli'. Buttery *et al.* (1997) also compared 17 common bean genotypes inoculated with various *Rhizobium* strains for N fixation and they found differences among genotypes in acetylene reduction activity and seed N content.

In contrast to the result in nodulation, the mean TBY and GY across locations were the highest with Gofta genotype. This genotype also produced the highest mean TBY and GY across treatments including the result in uninoculated control. Gofta genotype also produced the highest biomass and grain production in all experimental locations. This finding is supported by *Tsai et al.* (1993) who found that Mexico-309 was superior for nodulation parameters but poor for seed yield, while Preto Caruaru produced high seed yield, but was inferior in nodulation traits. The yield advantage of Gofta could be attributed to its later maturity when compared to other tested genotypes of common bean. Due to genetic makeup common bean genotypes, it may record high production though induced low nodulation (Pereira *et al.,* 1984).Conversely, Rodiño *et al.* (2011) found that genotypes with a big nodule phenotype showed a good plant response. They also indicate that this phenotype may be more beneficial for plant growth and seed yield in environmental conditions that may limit nodule development. In addition, in contrast to the current study, Farid and Navabi (2015) found the common bean genotypes-environment interaction for grain yield production.

Regardless of the tested genotypes, the highest TBY at Babillae site was recorded with inorganic N treatment. Similarly, Hungria *et al.* (2003) found further increase of common bean production on average by 132 kg ha^{-1} with a supplement of 15 kg N ha^{-1} over the inoculated plants. In other experimental sites, a significant increase in TBY was obtained with *Rhizobium* inoculation. Yield increase by 30 to 80% in common bean by *Rhizobium* inoculation when compared to N fertilizer plant has been found by Huntington *et al.* (1986). In contrast, Ruiz Diaz *et al.* (2009) found that no yield increase in soybean by inorganic N application with and without inoculation though plant N accumulation was improved. This result could be attributed to high N_2 derived from soybean when compared to common bean.

It was also found that none of inoculated *Rhizobium* significantly improved the plant accumulated N at Babillae soil when compared to the uninoculated control with all genotypes. This result could be attributed to dry condition and low soil moisture availability in Babillae (Saito *et al.*, 1984; Smith *et al.*, 1985, 1988) and cause early nodules senescence and decline in nitrogenase activity (Becana *et al.*, 1986) and low N_2 fixation. On the other hand, the *Rhizobium* inoculation at the other locations significantly increased plant N accumulation with all genotypes. This result could be attributed to the fact that more than 50% of its plant N accumulated derived from biological N_2 fixation when inoculated with effective *Rhizobium* under favorable condition (Pena-cabriales *et al.*, 1993).

Some of the tested *Rhizobium* with Dursitu accumulated significantly higher plant N than the uninoculated control but this result was not observed with the remaining genotypes. Similarly, tests performed under field conditions (Hobbs and Mahon, 1982; Rengel, 2002; Young *et al.*, 1982,) have shown that some *Rhizobium* isolates are more efficient when inoculated on some genotypes than on others. Huntington *et al.* (1986) conclude from their greenhouse study that the host/endophyte combination forms a relatively ineffective symbiotic association being primarily inherent in the host plant rather than the endophyte or the environment. This result is supported by Hungria and Neves (1987); Hardarson *et al.* (1993) and Neves *et al.* (1987) who found that accumulation of N in different pulse crops is influenced by the host plant cultivar as well as by *Rhizobium* strain. Graham (1981) and Amarger (1986) that nitrogen fixation depends on rhizobia x line interaction and that the process of selection of efficient rhizobia should be developed with adequate lines.

5. Conclusion

This experiment results showed the presence of *Rhizobium* strain x locations specificity. Besides, the result found the need of different *Rhizobium* isolate for tested genotypes of common bean. The result indicated similar performance of all common beans in most of investigated traits, except nodulation, regardless of the experimental locations. This suggests the need of specific *Rhizobium* strain development for biofertilizer production for different locations. Hence, we recommend the development of location based *Rhizobium* isolate for inoculate production.

Table 2. Nodule number of common bean investigated from three genotypes (Dursitu, Kufanzik and Gofta) over four experimental locations (Babillae, Fedis, Haramaya and Hirna), in eastern Ethiopian, in 2012/13 cropping season.

Inoculation	Dursitu				Kufanzik				Gofta			
	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis
NSCBR-14	226.67bcd	236.67bcd	101.33c	179.33ab	136.00abc	231.67bc	122.33a	140.67a	95.33cd	210.00ab	76.00abc	110.00bcd
NSCBR-$(25)_2$	290.00bc	235.00bcd	248.00a	166.67abc	135.33abc	280.00b	119.33ab	152.67a	130.67bc	153.33a-d	92.67a	160.00abc
NSCBR-59	117.00de	363.33a	85.67c	223.33a	96.67c	177.67cd	112.33abc	176.67a	268.00 a	160.33abc	75.00abc	213.33a
NSCBR-31	178.00cde	269.33abc	80.00c	139.67bcd	116.67bc	366.67a	110.33abc	130.00a	76.00de	110.67bcd	91.67ab	107.00bcd
NSCBR-16	463.33a	201.67cd	96.33c	171.67abc	145.00ab	246.00bc	98.33a-d	155.00a	93.67cd	215.00a	85.67ab	189.33ab
NSCBR-18	138.00e	148.33cd	162.00b	115.00cde	151.00ab	149.33de	90.33bcd	67.33b	100.33cd	198.33ab	89.33ab	106.87bcd
NSCBR-57	281.67bc	345.00ab	75.00c	151.67bc	143.67ab	199.33cd	86.00cd	166.00a	119.67bc	136.67a-d	79.67ab	89.33cd
NSCBR-25	333.00b	166.67cd	92.00c	213.33a	160.33a	284.33b	102.33a-d	73.67b	151.67b	183.33abc	86.00ab	132.33a-d
-Ve Control	79.33e	140.00d	61.67c	89.33de	47.33de	150.00de	73.00de	70.00b	49.67e	88.33cd	68.00bc	97.67cd
+Ve Control	54.67e	113.33d	60.33c	73.67e	38.33e	93.00e	53.00e	72.00b	42.67e	55.67d	52.67c	54.67cd
LSD	126.39	124.57	50.57	60.55	42.8	80.27	30.65	48.51	39.55	103.74	23.72	83.97d
CV (%)	20.22	19.41	16.46	13.74	12.65	12.74	10.95	13.93	12.13	23.74	10.30	23.04
Mean	216.17	221.93	106.27	152.37	117.03	217.80	96.77	120.00	112.77	151.17	79.67	126.03
P value	***	***	***	***	***	***	***	***	***	***	***	***
F value	25.61	11.70	32.37	16.55	25.37	25.20	12.55	21.16	66.02	6.68	6.85	8.32

****, significant at P<0.001; Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. -VE=negative control (no inoculation and N application), +VE control =20 kg N ha*$^{-1}$*; NSCBR= National soil Common Bean Rhizobium.*

Table 3. Nodule dry weight of common bean investigated from three genotypes (Dursitu, Kufanzik and Gofta) over four experimental locations (Babillae, Fedis, Haramaya and Hirna), in eastern Ethiopian, in 2012/13 cropping season.

Inoculation	Dursitu				Kufanzik				Gofta			
	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis
NSCBR-14	1.9527bc	0.9591b	0.1557b	0.4807b	0.8001ab	1.1745cd	0.1263bcd	0.5393c	0.2401b	0.2401ef	0.0661bc	0.6500bc
NSCBR-(25)2	1.3644cd	1.8193a	0.1396b	0.8717a	0.5716bc	1.5471bc	0.2061a	0.3117de	0.3697b	0.5116cd	0.0876ab	0.3970de
NSCBR-59	0.7400e	1.7659a	0.1451b	0.8983a	0.4329cde	0.5869e	0.1025cde	1.0351a	1.5093a	0.4133de	0.0830ab	1.1167a
NSCBR-31	0.5533e	1.5782a	0.1105b	0.1320c	0.3429cde	2.4817a	0.1670ab	0.5503c	0.2070b	0.4300d	0.0980ab	0.1965ef
NSCBR-16	3.6583a	0.7725bc	0.1449b	0.4413b	1.0363a	1.7070b	0.1107cd	0.7637b	0.2398b	0.8381b	0.0772ab	0.8187b
NSCBR-18	0.8420de	0.6835bc	0.3122ab	0.3270bc	0.5450cd	0.7108de	0.0850de	0.2520def	0.5243b	1.1180a	0.1072ab	0.2473def
NSCBR-57	1.3562cd	1.7729a	0.1380b	0.4897b	0.3997cde	1.2241bc	0.0623ef	0.4250cd	0.4911b	0.6549bc	0.0630a	0.1145f
NSCBR-25	2.0594b	0.9970b	0.1028b	0.7607a	0.3843cde	1.6599bc	0.1423bc	0.1106f	0.4867b	0.4978cd	0.0859bc	0.4743cd
-Ve Control	0.4963e	0.6788bc	0.1164b	0.1654c	0.3033de	0.6581e	0.1301bc	0.2500def	0.2405b	0.3342de	0.0640ab	0.3125def
+Ve Control	0.3183e	0.5108c	0.5703a	0.1210c	0.2317e	0.3532e	0.0217f	0.1410ef	0.1233b	0.1262f	0.0340bc	0.2661def
LSD	0.606	0.3691	0.3740	0.2659	0.2491	0.5016	0.0435	0.1948	0.4763	0.1896	0.0404c	0.2403
CV (%)	15.71	11.06	66.83	19.62	16.86	14.33	13.03	15.38	34.71	12.70	18.25	18.09
Mean	1.3341	1.1538	0.1935	0.4688	0.5048	1.2103	0.1154	0.4379	0.4746	0.5164	0.0766	0.4594
P value	***	***	**	***	***	***	***	***	***	***	***	***
F value	70.19	50.09	3.77	30.73	25.20	42.73	36.36	56.34	17.14	59.30	6.67	43.12

***and ***, significant at P<0.01and P<0.001, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. -VE-negative control (no inoculation and N application), +VE control -20 kg N ha-1; NSCBR- National soil Common Bean Rhizobium.*

Table 4. Total biomass yield of common bean investigated from three genotypes (Dursitu, Kufanzik and Gofta) over four experimental locations (Babillae, Fedis, Haramaya and Hirna), in eastern Ethiopian, in 2012/13 cropping season.

Inoculation	Dursitu				Kufanzik				Gofta			
	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis
NSCBR-14	5157.4cde	7370.1a	2095.4ab	3414.8cd	6870.4a	6500.0a	1722.2b	4222.2cd	6018.5ab	6944.4abc	2342.6cde	4568.5b
NSCBR-$(25)_2$	5000.0cde	5981.5bcd	1185.2d	3870.4abc	6473.1ab	6270.4a	3301.9a	4796.3ab	5859.3ab	5592.6d	2824.1bc	6111.1a
NSCBR-59	5240.7cde	7388.9a	1555.6bcd	4318.5a	6154.6ab	6740.7a	2591.6ab	3722.2e	6322.2ab	7611.1a	2722.2bcd	5185.2b
NSCBR-31	5101.9cde	6844.4ab	1975.9abc	3648.1bcd	5898.1ab	6837.0a	2000.0b	4481.5abc	5025.9b	6129.6cd	2705.6bcd	4800.0b
NSCBR-16	6388.9b	6314.8bc	1385.2cd	4179.6ab	5693.5b	6659.3a	3355.6a	4905.6a	5546.3ab	6414.8bcd	1888.9e	5222.2ab
NSCBR-18	4772.4de	5925.9bcd	1946.3bcd	3731.5a-d	5740.7b	6851.9a	2074.1b	4157.4cde	5450.0ab	7348.1ab	2220.4de	5422.2ab
NSCBR-57	7518.5a	5442.6cd	1694.3bcd	4148.1ab	5497.2b	5840.7a	2063.9b	3907.4de	6495.5a	5787.0d	2351.9cde	4557.4b
NSCBR-25	6064.8bc	5259.3d	1966.7abc	3190.7d	5713.0b	6533.3a	2527.8b	4083.3cde	5685.2ab	6263.0bcd	2948.1b	5074.1b
-Ve Control	4685.2e	5463.0cd	1407.4cd	3425.9cd	5675.9b	5492.6a	2037.0b	3851.9de	5954.6ab	5835.2cd	2294.4cde	4627.8b
+Ve Control	5851.9cd	6537.0ab	2534.7a	3805.6a-d	6023.1ab	6805.6a	3052.8a	4322.2bcd	6101.9ab	7333.3ab	3596.3a	4796.3b
LSD	1093.4	1002.8	617.77	648.73	1061.1	1421.3	918.92	487.25	1359.4	1143.8	579.93	9.9.78
CV (%)	6.78	5.55	12.04	5.95	6.14	7.62	12.85	3.97	8.04	6.06	7.75	6.25
Mean	5578.17	6252.78	1774.66	3773.3	5973.98	6453.15	2472.78	4245.0	5845.94	6525.93	2589.44	5036.48
P value	***	***	***	***	**	*	***	***	*	***	***	***
F value	16.39	15.02	10.78	8.07	3.99	2.61	10.31	16.18	2.56	10.25	17.07	7.04

, ** and*, significant at P<0.05, P<0.01and P<0.001, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. -VE-negative control (no inoculation and N application), +VE control -20 kg N ha-1; NSCBR- National soil Common Bean Rhizobium.*

Table 5. Grain yield of common bean investigated from three genotypes (Dursitu, Kufanzik and Gofta) over four experimental locations (Babillae, Fedis, Haramaya and Hirna), in eastern Ethiopian, in 2012/13 cropping season.

	Dursitu				Kufanzik				Gofta			
	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis
NSCBR-14	2323.1a	2554.35a	1340.6ab	1093.3b	3800.1a	2829.3ab	1098.6d	1607.0abc	3735.6a	3470.6ab	954.0e	2384.4ab
NSCBR-(25)2	2192.9ab	2107.69bcd	653.7c	1285.6ab	3206.5bc	2634.3ab	1987.1a	1801.3ab	3339.8abc	3201.4abc	1657.8abc	2570.0a
NSCBR-59	2059.8abc	2486.94a	796.5c	1616.8a	3105.8bc	2565.6ab	1619.8abc	1716.7abc	3608.7ab	3625.7a	1913.1a	1956.3abc
NSCBR-31	2239.1ab	2132.96bc	1101.2abc	1328.1ab	2915.8bc	2910.8a	1046.6d	1938.8a	3062.1bc	3092.9bc	1631.9abc	2497.4ab
NSCBR-16	2454.9a	2122.41bcd	786.9c	1285.2ab	2983.8bc	2657.0ab	1932.4ab	1693.1abc	3265.8ab	3334.4ab	1080.1de	1776.4bc
NSCBR-18	1683.9c	1803.06de	1054.2bc	1162.4ab	3007.3bc	2817.5ab	1411.9cd	1615.7abc	3215.7a	3648.0a	1232.8cde	2659.6a
NSCBR-57	2441.9a	1615.56e	892.7bc	1396.7ab	2761.6c	2433.8b	1442.5bcd	1333.8bc	3688.4bc	3096.4bc	1390.0b-e	1577.2c
NSCBR-25	2139.1ab	1918.89cde	1043.5bc	1097.6b	3195.3bc	2611.5ab	1474.8bcd	1248.6c	3241.9c	2759.7c	1774.9ab	2024.3abc
-Ve Control	1873.0bc	1750.93e	790.6c	1063.8b	2727.0c	2534.2ab	1221.9cd	1432.4abc	2932.3c	2739.8c	1490.0a-d	2065.6abc
+Ve Control	2263.1ab	2292.59ab	1543.0a	1238.9ab	3375.9ab	2776.4ab	1501.0a-d	1652.2abc	3498.4a	3609.4a	1868.1ab	2537.0a
LSD	408.32	325.07	473.22	517.43	537.01	452.72	500.47	529.87	582.44	502.05	484.42	734.04
CV (%)	6.51	5.40	16.36	14.24	5.98	5.85	11.75	11.43	6.00	5.33	11.18	11.51
Mean	2167.07	2078.54	1000.28	1256.83	3107.92	2677.03	1473.66	1603.96	3358.89	3257.82	1499.25	2204.82
P value	***	***	***	*	***	*	***	**	***	***	***	***
F value	8.86	22.88	8.56	2.65	8.66	2.80	9.87	4.00	5.30	11.44	11.66	6.51

, ** and*, significant at P<0.05, P<0.01and P<0.001, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. -VE-negative control (no inoculation and N application), +VE control -20 kg N ha-1; NSCBR- National soil Common Bean Rhizobium.*

Table 6. Total N accumulation of common bean investigated from three genotypes (Dursitu, Kufanzik and Gofta) over four experimental locations (Babillae, Fedis, Haramaya and Hirna), in eastern Ethiopian, in 2012/13 cropping season

Inoculation	Dursitu				Kufanzik				Gofta			
	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis
NSCBR-14	3.4633c	4.0600a-e	2.2967b	3.1533d	2.5300c	4.0300a	2.8933a	2.2733b	2.2567de	3.6667ab	2.2300d	2.7533abc
NSCBR-$(25)_2$	3.3000c	4.3667ab	3.1367abc	3.7767ab	2.3400c	3.3367bcd	2.3567b	3.0700a	2.5900bcd	3.8067ab	2.8800ab	2.9233a
NSCBR-59	3.6433bc	4.0933a-d	2.2700d	3.3067cd	2.7600bc	2.6667e	2.3600b	3.0733a	2.7400ab	3.8733a	2.5267a-d	2.7933ab
NSCBR-31	3.5267bc	3.4167f	2.5233cd	3.6433bc	2.5633c	3.5967abc	2.8733ab	3.1533a	2.7167abc	3.4300b	2.4667bc d	2.4367bc
NSCBR-16	3.9367ab	4.0333b-e	3.2400ab	3.7200ab	3.1633ab	3.3433bcd	3.1933a	3.0467a	3.0300a	3.6167ab	2.5567a-d	2.6200abc
NSCBR-18	4.1367a	3.7433def	3.3600a	3.5800bc	2.4333c	3.0567de	3.0967a	3.0767a	2.1567e	3.5967ab	2.6767ab	2.9533a
NSCBR-57	3.5800bc	4.4233a	3.0233abc	3.8300ab	3.1033ab	3.3000bcd	2.8567ab	2.7067ab	2.3267de	3.9200a	2.6667abc	2.5700abc
NSCBR-25	3.6033bc	4.1367abc	3.0233abc	3.6133bc	3.1533ab	3.2100cd	2.8333ab	3.0333a	2.5233cde	3.6533ab	2.6567abc	2.3867c
-Ve Control	3.7400abc	3.7033ef	3.0767abc	2.9133d	2.3100c	3.7500ab	3.7167ab	2.9933a	2.3400de	3.5600ab	2.8933a	2.6300abc
+Ve Control	3.3267c	3.9733cde	2.5933bcd	4.1000a	3.5767a	3.8567a	3.0300a	2.8567a	2.3467cde	3.7667ab	2.2467cd	2.9167a
LSD	0.4527	0.3881	0.6467	0.4105	0.4878	00.5133	0.5198	0.5252	0.3751	0.4025	0.4235	0.4047
CV (%)	4.32	3.36	7.84	3.98	6.04	5.20	6.37	6.20	5.18	3.77	5.68	5.19
Mean	3.6257	3.9950	2.8543	3.5637	2.7933	3.4147	2.8210	2.9283	2.5027	3.6890	2.5800	2.6983
P value	***	***	***	***	***	***	***	***	***	**	***	***
F value	8.23	15.55	9.47	18.10	19.54	15.58	7.29	6.33	12.88	3.55	7.16	6.26

*** and***, significant at $P<0.01$and $P<0.001$, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test. -VE-negative control (no inoculation and N application), +VE control -20 kg N ha-1; NSCBR- National soil Common Bean Rhizobium.*

6. Acknowledgement

This research was funded by the Ethiopia Institute of Agricultural Research, under Biofertilizer and Organic Fertilizer Research Project. The authors would like to express their sincere thanks to Field and technical assistants for their involvement in field experiment management and data collection.

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7. Effect of Fungicides and Resistant Genotypes on Severity of Potato Late Blight [*Phytophthora infestans* (Mont.) de Bary], Yield and Yield Components at Haramaya, Eastern Ethiopia

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Abstract: Potato is a high potential food security crop due to its high yield potential per hectare and nutritious tubers. Late blight of potato is a devastating disease with dramatic and disastrous economic consequences. A field experiment was conducted at Haramaya University in 2014 main cropping season to evaluate three potato genotypes and alternate spray sequences of different fungicides for late blight management and their effects on tuber yield and yield components and to identify an economical fungicide spray sequence(s). The potato genotypes included Bubu (highly resistant), Maracharre (moderately resistant) and the local potato cultivar namely Jarso (highly susceptible). The three fungicides included Mancozeb (Ethiozeb 80 WP) represented as M, Metalaxyl 8% + Mancozeb 64% WP (Matco) represented as MM, and Curzate RWP (Curzate) represented as C. The treatments that included the three potato genotypes and seven alternate and sole fungicide spray sequences, including the control, were laid out in a factorial arrangement in a randomized complete block design (RCBD) with three replications. Late blight severity, yield and yield component parameters, area under disease progress curves (AUDPC) and disease progress rates were measured and analyzed. Late blight severity, AUDPC and disease progress rates were reduced more in the highly resistant variety Bubu than in the other two potato genotypes. The sequential application of the MM-C-M proved to be the most effective fungicide schedule for late blight management on each potato genotype and Bubu, Maracharre and Jarso gave the highest yields of 43.8, 35.7 and 17.4 t ha^{-1}, respectively. Future research should be undertaken to evaluate alternate spray of more fungicides as an one option to protect the crop against late blight and to reduce the chances of the pathogen races in building resistance to the fungicides' active ingredients.

Keywords: AUDPC; Disease progress rate; Disease severity;Fungicides and Late blight.

1. Introduction

Potato is a global crop planted in a wider range of altitude, latitude, and climatic conditions. Nutrition analysis showed that potato is a healthy food in its contents of vitamins, minerals, proteins, antioxidants, essential amino acids and carbohydrates (Andre *et al.*, 2007). In Ethiopia, potato is a high potential food security crop due to its high yield potential per hectare and nutritious tubers. Potato production in Ethiopia is possible on about 70% of the arable land (Yilma, 1991; Medhin *et al.*, 2000; FAO, 2008). According to CSA (2013/14) estimate, potato production in Ethiopia has decreased from 863347.7 tons in 2012/2013 to around 784993.4 tons in 2013/2014. Several biotic stresses are expected to be the cause of yield reduction of which late blight, caused by *Phytophthora infestans*, constitutes a major threat. In the same year report(CSA 2013/14),the average yield of potato was about 19.3 t ha^{-1} in East Hararghe. Though the average yield in the region was higher than the national average the yield of the crop was not attained the maximum that could be attainable due to late blight as a major constraint. Potato late blight may be the best known, longest studied and still the most destructive agent of all plant diseases. The pathogen develops most rapidly at low temperatures and high relative humidity (Fry and Mizubuti, 1998). In Ethiopia, the disease caused 100% yield loss on unimproved potato local variety (Bekele and Yaynu, 1996).

Breeding for resistance to late blight has been an ongoing worldwide research focus in potato for several decades. Management of the disease mostly depends on repeated application of fungicides that could cause a slow erosion of disease control due to a gradual loss of sensitivity of the targeted pathogen population to the fungicide in addition to the increase in production costs and environmental risk. It is recognized that rotation, mixture and alternate application of fungicides can delay the development of resistance by *Phytophthora infestans* to the fungicide like ridomil. Development of effective management strategies for late blight resistance of potato usually requires tremendous genetic resources and efforts when traditional breeding approaches are considered. Since the pathogen is windborne, residue management and crop rotation alone may not be very effective as that of the use of potato resistant varieties and chemical protection.

Although *P. infestans* is the best known and long studied pathogen, the existing information gap on effective management of the disease in East Hararghe Zone called for research work. Therefore, this study was conducted to evaluate the integration of potato genotypes with alternate application of fungicides as management option against late blight in East Hararghe and elsewhere.

2. Materials and Methods

The study was conducted during 2014 cropping season and was dealt with three alternately sprayed fungicides on three potato genotypes for the management of late blight in the field under natural infection.

2.1. Description of the Experimental Site

The experiment was conducted at Haramaya University research field station (Raare) under rainfed condition during the 2014 main cropping season. Haramaya University is located 25 km northwest of Harar town located 525 km east of Addis Ababa. *Raare* research site is located at 9 ^{o}26' N latitude, 42 ^{o}3' E longitudes at an altitude of 1980 m.a.s.l. The area receives average maximum and minimum temperatures of 23.4 and 8.25 ^{o}C, respectively. The mean annual rainfall is 645 mm. The mean relative humidity is 61.7%, varying from 54 to 75% of the year 2014 (National Meteorological Agency, Jigjiga, 2014).

2.2. Experimental Materials and Arrangement of Treatments

Three potato genotypes, namely Bubu, Mara Charre and Jarso were used. Bubu, Mara Charre and Jarso were identified as resistant, moderately resistant and highly susceptible genotypes, respectively (Wassu, 2014). The description of the genotypes is presented in Table 1. Three fungicides, namely Mancozeb (Ethiozeb 80 WP), Metalaxyl 8% + Mancozeb 64% WP (Matco), and Curzate RWP (Curzate) at rates of 2 kg ha^{-1}, 2.5 kg ha^{-1} and 200 g ha^{-1},respectively, were used. These fungicides were previously screened and recommended for late blight management. A total of 21 treatments (three levels of genotypes x six sequences of three fungicides along with the untreated control as check) were applied which were arranged in factorial combinations (Table 2).

Table 3. Potato genotypes used in the experiment at Haramaya during 2014 main cropping season.

Variable	Bubu	Mara Charre	Jarso
CIP accession code	CIP-384321-3	CIP-389701-3	Farmers' cultivar
Year of release	2011	2005	Farmers' cultivar
Total tuber yield (t ha-1) at research field	39-42	33.3	NA
Total tuber yield (t ha-1) at farmers' field	35-39	28.4	NA
Recommended altitude (m.a.s.l.)	1700-2000	1700-2700	NA
Late blight resistance	Resistant	Moderately resistant	Susceptible
Breeder/maintainer ceneter	Haramaya University	Hawassa Agriculture Research Ceneter	Farmers' cultivar

Source: MoA, 2013 and 2012, Wassu (2014).

Table 2. Three potato genotypes and seven fungicides treatment combinations used to control late blight at Haramaya during 2014 main cropping season.

Genotype (Factor A)	Fungicides combinations & sequences of application (Factor B)	Treatment designation
Bubu	MCMM	Bubu +MCMM
Bubu	CMMM	Bubu + CMMM
Bubu	MMCM	Bubu + MMCM
Bubu	MMM	Bubu + MMM
Bubu	MMMMMM	Bubu + MMMMMM
Bubu	CCC	Bubu + CCC
Bubu	Control	Bubu + Control
Mara Charre	MCMM	Maracharre +MCMM
Mara Charre	CMMM	Maracharre + CMMM
Mara Charre	MMCM	Maracharre + MCMM
Mara Charre	MMM	Maracharre + MMM
Mara Charre	MMMMMM	Maracharre+ MMMMMM
Mara Charre	CCC	Maracharre + CCC
Mara Charre	Control	Maracharre + Control
Jarso	MCMM	3. Jarso + MCMM
Jarso	CMMM	Jarso + CMMM
Jarso	MMCM	Jarso + MCMM
Jarso	MMM	Jarso + MMM
Jarso	MMMMMM	Jarso + MMMMMM
Jarso	CCC	Jarso + CCC
Jarso	Control	Jarso + Control

MMM = Mancozeb + Curzate RWP + (Metalaxyl 8% + Mancozeb 64%WP), CMMM = Curzate RWP + Mancozeb + (Metalaxyl 8% + Mancozeb 64%WP), MMCM = (Metalaxyl 8% + Mancozeb 64 WP) + Curzate RWP + Mancozeb, MMM = Mancozeb + Mancozeb + Mancozeb, MMMMMM = (Metalaxyl 8% + Mancozeb 64 WP) + (Metalaxyl 8% + Mancozeb 64 WP) + (Metalaxyl 8% + Mancozeb 64 WP), CCC =) Curzate RWP + Curzate RWP + Curzate RWP and Control = no fungicide application. Curzate RWP. The "+" sign out of the parenthesis indicated the sequences of fungicide application and "+" in the parenthesis showed the combination of fungicides. For instances, MMM = Mancozeb + Curzate RWP + (Metalaxyl 8% + Mancozeb 64%WP) represented the fungicide Mancozeb 64%WP was sprayed first followed by Curzate RWP and the combination of Metalaxyl 8% + Mancozeb 64%WP was applied as the third time application.

2.3. Experimental Design and Procedures

The experiment was laid out as a Randomized Complete Block Design (RCBD) where each genotype was replicated three times. The medium sized and well sprouted tubers of the three potato genotypes were planted on 21 July 2014 at the start of the rainy season in a field. The tubers were placed 5 to 10 cm depth on well prepared ridges of four rows per plot at spacing of 75 cm between rows and 30 cm between plants. The gross plot size was 4.50 m x 3.60 m = 16.2 m2 and net plot size was 3.0 m x 3.6 m = 10.8 m2 wide. Each row accommodated 12 plants per row and thus 48 plants per plot. The spacing between plots and adjacent replications was 1 and 2 m, respectively. The infected tubers of known susceptible cultivar (Bate genotype) were planted around the borders of each plot as spreader rows to ensure uniform infection across all plot area.

Fertilizer was applied as the recommendation made by Haramaya University. According to HU recommendation, 75 kg N and 92 kg P2O5 ha-1was applied. The source of N and P was DAP (18% N, 46% P2O5), which was applied at the depth of 10 cm below the seed tuber at planting. Nitrogen was applied as urea (46 % N) 7-10 cm away from the plant as two side dressings for subsequent split applications (50% + 50% in two installment of 30 and 50 day after planting).This means half rate of nitrogen fertilizer was applied after full emergence and the remaining half of the nitrogen rate was applied at the initiation of tubers or start of flowering. All other agronomic management practices were applied as to the recommendation of Haramaya University for the production of the crop.

Fungicides application was started at 47 days after planting (DAP) and continued at two weeks interval with each fungicide as sole or combination of fungicides alternating with one another in each plot for each genotype. Three sprays in sequences of fungicides treatment arrangement indicated in Table 2 were applied. The check plots were sprayed with pure water only. Each plot was sheltered by plastic sheet during fungicide application to avoid spray drift to other plots.

The haulm was mowed two weeks before harvesting to thicken tuber periderm; as yellowing or senescence observed apparent on the lower leaves. For yield estimation, tubers were harvested from plants of the two middle rows, leaving the plants growing in the two border rows as well as those growing at both ends of each row to avoid edge effects.

2.4. Data Collection

Disease assessment: Disease incidence and severity were recorded from 10 pre-tagged plants in the central two rows of each treatment. Disease incidence was recorded two times and disease severity was recorded six times at seven days interval using the 1-9 disease scoring scale (Henfing, 1987). Severity scores were then converted into percentage severity index (PSI) for analysis (Wheeler, 1969).

$$\text{PSI (percentage severity index)} = \frac{\text{Summation of numerical rating}}{\text{No.plants examined} \times \text{Maximum disease score}} \times 100$$

Area under disease progress curve (AUDPC) and disease progress rate were calculated from the percent severity index.

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_i + 1}{2} \right) (t_{i+1} - t_i)$$

Where X_i is the cumulative disease severity expressed as a proportion at the i^{th} observation, t_i is the time (days after planting) at the i^{th} observation and n is the total number of observations. Since late blight severity was expressed in percent and time (t) in days, AUDPC values were expressed in percent-days (Campbell and Madden, 1990).

Disease progress rate: One useful approach for quantifying the variation in disease progress between plots was the application of growth curve models. Following the procedures used by Campbell and Madden (1990) to fit growth curve models to epidemics of potato, late blight selected disease progress data from each plot was fitted to two different models. The rate of foliar disease development was quantified by repeated assessments of the percentage of leaf and stem area affected by late blight in each plot beginning in mid- August during the disease onset. From a thorough evaluation of the models logistic and Gompertz, the conclusion was reached that the logistic model described the progress of foliar blight in most of the replicates better than Gompertz model. Two factors were considered in determining the adequacy of a model in describing disease development through time, the R^2 value, which is an indication of the degree of association between disease severity and date, was higher for the logistic than the Gompertz model and residuals (SE) was lower for the logistic than the Gompertz . Thus the epidemic moved rapidly through the logistic phase.

The logistic equation describes a S- shaped growth curve where dYt/dt (the absolute rate of disease increase) is proportional to the amount of disease at any given time (Yt) multiplied by a logistic rate constant (r_L) and correction factor dependent on the proportion of plants already infected (1-Yt).

$$Yt=1/ [1+\exp (-\{\ln[Yo/(1-Yo)]+r_L t\})]$$

The logistic curves for each replicate have been charted based on values of r_L and Yo generated by the regression analysis.

Total tuber yield (ton/ha): This was determined as the sum of the weights of marketable and unmarketable tubers from the net plot area and was converted to tons per hectare.

Yield loss was computed using the formula (Robert and James, 1991)

$$RYL= \frac{(YP-YT)}{YP} X100$$

Where RYL= Relative percent loss, YP= Yield from the maximum protected plot (in this study MMCM) sprayed plots and YT= Yield from other plots of treatments.

Marketable tuber yield (ton/ha): The total tubers weight which was free from diseases, insect pests, and greater than or equal to 20 g in weight were determined from the net plot area and was converted to tons per hectare.

Cost-Benefit-Analysis: Prices of potato tubers (Birr/ton) from local market and total sale from one hectare was computed. Price of tubers was collected from local market and farmers union in the localities. Price of mancozeb was 150 Birr/kg (300 birr/ha), Metalaxyl 8% + Mancozeb 64% WP was 320 Birr/kg (800 birr/ha) and Curzate RWP was 460 Birr/Kg (92 birr/ha) and total price incurred to spray one hectare of potato fields at all times was calculated. Labor cost for land preparation, different agronomic practices and to spray chemicals was computed. Costs of labor were 40 Birr/ man/day. Cost of spray and spray equipment to spray three times per hectare was calculated. Cost benefit analysis was performed based on the data obtained from the recorded field using partial budget analysis (CIMMYT, 1988). Accordingly, the following formula was used.

MRR =DNI/DIC

Where, MRR is marginal rate of returns, DNI, difference in net income compared with control, and DIC, difference in input cost compared with control.

Costs for all agronomic practices were uniform for all genotypes and treatments in each location. Price of tubers per kg for each genotype was the same within each locality at time of harvesting.Cost of labor and spraying equipment were taken based on the prevailing rates of payment in the locality,and costs, return and benefit, were calculated on hectare basis.

2.5. Data Analysis

All data were subjected to ANOVA to determine the treatment effect. All the disease reaction for each treatment was evaluated by averaging the data from the individual plants. Least significant difference (LSD at 5% probability level) was used for mean comparison where ever the mean square of treatments was obsereved. All the data analysis was done using the Statistical Analysis System (SAS) Version 9 (SAS Institute, 2002). The percentage data on disease incidence was transformed according to the data recorded using appropriate data transformation before statistical analysis was conducted. Relationship of final PSI and AUDPC with yield and yield components was examined using correlation analysis.

3. Results and Discussion

3.1. Disease Severity

The analysis of variance (ANOVA) showed that late blight disease severity was significantly ($p \leq 0.01$) affected by the main effects and interaction of genotypes and fungicides application schedules in all the disease severity assessment except at 45 and 66 days after planting (DAP). Disease severity was highly reduced on the genotypes Bubu, Mara Charre and Jarso when sprayed with MM-C-M sequence compared to other treatments (Figure 1).

The mean severity of plots treated with different fungicide spray schedules ranged from the least 17.4% for the genotype Bubu sprayed with MM-C-M sequence plots to the highest 97% for highly susceptible genotype Jarso (Figure 1). This result coincides with the findings of Olanya *et al.* (2001) who reported that the late-blight severity was very low, especially in fungicide-treated plots. In the control (untreated) plots, the disease was highest in the susceptible Jarso potato variety.

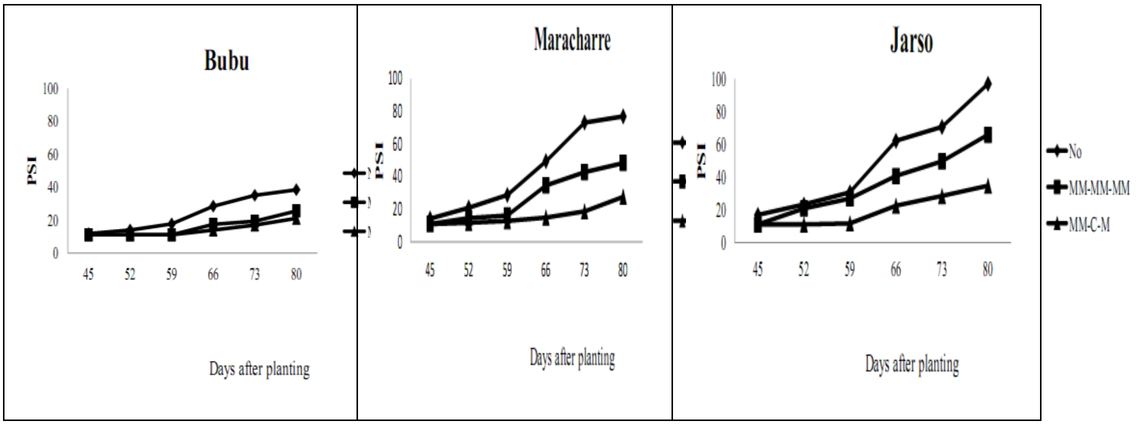

Figure 1. Potato late blight progress curve as affected by different fungicide spray sequences on three potato genotypes at Haramaya, during 2014 main cropping season.

3.2. Area under Disease Progress Curve (AUDPC)

The AUDPC values exhibited highly significant (p≤0.01) differences among the genotypes and fungicides or application schedules. On unsprayed plots, the Jarso genotype reached the highest (1708.4%-days) AUDPC, which varied significantly from the other two genotypes Mara Charre (1530.9%-days) and Bubu (845.1%-days). MM-C-M sequence sprayed plots significantly reduced the AUDPC values by 486.2, 548.2 and 679.0% on the genotypes Bubu, Mara Charre and Jarso compared to unsprayed plots, respectively (Table 2). Binyam *et al.* (2014) reported that the highest AUDPC value corresponded with the highest disease development on plots were not treated with any combinations of these varieties and rates of fungicide applications.

3.3. Disease Progress Rate

Disease progress rate was significantly (p≤0.01) affected by the interaction of genotypes and fungicides application. Disease progress rate (r) was highly significant for both main effects genotypes and fungicides application (Table 2).

The genotypes Bubu, Mara Charre and Jarso showed disease progress rate (r) of 0.048, 0.094 and 0.130 units per day, respectively, on unsprayed plots, while on plots sprayed with MM-C-M sequence the disease progress was 0.022, 0.030 and 0.046 units per day on the genotypes Bubu, Mara Charre and Jarso, respectively. Hence the rate of late blight progress was faster on unsprayed plots than on the sprayed plots, regardless of potato genotypes (Table 2). Bekele and Hailu (2001) reported that frequent application of fungicide could retard the rate of late blight progress in the field.

Table 2. Interaction effects of different fungicides application and potato genotypes on area under disease progress curve (AUDPC) and disease progress rate (r) at Haramaya, during the 2014 main cropping season.

Genotype	Fungicide	AUDPC	r
Bubu	No-fungicide	845.1i	0.048hi
	M-M-M	665.9j	0.042ij
	C-C-C	611.1jk	0.038ijk
	MM-MM-MM	541.6kl	0.030kl
	M-C-MM	520.2kl	0.027kl
	C-M-MM	503.8kl	0.024l
	MM-C-M	486.2l	0.022l
Mara Charre	No-fungicide	1530.9b	0.094b
	M-M-M	1224.8ed	0.076cde
	C-C-C	1063.6fg	0.063fg
	MM-MM-MM	971.2gh	0.063fg
	M-C-MM	900.5hi	0.057gh
	C-M-MM	682.6j	0.042i
	MM-C-M	548.2kl	0.030kl
Jarso	No-fungicide	1708.4a	0.130a
	M-M-M	1382.5c	0.086bc
	C-C-C	1307.8cd	0.079cd
	MM-MM-MM	1237.2ed	0.074def
	M-C-MM	1160.1ef	0.068defg
	C-M-MM	964.4gh	0.064efg
	MM-C-M	679.0j	0.046hi
LSD (0.05)		116.7	0.0116
CV (%)		7.6	12.03

Means within the same column followed by the same letter(s) are not significantly different, LSD (0.05) = Least significant difference at p≤0.05, CV (%) = coefficient of variation in percent, M = Mancozeb, MM = Metalaxyl 8% + Mancozeb 64% WP, C = Curzate RWP applied with the indicated sequence in the three successive sprays.

3.4. Marketable Tuber Yield and Relative Tuber Yield Loss

The main effects as well as the interaction of genotypes and fungicides highly (p≤0.01) influenced marketable tuber yields. The highest (36.7tons ha^{-1}) marketable tuber yield was recorded for Bubu, closely followed by the mean marketable tuber yield (28.5 tons ha^{-1}) of Mara Charre. The lowest (12.5 tons ha^{-1}) marketable tuber yield was recorded for the genotypeJarso from plots sprayed with MM-C-M sequence(Table 3).The highest levels of yield loss of 16.6, 43.4 and 51.0% occurred on the untreated plots of the genotypes Bubu, Mara Charre and Jarso, respectively, as compared to the best protected plots sprayed with MM-C-M sequence.

Table 3. Interaction effect of different fungicides application and potato genotypes yield and yield loss at Haramaya, during 2014 main cropping season.

Genotype	Fungicide	MTY	UTY	TY	RYL
Bubu	No-fungicide	31.7c	4.7	36.5defg	16.6de
	M-M-M	33.5bc	4.9	38.4cdef	12.2efg
	C-C-C	33.8bc	5.2	39.1bcde	10.6fg
	MM-MM-MM	34.1bc	5.4	39.6bcd	9.6fghi
	M-C-MM	34.9ab	6.1	41.0abc	6.3hijk
	C-M-MM	35.6ab	6.8	42.5ab	3.0jkl
	MM-C-M	36.7a	7.1	43.8a	0l
Mara Charre	No-fungicide	15.0f	5.1	20.1j	43.4b
	M-M-M	25.5e	5.5	31.0i	13.0def
	C-C-C	26.3ed	5.8	32.2hi	9.7fghi
	MM-MM-MM	26.6ed	6.2	32.9hi	7.8fghij
	M-C-MM	27.6ed	6.4	34.1gh	4.4ijkl
	C-M-MM	28.1d	6.8	35.0fgh	1.9kl
	MM-C-M	28.5d	7.2	35.7efgh	0l
Jarso	No-fungicide	5.2i	3.5	8.8m	51.0a
	M-M-M	10.0h	3.8	13.8l	22.8c
	C-C-C	10.5gh	4.0	14.6kl	18.1cd
	MM-MM-MM	11.2gh	4.2	15.5kl	12.9def
	M-C-MM	12.0gh	4.6	16.6jkl	6.8ghijk
	C-M-MM	12.3gh	5.1	17.4jk	2.5jkl
	MM-C-M	12.5fg	5.3	17.4jk	0l
LSD (0.05)		2.5	1.9	3.5	5.4
CV (%)		6.4	21.5	7.4	13.6

Means within the same column followed by the same letter(s) are not significantly different, LSD (0.05) = Least significant difference at p≤0.05, CV (%) = coefficient of variation in percent, M= Mancozeb, MM = Metalaxyl 8% + Mancozeb 64% WP, C = Curzate RWP applied with the indicated sequence in the three successive sprays, MTY = Marketable tuber yield, UTY = unmarketable tuber yield, TY = tuber yield, RYL = relative yield loss.

3.5. Association of Yield and Disease Parameters

Many associations were observed among severity, AUDPC and yield related characters. AUDPC and percentage severity index final were positivelyand highly significantly (p≤0.01) correlated (r = 0.99**). Yield and final severity were negatively, highly and significantly (p≤0.01) correlated (r = -0.831**) at final severity score (Table 4). This is in agreement with the findings of Biniam *et al.* (2014) who reported that the epidemiological parameters PSI at 66 DAP and AUDPC were highly correlated.

Table 4: Coefficients of correlation (r) between yield and AUDPC and PCI of final score on potato genotypes at Haramaya, during 2014 main cropping season

	AUDPC	PSI final	MTY	UTY
AUDPC				
PSI final (%)	0.990**			
MTY (t ha^{-1})	-0.795**	-0.825**		
UTY (t ha^{-1})	-0.381*	-0.400**	0.373**	
TY (t ha^{-1})	-0.799**	-0.831**	0.986**	0.522**

** and **, significant at P<0.05 and P<0.01, respectively. AUDPC = Area under disease progress curve, PSI final (%) = Percentage severity index, MTY (t ha^{-1}) = Marketable tuber yield ton per hectare, UTY (t ha^{-1}) = Unmarketable tuber yield ton per hectare and TY (t ha^{-1}) = Total tuber yield ton per hectare.*

3.6. Cost-Benefit Analysis

Partial budget analysis indicated that the MM-MM-MM sequence sprayed fungicides had the highest total cost but the unsprayed plots had the lowest cost (Table 4).Variation in net benefit was recorded among the three genotypes. The genotype Bubu had the highest net profit of ETB 163,338 ha^{-1} with marginal rate of return (MRR) 1141.72% from plots sprayed with MM-C-M, followed by plots treated with C-M-MM and M-C-MM, respectively. Increased variation was observed on the genotype Mara Charre, when treated with MM-C-M, followed by plots treated with C-M-MM spray sequence and M-C-MM spray sequence with a net yield benefit of ETB 126,438, ETB 124,638 and ETB 122,388 ha^{-1}, respectively, as compared to plots treated three times with Metalaxyl 8% + Mancozeb 64% WP, Curzate RWP and Mancozeb ETB 116,680, ETB 117,454 and ETB 113,230 ha^{-1}, respectively. But it still had higher gross yield benefit than the control (ETB 67,500 ha^{-1}) (Table 5).

Forbes (n.d.) showed that growers in the developing countries are managing the disease almost solely based on fungicide applications, but in many situations irrational use of chemicals results in serious economic, health and environmental problems. Epidemiological research should be fostered in the developing countries to optimize fungicide usage without compromising profit.

Table 5. Partial budget analysis for fungicide used for controlling late blight of potato at Haramaya during 2014 main cropping season.

Cost benefit data			Fungicides				
Bubu	C(3)	C=M-MM	M-C-MM	MM-C-M	M(3)	MM(3)	control
Adj.yield (t ha^{-1}) (yield*90)	30.42	32.04	31.41	33.03	30.15	30.69	28.53
Price (ETB ton^{-1})	5000	5000	5000	5000	5000	5000	5000
Sale revenue (1*2)	152100	160200	157050	165150	150750	153450	142650
Total input cost (ETB ha^{-1})	896	1812	1812	1812	1520	3020	0
Marginal cost (ETB ha^{-1})	896	1812	1812	1812	1520	3020	0
Net Profit (3-4)	151204	158388	155238	163338	149230	150430	142650
Marginal benefit (ETB ha^{-1})	8554	15738	12588	20688	6580	7780	0
Marginal rate of return (7/5) (%)	954.69	868.54	694.70	1141.72	432.89	257.62	0
Maracharre	C(3)	C-M-MM	M-C-MM	MM-C-M	M(3)	MM(3)	control
Adj.yield (t ha^{-1}) (yield*90)	23.67	25.29	24.84	25.65	22.95	23.94	13.5
Price (ETB ton^{-1})	5000	5000	5000	5000	5000	5000	5000
Sale revenue (1*2)	118350	126450	124200	128250	114750	119700	67500
Total input cost (ETB ha^{-1})	896	1812	1812	1812	1520	3020	0
Marginal cost (ETB ha^{-1})	896	1812	1812	1812	1520	3020	0
Net Profit (3-4)	117454	124638	122388	126438	113230	116680	67500
Marginal benefit (ETB ha^{-1})	49954	57138	54888	58938	45730	49180	0
Marginal rate of return (7/5) (%)	5575.22	3153.31	3029.14	3252.65	3008.55	1628.48	0
Jarso	C(3)	CMMM	MCMM	MMCM	M(3)	MM(3)	control
Adj.yield (t ha^{-1}) (yield*90)	9.45	11.07	10.8	11.25	9	10.08	4.68
Price (ETB ton^{-1})	5000	5000	5000	5000	5000	5000	5000
Sale revenue (1*2)	47250	55350	54000	56250	45000	50400	23400
Total input cost (ETB ha^{-1})	896	1812	1812	1812	1520	3020	0
Marginal cost (ETB ha^{-1})	896	1812	1812	1812	1520	3020	0
Net Profit (3-4)	46354	53538	52188	54438	43480	47380	23400
Marginal benefit (ETB ha^{-1})	22954	30138	28788	31038	20080	23980	0
Marginal rate of return (7/5) (%)	2561.83	1663.25	1588.74	1712.91	1321.05	794.04	0

M (3) = Mancozeb, MM (3) = Metalaxyl 8% + Mancozeb 64% WP, C (3) = Curzate applied with the indicated sequence in the three successive sprays, Adj. yield = Adjusted yield.

4. Summary and Conclusions

Late blight is an important disease that calls for due attention to achieve economical management with fungicides and highly resistant potato genotypes. The genotype Bubu appears to have an outstanding resistance to potato late blight and is a promising genotype against the late blight. It would be possible to recommend this genotype to be used without the need for any fungicide application as it is profitable even if not sprayed. Use of the genotype Maracharre by spraying the alternate fungicide spray sequence MM-C-M would be profitable for the farmers as high net profit (ETB 126,438 ha^{-1}) was obtained by spraying the fungicide schedule. However, ETB 67,500 ha^{-1} net benefit was obtained from unsprayed plots. Generally, the use of fungicides alternatively or timely scheduled application of fungicides, instead of using several fungicides arbitrarily, can substantially suppress potato late blight, thereby minimizing the cost of production, giving maximum net benefit and avoiding the risk of fungicide resistance development. In conclusion, the use of fungicides with MM-C-M spray schedule is recommended as it gave the best protection against late blight and the best monetary benefit as compared to the other treatments and the control. Future research should be undertaken to evaluate alternate spray of fungicides as an immediate protection against late blight. Additionally, reduction of the chances of the pathogen races in building resistance to the fungicides active ingredients.

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8. Effect of Seed Tuber Planting Depth and Nitrogen Rate on Yield and Yield Related Traits of Potato (*Solanum tuberosum* L.) at Haramaya and Hirna, Eastern Ethiopia

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Abstract: Potato (*Solanum tuberosum* L.) is an important cash and food security crop in the eastern highlands of Ethiopia. However, the yield of the crop is constrained by low soil fertility and poor agronomic practices. Therefore, field experiments were conducted during the 2015 main cropping season at the crop research fields located at the main campus of Haramaya University and at Hirna Research sub-station. The objective of the experiments was to elucidate the effect of seed tuber planting depth and nitrogen fertilizer rate on the yield and yield related traits of the crop. The treatments consisted of four seed tuber planting depths (5, 10, 15 and 20 cm) and five rates of nitrogen (0, 46, 92, 138 and 184 kg N kg ha-1). The experiment at each location was laid out as a Randomized Complete Block Design (RCBD) with three replications. The results of the experiment done at both locations revealed that the main effect of nitrogen application rate influenced total dry weight, unmarketable tuber yield, percentage of small-sized tubers and percentage of medium-sized tubers. Increasing the rate of nitrogen enhanced the total dry weight and percentage of medium-sized tubers while reducing percentage of small-sized tubers. Nitrogen rate and seed tuber planting depth also interacted to influence unmarketable tuber yield, percentage of medium-and large-sized tubers produced, and tuber starch content. The main effects of seed tuber planting depth significantly influenced shoot fresh weight, total dry weight, unmarketable tuber yield, total tuber yield, marketable tuber yield, percentage of small-sized tubers produced and tuber starch content. Increasing seed tuber planting depth generally enhanced the aforementioned parameters. However, for most parameters, the highest values were obtained already at the seed tuber planting depth of 10 cm. In conclusion, the optimum total and marketable tuber yields of 26.93 t ha-1and 26 t ha-1for Haramaya and 34.57 t ha-1and 32.65 t ha-1for Hirna were obtained in response to planting seed tubers at the depth of 10 cm.

Keywords: Marketable tuber yield; total tuber yield; tuber dry matter; tuber quality parameters; tuber size distribution.

1. Introduction

Potato (S*olanum tuberosum* L.) is the third most important food crop after rice and wheat for human consumption and over a million people on earth eat potatoes (CIP, 2014). Potato is also, the most important tuber crop, ranking first in volume produced among root and tuber crops, with an annual production of approximately 320 million tones grown on about 20 million hectares; it is followed by cassava, sweet potato, and yam (FAO, 2008). Potato is grown in more than 150 countries worldwide from latitudes 65^0 N to 50^0 S and from sea level to 4000 meters above sea level (Acquaah, 2007). In 2007 the potato production reached a record of 325 million metric tons becoming the first non-grain commodity for the humanity (FAO, 2009). However demand for both food and energy is rising and it is expected to keep the same trend with increases in global population and average income (Lobell *et al.*, 2009). Asia and Europe are the world's major potato producing regions, accounting for more than 80% of world production, while Africa produces the least, accounting for about 5% (FAO, 2008). North America is the clear leader in productivity at more than 40 t ha^{-1}, followed by Europe at 17.4 t ha^{-1}, while Africa lags at about 10 t ha^{-1} (FAO, 2008). The average yield of potato in Ethiopia ranges only between 8 to 10 t ha^{-1}, which is much lower than the yields obtained even in Sudan (17 t ha^{-1}) and Egypt (26 t ha^{-1}) (Haverkort *et al.*, 2012). The major contributing factors to the low yields of potato have been the use of poorly adapted varieties, high prevalence of diseases and pests, poor soils, unfavorable weather conditions, un-scientific cultural practices including tuber planting depth (Snapp and Kravchenko, 2010).

Soil conditions for potato growth are determined by soil structure, moisture and temperature. By adjusting the tuber planting depth, potato cultivation can be adapted to existing conditions of moisture and temperature. The effect of planting depth on potato was studied in the Columbia Basin of Washington State for three years and on two potato varieties. The results indicated that seed tuber planting depth of 10, 15, 20 and 25 cm did not affect tuber yield despite differences in the emergence rate due to planting depth. However, marketable yield and gross income typically declined when seed pieces were planted at a shallow depth of 10 cm (Pavek and Thornton, 2009).

In a sandy loam soil in the Tunisia, two potato varieties planted at the seed tuber depth of 15 cm outperformed those planted at a 10 cm depth (Sayed *et al.*, 2013). A field experiment done in Iran on clay loamy soil indicated higher overall performance at 10 cm planting depth than 15 and 25 cm depths (Laei *et al.*, 2012). This research result also revealed a decreasing trend in many of yield and yield related traits in response to increasing seed tuber planting depth above 10 cm. Similarly, Gholipour (1996) reported that number of tubers $plant^{-1}$ and unit $area^{-1}$ decreased as planting depth increased and the studies also revealed that the reduction of stem number was the cause for the reduction of tubers $plant^{-1}$ and unit $area^{-1}$.Several factors can affect the depth of planting potatoes which include seed quality, soil moisture and temperature (Van der Zaag, 1982).

In Ethiopia, some farmers use inorganic fertilizers for increasing potato yields. However, they use only nitrogen in the form of Urea and phosphorus (as DAP) since these are the only fertilizers commercially available in the local market. Application of these fertilizers to potato crop is also based on blanket recommendations made based on soils of certain sites in the country decades ago. The recommendation rates are 165 kg Ureaha^{-1} (111 kg Nha^{-1}) and 195 kg DAP ha^{-1} (40 kg P ha^{-1}). These recommendations wholly disregard the physio-chemical characteristics of the soils as well as the dynamic nature of soil nutrient status in specific growing area.

Potato is naturally a heavy feeder crop. Economically feasible fertilizer rate varies with soil type, fertility status, moisture level, other climatic variables, variety, crop rotation and crop management practices (Smith, 1977). Research results indicated that 108 69^{-1} and 81 69^{-1} kg ha^{-1} N $P_2O_5$$^{-1}$were economically feasible and optimum rate for potato production in south Gondar and Gojam areas, respectively (Tesfaye *et al.*, 2008). Application of 165 90^{-1}kg ha^{-1} N $P_2O_5$$^{-1}$ was found to be a feasible rate for potato production in the central Shewa, and this recommendation is still in use as blanket recommendation throughout the country (Berga *et al.* 1994). In the same way, 146 138^{-1}kg ha^{-1} N $P_2O_5$$^{-1}$ was recommended as economic and agronomic rate of fertilizer for the highlands of Hararghe (Teressa, 1995). However these recommendations may not be optimum rate for the current market, soil fertility status, and other climatic variables. Therefore, details soil test-based fertility studies should be carried out to provide appropriate local recommendations.

Nitrogen is one of the essential elements for plant growth and is one of main components of proteins. When plants were fertilized with overdose of nitrogen, protein production decrease and nitrogen was found be stored as non-protein form (Molerhagen, 1993). Nitrate is one of the non-protein forms which are poisonous for human or stock. On the other hand, different cultivars have different rates of nitrogen for maximum tuber yields (Molerhagen, 1993).

On the supply side, experts consider that maximum possible yields for major cereals achieved in farmers' fields might level off or even decline in many regions over the few decades to come (Lobell *et al.*, 2009). That means potatoes still have a high potential to solve the food shortage especially in countries where farmers' yields are still low from the potential ones – existence of huge yield gaps - since it's known that food supply is a mathematical product of crop area by yield.

In Ethiopia there is almost no recommended seed tuber planting depth in general. Farmers have been using haphazardly their own seed tuber planting depth which is not tested and proved through research. In Eastern Hararghe Zone, potato is one of the cash crops which play a significant role in improving farmers' income and food sustainability. Farmers in this region plant tubers at the average depth of 7.5 cm (personal communication). Apart from soil conditions, improper tuber planting depth has adverse effect on the performance and total yield of potato (Bohl and Love, 2005). In different countries, many scientists have indicated the significant effect of seed tuber planting depth on the yield and yield components of potato. Tuber weight depends primarily on the operating time of the leaf canopy (Snapp and Kravchenko, 2010), but also the conditions of operation and conditions of root growth. (Abdulla *et al.*, 1993; Bohl and Love, 2005) reported that potato yield increase when planting depth increases, with a small tuber greening effect. Seed tuber planting depth plays an essential role in formation of stolons and aerial potato stems so that stolon numbers and ultimately the yield itself will decrease with higher planting tuber depths (up to approximately 20 cm depth, depending on crop conditions) (Ezekiel *et al.,* 1992). However, few researches have been done on this topic in Ethiopia. These problems related to the use of appropriate rate of nitrogen fertilizer as well as seed tuber planting depth need a solution. This study was, therefore, initiated with the objective of assessing the effect of seed tuber planting depth and nitrogen fertilizer rate on the yield and yield related traits of potato.

2. Materials and Methods

2.1. Description of the Study Area

The field experiment was conducted during 2015 main rain season, at two locations, Haramaya University Rare Research farm and Hirna Research farm. Haramaya University is located 25 km northwest of Harar town. Haramaya University Rare Research farm is located at 2020 meters above sea level, 9°41"Nlatitude and42°03"Elongitude.The area receives an annual rainfall of760mm with bimodal rainfall pattern and average maximum and minimum temperatures of 23.4^{0}C and 8.25^{0}C,respectively.The area 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 soil of the experimental site is a well-drained deep alluvial with a sub-soil stratified with loam and sandy loam (Tamire, 1973). The chemical properties of the soil indicated that the soil has organic carbon content of 1.15%, total nitrogen content of 0.11%, available phosphorus content of 18.2 mg kg soil^{-1}, exchangeable potassium content of 0.65 c mol kg soil^{-1} (255 mg exchangeable K kg soil^{-1}), pH of 8.0. The physical properties of the soil indicated sand, silt, and clay contents of 63, 20, and 17 percentages respectively, which is sandy clay (Simret *et al.*, 2014).

The field experiment was replicated at another research sub-station of the University, which is located in Tullo district at Hirna. This research sub-station is situated at a distance of about 150 km west of Haramaya University. Geographically, the site is located at 9^{o}12' North latitude, 41^{o}4' East longitude, and at an altitude of 1870 meters above sea level. The area receives mean annual rainfall of 990 to 1010 mm (HURC, 1996). The mean maximum and minimum annual temperatures are 21.8°C and 8.6°C, respectively (Tekalign, 2011). The soil of Hirna is vertisol type with a silty clay texture, which contains 1.75% organic carbon, 0.18% total Nitrogen, 32 mg kg soil^{-1} available Phosphorus, 0.68 cmol$_{c}$ kg soil^{-1} exchangeable potassium, and has neutral soil pH of 7.09 (Nebret, 2011).

2.2. Experimental Materials

Improved potato variety, named "Bubu" (CIP-384321.3) was used as a planting material. Bubu was released by Haramaya University in 2011 which was adapted to the altitude of 1650-2330 metres above sea level, high yielding and resistant to late blight. Bubu takes to mature 95 to100 days to mature (MARD, 2009).

Urea ($CO [NH_2]_2$) (46% N) and (TSP) Triple-Super Phosphate ($Ca [H_2PO_4]_2$), which constitutes about 46% P_2O_5, were used as a source of inorganic fertilizer.

2.3. Treatments, Experimental Design and Procedures

The treatments consisted of four seed tuber planting depth (5, 10, 15 and 20 cm) and five rates of N (0, 46, 92, 138 and 184 kg N ha^{-1}). The experiment was laid out as a Randomized Complete Block Design (RCBD) in factorial arrangement and replicated three times per treatment. Thus, there were 5 x 4 treatment combinations, with a total of 20 experimental units (plots). The treatments were assigned to each plot randomly.

The land was prepared in accordance with a standard practice of Haramaya University (HURC, 1996). The experimental plot was cultivated to a depth of 25–30 cm using tractor. In order to create good seed bed for proper crop growth, the experimental field was cleared and ploughed and disked three times using tractor.

Medium sized potato tubers with sprouts measuring about 1.5 to 2.5 cm were planted on prepared ridges at the spacing of 75 cm between rows and 30 cm between plants at the depth of 5, 10, 15 and 20 cm on 07 and 08 July 2015 at Haramaya and Hirna, respectively. Plot size was 3.6m x 4.5m (16.2 m2), 6 rows per plot and 12 hills per row. Hence, there were 72 hills per plot. Regular hoeing was done for control of weeds.

Full dose of phosphorus was applied at planting time in prepared ridges by banding the granules at the depth of 10 cm below and around the seed tuber. Nitrogen fertilizer was side dressed in three splits: one-third at planting; one-third at active vegetative stage (about 50 days after planting) and the remaining one-third just before start of flowering or tuber initiation.

Weeding, cultivation and earthing-up and ridging were done at the appropriate time to facilitate root, stolon and tuber growth. Weeds were controlled by hoeing and earthing-up as required to prevent exposure of tubers to direct sun light and for promoting tuber bulking and for ease of harvesting. Other cultural practices were applied as per the usual practices used by Haramaya University to grow potato crops.

To avoid bruising and skinning of tubers during harvesting and post-harvest handling, the haulms were mowed two weeks before harvesting to thicken tuber periderm. Mowing of haulms was carried out when the plants reached physiological maturity, i.e. when yellowing or senescence was apparent on the lower leaves. Forty plants were harvested from a net plot area of 3 m x 3 m (9 m^2), leaving aside all plants at the border rows as well as those at both ends of each row to avoid edge effects, to estimate tuber yield and other yield-related parameters.

2.4. Data Collection and Measurement

Data on total biomass were determined from 5 plants randomly sampled from each plot just at physiological maturity. Shoot dry mass was determined by oven drying the fresh shoot biomass at 72°C for 24 hours to a constant mass. Tuber specific gravity was determined by the weight in air/weight in water method. 5 kg tubers of all shapes and sizes were randomly taken from each plot. The selected tubers were washed with water. The samples were then first weighed in air and then re-weighed suspended in water. Specific gravity was calculated using the following formula (Kleinkopf *et al*., 1987). All seed tubers from five randomly selected plants plot^{-1} were categorized into small (< 39 g), medium (39-75 g) and large (>75 g) according to (Lung'aho*et al*., 2007). The proportion of the weight of each tuber category was expressed in percentage and the average tuber weight of each category was recorded for a single plant. Seed tuber which was a healthy and weighed greater than or equalto 20g was considered as marketable while seed tubers which were blemishes due to diseases and weighed less than or equal to 20g was considered as unmarketable. Total tuber yields and numbers were recorded as the sum of marketable and unmarketable tuber yields and numbers, respectively. To determine tuber dry matter content (%) five potato seed 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 was taken for drying to constant weight. Each sub-sample was placed in a paper bag and put in an oven at 70^{o}C for 72 hours. Each sub-sample was immediately weighed and the mean dry weight was recorded. Percentage dry matter content for each sub-sample was calculated based on the formula described by Bonierbale and Forbes (2006).

$$\text{Dry matter} = \frac{\text{weight of sample after drying(g)}}{\text{initial weight of sample(g)}} x100$$

The percentage of starch was calculated from the specific gravity where specific gravity was determined as indicated above by the weight in air and weight in water method.

Starch (%)=17.546 +199.07 × (specificgravity-1.0988) (Smith and Talburt, 1959 as cited by Yildrim *et al*.,2005).

2.5. Data Analysis

The data were subjected to analysis of variance (ANOVA) of RCBD in factorial arrangements for each location using the general linear model of Genstat 15th edition updated version. Treatment means that exhibited significant differences was separated using Tukey test at 5%level of significance.

3. Results and Discussion

3.1. Effect of Seed Tuber Planting Depth and Nitrogen Fertilizer Rates on Dry Biomass

Analysis of the data on total dry biomass revealed that both tuber planting depth and nitrogen rate had significant effect on total dry biomass at Haramaya, but non-significant difference was observed from same treatments at Hirna (Table 1). Interaction of tuber planting depths and nitrogen application rates showed non-significant difference on total dry biomass at both locations.

Total dry biomass yield decreased in response to the increased rates of nitrogen from null to 92 kg ha^{-1} in turn from this, it significantly increased up to 138 kg N ha^{-1} and continued declining above this level. Thus, the highest total dry biomass yield was attained relatively at the highest rate of the nutrient, and was higher than the total dry biomass yield of plants in the 46 and 92 kg N ha^{-1} treatments by about 12.81 and 21.59%, respectively. However, there was non-significant difference between control and nitrogen application rate of 138 kg ha^{-1} treatments at Haramaya (Table 1).

Table 1. Effect of nitrogen fertilizer rate and seed tuber planting depth on total dry biomass weight of Bubu variety at Haramaya and Hirna during 2015 cropping season.

Treatment	Total dry biomass weight (g plant^{-1})	
Tuber planting depth (cm)	Haramaya	Hirna
5	187.20^{c}	232.30
10	194.50^{bc}	253.00
15	223.70^{a}	240.50
20	216.20^{ab}	245.00
LSD (5%)	25.25	NS
Nitrogen (kg N ha^{-1})		
0	219.30^{a}	212.10
46	196.70^{ab}	251.80
92	182.50^{b}	257.00
138	221.90^{a}	242.70
184	206.60^{ab}	250.00
LSD (5%)	28.22	NS
CV (%)	16.60	17.30
Location (means)	205.40^{b}	242.70^{a}
T-test	* *	

Means with the same letter(s) within a column are not significantly different at 5% level of significance. NS = non-significant difference, LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

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. Similarly, Zelalem *et al.* (2009) reported significant increase in biomass and shoot dry matter yields in response to nitrogen application. Yibekal (1998) also reported that higher.

N rates resulted in greater total dry biomass yield of potato. This result is also in accordance with the results of Mulubrhan (2004) who reported significant increase in underground and above ground dry matter yields of potato in response to increased nitrogen application.

Total dry biomass obtained from 15cm tuber planting depth was higher than other depths. As tuber planting depths decreased from 15cm to 10 and 5cm total dry biomass decreased by about 15.01 and 19.50%, respectively. The lowest total dry biomass was produced from tuber planting depth of 5cm treatment at Haramaya experimental site.

The significant highest total dry biomass recorded at 15cm might be due to deeper depth allowed optimum root growth and soil moisture was available which in turn was be accounted due to the fact that when helpful for high total dry matter accumulation. Tuber planting depth has an effect on the emergence, seedling establishment, survival of the plants and above ground biomass which is the function of plant height and number of stems. Sayed *et al.* (2013) found the highest above ground biomass was at tuber planting depth of 15 cm.

3.2. Effect of Seed Tuber Planting Depth and Nitrogen Fertilizer Rates on Tuber Yield

The analysis of variance revealed that seed tuber planting depth had significant ($P < 0.01$) main effect on total and marketable tuber yields at both locations. However, the rate of nitrogen application as well as the interaction of nitrogen application rate and seed tuber planting depth did not influence both total and marketable tuber yields (Table 2).

Increasing the depth of seed tuber planting from 5cm to 10cm increased the marketable tuber yield of the crop by about 28% at Haramaya and by about 35% at Hirna. The corresponding increase for total tuber yield at Haramaya and Hirna were 18 and 32%, respectively. Consistent with the results of this study, Kumar *et al.* (2015) reported that total tuber yield was significantly higher at 10cm seed tuber planting depth than 15 and 20 cm depths. Similarly, Pavek and Thornton (2009) observed the performance of two commercial varieties of potatoes were lower for shallower seed tuber planting depth.

Table 2. Effect of seed tuber planting depth and nitrogen fertilizer rates on marketable and total tuber yield of Bubu variety at Haramaya and Hirna during 2015 cropping season

Treatment	Marketable yield (t ha^{-1})		Total tuber yield (t ha^{-1})	
	Haramaya	Hirna	Haramaya	Hirna
Tuber planting depth (cm)				
5	20.33^{b}	24.11^{c}	22.89^{b}	26.13^{c}
10	26.00^{a}	32.65^{a}	26.93^{a}	34.57^{a}
15	26.26^{a}	30.10^{b}	26.89^{a}	30.82^{b}
20	24.88^{a}	29.86^{b}	25.42^{a}	30.66^{b}
LSD (5%)	2.29	2.33	2.31	2.35
CV (%)	12.7	10.8	12.3	10.4
Location (means)	24.37^{b}	29.18^{a}	1.17	1.36
T-test	*	*	*	*

Means with the same letter(s) within a column are not significantly different at 5% level of significance. LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

This increase in both marketable and total tuber yields in response to increasing the depth of planting seed tubers from 5cm to 10cm may be attributed to provision sufficiently loose soil for nourishment of seedlings through good proliferation of roots, resulting in optimum emergence of sprouts above the soil surface and their subsequent growth.

At Haramaya location, increasing the depth of seed tuber planting further to 15 and 20 cm did not change both the total and marketable tuber yields. However, further increase in seed tuber planting depth decreased both the total and marketable tuber yields at Hirna. This difference between the two locations in terms of response of the tuber yields to seed tuber planting depth could be attributed to inherent soil texture at the two locations. Thus, Hirna soil is more clayey and compact compared to Haramaya soil, which is light. Thus, deepening planting of seed tubers at Hirna could have the consequences of stressing seedlings when they emerge above the soil surface. Thus, in such soil, seedlings would require more energy to emerge, with curtailed potential of subsequent growth and productivity of the crop. However, at Haramaya, the lighter soil would enable it easier for the seedlings to emerge above the soil surface with better root proliferation with a relative ease, with less stress during emergence and subsequent growth. Consistent with this suggestion, Sayed *et al.* (2013) reported that deeper planting of seed tubers was a cause for more slow germination of potato seedlings.

In line with this finding, Lambion *et al.* (2006) advocated that deeper planting may also limit tuber bulking due to soil compaction or damage of tubers by certain pests. Rosen and McNearney (2003) argued that to get more marketable tubers extra nutrients were needed. Hence, deeper planting was important. Concurrent with the results of this study, Pavek and Thornton (2009) reported that marketable yield and gross income declined typically when seed pieces were planted at shallower depths. The authors also explained that the largest impact on marketable yield and gross income reduction might be due to shallower seed tuber planting depth which attribute to greening of tubers, when seed tuber pieces were planted deeper, the incidences of tuber greening was recorded.

3.3. Effect of Seed Tuber Planting Depth and Nitrogen Fertilizer Rates on Unmarketable Tuber Yield

Tuber planting depth, nitrogen fertilizer rate, and the interaction of the two factors had significant ($P < 0.01$) effect on unmarketable tuber yield at Haramaya (Table 3). The lowest unmarketable tuber yield was produced from tuber planting depth of 20 cm and nitrogen application rate of 92 kg ha^{-1} while tuber planting depth of 5 cm and nitrogen application rate of 46 kg N ha^{-1} produced the highest unmarketable tuber yield.

The small sized and greening tubers were high in tuber planting depth of 5cm at 46 kg N ha^{-1} nitrogen applications and this in turn increased unmarketable tuber yield. The major benefits of increased planting depth appeared to be better soil moisture, less green and surface exposed tubers, larger tuber size, and higher market yields (Stalham *et al.*, 2001). Kumar *et al.* (2015) studied the effect of tuber planting depth on processing potato and reported that green tubers were statistically higher at shallow depth plantings of 10cm and 15cm than 20cm depth.

Table 3.Effect of seed tuber planting depth and nitrogen fertilizer rates on unmarketable tuber yield of Bubu variety at Haramaya during the 2015 main cropping season.

	Seed tuber planting depth (cm)			
Nitrogen (kg N ha^{-1})	5	10	15	20
0	2.66^{b}	0.99^{d-f}	0.37^{g-i}	0.49^{f-i}
46	3.41^{a}	0.53^{f-i}	1.33^{de}	0.43^{g-i}
92	3.34^{a}	0.40^{g-i}	0.25^{hi}	0.22^{i}
138	1.42^{d}	0.28^{hi}	0.82^{fg}	0.74^{f-h}
184	1.98^{c}	2.45^{bc}	0.38^{g-i}	0.83^{e-g}
LSD (5 %)		0.51		
CV (%)		26.30		

Means with the same letter(s) within a column are not significantly different at 5% level of significance. LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

3.4. Effect of Seed Tuber Planting Depth and Nitrogen Fertilizer Rates on Tuber Size Distribution

The proportion of small, medium and large sizes tubers in each treatment was calculated and subjected to analysis of variance. The result revealed that the production of small sized tubers production was significantly ($P < 0.01$) influenced by tuber planting depth and nitrogen fertilizer rates and the interaction of the two factors at Haramaya. In this location, planting depth and nitrogen fertilizer rates had also significantly influenced the production of large and medium size tubers, respectively. At Hirna, tuber planting depth and nitrogen fertilizer rate interacted to influence significantly the production of medium and large size tubers while tuber planting depth had significant effect on the production of small size tubers.

The interaction of tuber planting depth at 5 cm and application of nitrogen fertilizer rate at 184 kg N ha^{-1} produced the highest and significant proportion of small size tubers while the interaction of tubers placed at 15cm and 138kg N ha^{-1} rate produced significantly lowest proportion of small size tubers at Haramaya (Table 4). At Hirna, the proportion of small size tubers produced from planting depth of 5cm and 10cm had non-significant difference. Plants grown from tubers placed at the depth of 5cm produced significantly highest percentage of small size tubers than tubers placed at 15 and 20cm depth that exceeded them by about 6.72 and 5.14 %, respectively. The percentage of small size tubers obtained from plants grown from tubers placed at 10cm depth exceeded 15 and 20cm tubers planting depth by about 3.44 and 1.86%, respectively (Table 7).

The highest proportion of small-sized tubers was obtained from the interactions of tuber planting depth at 5cm and nitrogen application rates at184 kg ha^{-1} at Haramaya and plants grown from tubers placed at 5cm depth produced the highest significant result in case of Hirna. This effect might be due to shallow soil cover of tubers that might have allowed fast growth of aerial parts of the plants and lower accumulation of assimilates in below ground part (tubers). The seed pieces planted closer to the soil surface that faced daily temperature extremes and fluctuation increased the production of small size tubers (Pavek and Thornton, 2009). On the other hand, the lowest proportion of small-sized tubers produced at planting depth of 15 cm and 138 kg N ha^{-1} at Haramaya and from the tuber planting depth of 15 and 20 cm at Hirna might be due to the deep planting that might have prevented the tubers from being infested by pests example potato tuber moth, absence of tuber greening due to absence of exposure to light, less production of small-sized tubers. Also deep soil has fine, loose soil (without compaction), optimum soil moisture, that facilitate root penetration. This may have increased the proportion of large and medium-sized tubers. These results clearly indicate that nitrogen application influenced potato tuber size distribution. It can, thus, be suggested that production of seed potato or ware potato could be optimized through manipulation of rates of mineral nutrients such as nitrogen. Consistent with the results of this study, the highest yield (7.56 t ha^{-1}) of small-sized tubers were obtained at 50 kg N ha^{-1} while the lowest yield (4.62 t ha^{-1}) of this tuber size category was obtained at 200 kg N ha^{-1} at Haramaya (Simret *et al.*,2014).

Table4. Interaction effect of nitrogen fertilizer and seed tuber planting depth on percentage of small-size tubers at Haramaya during the 2015 main cropping season

	Tuber planting depth (cm)			
Nitrogen (kg N ha^{-1})	5	10	15	20
0	23.16^{d-f}	17.86^{d-g}	21.19^{d-f}	20.80^{d-f}
46	35.32^{c}	25.83^{d}	15.92^{fg}	16.75^{e-g}
92	36.12^{c}	20.65^{d-f}	10.35^{gh}	16.45^{fg}
138	46.12^{ab}	37.49^{bc}	6.01^{h}	25.35^{de}
184	51.62^{a}	17.02^{d-g}	17.35^{d-g}	15.67^{fg}
LSD (5%)	8.83			
CV (%)	22.40			

Means with the same letter (s) are not significantly different at 5% level of significance. LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

The highest and significant medium sized tubers and the lowest medium sized tubers were obtained from the interactions of seed tuber planting depth of 15 and 20cm with nil applications of nitrogen fertilizer at Hirna (Table 5).The highest medium sized tuber percentage was recorded from 46 kg N ha^{-1} while the lowest percentage was obtained from 138 kg N ha^{-1} in the case of Haramaya.The percentage of medium sized tubers at tuber planting depth of 15 cm exceed tuber planting depth at 20 cm by about 23.02%in both case without application of nitrogen fertilizer at Hirna. The medium size tubers produced by the application of 46 kg N ha^{-1} exceeded 184 and138 kg N ha^{-1} by about 6.85 and 8.71%, respectively, at Haramaya. However, there were non-significant differences between the control, 46 and 92 kg N ha^{-1} treatments at Haramaya (Table 6). The highest and significant result recorded at Hirna from tuber planting depth of 15cm without application of nitrogen fertilizer might be due to the placing of tubers at this depth alone was sufficient to produce highest proportion of medium size tubers as compared to other planting depths either with or without application of nitrogen fertilizer.

Table 5. Interaction effect of seed tuber planting depth and nitrogen fertilizer on percent medium and large-sized tubers at Hirna during 2015 main growing season

	Medium-sized tubers (%)				Large-sized tubers (%)			
Nitrogen (kg N ha^{-1})	Tuber planting depth (cm)				Tuber planting depth (cm)			
	5	10	15	20	5	10	15	20
0	26.89^{b-e}	24.53^{c-e}	39.10^{a}	16.08^{e}	52.29^{a-d}	53.07^{a-d}	44.4c^{-f}	62.02^{a}
46	26.17^{b-e}	33.96^{a-d}	35.07^{a-c}	31.36^{a-d}	49.36^{b-e}	37.36^{e-f}	48.54^{b-e}	43.32^{d-f}
92	25.50^{c-e}	27.87^{a-e}	34.48^{a-d}	28.41^{a-d}	50.82^{a-d}	54.76^{a-d}	46.12^{c-f}	52.71^{a-d}
138	26.75^{b-e}	29.00^{a-d}	28.54^{a-d}	33.51^{a-d}	37.07^{e-f}	52.45^{a-d}	56.25^{a-c}	44.82^{c-f}
184	37.71^{ab}	32.71^{a-d}	22.47^{de}	28.61^{a-d}	35.88^{f}	43.79^{c-f}	58.67^{ab}	58.74^{ab}
LSD (5%)	8.83				12.54			
CV (%)	22.4				15.4			

Means with the same letter(s) are not significantly different at 5% level of significance; LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

Table 6. Effect of nitrogen fertilizer rates on percentage of medium-sized tubers of Bubu variety at Haramaya and Hirna during the 2015 cropping season

	Medium-sized tubers (%)	
Nitrogen (kg N ha^{-1})	Haramaya	Hirna
0	27.00^{ab}	26.65
46	29.60^{a}	31.64
92	26.88^{ab}	29.07
138	20.89^{c}	29.45
184	22.75^{bc}	30.37
LSD (5%)	5.89	NS
CV (%)	28.1	25
Location (means)	25.42^{b}	29.44^{a}
T-test	*	*

Means with the same letter(s) within a column are not significantly different at 5% level of significance. NS = non-significant difference, LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

The highest and significant medium-sized tubers obtained from 46 kg N ha^{-1} application at Haramaya indicated that nitrogen was efficiently used by potato plants to produce optimum sized tuber that are preferable for seed tubers and house consumption. Lung'aho *et al.* (2007) suggested that medium-sized tubers are appropriate for planting. The yield of medium-sized tubers remained significantly low at 0 and 50 kg N ha^{-1}, but increased significantly at the remaining higher levels of the nutrient reaching the maximum at 200 kg N ha^{-1} (Simret *et al.*, 2014). Similarly, Girma (2001) also reported as nitrogen application rates increased, there was a progressive shift of the small size tubers towards medium-sized ones.

The highest and significant large sized tubers were obtained from tuber planting depth of 20cm without nitrogen fertilizer application while the lowest proportion of large size tubers were obtained from planting depth of 5 cm and 184 kg N ha^{-1}applications at Hirna. Tuber planting depth of 20cm without nitrogen fertilizer application exceeded 5cm planting depth with 184 kg N ha^{-1}application by about 26.14% at Hirna (Table5). At Haramaya, the highest large size tubers as percent of total tubers was calculated for tuber planting depth of 15cm, while the lowest percentage was obtained from 5 cm depth. In this location, the proportion of large size tubers obtained from 15 and 20 cm depth had non-significant difference. In addition, the proportion of large size tubers obtained from 15 cm tuber planting depth exceeded 5 and 10cm depth by about 29.60 and 14.64%, respectively (Table 7).

Table 7. Effect of seed tuber planting depth on percentage of small- and large-sized tubers at Hirna and Haramaya during the 2015 cropping season.

	Small-sized tubers (%)		Large-size tubers (%)	
Tuber planting depth (cm)	Haramaya	Hirna	Haramaya	Hirna
5	38.47^{a}	25.38^{a}	33.50^{c}	45.08
10	23.77^{b}	22.10^{ab}	48.46^{b}	48.29
15	19.00^{c}	18.66^{b}	63.10^{a}	50.8
20	14.16^{d}	20.24^{b}	57.63^{a}	52.32
LSD (5%)	3.95	4.265	7.05	NS
CV (%)	22.40	26.70	18.8	15.4

Means with the same letter(s) within a column are not significantly different at 5% level of significance. NS = non-significant difference, LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

The highest and significant large sized tubers as percent of total tubers produced at 20cm planting depth without nitrogen application at Hirna might be due to less chance of greening of tubers and the low small sized tubers production. In contrast with this result, Herlihy and Carroll (1969) attributed increase in the number of medium and large tubers to increase in total tuber yield at the expense of small tubers up to the level of 150 kg N ha^{-1}. The highest proportion of large sized tubers obtained from tuber planting depth of 15 cm at Haramaya might be that root growth and development was better at this planting depth because of sufficient soil moisture availability than shallow and deeper tuber planting depth. Bohl and Love (2005) also reported that large size tubers yield of the cultivar 'Shurchip' was significantly higher when planted at a 15cm depth compared to 5 cm or 10cm.

3.5. Tuber Quality Related Traits

Nitrogen fertilizer rates had significant effect on tuber specific gravity and starch content at Haramaya, while the interaction of tuber planting depth and nitrogen fertilizer rate significantly influenced the two tuber quality related parameters (specific gravity and starch content) at Hirna. In addition, tuber planting depth had significant effect on tuber dry matter content and tuber starch content at Haramaya and Hirna, respectively. However, tuber dry matter content was not significantly influenced by nitrogen fertilizer rates and the interactions of two factors.

3.5.1. Tuber dry matter content

The mean comparison of tuber dry matter contents for different treatments showed that the highest and significant tuber dry matter content was measured from plants grown from tubers placed at 15cm during planting while the lowest was obtained from tuber planting depth of 5 cm at Haramaya (Table 8). Tuber dry matter content of plants grown from 5, 10 and 20 cm planting depth had non-significance.

Table 8. Effect of seed tuber planting depth on tuber dry matter and starch contents of Bubu variety at Haramaya and Hirna during 2015 cropping season.

	Tuber dry matter content (%)		Starch content g/100g	
Tuber planting depth (cm)	Haramaya	Hirna	Haramaya	Hirna
5	25.77[b]	24.44	16.39	16.71[b]
10	25.94[b]	24.80	16.77	17.07[ab]
15	28.13[a]	25.49	16.25	17.83[a]
20	26.76[b]	25.31	15.31	16.41[b]
LSD (5%)	0.10	NS	NS	0.99
CV (%)	5.10	6.8	9.10	7.90
Location (means)	26.33[a]	25.01[b]	16.18[b]	17.01[a]
T-test	*	*	*	*

Means with the same letter(s) within a column are not significantly different at 5% level of significance. NS = non-significant difference, LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

High tuber specific gravity, dry matter and starch content are important for processing by enhancing chip yield, crispness and reduces oil uptake in fried products (Freitas *et al.*, 2012). Beukema and Van der Zaag (1989) indicated that potato tuber dry matter content is influenced by a large number of factors the most important ones being cultivar and environmental conditions. Water and nitrogen supply, type and condition of soils are among the environmental factors that influenced tuber dry matter content. Habtamu (2013) reported average tuber dry matter content of 27.49% for Bubu at Haramaya and Hirna and also indicated that the average tuber dry matter content of 16 potato varieties and two farmers' cultivars was higher at Haramaya than Hirna. This might be due to the difference in altitude and soil type at two locations. Plants grown on silt soils have higher tuber dry matter content than plants grown on sandy soils. This is because of that the available water in silt soils is more tightly and exerts higher suction pressure of the water in leaves and consequently dry matter partitioning in tubers increased than sand soils (Beukema and Van der Zaag, 1989).Soil conditions are determined by soil structure, moisture, and temperature. By adjusting the planting depth, potato cultivation can be adapted to existing conditions of moisture and temperature. At Haramaya there was low rainfall and higher soil temperature which led to loss of soil moisture relatively at shallow depths (5 and 10 cm) while deeper depth (15 and 20 cm) accumulate more soil moisture. Hence tuber planting depth adjusted to soil moisture at deeper level tend to produce tubers of higher specific gravity while shallower depth affected by warm soil condition and lower specific gravity was observed. The soil difference of the two sites and the rainfall difference during the growing season (drought was sever at HU and higher suction pressure of the water in leaves at 15cm depth than 5 and 10 cm and beyond 15cm) and helped 15cm depth to produce higher specific gravity.

3.5.2. Specific gravity

The highest specific gravity was recorded from the interaction effect of 5cm tuber planting depth and 138kg N ha^{-1}but the lowest was obtained from the interaction effect of 5cm tuber planting depth and 46 kg N ha^{-1} at Hirna. The interaction of 5cm and 138 kg N ha^{-1} produced tubers with specific gravity that exceeded those tubers planted at same depth but with the application of 46kg N $ha^{-1}$by 2.69 % (Table 9). Tubers specific gravity showed consistent increase trend as rate of nitrogen fertilizer rate increased from 0 to 138 kg $ha^{-1}$and interacted with 10 and 15 cm planting depth but the interaction of 20cm planting depth and 46 kg N ha^{-1}showed an increased tuber specific gravity over 0 kg N ha^{-1} application. On the other hand, the interaction of 46kg N $ha^{-1}$and 10, 15 and 20 cm showed an increased tuber specific gravity parallel to interacted tuber planting depth but other rates of nitrogen fertilizer interaction to same tuber planting depth were lacking such linear increase. Whereas at Haramaya, significantly higher specific gravity in the range between 1.092 to 1.094 were measured from plots that received 0 to 138 without significant differences among treatment means while the higher rate of 184 kg ha^{-1} nitrogen application significantly reduced (Table 10).

Table 9. Effect of seed tuber planting depth and nitrogen fertilizer rates interaction on tuber specific gravity of Bubu variety at Hirna during 2015 cropping season.

	Tuber planting depth (cm)			
Nitrogen (kg N ha^{-1})	5	10	15	20
0	1.097^{a-e}	1.093^{c-e}	1.095^{b-e}	1.091^{c-e}
46	1.078^{f}	1.095^{b-e}	1.097^{a-e}	1.102^{a-c}
92	1.098^{a-e}	1.1^{a-d}	1.097^{a-e}	1.096^{a-e}
138	1.107^{a}	1.101^{a-c}	1.105^{ab}	1.088^{ef}
184	1.092^{c-e}	1.093^{b-e}	1.100^{a-e}	1.098^{d-f}
LSD (5 %)	0.01189			
CV (%)	0.70			

Means with the same letter(s) within a column are not significantly different at 5% level of significance. LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

Table 10.Effect of nitrogen fertilizer rates on tuber specific gravity of Bubu variety at Haramaya during 2015 cropping season.

	Specific gravity (gcm^{-3})	
Nitrogen (kg N ha^{-1})	Haramaya	Hirna
0	1.094^{a}	1.094
46	1.093^{a}	1.093
92	1.092^{a}	1.098
138	1.094^{a}	1.100
184	1.085^{b}	1.093
LSD (5%)	0.006167	NS
CV (%)	0.70	0.70
Location (means)	1.092b	1.096a
T-test	*	*

Means with the same letter(s) within a column are not significantly different at 5% level of significance. NS = non-significant difference, LSD (5 %) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

The higher tuber specific gravity recorded from plots that received 0 and 138 kg N ha^{-1} with significant difference at Haramaya might indicated the non-significant effect of nitrogen fertilizer application on this tuber quality parameter. This suggested application of nitrogen fertilizer was economically not visible in Bubu variety at Haramaya.

According to Tanyaradzwa *et al.* (2015), there were no significant differences for tuber planting depths (10, 15 and 20 cm) with respect to tuber specific gravity from the study on effects of planting depth and variety on potato. Non-significant differences in tuber specific gravity for planting depth could be attributed to physical restriction of the space for root growth which in turn affects carbohydrate metabolism and whole plant growth (Yang *et al.*, 2007).

Fitzpatrick *et al.* (1964) categorized specific gravity values of tubers as low (<1.077), intermediate (1.077≤x ≤ 1.086, and high (>1.086) specific gravity grades cited by Asmamaw (2007). Based on the above category, all treatments had an effect to produce tubers with high tuber specific gravity in Bubu variety at both locations except the interaction effect of 5 cm planting depth and 46 kg N ha^{-1}application at Hirna.

3.5.3. Starch content

According to the analysis variance result, the application of nitrogen had highly significant (P < 0.01) effect on starch content of tubers at Haramaya (Table 12). However, tuber seed planting depth and the interactions of the two factors showed non-significant difference (Table 11). On the other hand, the interaction of tuber planting depth and nitrogen rate had significant (P < 0.05) effect on tuber starch content at Hirna.

Table 11. Interaction effect of seed tuber planting depth and nitrogen fertilizer rates on starch content of Bubu variety at Hirna during 2015 cropping season.

Starch content (%)				
	Tuber planting depth (cm)			
Nitrogen (kg N ha^{-1})	5	10	15	20
0	17.27^{a-f}	16.29^{d-f}	16.82^{b-f}	16.06^{d-f}
46	13.40^{g}	16.83^{b-f}	18.61^{a-c}	18.04^{a-d}
92	17.45^{a-f}	17.86^{a-d}	17.26^{a-f}	16.97^{b-f}
138	19.24^{a}	17.95^{a-d}	18.73^{ab}	15.43^{fg}
184	16.21^{d-f}	16.41^{c-f}	17.72^{a-e}	15.56^{e-g}
LSD (5%)	2.212			
CV (%)	7.9			

Means with the same letter(s) within a column are not significantly different at 5% level of significance. LSD (5%) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

The highest and lowest tuber starch contents were measured from plots that received 138 and 46 kg N ha^{-1}, respectively, both interacted with 5cm tuber planting depth at Hirna (Table 11). Whereas at Haramaya, the highest tuber specific gravity was recorded from plots that did not received nitrogen and 138 kg ha^{-1} nitrogen rate (Table 12). The lowest specific gravity was deserved from 184kg ha^{-1} nitrogen rate application. There were non-significant differences among 0, 46, 92 and 138 N ha^{-1} nitrogen rates application.

Table 12. Effect of nitrogen fertilizer rates on tuber starch content of Bubu variety at Haramaya and Hirna during 2015 cropping season.

	Starch content g/100g	
Nitrogen (kg N ha^{-1})	Haramaya	Hirna
0	16.90^{a}	16.61
46	16.41^{a}	16.72
92	16.17^{a}	17.39
138	16.60^{a}	17.84
184	14.82^{b}	16.47
LSD (5%)	1.22	NS
CV (%)	9.10	7.90
Location (means)	16.18b	17.01a
T-test	*	*

Means with the same letter(s) within a column are not significantly different at 5% level of significance. NS = non-significant difference, LSD (5 %) = Least significant difference at P = 0.05, and CV (%) = Coefficient of variation in percent.

The T-test indicated that the growth and tuber yield of the crop observed at Hirna were significantly higher than that of Haramaya. The main cause for the superior growth and yield parameters of the crop at Hirna than Haramaya may be attributed to variation in soil chemical properties between the two locations, mainly soil pH. Potatoes can grow under a wide range of soil pH varying from neutral to alkaline reaction (Jadhav and Kadam, 1998; Fageria*et al*., 2011). However, the soil pH for optimum yield ranges from 5.0 – 6.5 (McLean and Brown, 1984). It was indicated that the pH of Hirna soil is neutral whereas that of Haramaya is alkaline. This means that the crop is grown under sub-optimal pH in most of the surveyed farms. The effects of high soil pH on potato include low availability of phosphorus (Holford, 1997). Alkaline soils also favour potato skin diseases such as common scab (Streptomyces scabies) (Jadhav and Kadam, 1998)

4. Conclusion

In this study, the yield and yield components of potato, Bubu variety was significantly different in response to the seed tuber planting depths and different nitrogen rates as well as their interaction. Seed tuber planting depth caused significant variations in the number of stem, fresh shoot weight, tuber dry biomass, total dry biomass, percentage of dry shoot, tuber number hill^{-1}, large tuber percentage, marketable tuber yield, unmarketable tuber yield, total tuber yield, mean tuber weight, small tuber percentage at Haramaya. Also, the application of nitrogen fertilizer caused significant variations in the number of stem, total dry biomass, unmarketable tuber yield, small tuber percentage, medium tuber percentage and specific gravity. There were interaction effects of tuber planting depth and nitrogen application on unmarketable tuber yield and small tuber percentage.

Seed tuber planting depth caused significant variations in plant height, tuber number hill^{-1}, marketable tuber yield, unmarketable tuber yield, total tuber yield, small tuber percentage and tuber starch percentage at Hirna. Also, the application of nitrogen fertilizer caused significant variations in the fresh shoot biomass, dry shoot biomass and unmarketable tuber yield. There were significant interaction effects of seed tuber planting depth and nitrogen application on unmarketable tuber yield, medium tuber percentage, large tuber percentage, specific gravity and tuber starch content.

There were significant effects of seed tuber planting depth on the total tuber yield of the crop at both locations, Haramaya and Hirna. Total tuber yield was highest at 10cm depth and decreased in both directions, below and above 10cm depth. However, the interaction of seed tuber planting depth and nitrogen application showed non-significant difference at both locations. Thus, the maximum total tuber yield recorded from 10cm depth was 26.93 t ha^{-1} and 34.57 t ha^{-1} at Haramaya and Hirna respectively. In contrast to the significant effect of seed tuber planting depth on total tuber yield, the application of nitrogen fertilizer showed no significant difference on total tuber yields at both locations. The main cause for the superior growth and yield parameters of the crop at Hirna than Haramaya may be attributed to variation in soil chemical properties between the two locations, mainly soil pH. Highest total and marketable tuber yield of 26.93 t ha^{-1} and 26 t ha^{-1} for Haramaya and 34.57 t ha^{-1}and 32.65 t ha^{-1} for Hirna were already obtained at the seed tuber planting depth of 10 cm. In conclusion, potato responded well to the seed tuber planting depths in terms of growth and yield in the study areas.

Therefore, smallholder farmers in the area could be advised to use seed tuber planting depth of 10 cm to optimize potato tuber yield.

5. Acknowledgements

The authors thank the Ethiopian Ministry of Education (EME) for providing the financial support for the research. The supports given by technical and field assistants of crop research programme of Haramaya University are acknowledged.

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9. In vitro Screening of Potato (*Solanum tuberosum* L.) Genotypes for Osmotic Stress Tolerance

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Abstract: Potato produces nutritious food more quickly per unit land than many cereal crops and considered as one of the food security crop in Ethiopia. However, despite the domestication and area expansion of high land potato varieties to marginal areas, lack of suitable potato varieties adaptable to drought prone areas deprives farmers to produce and use the crop as source of food and income. This research was conducted with the objectives of evaluating different potato genotypes for osmotic stress tolerance under in vitro condition and to identify drought tolerant potato genotypes for future field evaluation. The experiment was conducted at Leibniz University of Hannover, Germany by inducing moisture stress using Sorbitol at three concentrations of -0.8 (control), -1.1 and -1.35 MPa Ψw, respectively, in the growing medium. A total of 43 potato genotypes collected from farmers', research centers and Centre for International Potato (CIP) were used in the experiment. The experiment was set in completely randomized design in eight replications having six plantlets per replicate and 1032 treatment combinations. Data was collected on days to shoot and root initiation, shoot height, leaf number, shoot fresh and dry weight, root number, root length, root fresh and root dry weight and subjected to analysis using statistical soft ware. The dendrogram obtained from UPGMA cluster analysis was constructed to group into moisture stress tolerant, moderately tolerant, and susceptible once at sorbitol treatment of -1.35 MPa Ψw. The results revealed that, the tested genotypes, treatment affect (Sorbitol) and their interactions adversely affected all root and shoot related growth parameters. From the tested genotypes Dadefa (farmers' cultivar), Zemen and Belete (improved varieties) and clones

CIP304350.100, CIP304405.47, CIP392745.7, CIP388676.1 and CIP388615.22 were performed better under sever moisture stress treatments in early root and shoot initiation, increased root number plantlet-1, root length (cm plantlet-1), shoot and root weights (mg plantlet-1) than the susceptible genotypes.

Keywords: Drought; In vitro; Osmotic stress; Potato and Sorbitol.

1.Introduction

Potato (*Solanum tuberosum* L.) plays an increasing role in the livelihood of people in Eastern Africa as a cash and food security crop (Lung'aho *et al*., 2007). In Ethiopia, demand for potato is increasing because of increase in urbanization and change in consumption patterns of the urban population towards processed products like chips (Tesfaye *et al*., 2010). Due to these significant roles, the area under potato production is expanding, especially to lowland areas. Drought and heat stress are the main environmental factors limiting potato production worldwide (CIP, 2011). Drought caused due to moisture stress affects potato yield and tuber quality (Costa and Giovanardi, 1994; Cabello *et al*., 2013). Water stress begins when transpiration demand exceeds root water uptake, resulting in a loss of turgor (Saseendran *et al*., 2008).

Potato is a cool season crop and sensitive to both moisture and heat stresses. Modern potato varieties are generally highly susceptible to drought (Monneveux *et al*., 2013). Thus, there is an increased interest in exploring and exploiting the wide genetic variation that exists for abiotic stresses tolerance in local farmers cultivars and native potatoes. Traits for potato breeding in developing countries as carried out by CIP comprise high yield, earliness, adaptation to long photoperiod, processing and tuber nutrient quality, heat and drought tolerance as well as resistance to viruses and late blight (Raymundo *et al*., 2014).

In Ethiopia, the nationally released potato varieties and local cultivars are less productive in the mid and low lands of the country, where there are frequent moisture and heat stresses. Therefore, developing potatovarieties tolerant to drought would enable not only to sustainably produce the crop in the highlands but also to expand its agro-ecology to the lowlands of the country. As a climate adaptation strategy, this would make the crop resilient to moisture stress and heat for improved yield to attain targeted food requirements. Hence, the objectives of this experiment were to evaluate different potato genotype for moistures stress tolerance, identify genotypes tolerant to moisture stress and traits responsible for the tolerance under *in vitro* condition.

2. Materials and Methods

2.1. Potato Genotypes Descriptions

Forty-three potato genotypes (local varieties, improved varieties, and CIP clones) were collected from three different sources and used *in vitro* screening for osmotic stress tolerance (Table 1). The genotypes include CIP collections reported for drought or heat tolerance or combination of both traits; improved varieties collected from national potato breeding centers in Ethiopia and local (farmers) cultivars collected from seed producers' cooperatives. These collected genotypes were first grown under greenhouse using pots in Ethiopia and explants from each genotypes were cultured *in vitro* in a test tubes containing 20 ml Murashige and Skoog (1962), supplemented with 30 g l^{-1} sucrose and 7 g l^{-1} agar. *In vitro* propagated genotypes were taken to Leibniz University of Hannover and further multiplied in tissue culture laboratory to provide sufficient plantlets for the osmotic screening experiment.

Table 1. List of potato genotypes collected from different sources and used for *in vitro* screening to osmotic stress tolerance.

Genotype	Type	No.	Genotype	Type
CIP302499.30	Accessions	23	CIP397006.18	Accessions
CIP303381.106	Accessions	24	CIP397016.7	Accessions
CIP303381.30	Accessions	25	CIP397036.7	Accessions
CIP304350.100	Accessions	26	CIP397077.16	Accessions
CIP304350.18	Accessions	27	CIP397079.6	Accessions
CIP304366.46	Accessions	28	Bubu (CIP384321.3)	Improved variety
CIP304368.46	Accessions	29	Belete (CIP393371.58)	Improved variety
CIP304371.20	Accessions	30	Bulle (CIP38722425)	Improved variety
CIP304371.67	Accessions	31	Chala (CIP387412.2)	Improved variety
CIP304383.80	Accessions	32	Gorebella (CIP382173.12)	Improved variety
CIP304387.39	Accessions	33	Jalene (CIP37792.5)	Improved variety
CIP 304394.56	Accessions	34	Zemen (AL-105)	Improved variety
CIP304405.42	Accessions	35	Chiro (AL-111)	Improved variety
CIP304405.47	Accessions	36	Gudane	Improved variety
CIP304406.31	Accessions	37	Bate	Local
CIP388615.22	Accessions	38	Dadefa	Local
CIP388676.1	Accessions	39	Jarso	Local
CIP388972.22	Accessions	40	Local-Chiro	Local
CIP390478.9	Accessions	41	Tulema	Local
CIP392745.7	Accessions	42	Samune	Local
CIP395436.8	Accessions	43	Matahara	Local
CIP396311.1	Accessions			

2.2. Treatments and Experimental Design

The screening experiment was conducted at Leibniz University Hannover, Germany. Three treatments induced *in vitro* using Sorbitol having a molecular weight of 182.17

g/mole were used. Treatment one was non stress environment without addition of Sorbitol, Treatment two and three were stress environments induced by addition of 0.1 and 0.2 Molar (M) Sorbitol, respectively into the growing medium. A given weight of Sorbitol powder (0, 18.219 and 36.348 grams were added to Murashige and Skoog (1962) medium supplemented with 30 g l^{-1} sucrose and 7.5 g l^{-1} agar to prepare the three treatments used for *in vitro* screening. The water potential (Ψ_w) of the three treatments was expected to be -0.8, -1.1 and -1.35 MPa, respectively in the culture medium according to previous authors (Gopal and Iwama, 2007). The pH of the medium was adjusted to 5.8 before autoclaving for two hours at 120 °C.

The genotypes first multiplied and maintained *in vitro* for two to three weeks and then stem cuttings having 1.5-2.0 cm length were prepared having one auxiliary bud by excluding the basal and apical portions of the plantlets. Six cuttings of each genotype were cultured in a vessel having approximately 30 ml of the growing medium (treatments). The experiment was laid out as randomized complete design in factorial arrangement replicated eight times having 1032 treatments combinations. The experimental materials were incubated in tissue culture laboratory at the Institute of Horticultural Production Systems-Woody Plant and Propagation Physiology of the Leibniz University of Hannover, Germany for 30 days before data collection. The *in vitro* growing room was maintained at the temperature of 18±2 °C, 16/8 hours light/dark photoperiod with 3000 to 4000 lux light intensity.

2.3. Morphological Related Data Collection

On the 30th day of *in vitro* culturing, data were collected on morphological osmotic stress related traits described as follows. Days to shoot and root initiation were recorded by counting the number of days from culturing to when 75% of the plantlets started to produce primary shoots and roots, respectively. Shoot height (cm) was measured as the length of the main stem from the base to the tip of the plantlet using a ruler after carefully removing the shoot part from the root part. Leaf number per plantlet was recorded by counting the total number of new leaves produced from the plantlet. Shoot fresh weight (mg plantlet^{-1}) was measured by weighing shoot parts of the plantlets using a sensitive balance. Root fresh weight was measured after carefully removing root portion of the plantlets from the growing medium, removing or cleaning all agar traces using tissue paper and measured using a sensitive balance (mg plantlet^{-1}). Shoot and root dry weight (mg plantlet^{-1}) was recorded after drying fresh samples in a drying cabinet (oven) at 70 °C for 72 hours according to the procedure suggested by Schafleitner *et al.* (2007). Root number per plantlet was recorded after carefully removing plantlets from the growing medium, separating agar and counting the total number of roots (all types of roots) produced at approximately one centmetre from basal tip of the plantlets. Root length (cm) was measured by considering the maximum length of the root produced per plantlets using a ruler.

2.4. Statistical Analysis

Morphological data collected was subjected to analysis of variance (ANOVA) using SAS (2004). Passion correlation coefficients were calculated among all traits to determine the relationship between traits. The unweighted pair group method with arithmetic averages (UPGMA) of cluster analysis was used to construct the dendrogram of 43 potato genotypes based on the Euclidean distance matrix according to Sneath and Sokal (1973).

3. Results

Analysis of variance showed significant differences among the genotypes for all morphological traits measured (Table 2). The results revealed that, the tested genotypes, treatment affect (Sorbitol) and their interactions adversely affected osmotic stress related morphological traits considered. The coefficients of determination (R^2) for these osmotic stress related morphological traits also ranged from 0.70 to 0.89, which indicated the fitness of the data to the statistical model.

Table 2. Mean square value of osmotic stress related traits of potato genotypes as affected by genotype, Sorbitol treatments and their interactions.

Source of variation	DF	Mean square										
		DRI	DSI	LN	SH	SFW	RFW	RL	RN	SDW	RDW	R:S(DW)
Genotype (A)	42	57.991***	42.42***	14.117***	12.796***	18857.096***	6685.300***	41.885***	83.596***	71.299***	71.859***	0.578***
Sorbitol (B)	2	2674.053***	3056.12***	310.140***	524.735***	907964.584***	81443.510***	849.035***	298.278***	1244.254***	435.198***	0.143***
AXB	84	11.442***	10.05***	2.543***	2.526***	4915.430***	425.929***	5.403***	12.391***	14.209***	3.687***	0.037***
Error	903	1.474	1.615	0.472	0.449	382.582	133.51	1.186	2.343	3.768	1.180	0.011
CV (%)		10.61	11.19	12.55	23.16	21.12	19.97	15.64	22.59	22.74	17.45	22.07
R^2		0.87	0.86	0.77	0.82	0.89	0.80	0.79	0.71	0.78	0.80	0.74

DF=degree of freedom; DRI=days to root initiation; DSI=days to shoot initiation; LN=leaf number (plantlet^{-1}); SH=shoot height (cm plantlet^{-1}); SFW=shoot fresh weight (mg plantlet^{-1}); FB=Fresh biomass (mg plantlet^{-1}); RFW=root fresh weight (mg plantlet^{-1}); RL=root length (cm plantlet^{-1}); RN=root number (plantlet^{-1}); SDW=shoot dry weight (mg plantlet^{-1}); RDW=root dry weight (mg plantlet^{-1}); R:S=root to shoot ratio; DW=dry weight; CV=coefficient of variation; R^2=coefficent of determination.

The pears coefficients of correlation among traits under control verses 0.2 M Sorbitol induced water stress are presented in Table 3. All osmotic stress related morphological traits measured under osmotic stress conditions were induced by 0.2 M Sorbitol positively and strongly correlated with each other. However, the highest positive correlation coefficient ($r=0.601^{***}$,0.614^{***}, 0.612^{***} and 0.614^{***}) were recorded between root fresh weight to leaf number, root fresh weight to shoot height, root fresh weight to shoot fresh weight and root fresh weight to root fresh weight, respectably. Root dry weight has also high correlation coefficient (0.621^{***} and 0.655^{***}) with shoot dry weight and root dry weight, respectively.

Mean distribution of osmotic stress related morphological traits under three different growing environments and the deviation of the genotypes trait from the population mean are indicated in Table 4. Considerable variability exists among the genotypes in all morphological evaluated parameters as was revealed by big differences between minimum and maximum values. For instance, under the non-stress environments, the genotypes were able to produce root in the range of 5 to 14 days and shoots in the range of 5 to 13 days. However, under the osmotic stress environments induced by 0.2 M Sorbitol the time taken for days to root initiation is in the range of 8 to 22 days and time for shoot initiation range from 8 to 25 days. On the contrary, leaf number, shoot height, root length, root number, shoot dry weight, and root dry weight were drastically reduced as the stress in the growing environment increased. The deviation of the traits from the population mean was also variable for non-stressed and stressed environments, indicating the variability of the genotypes in different traits under normal and moisture stress conditions. For instance, population mean value of leaf number was reduced from 6.3 to 4.5, shoot height from 4.1 cm to 1.6 cm, root length from 8.6 cm to 5.4 cm and root number from 11.1 to 5.4 as the concentration of sorbitol increased in the growing medium. Similarly, shoot dry weight were decreased from 4.9 (mg^{-1} plantlet) to 2.8 (mg^{-1} plantlet) as the stressed in the growing environment increased.

The dendrogram obtained from UPGMA cluster analysis of the genotypes on the basis of combined morphological osmotic stress related data classified the genotypes into three main clusters and one outlier genotype (Figure 1).

The outlier genotype Jarso is a local potato cultivar collected from farmer field. Among the three other clusters, cluster-I contained six potato genotypes (14% of the tested genotypes) all from CIP clones. Cluster-II contained 12 potato genotypes [(one local variety (Dadefa), two improved varieties (Zemen and Belete) and ten CIP clones. Cluster-III contained the largest proportion (53%) of potato genotypes (five local varieties, seven improved varieties and 12 CIP clones).

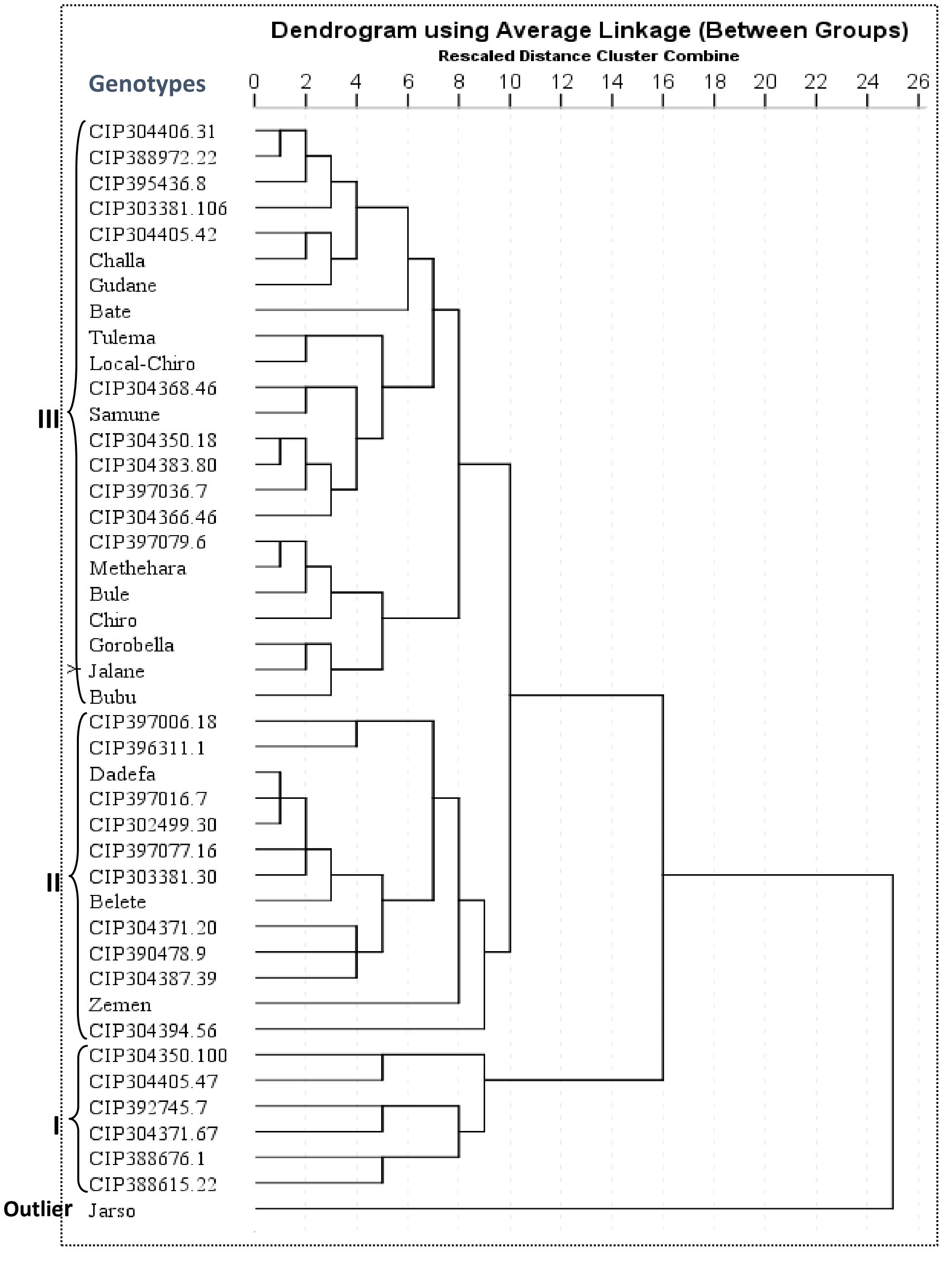

Figure 1. Dendrogram of 43 potato genotypes revealed by UPGMA cluster analysis based on morphological osmotic stress related data under moisture stress induced by 0.2 M Sorbitol *in vitro*.

Table 3. Pearson correlation coefficients between morphological traits under normal and 0.2M Sorbitol induced osmotic stress-environment of potato genotypes grown *in vitro*.

Treatments	Moisture stress induced by 0.2M Sorbitol									
	Traits	Leaf number	Shoot height	Shoot fresh weight	Root fresh weight	Root length	Root number	Shoot dry weight	Root dry weight	R:S (dry weight)
Control	Leaf number	0.407***	0.214**	0.367***	0.151ns	0.099ns	0.340***	0.398***	0.238**	-0.106ns
	Shoot height	0.294***	0.527***	0.329***	0.239**	0.218**	0.238**	0.299***	0.196*	-0.039ns
	Shoot fresh weight	0.200**	0.270***	0.401***	0.113ns	0.148ns	0.235**	0.340***	0.098ns	-0.140ns
	Root fresh weight	0.601***	0.614***	0.612***	0.614***	0.515***	0.545***	0.529***	0.581***	0.336***
	Root length	0.280***	0.182*	0.189*	0.300***	0.497***	0.202**	0.196*	0.371***	0.389***
	Root number	0.371***	0.328***	0.493***	0.245**	0.166*	0.522***	0.482***	0.238**	-0.100ns
	Shoot dry weight	0.245**	0.280***	0.429***	0.132ns	0.196*	0.317***	0.447***	0.167*	-0.145ns
	Root dry weight	0.573***	0.551***	0.599***	0.570***	0.550***	0.578***	0.621***	0.655***	0.397***
	R:S (fresh weight)	0.323***	0.303***	0.099ns	0.389***	0.386***	0.185*	0.079ns	0.373***	0.457***
	R:S (dry weight)	0.406***	0.387***	0.265***	0.491***	0.497***	0.339***	0.252**	0.532***	0.610***

****, **, * Significant at $P \leq 0.001$, 0.01 and 0.05, respectively; ns=non significant at $P \leq 0.05$; R:S=root to shoot ratio.*

Table 4. Mean, standard deviation of the mean±SD(m), range, F-test and variance (σ^2) of morphological growth traits of potato genotypes evaluated *in vitro* under different osmotic stress environments.

Traits	Control				0.1 M Sorbitol				0.2 M Sorbitol			
	Mean±SD	Range	σ^2	F-test	Mean±SD	Range	σ^2	F-test	Mean±SD	Range	σ^2	F-test
DRI	8.8±1.5	5.0 -14.0	1.32	***	11.1±2.17	7.0-24.0	3.14	***	14.4±2.8	8.0-22.0	4.22	***
DSI	8.6±1.3	5.0 -13.0	1.5	***	10.9±1.7	7.0-15.0	3.56	***	14.5±2.7	8.0-25.0	3.83	***
LN	6.3±0.8	4.17-8.8	0.79	***	5.7±0.1	2.0-8.7	1.79	***	4.5±1.4	1.0-7.6	1.52	***
SH	4.1±1.3	1.6-8.2	1.09	***	2.9±1.1	1.0-7.3	0.76	***	1.6±0.7	0.2-4.6	0.28	***
RL	8.6±1.9	2.9-12.7	2.74	***	6.9±1.7	1.2-12.6	2.38	***	5.4±1.6	0.5-9.6	0.98	***
RN	11.1±3.7	4.8-27.5	3.45	***	9.3±3.2	1.5-18.8	5.79	***	5.4±2.2	1.1-13.8	4.70	***
SDW	4.9±2.2	0.2-15.6	7.22	***	4.3±2.3	0.1-15.1	5.66	***	2.8±1.7	0.04-9.1	3.03	***
RDW	0.4±0.2	0.1-0.9	12.72	***	0.5±0.2	0.1-1.3	1.25	***	0.5±0.3	0.2-1.8	1.01	***
R:S	7.2±2.7	2.4-15.5	0.97	***	7.6±2.5	1.5-13.8	0.98	***	5.8±2.4	1.2-11.6	1.12	***

*DRI=days to root initiation; DSI=days to shoot initiation; LN=leaf number (plantlet^{-1}); SH=shoot height (cm plantlet^{-1}); RL=root length (cm plantlet^{-1}); RN=root number (plantlet^{-1}); SDW=shoot dry weight (mg plantlet^{-1}); RDW=root dry weight (mg plantlet^{-1}); R:S=root to shoot ratio; SD=standard deviation; σ^2-variance; ***-Significant at $P \leq 0.001$.*

4. Discussion

Water stress induced by Sorbitol decreased significantly affected the overall growth of plantlets *in vitro*, including the aerial part and roots (number, length, and weight) in this study. Significant difference observed among the genotypes for all measured morphological traits (Table 2 and 3), indicated the presence of high genetic variability in the genotypes. Morphological trait variations among potato genotypes evaluated *in vitro* for moisture stress tolerance have been also previously reported by different researchers. The differential response of potato cultivars to water stress indicates that there is genetic variability for osmotic stress tolerance among the potato genotypes (Levy, 1983). Similarly, Wishart *et al.* (2013) and Wishart *et al.* (2014) reported the presence of genotypic variation in drought tolerance among potatoes. Response of potato germplasm to drought stress was reported in some potato varieties (Arvin and Donnelly, 2008; Vasquez-Robinet *et al.*, 2008). Consistent with the results of this study, Gopal and Iwam (2007) revealed that addition of Sorbitol or Polyethylene glycol (PEG) to the MS medium decreased the water potential of the medium, inducing water-stress that adversely affected both shoot and root growth of plantlets. The authors further reported that all root and shoot characteristics of potato genotypes evaluated for drought tolerance using PEG were also adversely affected. Earlier research indicated that moisture stress inducing chemicals such as Sorbitol at higher concentrations could help to rapidly identify large number of potato genotypes for drought tolerance. According to Albiski *et al.* (2012), screening a large number of genotypes for drought tolerance in the field is difficult due to spatial heterogeneity of soil chemical and physical properties and seasonal fluctuations. *In vitro* screening of potato genotypes for water stress tolerance has been proposed as an alternative approach to costly, labor-intensive and sometimes problematic field-based screening (Rahman *et al.*, 2008). Gopal and Iwama (2007) reported that addition of Sorbitol to Murashige and Skoog medium decreased water potential, inducing drought stress affecting shoot and root growth.

The existence of a wide range between minimum and maximum values of each trait evaluated is an opportunity for potato breeders to identify traits of interest especially for abiotic stress tolerance through selection. UPGMA cluster analysis used also clearly depicts the genotypes into different drought tolerance categories. For instance, the outlier genotype was characterized by thin stem and weak vegetative growth and the most poorly performed in morphological growth parameters, took 14 and 13 days to root and shoot initiation, respectively under moisture stress conditions. The genotypes under cluster-I had the highest values of morphological traits measured; shortest days to root and shoot initiation and are the best performed genotypes under moisture stress conditions *in vitro*. These genotypes are also considered to be the most drought tolerant once *in vitro*. Genotypes under cluster-II had medium to height value in all measured morphological traits and are considered to be moderately tolerant to drought. Genotypes categorized under cluster -III had yielded lowest to medium values for morphological growth traits and extended time for days to root and shoot initiation next to the outlier and drought susceptible genotype Jarso.

5. Conclusions

In vitro screening of potato genotypes for osmotic stress or drought tolerance evaluated in the current study demonstrated that, increased osmotic stress level due to Sorbitol treatments result in reduced shoot and root related growth morphological traits and extended root and shoot initiation days. However, differential response of evaluated potato genotypes toward osmotic stress tolerance indicated that there could be the presence of potato genotypes tolerance to drought. Increased root related traits such as number, length, weight and root to shoot ratio were an important morphological traits contributed for drought tolerance mechanism of potato genotypes evaluated in this study.

On the other hand, water stress tolerance of potato genotypes can be evaluated *in vitro*, and screening growth parameterssing drought inducing chemicals such as Sorbitol at a given concentration. The *in vitro* method could also help to screen large number of plant genotypes within a short period of time and to identify promising genotypes for further field evaluation. The similarities in the effects of water-stress under *in vitro* and in vivo conditions suggested by Gopal and Iwam (2007) could seems that *in vitro* screening system is an alternative method to field evaluations for studying the general effect of water-stress on plant growth and development. However, the effectiveness of *in vitro* screening should be further tested under field conditions on promising potato genotypes for better root yield and quality production capacity under different moisture stress regimes.

6. Acknowledgments

The authors gratefully acknowledge potato breeding centers, CIP and farmers and who for their support to access potato genotypes. The authors would also like to acknowledge Deuscher Akademischer Austauschdienst (DAAD) for financial support and Institute of Horticultural Production Systems-Woody Plant and Propagation Physiology of the Leibniz University of Hannover for hosting to execute the experiment and providing laboratory facilities.

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10. Effect of Dormancy-breaking Methods on Seed Tuber Sprouting and Subsequent Tuber Yield of Potato *(Solanum tuberosum* L.*)* Cultivars

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Abstract: Potato is an important food and cash crop in Eastern Ethiopia. However, its productivity is low due to a number of constraints. Shortage of quality planting materials and poor tuber sprouting due to long dormancy period of improved varieties at a time of planting are among the factors known to affect production cycle and productivity of the crop in Easter Ethiopia. Two separate experiments were conducted from November 2013 to June 2014. The treatments in the first experiment consisted of two potato varieties (Bubu and Bate), three level of Gibberellic acid (GA3) (0, 10 and 20 ppm) and three level of storage conditions [(Diffused light store (DLS), pit and farm-yard manure heap)] aimed at assessing the effect of the treatments on seed tuber dormancy breakage. The experiment was laid out as a Randomized Complete design with four replications and conducted in the horticulture laboratory of Haramaya University. The second experiment consisted of the same treatments laid out in the field with the objective of studying the effects of the treatments on the subsequent growth, yield and yield related traits. The experiment was laid out in Randomized Complete Block Design with three replications, and conducted on a farmer's field. The results of the experiments showed that, genotypes, exogenous application of GA3 and storage conditions as well as the interaction between them significantly affected both seed tuber dormancy period, sprouting characteristics, and subsequent yield of tubers. Dormancy period, sprouting percent, sprout length, length of lateral axillary sprouts and sprout vigor were significantly affected by the treatments. However, parameters like days to 50% emergence, days to 50% flowering, number and weight of very small-sized and small-sized tubers showed highest values for seed tubers either treated with GA3 or not and stored under heap of farm yard manure (FYM) and pit storage conditions as compared to tuber treated and stored in DLS. In general, the study indicated that the interaction between genotypes, exogenous application of GA3 and storage conditions resulted in early dormancy termination, early emergence of shoots and high marketable tuber yield.

Keywords: Dormancy breaking; Gibberellic acid; Potato; Tuber yield.

1. Introduction

Potato (*Solanum tuberosum* L.) is the world's number one non-grain food commodity (Rykaczewska, 2013) and it is the third most important food crop in terms of consumption in the world after rice and wheat (Birch *et al*., 2012; Hancock *et al*., 2014). Potato is regarded as a high-potential food security crop because of its ability to provide a high yield of high-quality product per unit input with a shorter crop cycle (Adane *et al.,* 2010). It is a major part of the diet of half a billion consumers in the developing countries (Mondal, 2003). Potato is an important food and cash crop in Eastern and Central Africa, playing a major role in national food security and nutrition, poverty alleviation and income generation, and provides employment opportunities in the production, processing and marketing sub-sectors (Lung'aho *et al*., 2007).

Timely availability of well-sprouted seed potato tubers at the onset of rain is a pre-requisite for attaining high yield. In areas with a tradition of more than one production system and a bimodal rainfall pattern in the country, there is little time between growing seasons to permit adequate tuber sprouting of improved potato varieties released in Ethiopia. However, limited supply of high quality seed tubers is one of the major constraints to potato production in many developing countries, like Ethiopia (Gildemacher *et al*., 2009). One major problem contributing to regular supply of quality seed to year round production of potato by farmers is poor tuber sprouting techniques by farmers and prolonged tuber sprouting of many improved potato varieties in Ethiopia. For instance, most of the improved potato varieties released by Haramaya University in eastern Ethiopia have a dormancy period of more than two months (Helen, 2012). On the contrary, farmers' potato varieties have a short dormancy period (a month and little more than a month). However, local varieties are not recommended for production using rain fed agriculture due to the problem of late blight infestation. Hence this research aimed to study dormancy breaking methods using GA_3 under diffused light storage (DLS), heap of farm yard manure (FYM) and pit storage (PS) practices to enhance tuber sprouting and subsequent field evaluation for tuber growth and yield.

2. Materials and Methods

2.1. Description of the Study Sites

The tuber dormancy breaking experiment was conducted at Haramaya University Rare Research station and the field experiment was conducted at Rare Hora Seed Producer Cooperative (RHSPC) farmer field at Haramaya Woreda. Haramaya University Rare research station is located at 9 °29' N latitude, 42 °7' E longitudes and altitude of 2022 m.a.s.l. The mean annual rainfall is 700 mm and the average annual maximum and minimum temperature is 25.4 °C and 8.6 °C with mean relative humidity of 50%, but that varied from 25 to 85%. Rare Hora Seed Producer Cooperative farmer field is located in the vicinity of Haramaya University.

2.2. Description of Experimental Materials

One improved potato variety Bubu, released from Haramaya University and one local potato cultivar Bate grown by the farmers were used for the experiment. Bubu was released in 2011, having yield potential of 39.5 t ha^{-1}, an average dormancy period of 3 months, late blight resistant and has high tuber quality. Bate is considered as farmers' cultivar having yield potential of 35 to 39 t/ha under irrigation in the absence of late blight, an average dormancy period of 1.5 months, susceptible to late blight, short storability of tubers for ware potato, low dry matter content as well as low specific gravity.

For both dormancy breaking and field evaluation experiments, factorial arrangement of potato varieties (Bubu and Bate), storage conditions (DLS, FYM and PS) and GA3 concentrations (0,10 and 20 ppm) were combined having 18 treatment combinations. Tuber sprouting experiment was laid out in completely randomized design (CRD) with four replications while the field evaluation experiment was laid out in randomized complete block design (RCBD) with three replications.

2.3. Experimental Procedures

Medium sized seed tubers of the two potato varieties (Bubu and Bate) seed tubers were used to conduct the experiments. The seed tubers were washed with distilled water to remove dirty materials before treating with GA_3. The seed tubers were grouped into three replicates, each having equal number, and treated with GA_3 concentration (0, 10 and 20 ppm) by keeping in the solution for 20 minutes, taken out from the solution, drained and dried for 10 minutes. The seed tubers with 0 ppm GA_3 treatment was treated with distilled water only for 20 minutes.

From each GA_3 concentration treated tubers, 30 seed tubers for each treatment combination in four replicates were weighed and put in 0.5 x 0.4 mm nylon woven mesh bags and stored on DLS bed, FYM and PS for dormancy breaking experiment. Similarly, 90 seed tubers for each treatment combination in three replicates were put in 0.5 x 0.4 mm nylon woven mesh bags and stored on DLS bed, FYM and PS for field experiment. Seed tubers that were used for dormancy breaking and field experiment were kept randomly in the same DLS, FYM and PS. For this purpose a total of six pits, each 1x1x1 m^3 area were dug, layered with maize straws and seed tuner were kept at the bottom of the pits. After placing the seed tubers, the pits were covered with a layer of soil 30 cm deep above the surface. Six a 1 x 1 m area which were 50 cm deep was dug, seed tubers placed in it and then after the area was covered with 50 cm thick heap of FYM above the surface. Similarly, treated seed tubers were randomly placed on six seed tuber beds in DLS. The seeds tubers were kept in potato tuber seeds storage house of Haramaya University, which was constructed and used for decades for the purpose. The pits and heap of farmyard manure storage were prepared around the DLS of Haramaya University.

2.4. Field Experiment

Land for potato planting was prepared as per the recommended practices. The seed tubers treated with different concentration of GA_3 and stored in DLS, FYM and PS were planted at a spacing of 75 x 30 cm in RCBD. For the field experiment, each treatment was assigned to one plot in each replication. Six rows with 12 plants were planted in each plot. Each plot was 3.60 x 4.50 m (16.2 m^2 area), which accommodated 72 plants per plot. The spacing between plots and adjacent blocks was 1m and 1.5m, respectively. Data were collected from the middle rows of each plot leaving one plant at both end of each row, to avoid border effects. As a crop protection measure Ridomil® MZ 68% WP was sprayed twice at a rate of 2 kg/ha before the occurrence of late blight to control the disease. Other cultural practices such as cultivation, pest and disease control, fertilization, weeding and irrigation were done according to the local recommendation.

2.5. Data Collection and Analysis

2.5.1. Dormancy breaking and sprouting parameters

Dormancy period: was counted as the number of days from dehaulming (haulm cutting) to sprouting of 80% of the total tubers with at least one sprout longer than 2 mm; sprouting (%): was calculated as the percentage of the number of sprouted tubers in the sample; number of sprouts per tuber; sprout length (mm); length of lateral axillary sprouts (mm); sprout thickness (mm) and sprout vigor. Sprout vigor score were evaluated based on the thickness of the base of the sprout and sprout length. The evaluation were based on a 5-point rating scale, where 1 = very low vigor (where more than half of the tubers in a sample had sprouts of $\leq$ 1 mm thick and a length of $\leq$ 3 mm), 2 = low vigor (where more than half of the tubers in a sample had sprouts of $\leq$ 2 mm thick and a length of $\leq$ 4 mm), 3 = good vigor (where more than half of the tubers in a sample had sprouts of $\leq$ 4 mm thick and a length of $\leq$ 4 mm), 4 = high vigor (where more than half of the tubers in a sample had sprouts of $\leq$ 4 mm thick and a length of $\geq$ 4 mm but were not firm and had not acquired the green colorations) and 5 =very high vigor (as described for score 4 but have acquired the green coloration, were firm and have no visible defects (Shibario *et al.*, 2006).

2.5.2. Growth, yield and yield components

Growth and phenological parameters data were collected for days to 50% flowering and maturity; number of main stem per hill and leaf area index (LAI). Leaf area index was determined by dividing the total leaf area of a plant by the ground area covered by a plant. Log 10 (leaf area in cm^2) = 2.06 x log10 (leaf length in cm) – 0.458.

Shoot dry mass weight (g): The shoot dry mass was measured from plants grown in five randomly selected/hills from the central rows of each experimental plot. The dry mass was obtained after drying the samples in open sun for 8 days and after oven drying at 72 °C for 24 hours to a constant mass.

Underground dry mass (g):underground parts of the plants in five randomly taken /hills (root, stolon, and parts of the stem remaining underground) were measured, and the dry weight was obtained after, open sun drying the samples for eight days and further oven-drying at 72 °C for 24 hours to constant mass.

Average tuber number per hill and tuber weight (g/tuber) and marketable tuber yield (t ha^{-1}): weredetermined from the weight of tubers obtained from the net plot after the sorting tubers and count number of tubers which were free from diseases, insect pests and greater than or equal to 20 g and weighted tubers per hill which and calculated as ton/marketable tuber yield (t ha^{-1}).

2.5.3. Statistical analysis

The data were subjected to analysis of variance using Gen Stat, 13th Edition (VSN Ltd, Oxford UK) statistical software package. Least significant difference (LSD) test at 5% probability was used to separate means when the analysis of variance indicated the presence of significant differences among treatments.

4. Results and Discussion

4.1. Effect of Dormancy Breaking Methods on Seed Tuber Sprouting

4.1.1. Dormancy period

Cultivar, GA_3 and storage condition interacted to reduce dormancy period significantly to 31 days for local variety and 36.5 days for improved variety as compared to GA_3 untreated tubers stored at 102.5 (Table 1). According to the result, increasing concentration GA_3 to 20 ppm reduced dormancy period for both cultivars stored in the three storage conditions. This reduction of dormancy period especially under pit and farm-yard manure heap may be due to the interaction of high temperature and low light intensity with exogenous application of GA_3.

Tubers of the Bubu variety experienced the shortest dormancy period in response to the application of 20 ppm GA_3 and storing under FYM. Thus, compared to tubers that were stored in the DLS without treating with GA_3, Bubu tubers treated with 20 ppm GA_3 and stored under FYM had their dormancy period shortened by more than three-fold. Similarly, tubers of Bate treated the same way had their tuber dormancy shortened by about half (Table 1). In general, the results of this study showed that tuber dormancy was significantly shortened with increase in the rate of GA_3 and storage under FYM and Pit.

Faster dormancy breaking and promotion of sprouting in potato tubers under dry heap of animal dung, pit and dark conditions was reported previously (Hunt, 1982; Bencini, 1991; MoA and GTZ, 1998; Shibairo *et al.*, 2006). It was reported that the use of different concentrations of GA_3 under pit and DLS induce dormancy breakage and promote sprouting of seed potato tubers (Suttle, 1996; Shibairo *et al.,* 2006). However, dormancy period for potato cultivars varieties due to the genetic nature of the cultivars as reported by Fuglie (2007). Hence, applications of different dormancy breaking techniques are an important approach to fit potato varieties into cropping calendars of farmers. The present research results are also in agreement with the findings of other researchers (Van Ittersum and Scholte, 1993) who reported that, the interaction of different concentration of GA_3 with different storage condition significantly reduced tuber dormancy period of different genotypes. Dormancy termination occurred much earlier when stored in pit than in DLS (Shibairo *et al.,* 2006). Faster dormancy breakage under pit conditions is due to buildup of high heat and low light penetration (Bencini, 1991). Abebe (2010) showed that, application of GA_3 with high concentration of 40-50 ppm significantly reduced tuber dormancy period. Lim *et al*. (2004) reported that minitubers derived from GA_3 sprayed treatment shortened the tuber dormancy period by 5-80% compared to the untreated control. Application of GA_3 at a later stage may be used to break tuber dormancy for early planting (van Ittersum and Scholet, 1993) without affecting tuber production in the next generation. Allen *et al*. (1992) reported that in case of potatoes, exogenous application of GA_3 effectively terminates tuber dormancy in most cultivars and is used in seed certification trials where sprouting of immature tubers is essential for latent disease evaluation.

Table 4. Interaction effect of GA_3, storage condition and potato cultivars on dormancy period, sprouting percentage and sprout vigor of potato cultivars.

Character		Dormancy period (days)			Sprouting percentage			Sprout vigour (score)		
Storage										
Cultivar	GA_3(ppm)	DLS	PS	FYM	DLS	PS	FYM	DLS	PS	FYM
	0	102.50a	48.25e	46.75ef	91.67d	90.83d	92.50d	2.59gh	2.71g	2.03i
Bubu	10	83.00b	43.00g	41.00gh	99.17ab	95.84c	98.34ab	3.51c	3.24def	3.33cde
	20	70.50c	39.50h	36.50i	100a	99.17ab	100a	4.50a	3.92b	3.96b
	0	54.25d	35.00ij	33.25jk	91.67d	85.00e	95.84c	2.37h	1.75j	1.34k
Bate	10	46.00f	34.00jk	32.00kl	95.84c	97.50bc	99.17ab	3.05f	2.51gh	2.00i
	20	39.50h	31.00l	31.00l	100a	100a	100a	3.36cd	3.17ef	2.54gh
LSD (5%)			2.22			2.32			0.25	

Means in rows and columns in each character with similar letter(s) are not significantly different, DLS=Diffused light store, PS= Pit storage, FYM= Farm-yard manure, LSD (5%) = Least significant difference at 5% probability level, CV (%) = Coefficient of variation in percent.

4.1.2. Sprouting percentage

Sprouting of tubers of all varieties generally increased across the increasing levels of GA_3 under all storage conditions. Significantly highest sprouting (100%) of both potato cultivars was observed at the application of 20ppm GA_3 under all storage conditions (Table 1). The lowest sprouting percentage (85%) was obtained for Bate cultivar in non-GA_3 treated tubers stored under pit, which was lower by 15% sprouting than tubers treated with GA_3 and stored in all storage conditions. Bubu variety without GA_3 treatment and stored under all storage conditions exhibited lower sprouting percentage as compared to the higher dose of GA_3 treated tubers. This may be due the lower response of the variety to different storage temperatures and exogenous application of GA_3.

Sprouting is associated with many physiological changes, including the conversion of starch to sugars, respiration, water loss, and glycoalkaloid content (Burton, 1989). Similarly, the physiological age of the tuber and post-harvest storage conditions has a great effect on the pattern of sprout growth but the basis is genetic factor (Carli *et al.,* 2012). In turn, the physiological age of the tuber is greatly influenced by growing and storage conditions, and length of storage period. Seeds, which have high sprouting capacity, produce more sprouts and therefore produce more stems (Kustiati *et al*., 2005). Increasing GA_3 concentrations increased sprouting (%), number of sprouts per tuber, and sprout length and vigor score (Gachango, 2006). The result of the current experiment is in agreement with the findings of other authors (Van Ittersum and Scholet, 1993; Shibairo *et al.,* 2006 and Menza *et al*., 2008). Shibairo *et al.* (2006) reported the presence of significant sprouting different among genotypes and GA_3 application both under DLS and pit storage condition.

4.1.3. Sprout vigour

Tubers of the potato cultivars treated with 20 ppm GA_3 and stored under DLS had recorded the highest sprout vigour of 4.5. On the other hand, both cultivars tuber without treating with GA_3 and stored under all storage conditions exhibited lowest sprout vigour. However, Bubu variety without GA_3 application stored under FYM significantly resulted in lowest sprout vigour of 1.34 (Table 1). High vigour score under DLS for both cultivars treated or untreated with GA_3 could be due to long storage time with an ambient temperature as compared to pit and FYM storage conditions. In line with the current observation, seed potatoes stored in diffuse light gave a more sprout vigor than seed stored for relatively long periods in the dark at higher temperatures (FAO, 2009). Similar results showed that increase in dark pre-storage treatment duration followed with storage under DLS led to increase in sprout vigor (Menza *et al*., 2008). Storage of seed potato tubers under dark conditions leads to weak and poor vigor sprouts (CIP, 1983; Crissman *et* al., 1993). A linear increase in sprout vigor of potato was observed by increasing concentration GA_3 both under DLS and pit storage condition (Shibairo *et al.,* 2006). These authors also observed difference in sprout vigor score among genotype treated with GA_3 and stored in DLS and pit storage conditions due to difference in internal factors like endogenous gibberellins, starch content, glucose and sensitivity of genotype to the exogenous application of GA_3. A significant interaction among genotype, GA_3 and different storage temperatures regimes on sprout vigour score was observed by Van Ittersum and Scholte, (1993a).

4.1.4. Length of lateral axillaries sprouts

The longest lateral axillaries sprout was recorded from Bubu variety tubers treated with 20 ppm (5.71mm) and 10 ppm (5.67mm) GA_3 and stored under FYM heap storage. Similarly, tubers of the Bate cultivar treated with 20 ppm GA_3 and stored under FYM heap produced longer lateral axillaries sprout (4mm) than untreated tubers and stored under all storage (Table 2). Generally, Bubu variety tubers produced longer lateral axillaries sprout than Bate cultivar in all treatment combinations. This may be due to interaction between genetic makeup of cultivars as well as difference in physiological aging among cultivars and response to exogenous application of GA_3 and storage temperatures.

The present result is consistent with the report of other researchers (Shibairo *et al.*, 2006; Carli *et al.*, 2012). Carli *et al.* (2012) who observed that genotype with long dormancy periods shows long multiple sprouting compared to genotype with short dormancy periods. These authors also indicated that the length of the longest lateral axillaries sprout among cultivars depended on number of sprout per tuber and length of longest sprout. In this experiment, the longest sprout and more number of sprouts per tuber were observed for Bate tubers treated with GA_3 and stored under farm yard heap. Similarly, Alexander (2004) stated that physiologically older seed tubers ("middle-aged") are characterized by the loss of apical dominance between eyes, i.e., effective IAA levels in tubers are decreased. This develops gradually in time but the time can be shortened by heat or the dominance can be disrupted by cutting the tuber and thereby breaking the translocation path of IAA in the tuber. Sprouts of physiologically old seed tubers are long and branched (Iritani *et al.,* 1983). Shibairo *et al.* (2006) suggested that application of exogenous GA_3 at a concentration of 10 mg^{-kg} and 20 mg^{-kg} will break apical dominance of seed tuber that is common to pit storage. Similarly, Dani *et al.* (2012) observed difference on length of lateral axillaries sprout between Nicola and Désirée cultivars stored at 20 °C and 6 °C for 45 and 60 days, respectively.

Table 5. Interaction effect of GA_3, storage condition and potato cultivars on length of lateral axillaries sprout and sprout length of potato cultivars.

Character	Length of lateral axillaries sprout (mm)				Sprout length (mm)		
	Storage						
		DLS	PS	FYM	DLS	PS	FYM
Cultivar	GA_3(ppm)						
Bubu	0	2.40k	3.86ef	4.19cd	2.92m	4.64k	5.84i
	10	2.89j	4.36c	5.67a	4.17l	6.48h	9.18d
	20	3.23hi	4.85b	5.71a	5.28j	8.75e	10.67b
Bate	0	3.12ij	3.35ghi	3.49gh	4.14l	5.31j	7.08g
	10	3.65fg	3.53gh	3.98de	5.97i	8.14f	9.16de
	20	3.88ef	3.64fg	4.00de	8.78de	10.30c	14.19a
LSD (5%)			0.31			0.33	
CV (%)			5.60			3.20	

Means in rows and columns in each character with similar letter(s) are not significantly different, DLS= Diffused light store, PS= Pit storage, FYM= Farm-yard manure, LSD (5%) = Least significant difference at 5% probability level, CV (%) = Coefficient of variation in percent.

4.1.5. Sprout length

The longest sprout length of 14.9 mm was recorded for Bate cultivar followed by Bubu variety, which resulted in sprout length of 10.67 mm when tubers treated with 20 ppm GA_3 and stored under FYM heap. Shortest sprout length for both cultivars recorded at tubers treated with10 ppm GA_3 and stored in DLS and pit (Table 2).

According to Mehta *et al.* (2010), potato seed tuber stored under traditional storage method like pit and heap of dry animal dung shows short dormancy period and long sprout as compared to improved storage system. Any traditional pre-sprouting treatment like pit storage, leaving tuber in field and storing in plastic sack in Ethiopia results in longest sprout length and more number of sprout per tuber due to high heat temperature in the storage system (Ayalew *et al.*, 2014). Temperature and exogenous application of GA_3 on potato seed tuber immediately after harvest influenced dormancy period and produce long etiolated sprout length (Hemberg, 1985; Burton, 1989). A relatively significant difference between cultivars were found by Carli *et al.* (2012) that indicates clones with shorter dormancy often show a greater length of their longest sprout. The results of the current experiment is also consistent with the report of Lommen and Struik (1992a) who found significant interaction between cultivars and exogenous application of GA_3 on sprout length under different storage condition. According to the authors, the effects of exogenous application of GA_3 on sprout growth depends on the internal gibberellins contents among variety and storage temperatures. Similarly, Gachango (2008) observed longest sprout length for GA_3 treated tuber stored under pit and black polyethylene bag in dark condition compared to DLS storage condition.

4.1.6. Number of sprouts per tuber

Significantly highest number of sprouts per tuber (6.57) was recorded at the treatment combination of 20 ppm GA_3 and FYM heap storage. On the other hand, interaction effect of variety by GA_3 indicated highest number of sprouts (6.99) per tuber for variety Bate at 20 ppm GA_3 and FYM heap storage. Interaction of GA_3 and storage condition indicated that, the lowest sprouts number of 3.43 and 3.57 per tuber was observed in GA_3 untreated tuber and stored in DLS and pit storages, respectively with no significant difference between the two values. On the other hand, the interaction effect of variety by GA_3 indicated that, Bubu variety tubers produce lower number of 3.49 sprouts per tuber when treated with 0 ppm GA_3 while the highest number of 6.99 sprouts per tuber obtained from Bate variety at 20 ppm GA_3 application. Cultivar by storage condition was also significantly affected number of sprouts per tuber. Highest number of 6.24 sprouts per tuber was recorded at the interaction of variety Bate by FYM heap storage condition while the lowest value of 4.18 and 4.12, number of sprouts per tuber was recorded from variety Bubu respectively when stored in DLS and pit, respectively with no significant difference between the two values (Figure 1). Generally, tuber treated with higher followed by lower concentration of GA_3 and stored under farm-yard manure heap followed by pit storage produced higher number of sprouts per tuber. This could be due to varietal difference in response to storage temperature and light.

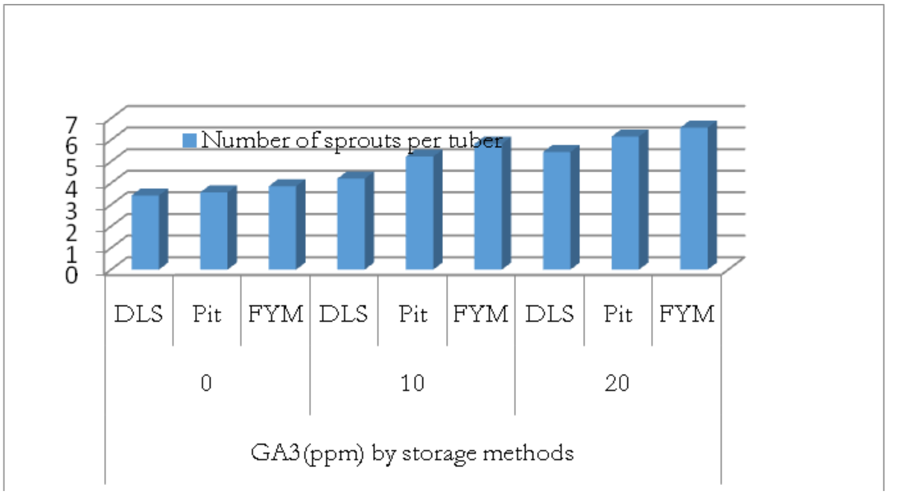

Figure 1. Number of sprouts per tuber.

Previous study indicated that, number of sprout per tuber depends on genetic factors, physiological age of seed tubers, tuber weight class or size, and storage temperature and storage duration (Andre *et al*., 2007). This author pointed that, tuber stored at high temperature in dark condition for long period produce more number of sprouts per tuber than tuber stored for short period in the same condition. On the other hand, Carli *et al*. (2012) observed that under traditional storage system tuber with short dormancy period shows more number of sprouts per tuber than tuber with long dormancy period. Similarly, Ayelew *et al*. (2014) and Agajie *et al*. (2007) reported that different traditional seed tuber storing techniques in Ethiopia like placing in bags, warm place in the house, in pit covering with soil, covering the tubers with crop residues or dry animal dung, spreading on the sun, mixing with ash and putting in bags and spreading on the ground. These methods of potato tuber storage have been used in Ethiopia to induce early tuber sprouting. They also reported that due to high temperature, all traditional storage facilities lead to faster dormancy break and many sprout than improved storage facility. Many authors have indicated that temperature promotes enzyme activity in a potato tuber, accelerates eye sprouting, shortens sprouting period and speeds up plant development (Scholte, 1986; Allen *et al*., 1992; Struik *et al*., 1999; Struik *et al*., 2006; Eremeev *et al*., 2008). Diffuse light may prevent rapid ageing of seed tubers as compared with improved pit storage (Virtanen *et al*., 2013).

GA_3 application as liquid solutions accelerates eye growth via sprout emergence and produces more slim accessory shoots (Rehman *et al*., 2003). It was also reported that using 1% thiourea and 5 ppm application of GA_3 is more efficient in increasing sprout number and length in five varieties of normal tubers of potato, than control treatments. Mani *et al*. (2014) reported that, increasing GA_3 concentration from 0, 5, 10, 20, 40 and 60 ppm increased number of sprout per tuber indicating that GA_3 has the potential to relieve dormancy and improve sprouting of many potato cultivars. The only restrain for GA_3 application is its efficient penetration into the internal tissues of tuber (Otroshy and Struik, 2006). Since the tuber skin is a main hindrance for chemical permeation, it is advisable to apply these chemicals on tuber slices and or tubers with damaged or cracked skin (for example on newly harvested tuber) (Shekari *et al.,* 2010). The duration of apical dominance as well as the number of sprout per tuber both under traditional cellar storage system and improved DLS storage is a varietal characteristic (Carli *et al*., 2012). The present results was also in agreement with many authors (Bodlaender and Marinus 1987; Jenkins *et al*., 1993) who observed significance difference on number of sprout per tuber between two cultivars stored at higher temperature for long period as compared to short storage duration of at the same temperature. Van Ittersum (1992) found significant interaction between variety and storage temperatures and suggested that high temperatures create favorable condition to endogenous gibberellins synthesis and starch can break down to sucrose and then all eyes on tuber start to produce sprouts. Also in line with the current investigation, Van Ittersum and Scholte (1993b) reported that sprout number of tubers from plants sprayed with a high GA_3 concentration was significantly higher than that of tubers from control plants. Similarly, Alexopoulos *et al.* (2008) found that irrespective of the concentration, GA_3 treatments (1, 5, 10 and 50 ppm) significantly increased the number of sprouting buds per tuber compared to the control. Shibairo *et al.* (2006) indicated that increasing concentration of GA_3 increased number of sprout per tuber under pit storage condition as compared to DLS.

Table 3. Interaction effect of cultivar and GA_3, cultivar and storage condition and GA_3 and storage condition on number of sprouts per tuber of potato cultivars.

Number of sprouts per tuber			
		Storage	
GA_3(ppm)	Diffused light store	Pit storage	Farm-yard manure
0	3.43g	3.57g	3.86f
10	4.21e	5.23d	5.82c
20	5.44d	6.16b	6.57a
LSD (5%)		0.21	
		GA_3 (ppm)	
Variety	0	10	20
Bubu	3.49f	4.28d	5.12c
Bate	3.75e	5.89b	6.99a
LSD (5%)		0.17	
		Storage	
Variety	Diffused light store	Pit storage	Farm-yard manure
Bubu	4.18d	4.12d	4.59c
Bate	4.54c	5.85b	6.24a
LSD (5%)		0.17	
CV (%)		4.20	

Means in rows and columns in each interaction effect with similar letter(s) are not significantly different. LSD (5%) = Least significant difference at 5% probability level, CV (%) = Coefficient of variation in percent.

4.1.7. Sprout thickness

Thicker sprouts of 2.94mm and 2.67mm were produced from tubers of Bubu variety treated with 20 and 10 ppm GA_3, respectively (Table 4). In case of the interaction effects of GA_3 and storage, thicker sprouts of 3.55 mm was observed from tubers treated with 20 ppm GA_3 treated tubers stored in DLS while the thinner sprout of 1.55mm was observed in non-GA_3 treated tubers and stored under FYM heap. Difference in sprout thickness between two cultivars in response to storage condition and external GA_3 application may be due to difference in internal contents of starch and other nutrients between the two cultivars.

Table 4. Interaction effect of GA_3 and storage condition and cultivar and GA_3 on sprout thickness (mm) of potato cultivars.

	Sprout thickness (mm)				
GA_3(ppm)	Diffused light	Pit	Farm-yard manure	Bate	Bubu
0	2.40d	1.73g	1.55h	1.55d	2.24c
10	3.34b	2.21e	1.91f	2.29c	2.67b
20	3.55a	2.69c	2.13e	2.64b	2.94a
LSD (5%)		0.17		0.14	
CV (%)		7.00		7.00	

Means in rows and columns in each interaction effect with similar letter(s) are not significantly different. LSD (5%) = Least significant difference at 5% probability level, CV (%) = Coefficient of variation in percent.

Potato seed tuber treated with GA_3 effectively terminates dormancy and results in sprouts having short, thick, robust, and resistant to breakage (Suttle, 2008). Moreover, Struik *et al* (2006) indicated that, high concentration of GA_3 was able to break down the internal starch to glucose due to high respirations caused by external gibberellins that in turn lead to faster sprouting and thick sprout. Additionally, some potato varieties reacted strongly against gibberellic acid treatment. Also treating tubers with gibberellic acid in DLS cause to sprouts increasingly elongations, which lead to creating thicker, strong and unbreakable sprouts (Salimi *et al.*, 2010; Mohammadi *et al.*, 2014). Treating potato tubers with high temperature in dark storage duration induces excessive sprout elongation resulting in sprouts that are thin, fragile, and prone to breakage during handling (Shekari *et al.,* 2010). Salimi *et al.* (2010) also reported difference on sprout thickness on potato tubers treated with GA_3 and Carbon disulphide at different storage duration, which are in agreement with the present research finding. Formation of thicker sprouts can be induced when higher concentrations were applied, especially with longer duration of exposure. They suggested that, in addition to GA_3 and Carbon disulphide application thicker sprout was due to internal nutrient contents. Similarly, Struik (2007) found positive correlation between internal contents of potato tuber and sprout thickness. Gudeva *et al.* (2012) observed thicker sprout on two potato variety treated by GA_3 and stored in DLS condition for long duration. These authors concluded that the more storage durations more nutrients could move to sprout and create high vigor and thicker sprout on tuber.

4. 2. Growth and Phenological Parameters

4.2.1. Days to 50% physiological maturity

Days to 50% physiological maturity was reduced by about 16.8% for Bubu variety when treated with 20 ppm GA_3 and stored under DLS storage as compared to Bate variety which took longest days of 103 to 50% physiological maturities at 0 ppm GA_3 application and stored under DLS (Table 5). Hence, high concentration of GA_3 application combined with DLS resulted in shortest period to reach 50% physiological maturity for both cultivars even though the two cultivars response is significantly different. This might be due to the inherent characteristics of genotypes and the combined application of storage duration and storage temperatures with the exogenous concentrations of GA_3 on the physiological aging of seed tuber. This result is supported by the previous findings that showed significant difference in days to 50% physiological maturity between cultivars (Burton *et al.*, 1992; Yibekal, 1998; Lim *et al.*, 2008; Helen, 2012). In agreement with the present research results, Van Ittersum, (1993) also indicated that a combination of GA_3 and storage condition at room temperature for long durations shortened days to physiological maturity, whereas the same treatment combination for short storage durations prolonged days to physiological maturity. This supports the suggestion that for cultivars with a rapid rate of physiological ageing, the storage period at room temperature should be more limited (Van Ittersum *et al.*, 1990). Moreover, Menzel (1983) and Van der Zaag *et al.* (1990) reported that a rapid onset of senescence in normal growing potato plants could be due to high concentration of GA_3 in response to higher temperature, which in turn favors rapid growth and development and shortens the growing period. In addition, Lim *et al.* (2004) examined that leaves from GA_3 sprayed potato plants showed pale- green color and senescence early than the untreated controls.

Table 5. Interaction effect of GA_3, storage condition and potato cultivars on leaf area index and days to 50% physiological maturity.

Character		Leaf area index			Days to 50% physiological maturity		
Storage		DLS	PS	FYM	DLS	PS	FYM
Variety	GA_3 (ppm)						
	0	2.27f	2.23g	2.23g	96.00e	97.70d	97.70d
Bubu	10	2.50b	2.30ef	2.30ef	90.30j	94.30g	94.30g
	20	2.63a	2.36d	2.36d	85.70m	89.70l	90.00jk
	0	2.18h	2.18h	2.18h	103.00a	101.70b	101.70b
Bate	10	2.30e	2.21g	2.20gh	95.30f	99.00c	99.00c
	20	2.38c	2.30ef	2.30ef	91.00i	94.00h	94.00h
LSD (5%)		0.03			0.92		
CV (%)		0.70			0.60		

Means in rows and columns in each character with similar letter(s) are not significantly different, DLS= Diffused light store, PS= Pit storage, FYM= Farm-yard manure, LSD (5%) = Least significant difference at 5% probability level, CV (%) = Coefficient of variation in percent.

4.2.2. Yield and yield components

4.2.2.1. Shoot dry mass

The highest shoot dry mass was recorded for Bubu variety treated with 20 ppm GA_3 and the same variety stored in DLS storage condition with no significant difference between the two interaction effects. The lowest shoot dry mass was recorded for non-GA_3 treated Bate cultivars closely followed by the same cultivars stored under the three storage conditions with no significant difference between the three storage conditions (Table 6). Generally, the highest shoot dry mass was recorded for tubers treated with 20 ppm GA_3 and tubers stored in DLS. This might be attributed to the combined application of exogenous GA_3 with longer storage duration in DLS as well as the higher number of stem and leaves associated at this treatment combination. In connection to this, Iritani (1968) and Struik and Wiersema (1999) reported that storing seed tubers at an ordinary temperatures for longer duration may lead to increased physiological age of the tubers with a resultant increase in stem numbers and leave number which in turn increase haulm dry mass. On the other hand, Faten *et al.* (2008) reported that increasing foliar application of GA_3 resulted in highest above ground dry biomass yield. Reports of Ewing (1997) and Vander Zaag and Van Loon (1987) indicted that planting GA_3 treated and physiologically older seed tubers results in smaller plants with more stems and promotes highest shoot dry mass. In accordance with the current results, other authors Shashirekha *et al.* (1991) reported that interaction of exogenous GA_3 and storage condition along with storage durations significantly influenced above ground dry biomass yield.

Table 6. Interaction effect of cultivar and storage condition and cultivar and GA_3 on shoot dry mass.

Shoot dry mass (g)		
	Cultivar	
GA_3(ppm)	Bubu	Bate
0	248.10c	55.70e
10	251.60b	56.90d
20	253.20a	56.90d
LSD (5%)	0.32	
CV (%)	0.20	
Storage	Bubu	Bate
Diffuse light storage	251.60a	56.60c
Pit storage	250.60b	56.40c
Farm yard manure storage	250.70b	56.50c
LSD (5%)	0.32	
CV (%)	0.20	

Means in rows and columns in each interaction effect with similar letter(s) are not significantly different. LSD (5%) = List significant difference at 5 percent and CV (%) = Coefficient of variation percent.

4.2.2.2. Underground dry mass

The highest undergrounddry mass was recorded for Bubu variety treated with 20 ppm GA_3 and for 20 ppm GA_3treated tubers stored in DLS storage condition. The lowest undergrounddry mass was recorded for non-GA_3 treated Bate cultivar and for non-GA_3treated tubers stored under pit and FYM heap storage conditions with no significant difference between the two storage conditions (Table 7). Difference among two cultivars in undergrounddry mass may be attributed to the proportionally lower tuber yield of the local cultivars as compared to improved variety as well as difference in response to exogenous GA_3 and storage conditions. Virtanen *et al.* (2013) found that the fresh weight and dry weight of roots and stolons were more in GA_3 treated plants than untreated. Kumar *et al.* (2003) found significant increase in undergrounddry mass yield with the increase in GA_3 concentration and prolonged storage duration partly attributed to the positive effects on root growth of potato tubers. Alemu *et al.* (2011) indicated that storage condition and duration significantly influenced undergrounddry biomass yield among two cultivars at Holeta. The present research results are in agreement with Kustiati *et al.* (2005) who reported that external GA_3 and different storage durations of potato seed tuber enhanced undergrounddry mass and stolon number per plant as well as the dry weight of whole plant organs. They further indicated that increase in undergrounddry mass in response to increasing rate of external GA_3 and long storage duration may attributed to faster physiological aging of long stored seed tubers and external GA_3 experience particularly break down of endogenous GA, which probably resulting in much higher rate of underground dry biomass yield.

Table 7. Interaction effects of cultivar and GA_3 and GA_3 and storage condition on undergrounddry mass.

Undergrounddry mass (g)					
	Storage condition			Cultivar	
GA_3 (ppm)	DLS	PS	FYM	Bubu	Bate
0	245.60d	244.00e	244.00e	319.60c	169.60f
10	252.20bc	248.40c	248.40c	323.30b	176.00e
20	255.60a	252.80b	252.80b	326.70a	180.80d
LSD (5%)	0.90			0.70	
CV (%)	0.30			0.30	

Means in rows and columns in each interaction effect with similar letter(s) are not significantly different. LSD (5%) = List significant difference at 5 percent and CV (%) = Coefficient of variation percent.

4.2.2.3. Average tuber weight

Bubu variety treated with 20 ppm GA_3 and 20 ppm GA_3 treated tubers stored in DLS had significantly higher average tuber weight with no significance different between the two. The lowest average tuber weight was registered for non-GA_3 treated Bate cultivars and the non-GA_3 treated tubers stored under pit and FYM heap storage conditions with no significant difference between the latter two treatments (Table 8). The observed average tuber weight increase may be attributed to an increase in photosynthetic area in response to GA_3 treatment and DLS storage condition that maximized the rate of assimilates production. Storage in DLS has been shown to delay the physiological ageing of the tubers and to reduce apical dominance resulting in more, short and firm sprouts per tuber (CIP, 1985). This translates into more stems and hence more yields since potato is a specialized underground stem. In addition, there are less storage losses from pests and diseases as compared to traditional storage methods because the crop can be easily monitored. Cutting seed tubers breaks dormancy and apical dominance and promotes an increase in stem and tuber number, and seed yield when compared with whole seed planted at the same rate (Caldiz, 1996). This is believed to be due to the production of endogenous "wound gibberellins" (Rappaport and Sachs, 1967). Similar with the current findings, Reeve *et al.* (1973) reported that tuber weight is affected by variety, storage and growth conditions such as environmental factors, which favored cell division and cell expansion, mineral nutrition uptake, optimum water supply etc. which enhance tuber size. The authors further described that the growth of tuber tissue is the function of both cell division and expansion.

Table 8. Interaction effect of GA_3 and storage condition and cultivar and GA_3 on average tuber weight.

Interaction effects	GA_3 and storage condition			cultivar and GA_3	
	Storage condition			Cultivar	
GA_3 (ppm)	DLS	PS	FYM	Bubu	Bate
0	60.50bc	57.80d	57.80d	60.00c	57.20f
10	61.10b	59.90c	59.60c	62.00b	58.3e
20	62.50a	61.20b	60.90bc	63.50a	59.50d
LSD (5%)	0.44			0.40	
CV (%)	0.60			0.60	

Means in rows and columns in each interaction effect with similar letter(s) are not significantly different. LSD (5%) = List significant difference at 5 percent and CV (%) = Coefficient of variation percent.

4.2.2.4. Total tuber numbers per hill

The highest total tuber numbers hill-1 were recorded for Bubu variety treated with 20 ppm GA3 and for the combined application of 20 ppm GA3 with DLS storage condition. The lowest total tuber numbers hill-1 were recorded for non-GA3 treated Bate cultivar and the non-GA3 treated tubers stored under pit and FYM heap storage conditions with no significant difference between the latter two treatments (Table 9). This showed that the cultivars markedly differ in the number of tubers they produce and in response to external GA3. The total tuber numbers produced at the combined application of each GA3 concentrations with DLS and pit and farm-yard manure heap storage conditions were significantly different. However, non-significance differences were observed for tubers in each GA3 treatment combined with pit and FYM heap storage condition. This showed that the external GA3 application and storage condition in which tubers stored may also influence the number of tuber produced per hill.

Yibekal (1998) observed a significant difference in total tuber numbers produced between the improved cultivar Al-624 and the local check. Gautam and Bhattarai (2006) stated that post-harvest storage condition and duration along with exogenous GA3 significantly influenced total tuber numbers per plant.They concluded that exogenous GA3 is more active in long stored or more aged seed tubers, which produce tall and higher stem per plant that lead to more total tuber number per plant.

Table 9. Interaction effect of cultivar and GA_3 and GA_3 and storage condition on total tuber number per hill.

Interaction effects	GA_3 and storage condition			cultivar and GA_3	
	Storage condition			Cultivar	
GA_3 (ppm)	DLS	PS	FYM	Bubu	Bate
0	12.80d	12.20de	12.20de	14.30c	10.40f
10	14.00b	13.10c	13.10c	15.30b	11.60e
20	15.00a	13.90b	13.90b	16.10a	12.50d
LSD (5%)	0.21			0.20	
CV (%)	1.30			1.30	

Means in rows and columns in each interaction effect with similar letter(s) are not significantly different. LSD (5%) = List significant difference at 5 percent and CV (%) = Coefficient of variation percent.

According to Bodlaender and Marinus (1984), tubers stored in traditional storage system resulted in low total tuber number and yield due to high disease and potato tuber moth occurrence during storage as compared to DLS. However, in the present experiment, either disease or potato tuber moth occurrence on tubers stored under pit or farm-yard manure heap storage condition leading to low yield was not observed as compared to tubers stored in DLS. In agreement with the current findings, Allen *et al.* (1979) and Arsenault and Christie (2004) reported that post-harvest storage condition in combination with exogenous GA_3 application on different cultivars resulted significant difference in average and total tuber numbers per plant produced among cultivars. This is due to genetic and physiological ageing difference between cultivars in response to exogenous GA_3 application.

4.2.2.5. Marketable tuber yield

The highest marketable tuber yield t ha^{-1} was recorded from 20 ppm GA_3 treated Bubu variety and stored in DLS closely followed by the same variety and treatment but stored under pit and FYM heap. The lowest marketable tuber yield t ha^{-1} was recorded for non-GA_3 treated Bate cultivars stored in DLS closely followed by the same non-GA_3 treated cultivar but stored under FYM heap and pit. For each GA_3 treatment on both cultivars non-significant differences were observed between pit and farm-yard manure heap storage conditions (Table 10). Increasing GA_3 concentration from 0 to 20 ppm significantly resulted in increased marketable tuber yield t ha^{-1} for both cultivars in all storage conditions. But in all treatment combinations, Bubu variety produced highest marketable tuber yield t ha^{-1} as compared Bate cultivar treated the same. This could be due to the production of higher above ground biomass caused by the combined application of exogenous GA_3 with different storage conditions as well as tuber ageing that encourages higher dry matter production and assimilation in the tuber.

Various factors are affecting tuber formation and tuber growth like storage condition (Burt, 1964) and interaction between exogenous and endogenous hormones (Kumar & Wareing, 1974). The average number of active haulms per plant is a variable that is most affected by cultivar characteristics in responding to exogenous GA_3 during storage or at a later stage of developments, which in turn affected the yielding potential of potato plants (Susnochi, 1982; Morena *et al*., 1994). However, according to Helen (2012) diseases and environmental stresses both under rain fed and irrigation affects more the local cultivars that lead to lowest production of marketable and total tuber yields compared to improved ones. The relation between potato yield and the physiological age of the tubers depends on several factors, especially the cultivar, exogenous GA_3 and the storage condition (Van der Zaag and Van Loon, 1987; Van Ittersum *et al*., 1990; Krijthe, 1992b). The result of this experiment is in conformity with the results of Van Ittersum *et al*. (1993) who also reported that interaction between exogenous GA_3, cultivar and storage temperature regimes make significant difference in total and marketable tuber weight ha^{-1}. Similarly, Lovell and Booth (1967) also reported that application of GA_3 has increased dry matter production, LAI, improved tuber bulking rate, produced medium sized tubers and hence the potato yield. Alexopoulos *et al*. (2008) used GA_3 at a concentration of 1–50 mg/l and found that treatment duration appears to be more important than GA_3 concentration on yielding potential of potato plant.

Table 10. Interaction effect of GA_3, storage condition and cultivar on marketable tuber yields.

Character		Marketable tuber yield t ha^{-1}		
Storage		DLS	PS	FYM
Cultivar	GA_3 (ppm)			
	0	25.98e	26.00e	26.20e
Bubu	10	30.50c	29.80d	29.80d
	20	34.20a	33.70b	33.60b
	0	12.90j	13.30i	13.10i
Bate	10	19.90h	20.50h	20.00h
	20	24.97f	23.70g	23.70g
LSD (5%)		0.23		
CV (%)		0.60		

Means in rows and columns with similar letter(s) are not significantly different. DLS= Diffused light store, PS= Pit storage, FYM= Farm-yard manure, LSD (5%) = List significant difference at 5 percent and CV (%) = Coefficient of variation percent.

5. Summary and Conclusion

There were a number of differences in dormancy and sprouting, and agronomic characteristics among and within genotypes, exogenous application of GA_3 and storage conditions. However, the improved cultivars were superior in yield and quality over the farmers' cultivars. Although the experiment was conducted in one location and season using only two cultivars, it is reasonable to point out that the combined application of exogenous gibberellic acid at 20 ppm with genotypes and different storage conditions immediately after harvest resulted in shortened dormancy period, increased sprout mass and improved both yield and quality of the subsequent potato generation. This suggested the rapid translocation of gibberellins in potato plants. It is anticipated that this treatment combination is more beneficial for early breaking dormancy of seed tubers for rapid use of tubers after harvest for next planting. This will be of great importance in the potato seed production for rapid seed multiplication as well as certification system and speeding up the process of virus testing. From the study, to promote early dormancy termination, early emergence of shoots and high marketable tuber yield production, the combined application of exogenous gibberellic acid at 20 ppm with improved variety and different storage conditions were found to be optimum. Therefore, these treatment combinations offer very good prospects for improving the performance of seed potatoes that are planted soon after harvest. Since the experiment was conducted for one season using irrigation with only two cultivars, 10 and 20 ppm concentration of GA_3 solution and the three storage conditions, it deserves further study including more potato varieties and GA_3 concentrations in main cropping seasons and various agro-ecologies to make a conclusive recommendation.

6. Acknowledgement

The authors' thanks Haramaya University and Raare Horaa Seed Producer Cooperative (RHSPC) for the financial support, for providing planting materials and allowing field experimental plots, and technical staff of Horticulture Program at Haramaya University for technical support and data collection.

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11. Effect of Depth and Rate of Phosphorus Fertilizer Application on Yield and Yield Related Traits of Potato at Haramaya, Eastern Ethiopia

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Abstract: Potato (*Solanum tuberosum* L) is an important food security and cash crop in the eastern highlands of Ethiopia. However, its productivity is constrained by poor soil fertility and fertilizer management practices, such as phosphorus deficiency in the soil and inappropriate placement of the fertilizers around the root zone. Therefore, an experiment was conducted at Haramaya University during the 2015 cropping season with the objective of elucidating effect of rate and depth of phosphorus fertilizer application on yield and yield related traits of the crop. The treatment consisted of four phosphorus fertilizer rates (0, 46, 92 and 138 kg P_2O5 ha^{-1}) and five phosphorus fertilizer placement depths below the seed tuber (2.5 cm, 5 cm, 7.5 cm, 10 cm and 12.5 cm). The experiment was laid out as a randomized complete block design (RCBD) with three replications in a factorial arrangement. The improved potato variety "Bubu" was used.The results showed that the main effects of rate and depth of placement of phosphorus fertilizer had significant ($P < 0.01$) influence on plant height, average tuber number per hill, average tuber weight, tuber size distribution, marketable tuber number, and unmarketable tuber number. For all of these parameters except unmarketable tuber number and yield, the highest values were obtained in response to the application of the highest phosphorus rate and phosphorus fertilizer placement at depth of .7.5.and .5 cm. However, neither the main factors nor the interaction effects had significant influence on stem number produced per plant and tuber quality parameters. The rate and placement depth of P interacted to influence significantly both total and marketable tuber yields. The optimum total and marketable tuber yields of 42.06t $ha^{-1}$and 39.65t ha^{-1}, respectively, were obtained in response to the application of 138 kg P_2O5 ha^{-1} and placing the phosphorus fertilizer at the depth of 5.0 cm below seed tubers. The cost- benefit analysis indicated that application of 138 kg P_2O5 ha^{-1}at the depth of 7.5.cm resulted in 132148.5Birr net benefit and 93.64% marginal rate of return. It could, thus, be concluded that application of 138 kg P_2O5 $ha^{-1}$and placing the P fertilizer in band at the depth of 5.0 cm below the seed tuber had significant contribution for maximum growth, yield and economic return of potato.

Keywords: Average tuber weight; Tuber size distribution; *Solanum tuberosum* L; Tuber yield.

1. Introduction

Potato (*Solanum tuberosum* L.) belongs to the family *Solanaceae*, genus *Solanum*, and accommodated in series *Tuberosa* (van den Berg and Jacobs, 2007). It is originated from the Lake Titicaca region in Peru and Bolivia which are at the high elevation area of South America (Hoops and Plaisted, 1987). It was introduced to Ethiopia in 1858 by the German botanist Schimper (Pankhurst, 1964). Since then, potato has become an important crop in many parts of the country.

Potato production is one of the most important agricultural activities in Hararghe Zone in Eastern Ethiopia. Increased potato production in this area is spurred by ever-increasing population pressure, land fragmentation and, thus, difficulty in deriving livelihoods from cereal production (Eshetu *et al.,* 2005). Eshetu *et al.* (2005) indicated that potato is the second most advantageous crop next to khatt (*Chata edulis* Forsk.) in supporting farmers' welfare with 759% increase in income over sorghum which is the main staple cereal in Hararghe region. The average yield of potato on farmers' fields in eastern highlands of Ethiopia ranges between 11-13 tons ha^{-1} whereas the yield on the research station ranges between 30-40 tons ha^{-1}. The continuously increasing and intensive potato production in eastern Ethiopia through improved agronomic practices have been stimulated by accessibility to export market to neighbouring countries such as Djibouti and Somalia as well as availability of improved cultivars released by Haramaya University (Eshetu, *et al*., 2005).

The rate, time, and method of phosphorus fertilizer application determine potato yield (Lung'aho *et al.,* 2007). Band application of phosphorus fertilizer at depths varying from 5cm to 15cm is commonly practiced depending upon soil texture, with shallower planting commonly done for heavier soils and deeper planting for lighter soils. However, there is no specific recommendation for placement of phosphorus fertilizers to produce potatoes in Ethiopia. Therefore, farmers place the fertilizer at haphazardly varying depths below the tuber during planting. Appropriate placement of phosphorus fertilizer reduces the contact area with the soil, thus avoiding soil binding (Marschner, 1995). Placement may decrease the phosphorus sorption rate, affecting phosphorus acquisition positively and increase the phosphorus fertilization requirement by approximately 50% (Grewal *et al*., 1993). Lack of appropriate phosphorus fertilizer placement may, therefore, be a major cause for the low yield of the crop in the country. In eastern Ethiopia in particular and in the country in general, no research has so far been done to elucidate the effect of depth of phosphorus fertilizer placement on growth and yield of potato. Therefore, this study was conduct to assess the effect of depth and rate of phosphorus fertilizer application on tuber yield and yield related traits of potato and to evaluate the economic feasibility of different rates of phosphorus fertilizer application on potato.

2. Materials and Methods

2.1. Description of the Study Site

The study was conducted at Haramaya University. The Haramaya research site is located at 42^0 3' E longitudes, 9^0 26' N latitude at an altitude of 2006 meters above sea level. 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. The mean annual rainfall is 760 mm (Belay *et al.*, 1998). The mean maximum and minimum annual temperatures are of 23.4^0C and 8.25^0C, respectively. The soil of the experimental site is a well-drained deep alluvial with a sub-soil stratified with loam and sandy loam (Tamire, 1973). Analysis of the chemical and physical properties indicated that the soil has organic carbon content of 1.15 %, total nitrogen content of 0.11%, available phosphorus content of 18.2 mg kg soil^{-1}, exchangeable potassium content of 0.65 c mol kg soil^{-1} (255 mg K kg soil^{-1}), pH of 8.0, and percent sand, silt, and clay contents of 63, 20, and 17, respectively (Simret, 2010).

2.2. Description of Experimental Materials

In this experiment, improved potato variety, Bubu was used as a test crop. The planting material of this variety was acquired from the Potato Improvement Program of Haramaya University. The description of the variety is given in Table 1.

Table 1. Specific characteristics of Bubu potato variety

Days to flowering (after planting)	45-55
Days to maturity	90-120
Skin colour	White
Flower colour	White
Yield (t ha^{-1}) at time of released	
Research field	32-40
Farmers' field	25-35
Adaptation (agr o-ecology)	1700-2000 meters above sea level

Source: Haramaya University Potato Improvement Program, 2011.

2.3. Treatments and Experimental Design

The treatments consisted of four phosphorus fertilizer rates (0, 46, 92, 138 kg P_2O_5 ha^{-1}) and five phosphorus fertilizer placement depths below the seed tuber (2.5 cm, 5.0 cm, 7.5 cm, 10 cm and 12.5 cm). Triple-Super Phosphate (TSP) (Ca $[H_2PO_4]_2$), which constitutes about 46% P_2O_5, was used as a source of inorganic fertilizer. The experiment was laid out as a randomized complete block design (RCBD) in a factorial arrangement and replicated three times per treatment. Treatments were assigned to each plot randomly. Each plot had 3.60m length x 4.50m width = 16.2m^2 in size consisting of six rows, accommodating 12 plants per row, and 72 plants per plot. The spacing between plots and adjacent blocks were 1.0m and 1.5m, respectively. The gross size of the whole experimental site was 669.3m^2. Plants in the two outer rows as well as those at both ends of each row were not considered for data collection to avoid border effects.

2.4. Experimental Procedure

The land was prepared in accordance with the standard practice. The experimental plot was cultivated to a depth of 25–30cm by a tractor. The land was levelled and ridges were made according to the proposed layout of the experiment.

Uniform and medium-sized (39-75g) tubers of the test variety with sprout lengths of 1.5 to 2.5cm (Lung'aho *et al.,* 2007) retained from the previous season's harvest and stored under a diffused light storage condition, were planted. The seed tubers were planted at the depth of 5.0 cm in the soil below the seed tuber at the spacing of 75cm between rows and 30cm between seed tubers.

All phosphorus fertilizer was applied at planting at the specified rate and depth of placement in the soil. Nitrogen was applied to all plots equally at the blanket recommendation rate of 111 kg N ha^{-1} in three splits: $1/4^{th}$ at planting, $1/2^{nd}$at active stage of vegetative growth (30 days after planting the tubers) and the remaining $1/4^{th}$ just before tuber invitation (50 days after planting the tubers).

2.5. Data Collection and Cost Benefit Analysis

Plant growth, yield data and tuber quality attributes were collected by the researchers. The data collected included plant height (cm), average tuber number/hill, average tuber weight (g/tuber), tuber size distribution in weight, marketable tuber number/hill, unmarketable tuber number/hill, marketable tuber yield (t ha^{-1}), unmarketable tuber yield (t ha^{-1}) and total tuber yield (t ha^{-1}).

Plant height was determined by measuring height from the base of the main shoot to the apex at full blooming. Average tuber number/hill was recorded at harvest as the actual number of tubers to be collected from all experimental plants in each plot and divided by the number of harvested plants that were recorded as average tuber number per hill. Average tuber weight was recorded by dividing the total f tuber weight collected in each plot to the respective total tubers number. Tuber size distribution in weight was recorded at harvest, tubers were collected from five randomly selected plants from each plot and were categorized as very small (< 25g); small (25-38g); medium (39-75g); and large (>75 g) (Lung'aho *et al.,* 2007). Tuber number and yield were collected from each plot. Healthy tuber with a size more than or equal to 25 g (weighed using sensitive balance) was considered marketable while rotten, diseased, insect attacked, deformed tuber and those having a weight less than 50g was categorized as unmarketable. Total tuber yield (t ha^{-1}) was determined as the sum of the weights of marketable and unmarketable tubers from the net plot area.

Simple partial budget analysis was employed for economic analysis of fertilizer application and it was carried out for combined tuber yield data. The potential response of the crop towards the added fertilizer and price of fertilizer during planting ultimately determine the economic feasibility of fertilizer application (CIMMYT, 1988).

2.6. Data Analysis

The data were subjected to analysis of variance (ANOVA) of randomized complete block design (RCBD) using GenStat 15th edition statistical software package. The Least Significant Difference (LSD) test at the 5% level of probability was used to compare means.

3. Result and Discussion

3.1. Plant Growth and Yield Components

3.1.1. Plant height

In response to increasing the rate of phosphorus fertilizer from nil to 46, 92, and 138 kg P_2O_5 ha^{-1}, the height of potato plants increased significantly and linearly by 29, 38, and 45%, in the order mentioned here. Thus, the tallest plants were obtained for the rate of 138 kg P_2O_5 ha^{-1} whereas the shortest were obtained for those that did not receive the phosphorus fertilizer (Table 2).

Increasing the depth of phosphorus fertilizer application from 2.5 to 5.0, and 7.5cm increased plant height by about 3.0 and 4%, respectively. However, increasing the depth of phosphorus fertilizer placement further from 7.5 to 10 and 12.5cm decreased plant height significantly, indicating the optimum placement depth of the fertilizer for growth of potato plants in this type of soil (sandy clay loam soil).Thus, the shortest plants were observed in plots that had phosphorus fertilizer placed at the depth of 2.5cm below the seed tuber, which was too shallow, and 12.5cm below the seed tuber, which was too deep for the roots to access and take up the nutrient sufficiently. The tallest plants were obtained from plots that had phosphorus fertilizer placed 7.5cm below seed tubers (Table 2).

Table 2. Effect of rate and depth of phosphorus fertilizer application on plant height of Bubu variety during 2015 cropping season at Haramaya

Phosphorus (kg $P_20_5ha^{-1}$)	PH(cm)
0	53.23 d
46	68.55c
92	73.46b
138	77.04a
LSD (5%)	0.91**
CV (%)	1.7
Depth (cm)	
2.5	71.78c
5	73.73b
7.5	74.96a
10	73.31b
12.5	71.3c
LSD (5%)	1.18**
CV (%)	1.7

Means sharing the same letter(s) are not significantly different at 5% of level of significance. LSD (5%) = least significant difference; CV = coefficient of variation in percent. PH=Plant height.

Consistent with the results of this study, Isreal *et al.,* (2012) found that increasing application of nitrogen and phosphorus significantly increased plant height. The high response of potato to phosphorus application in terms of growth in height may also be consistent with the findings of (Nigussie *et al.,* 2003) that the crop is low in phosphorus, and would require application of ample amounts of phosphorus for maximum growth and productivity. The availability and absorption these nutrients have enhancing effect on the vegetative growth of plants by increasing cell division and elongation (El-Tohamy *et al*., 2006). Many researchers have found the relationships between phosphorus availability and potato growth (Johnston *et al*., 1986; Maeir *et al*., 1989; Payton *et al*., 1989; Jenkins & Ali, 2000; Ali *et al*., 2004).

3.1.2. Average tuber number per hill

Average tuber number per hill increased significantly and linearly in response to increasing the rate of phosphorus application from nil up to the highest rate of the fertilizer. Thus, increasing the rate of the fertilizer from nil to 46, 92, and 138 kg P_2O_5 increased the average tuber number per hill by about 14, 37, and 55%, respectively. Thus, the lowest number of tubers per hill was obtained for the nil P treatment whereas the highest was received for the138 kg P_2O_5 (Table 3).

Table 3. Effect of rate and depth of phosphorus fertilizer application on average tuber number per hill and average tuber weight of Bubu variety during 2015 cropping season at Haramaya

Phosphorus (kg $P_20_5ha^{-1}$)	ATNPH	ATW (g)
0	4.609d	62.47c
46	5.241c	68.16b
92	6.322b	68.29b
138	7.148a	72.23a
LSD (5%)	0.2986	2.864
CV (%)	6.4	5.5
Depth (cm)		
2.5	5.973b	69.17
5.0	6.27ab	69.82
7.5	6.592a	69.00
10	6.273ab	71.19
12.5	6.114b	68.63
LSD (5%)	0.3854	NS
CV (%)	6.4	5.5

Means sharing the same letter(s) are not significantly different at 5% of level of significance. LSD (5%) = least significant difference; CV = coefficient of variation in percent. ATNPH=Average tuber number per hill, ATW=Average tuber weight.

Average tuber number per hill increased in response to increasing the depth of phosphorus fertilizer placement from 2.5 to 5.0cm increasing the depth of P fertilizer placement below the seed tuber further from 5.0 to 7.5 and 10cm did not change the number of tubers produced per hill. However, increasing the depth of P placement further to 12.5cm significantly reduced the number of tubers produced per hill. Thus, the optimum P fertilizer placement depth for number of tubers produced per hill was 5.0cm (Table 3).

The results of this study are concurrent with the findings of Firew (2014), who reported that increasing the rate of phosphorus from 0 to 138 kg ha^{-1} significantly increased total tuber number $hill^{-1}$ from 6.392 to 7.942, which was an increment of about 12.4 %. Similar to the results of this study, Sommerfeld and Knutson (1965), Sparrow *et al.* (1992), Israel *et al.* (2012) and Zelalem *et al.* (2009) reported that increasing the rates of phosphorus increased the number of potato tubers set per hill. Similarly, Rosen and Bierman (2008) reported that application of phosphorus significantly increased tuber number per hill.

3.1.3. Average tuber weight

Increasing the rate of phosphorus application from nil to 46 and 92kg P_2O_5 ha^{-1} increased average tuber weight by about 9%. However, increase the rate of the fertilizer from nil further to 138 kg P_2O_5 ha^{-1} increased the average tuber weight by about 16%. This may be due to the phenomenon that phosphorus also increases aboveground biomass. This is important for photosynthesis and net assimilation processes and no re-absorption evidently took place from the tubers, leading to increased tuber size and weight so the tuber could be marketable (Boral and Milthorpe, 1962).In contrast to this result, Bereke (1994) a non-significant response in average tuber weight to phosphorus application.

3.1.4. Tubers size distribution in weight

The increased phosphorus application from 46 to 138 P_2O_5 kg ha^{-1} increased the proportion of large and medium-sized tubers produced in the range between 49.42 to 53.72% and 36.62 to 41.26%, respectively, whereas it reduced the proportion of small and very small size tubers ranged from 10.22 to 3.83% and 6.298 to 2.24%, respectively (Table 4).

Increasing the depth of phosphorus application from 2.5 to 7.5cm increased large and medium size tubers categories in weight while from increasing the depth from 7.5 to 12.5cm decreased it. Significantly and highest proportion of large size tubers were produced from plants grown where phosphorus was placed at 7.5 and 5.0cm while significantly highest percent of medium sized tubers were produced at 7.5, 5.0 and 10cm. The lowest proportions of large-sized tubers were produced from plots where phosphorus was placed at 12.5, 2.5 and 10cm whereas the lowest percent medium size tubers were produced at 2.5 and 12.5cm. The lowest percent small-sized tubers were produced where phosphorus was placed at 12.5cm but the proportion of small sized tubers produced at all others depth of phosphorus application produced statistically equal proportion (Table 4).

Many researchers reported that applied phosphorus increased the proportion of large tubers harvested (Benepal 1967; Freeman *et al.* 1998). Other researchers observed that the increase in the number of small tubers was offset by a decrease in number of large tubers (Sharma and Arora 1987; Jenkins and Ali, 2000; Rosen *et al.*, 2008; Rosen and Bierman, 2008). A few results reported non-significant effects of phosphorus rate on total tuber number in spite of yield responses (Benepal, 1967; Dubetz and Bole, 1975; Sharma and Arora 1987; Mohr and Tomasiewicz, 2011).The present result showed that increasing the rate of phosphorus fertilizer application decreases the proportion of very small and small-size tubers but increased that of medium and large-sized tubers. This indicated that phosphorus is a critical nutrient in determining tuber size of potato varieties. This may be attributed to the enhanced metabolic role the nutrient plays in tuber cell growth and development (Marschner, 1995).

Table 4. Effect of depth and rate of phosphorus fertilizer application on large, medium, small and very small size of tubers in weight of Bubu variety during 2015 cropping season at Haramaya.

(P_20_5) kg ha^{-1}	LTW_%	MTW_%	STW_%	VSTW_%
0	49.16c	36.62d	8.22a	6.00a
46	49.42c	38.65c	6.52a	5.41a
92	50.45b	40.55b	4.80b	4.20b
138	52.72a	41.28a	3.73c	2.27c
LSD (5%)	0.701**	0.616**	0.39**	0.581**
CV (%)	1.8	2.1	10.4	19.6
Depth (cm)				
2.5	50.77c	39.24c	5.42c	4.57
5	51.67ab	40.34ab	4.32bc	3.67
7.5	52.49a	40.52a	3.51c	3.48
10	51.33bc	40.29ab	5.00bc	3.38
12.5	51.2bc	39.45bc	5.23ab	4.12
LSD (5%)	0.91*	0.80*	0.51*	NS
CV (%)	1.8	2.1	10.4	19.6

Means sharing the same letter(s) are not significantly different at 5% of level of significance. LSD (5%) = least significant difference; CV = coefficient of variation in percent.LTW=large tuber weight, MTW=medium tuber weight, STW= small tuber weight, VSTW=very small tuber weight.

3.1.5. Number of marketable and unmarketable tubers per hill

The depth and rate of phosphorus application significantly influenced the production of marketable tubers increasing the rate of phosphorus from 0 to 138kg P_2O_5 ha^{-1} significantly increased marketable tuber number per hill. On the other hand, increasing the depth of phosphorus from 2.5 to 7.5cm increased marketable tuber number per hill while from 7.5 to 12.5cm decreased marketable tuber number per hill. The highest marketable tuber number was counted from phosphorus placed at the depth 7.5 and 5.0 cm and the application of 138 P_2O_5 kg ha^{-1}. The lowest marketable tuber number (3.33) was counted from plants that did not receive phosphorus fertilizer followed by 46 kg $P_2O_5ha^{-1}$ (Table 5). The increase in marketable tuber number was from nil to138 kg

P_2O_5 ha^{-1}amounted to 129.13%. Similar to the results of this study, Sommerfeld and Knutson (1965), Sparrow *et al.* (1992), Israel *et al.* (2012) and Zelalem *et al.* (2009) reported the increased rates of phosphorus increased the number of potato tubers set per hill. The increase in tuber numbers per hill in response to increased application of phosphorus is consistent with the results of Rosen *et al.* (2008).

Significantly highest unmarketable tuber numbers per hill were counted for control treatment (without phosphorus) (1.73) and the lowest unmarketable tuber numbers per hill were counted in response to the application of 138Kg P_2O_5 ha^{-1}(0.907). This may be due to the phenomenon that phosphorus also increases aboveground biomass and also factors that increase marketable tuber per hill was decrease the un marketable tuber per hill. This was important for photosynthesis and net assimilation processes and no re-absorption evidently took place from the tubers, leading to increased tuber size and weight so the tuber could be marketable (Boral and Milthorpe, 1962).

Table 5. Effect of depth and rate of phosphorus fertilizer application on marketable and unmarketable tuber number per hill of Bubu variety during 2015 cropping season at Haramaya.

(P_20_5) kg ha^{-1}	MTNPH	UMTNPH
0	3.333d	1.733a
46	5.28c	1.187b
92	6.45b	1.107b
138	7.63a	0.907c
LSD (5%)	0.338	0.1528
CV (%)	7	19.1
Depth (cm)		
2.5	5.98c	1.178
5	6.6ab	1.022
7.5	6.82a	0.867
10	6.49ab	1.089
12.5	6.38bc	1.178
LSD (5%)	0.437	NS
CV (%)	7	19.1

Means sharing the same letter(s) are not significantly different at 5% of level of significance. LSD (5%) = least significant difference; CV = coefficient of variation in percent. MTNPH=marketable tuber number per hill, UMTNPH=unmarketable tuber number per hill.

3.2. Tuber Yields

3.2. 1. Total and marketable tuber yields

Increasing the depth of placement of the phosphorus fertilizer increased total tuber yield across the increasing rate of the fertilizer. Thus, the lowest total and marketable tuber yields were obtained in response to placing the phosphorus fertilizer at all depths and applying 46 kg P_2O_5 ha^{-1}.However, the highest total and marketable tuber yields were obtained at the depths of 5.0, 7.5, 10, and 12.5cm at the rate of 138 kg $P_2O_5ha^{-1}$. Thus, for example, the total tuber yield obtained from the treatment of placing the P fertilizer at the depth of 5.0cm and 138kg $P_2O_5ha^{-1}$a exceeded the total and marketable tuber yields obtained from the treatment of 2.5cm P placement depth and nil rate of P application by about 50 and 69%, respectively (Table 6).

Table 6. Interaction effect of phosphorus rate and application depth on total tuber yield and marketable yield of Bubu potato variety in 2015 at Haramaya.

TTY (t ha^{-1})				MTY (t ha^{-1})		
Treatment	Phosphorus (kg P_2O_5 ha^{-1})					
Depth (cm)	46	92	138	46	92	138
2.5	27.97c	33.33b	33.76b	23.5c	29.84b	30.71b
5	28.00c	33.78b	42.06a	23.78c	30.59b	39.65a
7.5	28.21c	34.11b	43.7a	24.81c	30.68b	41.03a
10	27.69c	33.44b	41.82a	23.54c	30.14b	39.58a
12.5	27.51c	33.43b	41.33a	23.42c	30b	39.31a
LSD (5%)		3.338*			3.95*	
CV (%)		5.9			7.7	

Means sharing the same letter(s) are not significantly different at 5% of level of significance. LSD (5%) = least significant difference; CV = coefficient of variation in percent. TTY=Total tuber yield, MTY=Marketable tuber yield.

A substantial number of experiments directly compared the performance of various method of phosphorus fertilizer application on potato. Banding application of a given rate of phosphorus in non-Andisol soils generally resulted in higher yields as compared to broadcasting application (Dudley, 1930; Welch *et al.*, 1949; Garg and Welch, 1967). This is because when phosphorus fertilizer is placed close to the roots, plant absorption of phosphorus frequently increases and consequently yield increases (Zhang and Barber, 1992). Crops with limited root systems show a higher response to fertilizer if placed near the seed than crops that have an extensive root system, particularly in soils with low concentrations of available phosphorus (Rodriguez, 1993).

The result showed that application of phosphorus at depth of 5.0cm and rate of 138 kg P_2O_5 optimum total tuber yields. Consistent with the results of this study, several studies showed that high rates (≥270 kg P_2O_5 ha^{-1}) of DAP or UAP placed near ≤5 cm or in contact with the seed piece delayed emergence, reduced stand, and negatively affected yields (Meisinger *et al.* 1978; Chu *et al.* 1984). Phosphorus is the nutrient that contributes the most for the increases in tuber yield (Freeman *et al.,* 1998; Alvarez-Sanchez *et al.,* 1999; Nava *et al.,* 2007; Rosen and Bierman, 2008; Fleisher *et al.*, 2013).

Placing the fertilizer near the active root zone reduces the contact with soil, thus avoiding fixation (Marchner, 1995), increase uptake efficiency and leads to a decreased phosphorus fertilization requirement with ~ 50 % (Grewal and Trehan, 1993). Sharma and Arora (1978) reported that placement of phosphorus fertilizer near the active root zone of the plant is recommended because most of the applied phosphorus is fixed by the soil and is not mobile. It was proved through experiments that with banding and placement method of fertilizer application potato yield increased up to 15 and 30% over broadcast, respectively. Malik (1995) reported that by broadcasting, fertilizer is less concentrated in the root zone of the plant. using the banding or placement method will reduce the amount of fertilizer used .According to Pruger and Hadacova (1996), the best yield was obtained from placement of fertilizer because it is attributed to the fact that no fertilizer is wasted as all nutrients come in close contact with the feeding roots and plants use these nutrient effectively.

3.2.2. Unmarketable tuber yield

The highest unmarketable tuber yield ha^{-1} was obtained from the plots that did not receive fertilizer followed by 46 Kg P_2O_5 ha^{-1}application. As the rate of phosphorus application was increased unmarketable tuber yield ha^{-1} was reduced. Regarding the depth of phosphorus application, the highest unmarketable tuber yield $ha-^{1}$ was obtained from the plots where the fertilizer was placed 2.5 and 10cm far from tubers (Table 7). The result shows that unmarketable tuber yield was decreased significantly when the rate of phosphorus was increased. This may due to the decreased the level of phosphorus, might have decreased the growth above ground biomass, tuber growth, leading to reduced tuber size and there by high unmarketable tuber yield.

Table 7. Effect of depth and rate of phosphorus fertilizer application on unmarketable tuber yield of Bubu variety during 2015 cropping season at Haramaya

P rate (kg $P_20_5ha-^1$).	UMTY
0	4.992a
46	2.601b
92	2.504b
138	1.942c
LSD (5%)	0.3106*
CV (%)	17.7
Depth (cm)	
2.5	2.45
5	2.275
7.5	2.164
10	2.453
12.5	2.403
LSD (5%)	NS
CV (%)	17.7

Means sharing the same letter(s) are not significantly different at 5% of level of significance. LSD (5%) = least significant difference; CV = coefficient of variation in percent, UMTY=unmarketable tuber yield.

In this experiment, unmarketable tuber refers to diseased, insect attacked and undersized tubers (less than 25 g). Thus, most of the tubers that were discarded as unmarketable tubers included the ones that were too small in size and rotten. This indicates that factors that increase percentages of small sized tubers would significantly increase unmarketable tuber yield. Small-sized tubers can be unmarketable and their increment in number would significantly increase unmarketable tuber yields. Highly and positive association were reported between the proportion of small sized tuber yield and unmarketable tuber yield (Helen, 2012). Unmarketable tuber yield might be controlled more importantly by manipulating other factors such disease incidence, harvesting practice, etc. other than mineral nutrition (Berga *et al.,* 1994).

3.2. Cost benefit analysis

The results of the partial budget analyses revealed that maximum net benefit of Birr 132148.5 ha^{-1} with an acceptable marginal rate of returns (MRR) of 93.68% was recorded in the treatment that depth of 7.5cm and received phosphorus at 138 kg P2O5 ha^{-1} (Table 8).This combination generated Birr 43572.62 ha^{-1} compared to the control treatment. On the other hand, the next maximum net benefit of Birr 127583.7 ha^{-1} with an acceptable MRR of 86.70% was recorded in treatment that depth 5cm and received phosphorus at 138kg P2O5 ha^{-1}. This combination generated Birr 39007.82 ha^{-1} more compared to control treatment.

Table 8. Interaction effect of rate and fertilizer depth of phosphorus application on the marginal economic analysis of the undonimated treatments of marketable tuber yield of Bubu potato Variety at Haramaya, in 2015 main cropping season

P rate (kg P_2O_5 ha^{-1}) and depth(cm)	AVMTY kg ha^{-1}	ADMTY	GFB Birr	TC Birr	NB Birr	MRR	MRR (%)	C:B
0, -	21.274	18.65	93237.77	4661.89	88575.88			19
92,2.5	29.836	26.5	132511.1	23464.67	109046.5	1.0887	108.87	4.65
92, 5	30.587	27.21	136044.5	30796.89	105247.6	0.6379	63.79	3.42
92, 7.5	30.685	27.27	136367.4	30861.48	105505.9	0.6462	64.62	3.42
138,2.5	30.714	27.34	136691.1	32681.22	104009.9	0.5508	55.08	3.18
138, 5	39.653	35.45	177235.5	49651.89	127583.7	0.867	86.7	2.57
138, 7.5	41.034	36.66	183322	51173.5	132148.5	0.9368	93.68	2.58
138,10	39.577	35.4	176977.8	58436.33	118541.4	0.5572	55.72	2.03
138,12.5	39.311	35.18	175886.7	58109.01	117777.7	0.5464	54.64	2.03

AVMTY kg ha^{-1} = Average marketable tuber yield kg per hectare, AdMTY kg ha^{-1} =Adjusted marketable tuber yield kg per hectare, GFB Birr = Gross field benefit in Birr, TC Birr = Total cost in Birr, NB Birr = Net benefit in Birr, MRR = Marginal rate of return and MRR (%) = Marginal rate of return in percent, C:B= Cost benefit ratio . During experimental period the price of TSP fertilizers were 39 Birr kg^{-1} and selling price of potato tuber

When the new technology surpassed the conventional practice, it is said to be undominated (CIMMYT, 1988). MRR % measures the increase in the net income. MRR% becomes unnecessary when the new technology costs less than the existing farmers, technology. When the new technology yield is.lower benefit, then the technology is said to be dominated. MRR is calculated by dividing the marginal increase in net benefit with the marginal increase in variable cost and multiplying the result by

100.In the present study, treatment depth with 7.5 cm and 138 kg P_2O_5 ha^{-1} application rates were more profitable. The highest MRR% was 93.68%, the computed MRR % gives an indication of what a producer can expect to receive by switching technologies.

Hence, a 93.68% MRR in switching from technology 1 to technology 2 (from farmers practice to improved new one) implies that for each Birr invested in the new technology, the producer can expect to recover the Birr one invested plus an additional return of Birr 2.58. Based on cost-benefit analysis, it is advisable to apply P_2O_5 to get optimum yield for the specific area of the experiment sit. High yield and low cost evidently leads to high income.

4. Summary and Conclusion

Sustaining soil and soil fertility in intensive cropping systems for higher yields and better quality of crops could be achieved through optimum levels of fertilizer application and fertilizer management. Thus, information on fertility status of soils and crop response to different soil fertility management is very important to come up with profitable and sustainable crop production. Therefore, this experiment was carried out with the objective of studying the effect of rate and depth of phosphorus fertilizer application on yield and yield related traits of potato (S*olanum tuberosum* L.) at Haramaya, Eastern Ethiopia. The experiment consisted of four rates of phosphorus (0, 46, 92 and 138 kg P_2O_5 ha^{-1}) and five phosphorus placement depths below the seed tuber (2.5cm, 5.0 cm, 7.5 cm, 10 cm, and 12.5 cm). The experiment was laid out as a randomized complete block design (RCBD) in a factorial arrangement and replicated three times per treatment. The improved variety Bubu was used as a test crop.

The analysis of variance indicated that rate and depth of P placement below the seed tuber had significant ($P < 0.01$) main effects on plant height, average tuber number per hill, average tuber weight, tuber size distribution, marketable tuber number, and unmarketable tuber number. Almost all parameters except unmarketable tuber number and yield had the highest values when the highest phosphorus rate of 138 kg P_2O_5 ha^{-1} was applied at the depths of 5.0 and 7.5cm below the seed tuber. The interaction effect of P rate and placement depth of phosphorus fertilizer also significantly influenced total and marketable tuber yields.

The optimum total and marketable tuber yields were obtained in response to the application of 138 kg P_2O_5 ha^{-1} and placing the phosphorus fertilizer at the depth of 5.0 below seed tubers. The cost- benefit analysis also indicated that application of 138 kg P_2O_5 ha^{-1} at the depth of 5.0 cm resulted in highest net benefit and marginal rate of return.

The results of the study clearly demonstrated that potatoes respond vigorously to phosphorus application and farmers in the study area have to apply higher rates than currently recommended by the research system in the country, which is only about 92 kg P_2O_5 ha^{-1} to reap higher tuber yields and economic benefits. However, the results also revealed that it is not only increasing the rate of phosphorus fertilizer application that would lead to the realization of increased tuber yields and economic benefits, but also placing the fertilizer at the right and optimum depth below the seed tuber is important.

In this study, placing the phosphorus fertilizer at the depth of 5.0 and 7.5 cm resulted in superior growth and yield of the crop. However, given that placing the fertilizer at 7.5cm is apparently more laborious than placing at 5.0 cm, the latter depth is optimum for the sandy clay loam soil of Haramaya. Therefore, farmers in the study area should consider placing P fertilizer at 5.0cm depth below the seed tuber upon planting the crop. However, to reach a conclusive recommendation, it will be necessary to repeat the experiment in different years and locations using more rates of P fertilize and more varied depth of placing the fertilizer below the seed tuber.

5. Acknowledgments

The authors acknowledged SIDA (Swedish International Development Agency) project for providing the financial support required to accomplish this study. Special thanks extend to Haramaya University for allowing experimental plot for field experiments field site and other facilities to execute the experiment on time.

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12. Participatory Variety Selection and Evaluation of Twelve Sorghum (*Sorghum bicolor (L) Moench*) Varieties in Low Land of Eastern Hararghe

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Abstract: Participatory variety selection was carried out at District of East Hararghe, Fedis and Babile during the main cropping season of 2015. The objectives were to select the best performing and adaptable sorghum varieties in the target areas. The result of the current study clearly showed a significant difference among the released varieties for most agronomic traits at both Districts. Grain yield and biomass were the dominant selection criteria of the farmers at both Districts. Accordingly, farmers overwhelmingly selected ESH-1 sorghum variety. Moreover, Farmers gave priority for grain yield at both districts but secondly, they gave equal emphasis to biomass and striga at Fedis and earliness and biomass at Babile District. In general, developing of high yielding, biomass and Striga resistant for Fedis and early maturing for Babile district can enhance the adoption rate of improved sorghum varieties.

Keywords: Participatory Variety selection; Sorghum; East Hararghe

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a monocotyledon crop belonging to the family Gramineae. It is naturally self-pollinated short day plant with the degree of spontaneous crosspollination, in some cases, reaching up to 30% depending on panicle types (Poehlman and Sleper, 1995).

Sorghum is the fifth most important cereal crop in the world after wheat, rice, maize, and barley. It is cultivated annually over approximately 45 million ha, producing approximately 60 million tones of grain. Sorghum grain is a major food in much of Africa, South Asia, and Central America, and an important animal feed in the USA, Australia, and South America. In addition to these uses, sorghum crop residues and green plants also provide sources of animal feed, building materials, and fuel, particularly in the semi-arid tropics (ICRISAT, 1997).

Sorghum is one of the major crops produced in Ethiopia, and it is the fourth important crop in terms of area coverage and volume of production. It is adapted to a wide range of environment, and hence can be produced in the highlands, medium altitude and lowland areas. It is widely produced more than any other crops in the areas where there is moisture stress. In 2014/15 cropping season, sorghum is produced on about 1,834650.81 hectare of land from which 43,391,342.6 quintals of yield are obtained (CSA, 2015).

Sorghum is a high potential crop in Hararghe region in general and at Babile and Fedis district in particular. Even if the crop is important in the target area, a number of factors constrained productivity of sorghum in the target areas. This is associated with the lack of improved varieties and their accessibility associated with edaphic and biotic factors that have been appreciated as one of the primary sources of lower sorghum production in the target areas. Therefore, to tackle the aforementioned problems, in addition to the existing varieties, introducing of nationally released improved varieties to the areas and testing for their performance could be taken as one of the options.

Different improved sorghum varieties have been released at national and regional levels; however, only few of these varieties are being cultivated by the farmers for a long period of time in the most sorghum growing areas of Hararghe Region. This is mainly due to lack of availability of improved varieties and also low involvement of farmers during the variety selection processes. Especially, the later reason makes the farmers to have low impact on the new released varieties. Even if this situation is true for Hararghe Region, there were limited studies so far done in the area. Therefore, this study was conducted through participatory variety selection experiment so as to test and select among the nationally released varieties of sorghum that adapt to Eastern Hararghe Region and similar agro ecologies.

Therefore**,** this experiment was conducted to select best performing sorghum varieties, enhance accessibility of improved varieties to the producers, find out farmers indigenous knowledge used for the future sorghum research, and enhance farmers experience towards varietal selection.

2. Material and Methods

The study was carried out at two potential areas of Eastern Hararghe namely: Babile and Fedis in the main cropping season of 2014/15.The experiment consisted of eleven released and one local sorghum varieties. Farmers participating in variety selection trials were selected in collaboration with the development agent of the two districts based on their experience in sorghum production. Accordingly, 41 farmers were invited to the stations to evaluate the varieties near harvesting. Out of the 41 participants, 20 were women. The experiment was designed as RCBD with 3 replication and the entire varieties were grown with a plot size of 4 rows (with 40 cm between rows and 15 cm between plants) and 5 m long.

Table 1. Lists of Varieties used in the study.

Variety name	Year of release	Responsible Center
Chemeda	2013	BARC/OARI
TAC-537	2013	BARC/OARI
Deqeba	2013	MARC/EIAR
Mesay	2011	SARC/ARARI
Dagem	2011	MARC/EIAR
Chare	2011	DBARC/ARARI
Melkam	2009	MARC/EIAR
ESH-1 C=nL-1	2009	MARC/EIAR
MACIA	2007	MARC/EAIR
EMAHOY	2007	PARC/EIAR
RAYA	2007	SRARC/ARARI
MISKIR	2007	SRARC/ARARI

Source: MoA, 2013.

3. Result and Discussion

3. 1. Farmers Preference Analysis

Based on the obtained result from pair wise matrix (Table 1), farmers at Babile district gave priority to earliness during sorghum variety selection. Secondly, they focused on seed and biomass yield. Therefore, developing of early sorghum varieties with high seed and biomass yield will enhance the adoption rate of improved sorghum varieties at Babile district. Accordingly, Variety ESH-1 has been preferred by the farmers (Table 2).

Table 1. Pair-wise matrix comparison for farmers selection criteria at Babile District

type of parameter	drought	seed yield	earlyness	disease resistant	biomass	height
drought		Drought	earliness	disease resistant	biomass	Drought
seed yield			earliness	seed yield	seed yield	seed yield
earliness				earliness	earliness	Earliness
disease resistant					biomass	disease resistant
biomass						Biomass
height						

Drought 2(3), disease resistant 2(3), seed yield 3 (2), biomass 3(2), earliness 5 (1) & height 0 (4). Values in the parenthesis refer to the selection criteria given by farmers.

Farmers at Fedis District gave priority to seed yield during sorghum variety selection, secondly they gave equal emphasis to biomass and Striga resistant varieties. Therefore, developing of high yielding sorghum varieties which can resist Striga and having good biomass yield enhance the adoption rate of improved sorghum varieties at Fedis district.

Table 2. List of improved sorghum Varieties ranked by farmers at Babile District.

Plot no	name of Variety	Rank
1	ESH-1	1
2	Dagim	7
3	Melkam	8
4	Dekeba	9
5	Macia	3
6	Raya	10
7	Charia	6
8	Mesay	4
9	Grana-1	5
10	Miskir	2
11	Emahoy	11
12	Local check (chame)	12

Table 3. Pair-wise matrix comparison for farmers selection criteria at Fedis District.

	yield	seed size	seed color	biomass	striga resistant	earlyness
Yield		Yield	yield	yield	yield	yield
seed size			seed size	biomass	striga resistant	earliness
seed color				biomass	striga resistant	earliness
biomass					striga resistant	biomass
striga resistant						
earlyness						

Yield 5 (1) seed size 1 (4) seed color 0 (5) biomass 3 (2) Striga Resistant 3 (2) earliness 2 (3) & Values in the parenthesis refer to rank of the selection criteria that given by farmers.

Table 4. List of improved sorghum Varieties ranked by farmers at Fedis District.

plot no	Variety name	rank
1	ESH-1	1
2	Dagim	12
3	Melkam	9
4	Dekeba	5
5	Macia	6
6	Raya	7
7	Charia	2
8	Mesay	3
9	Grana-1	8
10	Miskir	4
11	Emahoy	11
12	Local check (chame)	10

3.2. Agronomic Analysis

As indicated in Table 5, analysis of variance revealed significant difference for plant height, grain yield, above ground biomass yield and harvest index. The highest gy and agby recorded by ESH-1 variety and highest plant height obtained by the local variety Chame. According to Eshetu and Ketema (2001), in a good rainfall condition, Chame can extend its physiological maturity up to 240 days. But in this experimentation period due to moisture stress, it revealed only about 148.

Table 5. Analysis of variance for six characters of 12 varieties at Fedis District.

Trt no	Variety	ph	dm	tsw	Gy	agby	HI
1	ESH-1	147.33	143.67	22.43	4733.3	17556	0.27
2	Dagim	135	150.67	23.7	700	6444	0.11
3	Melkam	125.27	143.33	22.63	4433.3	7778	0.58
4	Deqeba	120.33	149	21.5	2844.4	8267	0.37
5	MACIA	114.2	150	18.77	2622.2	11333	0.23
6	Raya	165.67	145.67	21.1	2888.9	8267	0.37
7	Charie	159.53	145.33	25.967	2133.3	14311	0.15
8	Mesay	154.33	143.33	22.73	2200	7333	0.3
9	Grana-1	165.2	149.67	22.4	3533.3	7022	0.53
10	Misikir	167.4	147	20.13	2600	7156	0.37
11	Emahoy	182.33	142.67	20.3	3266.7	9467	0.34
12	Chame (check)	242	148.67	22.2	1040	10356	0.10
	CV(%)	8.73	2.79	19.14	23.07	15.62	29.59
	LSD	23.14	6.92	7.13	1074.3	2540.7	0.16
	Grand mean	156.55	146.58	21.99	2749.63	9607.41	0.31

Where: ph=plant height, dm=days to maturity, tsw=thousand seed weight (gm), Gy=grain yield (kg/ha), agby=above ground biomass yield (kg/ha), HI= Harvest index

The analysis result at Babile showed that there is no significant difference for all the parameters except grain yield. High and significant gy were obtained by Emahoy, Misikir and ESH-1 varieties. But there is no significant difference among themselves (Table 6).

The combined result indicated high gy, abgy and HI obtained by ESH-1 variety. The performance of the variety over the two locations was consistent and stable (Table 7). Biomass and grain yield were the dominant selection criteria of the farmers at the two districts.

Table 6.Analysis of variance for six characters of 12 varieties at Babile District.

Trt no	Variety	Df	dm	Ph	tsw	agbm	yld
1	ESH-1	119	152.67	85	25.6333	4000b	1045.7
2	Dagim	123.3	161.67	96.67	24.8667	4000b	203.9
3	Melkam	119	151.67	96.67	24.8667	4066.7	571.4
4	Deqeba	121.67	162.67	100	24.3333	4533.3	897
5	MACIA	121.33	154.33	101.67	26.1333	4333.3	428.5
6	Raya	121.33	154	91	25.4667	4355.6	655.4
7	Charie	119.33	159	101.67	23.8	5000	852

8	Mesay	119.33	155	103.33	24.1	1800c	726.6
9	Grana-1	119.67	151	83.33	25.3667	4266.7	868.5
10	Misikir	119.33	151.67	100	24.6	4000b	1191.5
11	Emahoy	121	153.33	78.33	24.8667	4088.9	1229
12	Chame (check)	120b	153.33ba	108.33	24.8667	2200c	572.6
	CV(%)	1.41	4.39	26.93	4.55	13.94	18.44
	LSD	2.87	11.51	43.55	1.92	917.4	240.48
	Grand mean	120.36	155.03	95.5	24.91	3887.04	770.17

Table 7. Combined analysis for the two districts.

trt	variety	dm	ph	tsw	agbm	yld	HI
1	ESH-1	148.17	116.17	24.033	10777.8	2889.5	0.268
2	Dagim	156.17	115.83	24.283	5222.2	451.9	0.08
3	Melkam	147.5	110.97	23.75	5922.2	2502.4	0.363
4	Deqeba	155.83	110.17	22.917	6400	1870.7	0.282
5	MACIA	152.17	107.93	22.45	7833.3	1525.4	0.167
6	Raya	149.83	128.33	23.283	6311.1	1772.1	0.260
7	Charie	152.17	130.6	24.88	9655.6	1492.6	0.160
8	Mesay	149.17	128.83	23.417	4566.7	1463.3	0.355
9	Grana-1	150.33	124.27	23.883	5644.4	2200.9	0.365
10	Misikir	149.33	133.7	22.367	5577.8	1895.8	0.337
11	Emahoy	148	130.33	22.583	6777.8	2247.8	0.316
12	Chame (check)	151b	175.17	23.533	6277.8	806.3	0.189
	CV (%)	3.64	16.34	13.21	16.37	25.67	27.93
	LSD	6.39	23.93	3.6	1283.7	524.97	0.085
	Mean	150.81	126.03	23.45	6747.22	1759.9	0.263
	location (A)	**	**	*	**	**	**
	Variety(B)	NS	*	NS	**	**	**
	AB	NS	*	NS	**	**	**

*Where: *Significant difference at 5%, ** highly significant difference at 1% and NS= non-significant difference at 5%.*

4. Conclusion and Recommendation

Pair-wise matrix analysis indicated that farmers at Babile District gave priority to earliness during sorghum variety selection. Secondly, they gave emphasis to seed yield and biomass yield. Therefore, developing of early maturing sorghum varieties with high seed yield and biomass yield will enhance the adoption rate of improved sorghum varieties at Babile district. At Fedis District, farmers gave priority to seed yield during the sorghum variety selection. Secondly, they gave equal emphasis to biomass and Striga resistant varieties. Therefore, developing of high yielding sorghum varieties which can resist Striga and having good biomass yield will enhance the adoption rate of improved sorghum varieties at Fedis district. During the selection, there was no considerable preference difference between men and women farmers at both Districts. Both the agronomic and farmers preference data analysis indicated that ESH-1 has been selected on both districts. Therefore, the variety needs to be multiplied and distributed to the farmers. In general, developing of high yielding, biomass and; Striga resistant for Fedis and early maturing for Babile District can enhance the adoption rate of improved sorghum varieties.

5. Acknowledgments

The authors are grateful to the Office of the Vice-President for Research Affairs of Haramaya University for providing fund during the research period. A special thanks also goes to Melkassa Agricultural Research Center, Pawe Agricultural Research Center, Debrebrhan Agricultural Research Center for providing seeds. The authors would also like to thank all the participating farmers, agricultural experts and Development Agents of Babile and Fedis District for their cooperation in making the approach successful.

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Figure 1: Pictures taken during Group Discussion

Figure 2: Picture taken during variety selection.

Figure 3: The selected sorghum variety (**ESH-1**)

13. Land Use / Cover Change, Woody Plants above Ground Biomass and its' Regeneration Status in Dire Dawa Administration, Ethiopia

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Abstract: The study was conducted with the objective of investigating the land use land cover change,woody plants above ground biomass and its' regeneration status in Dire Dawa administration. To assess land use land cover change, time-series satellite images that included Landsat MSS, TM and ETM+, which covered the time frame between 1985 to 2000 and 2000-2014, were used. Review of secondary documents was carried out to understand historical trends, collect ground truth and other information required. A multistage stratified cluster sampling was used for above ground biomass assessment. Nine sample plots of 10m*10m size in each kebeles and a total of 36 sample plots in all clusters were randomly established. In all sample plots woody plants having > 5cm DBH were measured for their diameter at breast height, and biomass estimated using allometric equation. The land use land cover change has shown a major shift in the vegetation cover of Dire Dawa Administration. Substantial portions of the natural, plantation, and woodland forests were converted into agricultural land, settlements, and bare lands. The major change occurred in 1985-2000 compared with 2000-2014. Increased reliance on forests for energy, income generation, construction, and expansion of subsistent agriculture constitute a growing threat to the physical integrity, biomass and regeneration of woodlands and forests of the Administration. The number of regenerants of tree species was low and only 36% of the plots had tree regenerants. Similarly, 279 tree regenerants were recorded out of the total 1116 observation, while the remaining (837) regenerants were herbaceous plants. Out of the four clusters Jelidesa cluster was relatively higher above ground woody plants biomass (6.27ton/ha) than the other clusters and the lowest was recorded for Aseliso cluster (3.90ton/ha).

Keywords: Land use land cove change; Satellite Image; Above Ground Biomass; Regeneration.

Introduction

Dependence on forest for energy, food, medicine and other products posed pressure on physical integrity, diversity and productivity of forests and woodlands. Furthermore, grazing, and subsistence agriculture have also created intense, competing demands on the vegetation cover. In Sub-Saharan Africa changes in land cover are possibly driven by five categories of causes: long-term natural changes in climatic conditions; geomorphologic and ecological processes (e.g., soil erosion and natural vegetation dynamics); human-induced alterations of vegetation cover and landscapes (e.g., deforestation and land degradation); inter annual climatic variability (e.g., recurrent droughts and floods); and human-induced climatic changes (Lamhin and Ehrlich, 1997*).*

In Ethiopia steadily growing population pressure and agricultural expansion will inevitably increase the forest resources utilization (construction and fuelwood), and hence, different forms of unsustainable forest utilization will take place (fires, encroachment, logging, cultivation, urbanization) in coming decades ultimately leading to the total forest depletion. Based on the most recent estimates of the rates of deforestation, and assuming that 75 percent of forest losses are attributable to agricultural expansion, it is estimated that over the next 25 years, the agriculture sector will require an additional 250 to 300 million hectares of new land to accommodate the demands of commercial farming, subsistence cropping, pasture and range development. Most of this increase in land area will come at the expense of forests lands (Limenih and Mekonnen, 2011). Similarly deforestation and forest degradation are serious environmental challenge in Ethiopia. Destruction of the natural forests of Ethiopia results directly in the loss of unaccounted plant and animal species as well as in a shortage of fuelwood, timber and other forest products. It also indirectly leads to more aggravated soil erosion, deterioration of the water quality, reduction of agricultural productivity, and to an ever-increasing poverty of the rural population (Limenih and Mekonnen, 2011, CRGE, 2010). It is obvious that the depletion of forest resources contributes significantly to the climatic and physical changes of the environment (Bond, I *et al*., 2010; Schulz, J.J, 2011). Ethiopia's rural energy needs are predominately satisfied by biomass (>90%). This includes traditional energy sources such as fuelwood, charcoal, and branches, leaves, and twigs. Authorised and unauthorised logging, however, is currently a relatively minor driver of forest degradation (CRGE, 2010).

Currently forest and biodiversity degradation are attracting the attention of governments and the media (Singh *et al.,* 1997; Hegde and Enters, 2000). Additionally, drought and other natural phenomenon complicated the problem (Pimm *et al.,* 1995; Groombridge, 1992; Brook *et al.,* 2006). Hence, maintenance of plant species diversity in the understory should be an important goal of current silviculture prescriptions; not only because of their sensitivity to disturbance but also because of the essential role they play in ecosystem structure and function (MacLean and Wein, 1977; Roberts and Gillam, 1995). Biomass is an important element in the carbon cycle, specifically carbon sequestration. It is used to help to quantify pools and fluxes of greenhouse gases (GHG) from the terrestrial biosphere to the atmosphere associated with land use land cover changes (Cairn et al.2003). Changes in aboveground biomass were linked primarily with fire and harvesting. Fire was the most important single factor contributing for woodland degradation (~50%) compared with pole harvesting (12%), charcoal production (10%) and agriculture (31%) (Chidumayo E.N, 2013). At the more arid end of the spectrum, woody biomass is a key resource with respect to both ecosystem structure and functioning, as well as to rural communities, especially for fuelwood (Pandey, 2002; Shackleton *et al.*, 2004).

It is so important to assess the land use land cover, vegetation composition and regeneration potential, and above ground woody biomass for better management of the remaining vegetation and design appropriate policies for conservation of woodlands of Dire Dawa Administration. The objective of this study was to investigate the land use land cover change,woody plants above ground biomass and regeneration status of tree species in Dire Dawa administration.

1. Material and Methods

1.1. Description of Study Sites

The study was conducted in Dire Dawa administration, which is located at 515km east of the capital, Addis Ababa. It lies with latitude and longitude of 9°36'N 41°52'E and 9o60'N 41°86'E coordinates. Dire Dawa has an average high and low temperature of 31.8°C and 17.9°C, respectively. The annual average precipitation is 612mm. The administration is divided into four clusters and sub divided into 38 rural kebeles (the lowest administration unit).

1.2. Data Collection

1.2.1. GIS based land use/cover change assessment

To assess the land use/cover change, multi-temporal (Landsat MSS 1985, Landsat TM 2000, and Landsat ETM$^+$ 2014) remote sensing data of the area was used. Image enhancement, rectification and classification were also applied on the raw images. The land cover condition of two different periods was spatially compared (1985 to 2000 and 2000 to 2014) and the rate and quality of change was calculated. Image classification was only used for the extraction of distinct classes or land use/land cover categories from satellite imagery. Ground survey was also made to cross check some of the features identified from satellite image. Detail ground survey was conducted to assess the status of forests. Based on knowledge of the study area, visual interpretations of imagery and detailed reconnaissance field survey, different land use/land cover categories were distinguished.

1.2.2. Vegetation data collection

A multistage stratified cluster sampling was used in the study. First the four clusters of the administration were purposively selected. Secondly, each cluster was grouped into homogenous groups based on the type of vegetation and their coverage. Thirdly,sample kebeles (lowest administrative unit) were randomly selected from each homogenous group. Finally, after reconnaissance survey of the sample kebeles, actual sampling of vegetation was done based on homogeneity via preferential sampling method. In Each kebeles, three sample plots of 10mX10m were established. Hence, 9 plots for each clusters and a total of 36 sample plots (4 cluster * 3 kebeles * 3 replications) from the four clusters were established. The diameter of individual trees was measured using Diameter tape and recorded for trees whose diameter at breast height (dbh) is > 5cm. The DBH of irregular trees was measured using a method developed by Pearson *et al.*, (2005). Types of tree species, regeneration status and other physical features in the sample plots were also recorded.

1.3. Data Analysis

Analysis of land use land cover data was done through integrated use of ERDAS imagine (version 9.1), ENVI (version 4.3) and ArcGIS (version 9.2) software packages along with Microsoft office analytical tools. While, analysis of biomass and social data was done by using Statistical Package for Social Science (SPSS) software version 20. Hence, one Way ANOVA was used at 95% confidence interval.

For above ground biomass estimation, the equation developed by Martinez-Yrizar *et al.*, (1992), for tropical dry forest, was selected.

$Y = 10^{\{-0.535+\log_{10}(BA)\}}$....... (Euq...1)

Where; Y= biomass per tree in kg,

D = dbh in cm, and

BA = basal area in cm^2

Basal diameter of a tree is an excellent predictor of dry weight. Further, fitted linear regression equations after log transformations were excellent predictors of biomass from stem diameters (Okello B.D, *et al.*, 2001). The same authors reported the strong relationship between weight and stem diameter of many dry land species.

2. Result and Discussion

2.1. Land Use-land Cover Change in Dire Dawa Administration

The satellite image of 1985, 2000 and 2014, and comparison of the vegetation cover for the duration of 1985-2000 and 2000- 2014 has shown that natural, plantation and woodland forests have been steadily reduced since 1985. The 1985 satellite image has shown that Acacia woodland had covered 65.5% of the land mass of the administration followed by shrub land (19.93%) and plantation forests (11.07%). The coverage of agricultural land and natural forest were 1.32 and 0.38% respectively in the specified year. Similarly, though there was massive reduction in coverage, the acacia woodland (49.34%), shrub land (29.9%) and plantation forests (7.75%) were the largest land covers observed in 2000. In 2014, the acacia woodland, shrub land, and bare lands had a coverage of 48.74, 33.02 and 7.18%, respectively, of the total area of the administration. Hence, massive conversion of natural, plantation and woodland forests occurred during 1985-2000 than 2000-2014.

The small increment of the size of agricultural land during 2000 -2014 could be attributed to the absence of suitable land for crop production and inhospitable climatic condition of the area for agricultural activities at least under the existing agricultural technologies used in the administration; this is in line with the findings of Campbell *et al*., (2007); CRGE, (2010). The increment of shrub land during 1985-2000 was primarily because of the destruction of plantation and natural forests and their substitution by shrubs. But during 2000-2014 the size of shrub lands remained the same as most of them are found in inhospitable areas for agriculture and other related activities. The reduction in the size of bare lands (-105.7 ha) during 2000-2014 is attributed to the massive area enclosure and rehabilitation of degraded areas in the administration. It is, therefore, possible to conclude that anthropogenic factors are the driving forces for land use/cover change in Dire Dawa Administration. A study by Grime (1997) showed that human interference is the common factor affecting natural vegetation. As indicated (EFAP, 1994) annually Ethiopia losses about 150,000 to 200,000ha of forest primarily for expansion of rain feed subsistence agriculture, fuelwood collection and over grazing.

Agricultural land coverage is high for Awale and Wahile clusters compared with the other two. This could be attributed to the presence of water sources, fertile river banks and gentle slope ground conditions. Jeldesa cluster, on the other hand, has the highest acacia woodland and other shrub lands coverage due to its far distance from major towns and are less exploited for fuelwood and charcoal making (Schulz, 2011). *Prosopis juliflora* primarily found in Aseliso cluster has been intensively used for fuelwood and become major sources of income for some rural households. Hence, its expansion was checked as contrasting to the commonly observed invasiveness of the plant in other areas of the nation (Afar National Regional State, 2010, Rettberg, S. and Müller-Mahn, D. (2012).

Table 1. Land use land cover changes of Dire Dawa administration

Cover class	Cover area (ha)	Cover %	Cover area (ha)	Cover %	Cover area (ha)	Cover %	Change in area (ha)		Change in %		Annual rate of change	
	1985	1985	2000	2000	2014	2014	1985-2000	2000-2014	1985-2000	2000-2014	1985-2000	2000-2014
AL	1992.5	1.32	3780.22	2.42	3961.02	2.64	1787.72	180.8	1.18	0.12	119.18	12.05
AW	92757.2	61.39	74,549.67	47.76	73599.8	49.07	-18207.5	-949.8	-12.1	-0.6	-1213.8	-63.3
SM	2913.83	1.93	4783.59	3.06	7818.43	5.21	1869.76	3035	1.24	2.01	124.65	202.3
NF	572.82	0.38	139.68	0.09	2.04	0	-433.14	-137.6	-0.29	-0.09	-28.88	-9.18
SL	30112.6	19.93	52,982.35	33.94	52,978	35.32	22,869.7	-4.45	9.97	0	1524.6	-0.3

BL	6028.18	3.99	10949.18	7.01	10843.5	7.23	4921	-105.7	2.26	-0.07	328.07	-7.05
PL	16721.03	11.07	8921.03	5.72	797.28	0.53	-7800	-3113.8	-8.47	-2.07	-594.74	-207.5
Tot	151098	100	156105.7	100	150000.1**	100						

*AL=Agricultural land, AW=Acacia woodland, NF=Natural forest, PF= Plantation forest, SL= Shrub land, BL=Bare land, SM= Settlement *includes2546.37ha of Prosopis juliflora primarily found in Haseliso cluster.*

*** The area of the administration has shown variation in all assessment years due to inclusion and exclusion of some areas into Dire Dawa administration and to other neighboring regions.*

2.2. Estimated Biomass of the Four Clusters

Comparison of the mean above ground woody biomass of three kebeles from each cluster showed that Jeldesa cluster has the highest above ground woody biomass (6.27± 4.19) compared with other clusters, which is attributed to the presence of well protected riparian forests and less exploited for fuelwood and construction as it is far from major cities (Schulz, 2011) and less populated pastoral area. The lowest mean above ground biomass was recorded in Aseliso cluster (3.90±1.50) resulting from expansion of Dire Dawa town and exploitation of the vegetation for fuelwood both for own consumption and market by rural residents.

Table 2. Cluster wise comparison of land use land cover (in 2014).

Cluster	AL	AWL	SM	NF	SL	BL	PL	Sub Total
Awale	1692.21	16228.26	1838.38	0.09	7056.93	3029.8	503.49	30349.16
Aseliso	371.47	7126.13	1647.37	0	9994.59*	1796.03	0	20935.59
Wahile	1333.17	18644.98	1510.96	1.95	1632.42	2114.67	174.01	32631.55
Jeldesa	386.07	29976.36	2292.35	0	26948.16	3651.02		63253.96
DD town	178.1	1623.45	528.47	0	1111.32	284.02	119.78	3845.14
Grand Total								151015.4

* 3244.84 ha of *Prosopis juliflora* included

Table 3. Clusters mean above ground woody biomass (ton/ha).

Clusters	Kebeles			Total Mean
	1	2	3	
Aseliso	3.99 ± 0.69	4.36 ± 2.09	3.36 ± 1.82	3.90± 1.50 ^{b}
Awale	3.88 ± 1.45	5.60 ± 0.58	4.06 ± 1.12	4.51± 1.26 ^{ab}
Jalidesa	3.77 ± 2.17	4.39 ± 1.12	10.64 ± 4.56	6.27± 4.19 ^{a}

Wahil	5.13 ± 1.11	5.03 ± 0.52	5.05 ± 0.73	5.07± 0.72 [ab]
LSD				2.25

Means with the same letters are not significantly different at P=0.05.

2.3. Regeneration of Tree Species and Herbaceous Vegetation

The number of regenerants of tree species in Dire Dawa administration was low (Fig. 5). The poorly managed woodlands and forests experienced human interference that leads to disturbance and fragmentation of the existing vegetation. Selective harvesting of some particular species such as *Juniperus procera, Acacia tortiles,Acacia bussie and Acacia meliferaa*ffected species regeneration and diversity in the administration. It is well noted that disturbances are the primary causes of patchiness and heterogeneity in ecosystems (Turner *et al.*, 2003).

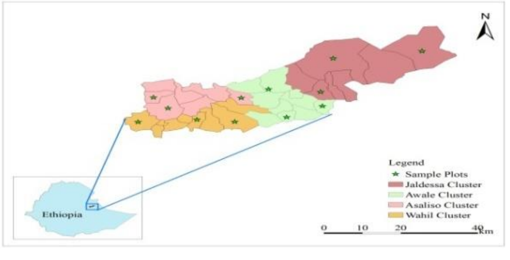

Figure 1. Map of Dire Dawa Administration and sample kebeles.

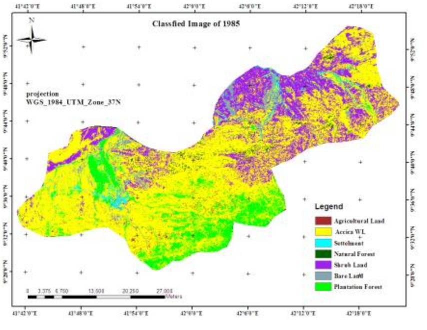

Figure 2. Satellite image of 1985.

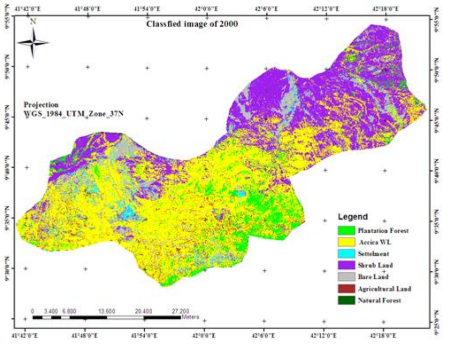

Figure 3 Satellite image of 2000.

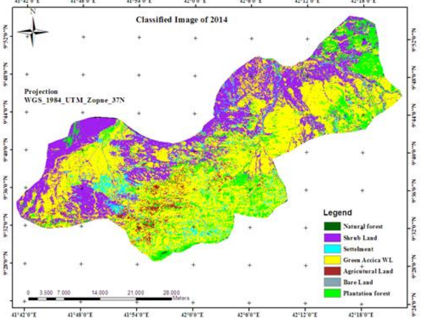

Figure 4. Satellite image of 2014.

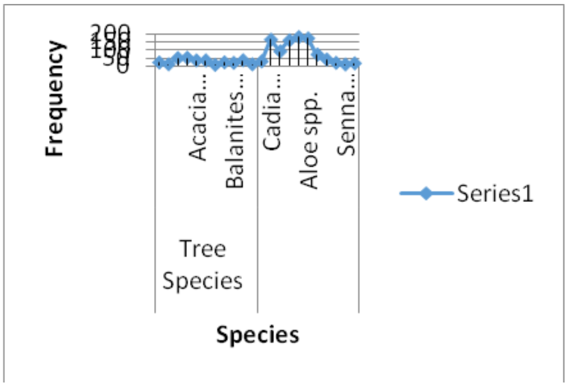

Figure 5. Tree and shrub and herbaceous regeneration in Dire Dawa Administration.

The number of regenerants of tree species in the forest was low and only 36% of the plots had tree regenerants. Similarly, 279 tree regenerants were recorded out of the total 1116 observation where the remaining (837) regenerants are herbaceous plants. The tree species with relatively higher number of regenerants were *Acacia tortilies* (46), *Acacia bussie* (48), *Acacia Senegal* (32), *Acacia melifera(31) and Acacia nilotica* (31).On the contraryherbaceous species such as *Opuntia spp* (178), Aloe spp. (170), *Cadia purpurea* (161) and *Lantana camara* (158) were found in abundant. The low number of tree regenerants is attributed to human activities such as fuelwood collection, charcoal making. Besids, the arid agro-ecology of the administration, limited germination and growth of seedlings (Shepperd *et al.,* 2001; Strand *et al.,* 2009a).

As noted by Okali and Eyong-Matig (2004) maintenance of natural regeneration requires sustainable management of natural resources which demands integration of protective, productive, social and environmental aspects of natural resources. In all surveyed plots regeneration of shrub and herbaceous is higher than tree species, which indicates the severity of degradation of woodlands, natural and plantation forests. Furthermore, low regeneration of tree species will have negative impact on the future biomass of the administration.

3. Conclusion and Recommendation

The vegetation cover of Dire Dawa Administration has shown reduction since 1985. Natural forests are almost vanished, plantation forests degraded, and woodlands became fragmented and intensively exploited. The presence of major cities and ever increasing population has exerted massive impact on the vegetation cover of the administration. The arid climate of Dire Dawa, where evapotranspiration is higher than precipitation, limits the availability of water for the growth of tree seedlings and diversity of species. Successful plantation may not be possible unless proper site preparation is in place, sustainable water supply (water budgeting) is secured and appropriate species were selected. Integration of tree planting in watershed development activities is important consideration to be taken by the administration. Avoidance of encroachment into protected areas should also be taken into account. Fuelwood collection and charcoal making may continue and damage important dry land vegetation unless the community well aware about its consequences. The Dire Dawa Administration should look alternative strategies such as homestead plantation, school greening and agroforestry practices to enhance the vegetation cover. Most importantly, however, is implementation of appropriate land use plan in the administration.

4. Acknowledgements

This research was sponsored by Dire Dawa Administration Agriculture, Water, Energy and Mine bureau (AWEMB) through Haramaya University Community Engagement and Enterprise Development vice president office. We are grateful for the technical support rendered by Mr. Merkebu Kassaw on land use land cover part.

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14. Genotype x Environment Interaction, Stability and Co-heritability of Tuber Internal Quality Traits in Potato (*Solanum tuberosum*L.) Cultivars in Ethiopia

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Abstract: Potato is a potential crop in ensuring food and nutrition security in Ethiopia because it yields more edible energy, protein and dry matter per unit area and time than cereal crops. In Ethiopia, potato varieties were developed mainly to fit preparation of traditional foods. However, French fries and chips are the emerging products of tubers that demanded breeders to identify cultivars fit to these processing. This study was conducted with the objectives of identifying wide adaptable potato varieties for internal quality of tubers, and to determine stability, coheritability and correlation among traits. A total of 17 improved and farmers' cultivars were evaluated in three locations during 2012 to 2014 with randomized complete block design and three replications. Specific gravity and starch content of tubers were significantly influenced by the interaction of cultivar x location x season while dry matter was significantly affected only by cultivar, location and growing season. The 15 improved varieties produced tubers with >1.08 gcm-3, >23% and >14% specific gravity, dry matter and starch content, respectively, that fit to French fries and chips processing, but the two farmers' cultivars failed to perform. Bubu and Gera were most stable varieties in producing tubers with uniform specific gravity, dry matter and starch content across environments and other four (Belete, Gudanie, Chirro, and Gorebela) produced tubers with high mean values for all the traits that may be considered for French fries and chips making. Tubers dry matter and starch contents were highly expressed by specific gravity ($R2>0.96$) with strong correlation ($r>0.98$) and high coheritability (>79.85%) of traits with specific gravity. Moreover, it was observed high coheritability (86.65%) of the three traits as covariate. This suggested tuber specific gravity is appropriate measurement in selection of varieties to determine the internal quality of tubers for processing. The significant effect of genotype x environment interaction on specific gravity and starch content suggested the importance of testing varieties across locations over years to identify high performing and wide adaptable varieties to benefit producers, processors and other consumers.

Keywords: Dry matter content; genotype x environment interaction; specific gravity; starch content, and tuber internal quality.

1. Introduction

The high yield potential of potato and its plasticity to environmental regimes makes it as one of the best crops for food and nutrition security in Eastern Africa (Kyamanywa *et al*., 2011). The potato is a versatile food crop and source of cheap human diet in many countries. It is the third most important food crop in terms of consumption in the world after rice and wheat ((Birch *et al*., 2012; Hancock *et al*., 2014). On average, 20% of a potato tuber is dry matter. Of this dry matter 60 to 80% or more composed of starches making a food rich in carbohydrates (Lutaladio and Castaidi, 2009). It surpasses wheat, rice and maize in the production of dry matter and protein per unit of area. The biological value of potato protein (about 71% that of whole egg) is better than of wheat (53%), maize (54%), peas (48%), beans (46%) and is comparable to cow's milk (75%) (Hawkes, 1990; FAO, 2001). Besides supplying of carbohydrates and a good source of quality protein (lysine), potato is a good source of minerals, nutrient salts and several vitamins (Horton, 1987). Potato also used for industrial purposes; starch in textile, paper making, glue, used as bioreactors for biopharmaceuticals for encapsulation and controlled release of functional ingredients (Li *et al*., 2009) and designer starches (Davies, 1998).

The use of potato is diverging from fresh product into commercial processed foods such as French fries and chips. The tuber specific gravity, dry matter and starch contents are critical in determining the quantity and quality of both products of industries (starch) and processed foods (French fries and chips). For French fry and potato chip industries, breeding for reduced sugar content, acceptable specific gravity, dry matter and starch contents is a primary goal (Lynch et al., 2003). Therefore, selection of varieties is not only for high yield, but also for internal quality of tubers.

Potato is a potential crop in Ethiopia to ensure food and nutrition security. Approximately, 1.3 million farmers grow potato in mid and highlands of Ethiopia and the area planted with potato increased from 30,000 to about 164,146 hectares between 2002 and 2007 (CSA, 2011). In Ethiopia, more than 27 potato varieties were developed and registered by the government research institutions starting 1987 (Gebremedhin, 2013). The varieties were developed for high yield and resistant to late blight and mainly for traditional meals and dishes not other processed products (chips and French fries). However, substantial amount of potato production is exported to neighboring countries, particularly from eastern Ethiopia (Adane *et al*., 2010) and the use of chips and French fries in cities is increasing (Elfnesh *et al*., 2011). Therefore, it is necessary to evaluate potato varieties for internal quality of tubers (specific gravity, dry matter and starch contents), assess the correlation and coheritability of these traits, and determine the effect of genotype, environment and genotype x environment interaction due to their implication on heritability of traits (Vermeer, 1990). Therefore, this research was conducted with the objectives; i) to evaluate potato cultivars for internal quality of tubers and identify wide adaptable varieties, ii) to determine the effects of genotype, environment, genotype x environment interaction and stability of tuber internal quality traits, and iii) to estimate the coheritability and correlation of quality traits.

2. Materials and Methods

2.1. Description of the Study Sites

The field experiment was carried out at three locations namely; Haramaya, Hirna and Arberkete which are considered as the representative potato growing areas of eastern Ethiopia. The experiment was conducted for two cropping season (2012 and 13) in all the three locations. In addition, at Haramaya, potato cultivars were evaluated during 2014 cropping season. This made a total of seven environments considering one location and one cropping season as one environment.

Haramaya University research farm is located at 2002 m.a.s.l., 9°41"N latitude and 42°03"E longitude. The area has a bimodal rainfall distribution with mean annual rainfall of 760 mm. The long rainy season extends from June to October and accounts for about 45% of the total rainfall. The mean maximum temperature is 23.4^{0}C while the mean minimum annual temperature is 8.25^{0}C (Tekalign, 2011). The soil of the experimental site is a well-drained deep alluvial with a sub-soil stratified with loam and sandy loam (Tamire, 1973). Hirna sub-station is situated at a distance of about 134 km to the west of Haramaya. The site is located at 9 ^{o}12' North latitude, 41 ^{o}4'East longitude, and at an altitude of 1870 meters above sea level. The area receives mean annual rainfall ranging from 990 to 1010 mm. The average temperature of the area is 24.0^o C (Tekalign, 2011). The soil of Hirna is vertisol. Arberekete field experiment was conducted on a farmer's filed, which is located at a distance of about 171 km to the west of Haramaya. The site is located at 9 ^{o}14' North latitude, 41 ^{o}2'East longitude, and at an altitude of 2280 meters above sea level.

2.2. Experimental Materials

A total of 17 potato cultivars of which 15 improved varieties and two farmers' cultivars were used. These varieties were released by five research centers and Haramaya University during 1987 to 2011 for different agroecologies of the country. The description of the varieties is given in Table 1.

Table 6. Name, accession code, year of release, maintainer center of potato cultivars.

Genotype	Accession code	Year of release	Breeding center	Recommended altitude (m.a.s.l.)
Araarsaa	CIP-90138.12	2006	Sinnana Research Center	2400-3350
Bedasa	AL-114	2001	Haramaya University	2400-3350
Belete	CIP-393371.58	2009	Holeta Research Center	1600-2800
Bete	Local cultivar			
Bubu	CIP-384321-3	2011	Haramaya University	1700-2000
Bulle	CIP-387224-25	2005	Hwassa Research Center	1700-2700
Chala	CIP-387412-2	2005	Haramaya University	1700-2000
Chirro	AL-111	1998	Haramaya University	2700-3200
Gabbisa	CIP-3870-96-11	2005	Haramaya University	1700-2000
Gera	KP-90134.2	2003	Sheno Research Center	2700-3200
Gorebela	CIP-382173.12	2002	Sheno Research Center	1700-2400
Gudanie	CIP-386423.13	2006	Holeta Research Center	1600-2800
Guasa	CIP-384321.9	2002	Adet Research Center	2000-2800
Jalenie	CIP-37792-5	2002	Holeta Research Center	1600-2800
Jarso	Local cultivar			
Mara Charre	CIP-389701-3	2005	Hwassa Research Center	1700-2700
Zemen	AL-105	2001	Haramaya University	1700-2000

Source: Plant Variety Release, Protection and Seed Quality Control Directorate, Crop Variety Register Issue No.16, pp.161-164 (Ministry of Agriculture, 2013, June, Addis Abeba, Ethiopia).

2.3. Experimental Design and Procedures

The experiment was laid out as a randomized complete block design with three replications in each environment. Each potato cultivar was assigned to one plot in each replication and six rows each with 12 plants. The gross plot size was 16.2 m^2 with 75 and 30cm spacing between rows and plants, respectively. The spacing of 1.5 and 1m was maintained between plots and replications, respectively.

Planting was at the end of June and first week of July during the main growing season after the rain commenced and when the soil was moist enough to support emergence. The planting depth was maintained at 10cm. The whole recommended rate of Phosphorus fertilizer (92 kg P_2O_5 ha^{-1})was applied at planting in the form of Diammonium Phosphate. Nitrogen fertilizer was applied at the rate of 75 kg N ha^{-1} in the form of Urea in two splits, half rate after full emergence and half rate at the initiation of tubers.Other agronomic managements were applied as per the recommendation made for the crop. The haulm was mowed two weeks before harvesting to thicken tuber periderm.

2.4. Data Collection

Tubers were carefully collected after the hills were hand dug. Tuber dry matter content (%) was measuredfrom five fresh tubers in each plot. The randomly selected tubers were weighed at harvest, sliced and dried in oven at 75^{o}C until a constant weight is obtained and dry matter percent were calculated as per the established procedure (William andWoodbury, 1968).

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

Specific gravity of tubers was measured using weight in air and weight in water method. Five kg tubers of all shapes and sizes were randomly taken from each plot and washed with water then weighed first in air and then in water. The specific gravity of tubers was calculated using the following formula (Kleinkopf *et al.*, 1987).

$$\text{Specific gravity}(gcm^{-3}) = \frac{Weight\ in\ air}{Weight\ in\ air - Weight\ in\ water}$$

Total starch content (g/100g) was estimated from specific gravity. Starch (%) =17.546 + 199.07 × (specific gravity-1.0988) (Yildrim and Tokusoglu, 2005) where specific gravity was determined as indicated above by the weight in air and weight in water method.

2.5. Data Analysis

Data were subjected to analysis of variance (ANOVA) for each location and combined over environments following the standard procedure using SAS software version 9.1 (SAS, 2007). The mean squares of cultivar x location x season were significant for tuber specific gravity and starch content and subjected to further genotype x environment interaction (GEI) and stability analyses. Mean separation was employed using Least Significant Differences at 5% probability.

Analyses of variances were computed for seven environments using Additive Main Effects and Multiplicative Interaction (AMMI) (Zobel *et al*., 1988) and regression (Eberhart and Russell, 1966) models. Regression coefficient (bi) and deviation from linear regression (Sdi^2) (Eberhart and Russell, 1966), and from AMMI model (Zobel *et al*., 1988), interaction principal component axes (IPCA) scores of genotype and environment stability parameters were computed. In AMMI biplot, main effects (genotype and environment means) were plotted on the abscissa and the IPCA 1 scores for the same cultivars and environments on the ordinate (Zobel *et al*., 1988). In addition, AMMI stability value (ASV) was calculated (Purchase *et al*., 2000).

ASV= $\sqrt{\left[\frac{\text{IPCA1SS}}{\text{IPCA2SS}}\left(IPCA\ 1score\right)\right]^2 + [\text{IPCA2score})^2}$

Where, ASV=AMI stability value; SS = sum of squares, IPCA1 and IPCA 2 = the first and the second interaction principal component axes, respectively, and thus cultivars with lower ASV was considered more stable than those with higher ASV values.

Analysis of covariance was also conducted for the three quality related traits over environments following the standard procedure(Gomez and Gomez, 1984). The coheritability of the covariate traits (specific gravity, dry matter and starch contents) was conducted. In this analysis, the total variation was partitioned into genotypic, phenotypic and environmental convinces as follows.

Environment convince between character x and y = $\sigma^2 exy$

Genotypic convince between character x and y ($\sigma^2 g$xy)

$$\sigma^2 g\text{xy} = \frac{Mean\ products\ of\ between\ genotypes - Mean\ products\ of\ within\ varieties\ (Error)}{Number\ of\ replications\ x\ Number\ of\ locations}$$

Mean products of between genotypes = Mean products of genotypes minus mean products due to genotype x season, genotype x location and genotype x season x location. Because the mean products of between genotypes is the sum of genotype x season, genotype x location and genotype x season x location since the experiment was conducted across locations and seasons.

Phenotypic convince between character x and y ($\sigma^2 p$xy) = $\sigma^2 g$xy + $\sigma^2 e$xy

Coheritability (CH2) = ($\sigma^2 g$xy/$\sigma^2 p$xy) x 100

Linear regression analysis for tuber specific gravity, dry matter, and starch contents was conducted for the mean performance of each cultivar at each location and overall mean performance of cultivars over locations. The regression was computed taking tuber specific gravity mean performance of cultivars over years and locations as dependent variable and over all mean performance of dry matter and starch contents as independent (response) variables. The regression equation for specific gravity, dry matter and starch contents were calculated and presented in graph.

3. Results

3.1. General Analysis of Variance and Mean Performance of Cultivars

The combined unbalanced analysis of variance over seven environments revealed the presence of significant influence of cultivar, location and season, the interactions of cultivar x location and location x season had significant influence on tuber dry matter content. Tuber specific gravity and starch content were significantly affected by the interaction of the three factors (cultivar x location x season) in addition to the three main factors and one or more of the two factors interactions (Table 2).

Table 2. Mean squares from unbalanced analysis of variance for tuber internal quality traits of 17 potato cultivars evaluated at three locations during 2012 to 2014 cropping seasons.

Source of variation	DF	DM (%)	SG (gcm^{-3})	Starch g/100g

Replication	2	0.456	0.00005058	1.085
Cultivar (G)	16	80.765**	0.00132426**	55.031**
Location (L)	2	51.237**	0.00003343	4.260*
Season (S)	2	8.196*	0.00013145*	4.964*
G x L	32	2.788*	0.00005739	2.555*
G x S	32	2.211	0.00008097*	2.669*
L x S	2	35.758**	0.00008907	7.062*
G x L x S	32	2.229	0.00006724*	2.815**
Error	236	1.782	0.00003885	1.291
CV (%)		5.5	0.57	7.65

** and **, significant at P<0.05 and P<0.01, respectively. DF= degree of freedom, DM (%) = dry matter content in percent, SG (gcm^{-3}) = specific gravity gram per cubic centimeter, Starch g/100g = starch content gram per 100 gram fresh tuber weight and CV (%) = coefficient of variation in percent..*

The tuber dry matter content of cultivars ranged from 19.08 (Jarso) and 27.18% (Belete) at Haramaya and Hirna, respectively, with overall mean of 24.22% (Table 3). The tuber specific gravity and starch content ranged from 1.065 to 1.097 and 10.71 to 16.88%, respectively (Table 6). Belete, Bubu,Gera, Gorebela, and Guasa had the highest specific gravity, dry matter and starch contents while the two farmers' cultivars had the lowest (Table 3 and 6). In general, none of the improved varieties had <1.08 <23% and <14%gcm^{-3} specific gravity, dry matter and starch contents.

Table 3. Mean tuber dry matter content of 17 potato cultivars at three locations during 2012 to 2014 cropping seasons (seven environments).

Cultivar	Haramaya	Hirna	Arberkete	Overall Mean
Bubu	25.79^{a-d}	25.35^{b}	26.82^{ab}	25.99
Belete	26.63^{a}	27.18^{a}	27.14^{a}	26.98
Chala	25.7^{a-e}	24.26^{bcd}	25.53^{cd}	25.16
Gudanie	25.25^{a-f}	24.75^{bc}	25.81^{c}	25.27
Bulle	24.84^{d-g}	22.33^{ef}	23.86^{efg}	23.68
Chirro	25.93^{a-d}	24.49^{bcd}	25.16^{cd}	25.19

Araarsaa	24.4^{efg}	21.81^{f}	23.26^{g}	23.16
Zemen	25.17^{c-f}	23.66^{cde}	24.58^{de}	24.47
Jalenie	24.87^{d-g}	23.05^{def}	25.41^{cd}	24.44
Jarso	19.08^{h}	19.27^{g}	20.11^{h}	19.49
Gabbisa	23.5^{g}	23.83^{cde}	24.57^{def}	23.97
Gorebela	26.56^{ab}	25.13^{bc}	25.52^{cd}	25.74
Mara Charre	25.21^{b-f}	22.34^{ef}	23.65^{efg}	23.73
Bete	19.88^{h}	19.12^{g}	20.99^{h}	20.00
Bedasa	24.23^{fg}	22.63^{ef}	23.58^{fg}	23.48
Gera	26.27^{abc}	25.55^{b}	25.84^{bc}	25.89
Guasa	25.59^{a-f}	23.77^{cde}	25.96^{bc}	25.11
Mean	24.64	23.44	24.576	24.22
LSD	2.39	2.123	1.4202	
Year				
2012	24.36	22.65^{b}	24.76^{a}	23.92
2013	24.62	24.23^{a}	24.39^{b}	24.41
2014	24.94	--------	--------	24.94
LSD (5%)	NS	0.515	0.344	

Means with similar letter(s) are not significantly different each other.

3.2. Genotype x Environment Analysis of Variance

The mean squares of all sources of variation (genotype, environment, genotype x environment interaction, IPCA I and II and residuals) were significant for specific gravity and tuber starch content in AMMI model analysis (Table 4). Eberhart and Russel's model analysis of variance also exhibited significant mean squares of genotypes, genotype x environment (linear), environment + (genotype x environment) and pooled deviation for starch content and specific gravity except the mean square of pooled deviation was nonsignificant for specific gravity (Table 5).

Table 4. AMMI analysis of variance for tuber specific gravity and starch content of 17 potato cultivars tested at seven environments (three locations during 2012 to 2014).

		Specific gravity (gcm^{-3})				Starch content g/100g			
		Sum of squares	Mean squares	Sum of square explained		Sum of squares	Mean squares	Sum of square explained	
Sources of variation	DF			% total	% G x E			% total	% G x E
Treatment	118	0.02827	0.0002396**	75.29		1165.2	9.875**	79.47	
Genotype	16	0.02119	0.0013243**	74.96		879.3	54.954**	75.46	
Environment	6	0.00051	0.0000847**	1.80		31.3	5.224**	2.69	
Rep within E	14	0.00038	0.000027	1.34		10.7	0.766	0.92	
G x E	96	0.00658	0.0000685**	23.28		254.6	2.652**	21.85	
IPCA 1	21	0.00269	0.0001279**		40.88	126.8	6.039**		49.8
IPCA 2	19	0.00215	0.0001131*		32.67	78.5	4.133**		30.83
Residuals	56	0.00175	0.0000312		26.60	49.3	0.88		19.36
Error	224	0.00889	0.0000397	23.68		290.3	1.296	19.8	
Total	356	0.03755	0.0001055			1466.3	4.119		

** and **, significant at P<0.05 and P<0.01, respectively. DF= degree of freedom, Rep within E= replication within environments, G x E=genotype by environment interaction, IPCA 1 and 2, interaction principal component axis one and two, respectively.*

Treatment sum of squares had highest contribution to total sum squares that accounted 79.47 and 75.29% for starch content and specific gravity, respectively. The error sum of squares contributed to total sum squares only 19.8 and 23.68% for starch content and specific gravity, respectively. Genotype sum squares accounted the highest proportion of 75.46 and 74.96% for treatment sum squares for starch content and specific gravity, respectively, while genotype x environment and environment sum squares had lower share. The IPCA 1 sum square contributed the highest share for genotype x environment sum squares as compare to IPCA 2 and residuals (Table 4).

Table 5. Analyses of variance from Eberhart and Russel's Model for specific gravity and starch content of 17 potato cultivars tested at three locations during 2012 to 2014.

Source of variation	DF	Specific gravity (gcm^{-3})	Starch content (g/100g)
Genotypes	16	0.0071**	18.32**
Environment + (Geno x Env.)	102	0.0024**	0.93**
Environment (linear)	1	0.0002**	10.45
Genotypes x Env. (linear)	16	0.0007*	1.55**
Pooled Deviation	85	0.0015	0.71**
Bubu	5	0.0003	0.10
Belete	5	0.0000	0.14
Chala	5	0.0000	0.78
Gudanie	5	0.0000	0.12
Bulle	5	0.0000	0.23
Chirro	5	0.0000	3.23**
Araarsaa	5	0.0001	1.05
Zemen	5	0.0000	0.12
Jalenie	5	0.0000	0.05
Jarso	5	0.0004	2.66*
Gabbisa	5	0.0000	0.04
Gorebela	5	0.0000	0.25

Mara Charre	5	0.0002	1.10
Bete	5	0.0001	0.84
Bedasa	5	0.0002	1.17
Gera	5	0.0000	0.04
Guasa	5	0.0000	0.10
Pooled Error	238	0.0031	0.42

** and **, significant at P<0.05 and P<0.01, respectively.*

3.3. Stability Analyses

Bulle, Araarsaa, Guasa, Bete, Bubu and Belet were ranked the lowest ASV for specific gravity, but only Belete, Bubu and Guasa had highest mean values ($\geq$1.09). Bedasa, Gorebela and Mara Charre were ranked the highest ASV but had high mean specific gravity. Mara Charre, Chala, Bubu, Bedasa and Gera were ranked the lowest ASV for starch content, however, only Bubu and Gera had highest mean starch content (>15%) (Table 6). For starch content, the deviation from regression (S^2di) was nonsignificant from unity for all cultivars, whereas regression coefficient (bi) was significantly different from zero for all except Belete, Chirro, Araarsaa, Gorebela, Mara Charre, Bedasa and Bete. Both S^2di and bi values were nonsignificant for tuber specific gravity in all cultivars.

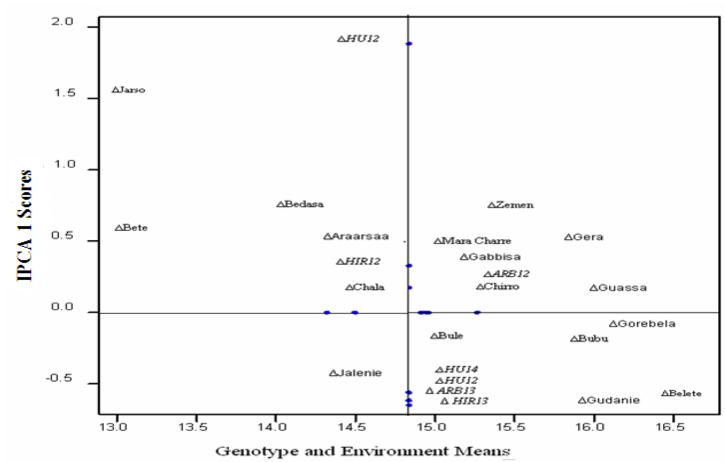

Figure 1. AMMI biplot of 17 potato genotypes evaluated for tuber starch content at seven environments in eastern Ethiopia.

The AMMI biplot for starch content was constructed and presented in Figure 1. The AMMI bi-plot showed that Gera, Mara Charre, Zemen, Gabbisa, Chirro and Gussa were displayed at top right, while Bedssa, Bete, Ararrsa and Chala at top left of the quadrant. All the others cultivars plotted at bottom right except Jalene were displayed at bottom left of the biplot quadrant. The five environments were plotted at bottom right whereas Arberkete and Hirna during 2012 cropping season were placed at top right and top left of the quadrants, respectively.

Table 6. Stability parameters for tuber specific gravity and starch content of 17 potato cultivars from AMMI and Eberhart and Russel's models analyses at seven environments (three locations during 2012 to 2014).

	Specific gravity (gcm^{-3})						Starch content g/100g tuber					
	Pooled	AMMI model stability			ER's stability	Model	Pooled	AMMI model stability			ER's stability	Model
Cultivar	Mean	IPCA 1	IPCA 2	ASV	b_i	S^2di	mean	IPCA 1	IPCA 2	ASV	b_i	S^2di
Bubu	1.091 (3)	0.093	-0.0003	0.028 (4)	0.0967	0.0003	15.84 (4)	0.1	-0.22	0.07 (3)	1.31**	-0.33
Belete	1.097 (1)	-0.021	0.0179	0.031 (5)	0.3451	0.0001	16.88 (1)	-0.22	0.28	0.20 (7)	0.44	-0.28
Chala	1.086 (7)	0.008	-0.1071	0.107 (13)	6.3783	0.0001	14.8 (11)	0.18	-1.45	0.06 (2)	4.41**	0.36
Gudanie	1.088 (5)	0.024	-0.0514	0.053 (10)	3.2309	0.0001	15.84 (4)	0.4	-0.67	0.31(8)	3.21**	-0.31
Bulle	1.085 (8)	0.016	-0.0188	0.023 (1)	1.7403	0.0001	14.97 (10)	0.28	-0.21	0.32 (9)	1.34*	-0.2
Chirro	1.090 (4)	0.014	0.0391	0.039 (8)	-1.015	0.0001	15.25 (7)	1.51	0.18	4.34 (16)	1.75	2.8
Araarsaa	1.083 (9)	-0.015	0.0101	0.023 (1)	0.3299	0.0001	14.76 (12)	0.72	0.46	0.90 (14)	1.09	0.63
Zemen	1.087 (6)	0.046	0.0559	0.067 (12)	-2.324	0.0001	15.35 (6)	-0.49	0.71	0.41(12)	-1.55**	-0.3
Jalenie	1.085 (8)	-0.014	-0.0521	0.052 (9)	3.6422	0.0001	14.6 (13)	0.39	-0.48	0.35 (10)	2.94**	-0.37
Jarso	1.065 (12)	-0.086	0.0011	0.066 (11)	2.0037	0.0004	10.71 (16)	-1.51	-0.22	3.96 (15)	-0.321	2.24
Gabbisa	1.085 (8)	0.023	0.0348	0.038 (7)	-0.975	0.0001	15.11 (8)	-0.24	0.39	0.18 (6)	-0.98**	-0.38
Gorebela	1.092 (2)	0.027	0.0035	0.203 (15)	0.5871	0.0001	16.15 (2)	-0.25	-0.09	0.41 (12)	0.04	-0.18
Mara Charre	1.083 (9)	-0.069	0.0092	0.521 (16)	1.2469	0.0002	14.98 (9)	0.06	0.46	0.02 (1)	1.68	0.68
Bete	1.066 (11)	-0.001	-0.0265	0.027 (3)	2.4519	0.0001	11.18 (15)	-0.65	-0.47	0.76 (13)	1.7	0.42
Bedasa	1.080 (10)	-0.059	0.0235	0.149 (14)	0.3875	0.0002	13.97 (14)	0.22	0.72	0.12 (4)	1.05	0.75
Gera	1.091 (3)	-0.001	0.0367	0.037 (6)	-0.823	0.0001	15.82 (5)	-0.24	0.49	0.16 (5)	-0.61**	-0.38
Guasa	1.090 (4)	0.015	0.0245	0.026 (2)	-0.304	0.0001	15.96 (3)	-0.27	0.13	0.39 (11)	-0.14	-0.33
Mean	1.085						14.83					

** and **, significant at $P<0.05$ and $P<0.01$, respectively.Numbers in parenthesis represent the pooled mean and ASV rank of cultivars in descending and ascending order, respectively. IPCA 1 and IPCA 1 = interaction principal component axis one and two, respectively, ASV = AMMI stability value, ER's = Eberhart and Russel's mmodel, biand S^2di, regression coefficient and deviation from regression, respectively.*

3.4. Analysis of Covariance and Coheritability

The analysis of covariance (ANCOVA) revealed the significant influence of cultivar, location and season as well as all possible interactions of these on covariate. However, dry matter and specific gravity as well as dry matter and starch content as covariate traits were not significantly influenced by the interaction of cultivar x location x season and cultivar x season (Table 7).

Table 7. Mean products from analysis of covariance (ANCOVA) for tuber internal quality traits in 17 potato cultivars tested at three locations during 2012 to 2014.

Source of variation	DF	DM x SG	DM x STAR	SG x STAR	DM x SG x STAR
Replication	2	0.437	992.12	1.583	1421.102
Genotype (G)	16	110.380**	84420.231**	71.983**	105609.111**
Location (L)	2	60.958**	19205.142**	5.488*	23197.012**
Season (S)	2	11.465*	9177.112**	6.424*	11440.104*
G x L	32	3.465*	2350.431	3.253*	2930.045*
G x S	32	2.861	2775.151**	3.548*	3567.131**
L x S	2	43.309**	19375.102**	9.129*	23678.103**
G x L x S	32	2.876	2295.132	3.610**	2901.005*
Error	236	2.179	1255.011	1.726	1619.002
CV (%)		5.6	9.73	8.15	10.17

** and **, significant at $P<0.05$ and $P<0.01$, respectively. DM = tuber dry matter content, SG = specific gravity and STAR = starch content*

The coheritability of tuber internal quality traits ranged from 79.85 (specific gravity and starch content) to 87.02% (dry matter and starch content). The three tuber quality traits (dry matter, specific gravity and starch content) as covariate traits had coheritability of 86.65% while dry matter and specific covariate had coheritability of 83.47% (Table 8).

Table 8. Genotypic and phenotypic covariance and coheritability of tuber internal quality traits in 17 potato cultivars.

Covariate traits	σ^2g	σ^2p	Coheritability (%)
Dry matter content x specific gravity	11.00	13.18	83.47
Dry matter x starch content	8416.06	9671.07	87.02
Specific gravity x starch content	6.84	8.57	79.85
Dry matter x starch contents x specific gravity	10510.21	12129.22	86.65

σ^2g = genotypic covariance of covariate traits x and y, σ^2p = phenotypic covariance of covariate traits x and y, and CH^2 (%) = coheritability of covariate traits x and y in percent.

3.5. Regression Analysis

The linear regression graph along with the equations is presented in Figure 2. Both tuber dry matter and starch contents were highly expressed by specific gravity. The coefficient of determination for starch content was higher (R^2= 0.97) than dry matter content (R^2= 0.96). The correlation between specific gravity and dry matter (r=0.98) and specific gravity and starch content (r=0.99) were highly approaching perfect association.

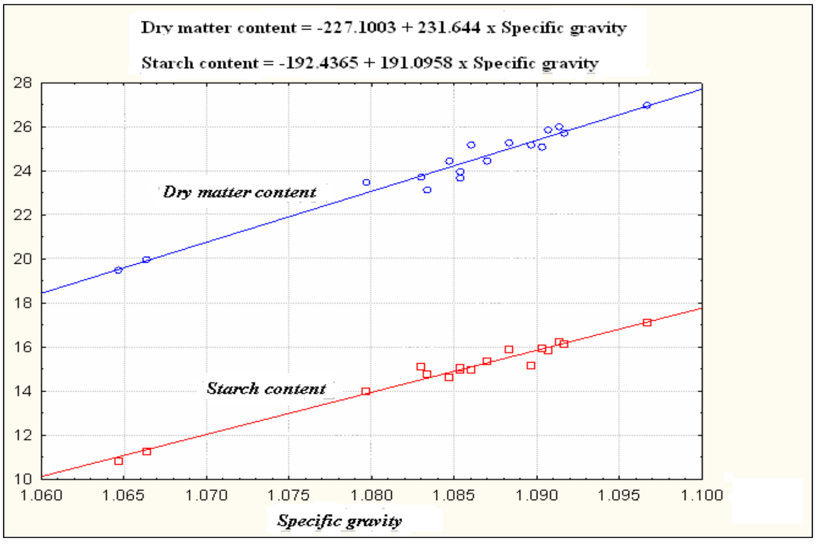

Figure 2. Linear regression of overall mean tuber specific gravity of 17 potato cultivars on overall mean tuber dry matter and starch contents of cultivars with equations of best-fit lines.

4. Discussion

Potato cultivars exhibited significant variations for all tuber internal quality traits. None of the improved varieties had <1.08 gcm^{-3}, <23% and <13% mean values of tuber specific gravity, dry matter and starch content, respectively, while the farmers cultivars failed to perform. Several other authors also reported the variations among varieties in producing tubers with varied quantity of specific gravity, dry matter and starch contents that determine the quality of tubers to be processed to quality French fries and chips(Elfnesh *et al.*, 2011; Hassanpanah *et al.,* 2011; Tesfaye *et al.*, 2013; Kaur and Aggarwal, 2014; Ismail *et al.*, 2015). Tubers with dry matter content of 20 to 24% are ideal for making French fries while those with up to 24% for preparing crisps. Potato tubers should also have specific gravity value of more than 1.080 and those with less than 1.070 are generally unacceptable for processing (Kabira and Berga, 2003). This showed that all improved potato varieties were suitable for French fries and chips making but the farmers' cultivars were not.

The significant influence of location and growing season on all tuber internal quality traits suggested the need to test cultivars across locations over seasons to identify varieties that fit for the intended end use. In addition, specific gravity and starch content were significantly influenced by the interaction of cultivar x location x season indicating the unstable expression of these traits in different cultivars across locations and seasons. These quality traits are genetically controlled and also influenced with growing locations and seasons (Dorota *et al*., 2011; Hassanpanah *et al.,* 2011; Tesfaye *et al*., 2013; Kaur and Aggarwal, 2014). Specific gravity and tuber dry matter content are influenced by both the environment and genotype (Elfnesh *et al*., 2011; Ismail *et al*., 2015). In the presence of significant influence of location and growing season, it is necessary to develop wide adaptable potato varieties. These varieties can produce the same specific gravity or dry matter when grown in differing environments and supply more uniform product that benefit producers, processors and other consumers (Johanson *et al*., 1967).

The predominant factor to determine the internal quality of tubers was the inherent characteristics of cultivars and locations. These quality traits are genetically controlled and also influenced with growing locations and seasons (Dorota *et al*., 2011; Hassanpanah *et al.,* 2011; Kaur and Aggarwal, 2014). However, from the two GEI analyses models, the significant influence of genotype x environment interaction on specific gravity and starch content was evident. This indicates some of the cultivars ranks different in different environments which emphasize the need to breed specific varieties better perform in specific environment(s) (Vermeer, 1990). But developing specific variety for specific environment is resource consuming that may not affordable in less developed countries. In such case, stability is becoming the key issue and the importance of developing varieties that outperform consistently other competing genotypes and perform well over a range of environments (Lin *et al*., 1986).The GEI analyses results suggested the importance of testing of many cultivars across locations and seasons to identify wide adaptable varieties that could produce tubers with uniform specific gravity and starch content in all environments.

All improved varieties had a required tuber quality for quality French fries and chips making. Potato cultivars producing tubers with dry matter content $\geq$20% and specific gravity $\geq$1.080(Kabira and Berga, 2003) as well as starch content $\geq$13% (Kirkman, 2007) were the most preferred for processed products. In this regard, potato improvement program in Ethiopia was in the right track since all varieties fit to general purpose including for French fries and chips processing. However, varieties were varied for stability of tubers internal quality. In this situation, it is necessary to consider the stability parameters along with high performance but the varieties can be responsive to changing environments (dynamic stability) (Yan and Kang, 2003). Bubu, Belete, Gera, Gudanie, Chirro, and Gorebela had higher mean values for all tuber internal quality traits across locations. These varieties can be recommended for production for all purpose including for French fries and chips. Moreover, Bubu and Gera were stable for internal quality of tubers suggested the wide adaptability of the varieties and can be recommended for production in both favorable and unfavorable environments. In choosing superior genotypes, a low or minimal genotype x environment interaction must exist (Cotes *et al*., 2002).

All locations at different cropping seasons plotted in three of four quadrants of the AMMI biplot indicated that the starch content of tubers was not static stability. Static stability refers when among environment variances are small and genotype always performs in a similar manner regardless of environmental conditions. Dynamic stability is when environmental responses are parallel mean genotypic responses (Lin *et al*., 1986). The regression coefficient (bi) being significant for most varieties also suggested that most of the varieties were responsive for the changing environments (Eberhart and Russell, 1966). This research result showed that the impossibility of avoiding either locations or growing seasons during the evaluation of varieties for starch content.

The highest coheritability of the three covariate traits (specific gravity, dry matter and starch contents) and strong correlations of the three traits suggested that the environment favoring or disfavoring one of the traits also had similar effects on the other two traits. It has been suggested that traits with high genotype x environment interaction have low heritability, which adversely affects the ability to select superior genotypes for all environments (Vermeer, 1990). However, this experiment result indicated that the significant effect of genotype x environment interaction on specific gravity and starch content did not adversely affects the coheritability of the three traits. The observed linear relationship of specific gravity with tuber dry matter and starch contents with high coefficient of determination suggested the selection of superior cultivars for tuber specific gravity was also simultaneous selection of the superior cultivars for other two traits. Tuber dry matter content and tuber specific gravity had high and positive correlation and the two traits reflect the amount of starch present which all used as crude indicators of processing quality (Miranda and Agulera, 2006).

Specific gravity is true indicator of the amount of tuber dry mater content due to positive and significant correlation of tuber dry matter content and specific gravity (Tekalign and Hammes, 2005).

5. Conclusion

The understanding of the tuber internal quality traits is important as equal to tuber yield since the improvement of varieties for high yield alone cannot guarantee the satisfaction of the end users. The potato varieties under cultivation in different agroecologies of Ethiopia showed significant variations for tuber specific gravity, dry matter and starch contents. All improved varieties were fit to be processed to quality French fries and chips. However, the observed significant effect of genotype x environment interaction on specific gravity and starch content indicated the importance of testing varieties across locations over years to identify high performing and wide adaptable ones to benefit producers, processors and other consumers. The research also showed that selection of varieties for high tubers specific gravity is the simultaneous selection for dry matter and starch contentsand thereby the determination of internal quality of tubers for processing. Therefore, it can be concluded that the measurement of tubers specific gravity is sufficient at each level of variety development to determine the suitability of genotypes for different purposes or end products.

6. Acknowledgements

The author thanks Haramaya University and National Potato Improvement Project for the financial support, Holeta, Adet and Sinna Research Centers for providing planting materials, and the technical staff of Horticulture Program at Haramaya University for their careful management of the experiment and data recording. The author also thanks Prof. Nigussie Dechassa and Dr. Mengistu Urge for their unreserved support for the success of the experiment particularly at the time when the researcher faced difficulties. The author also thanks Ms. Simret Burga, Mr. Habtamu G/selassie and Mr. Birhanu Blate who were involved in the first year research activities of the experiment.

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15. Internal Tuber Quality of Potato (*Solanum tuberosum* L.) Cultivars and Conversion Chart for Specific Gravity, Dry Matter and Starch Contents in Eastern Ethiopia

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Abstract: Research was conducted at Haramaya, Hirna and Arberkete in eastern Ethiopia during 2012 to 2014 in randomized complete block design with three replications to evaluate internal tuber quality of 17 potato cultivars, establish the relationship among traits and prepare specific gravity conversion chart. Tuber specific gravity (SG), dry matter (DM) and starch contents (SC) were significantly influenced by cultivar, location and year, while the interaction of the three significantly influenced SG and SC. All improved cultivarsproduced tubers with >1.085, >21% and >14% of SG, DM and SC, respectively, to the standard to fit French fries, chips and flakes making in all locations and years while farmers' cultivars tubers had <1.07 gcm2, <20% and <11% SG, DM and SC, respectively, which fit to boiling products. The dendrogram constructed using Unweighted Pair-group Method with Arithmetic means separated the cultivars into three clusters of which Cluster I with distinct Sub-group I consisted of eight (Ararsa, Bule, Marachere, Bedasa, Challa, Jalenine, Gabisa and Zemen) and Sub-group II with six (Bubu, Gera, Gorbella, Gudenine, Gusa and Chiro) improved cultivars, Cluster II and III consisted of one (Belete) and two farmers cultivars, respectively. The all purposes cultivarswere grouped in the Sub-group I as they produced tubers with SG, DM and SC contents to the acceptable standard for the products while cultivars in Sub-group II and Belete (Cluster II) produced tubers with high SG, DM and SC contents that may produce too hard, dry and brittle French fries and chips. The correlation of specific gravity with dry matter and starch contents being perfect or near to perfect with high coefficient of determination suggested that the appropriateness of using specific gravity and the conversion chart to estimate the other two traits and determine the quality of tubers for processing.

Keywords: Conversion chart; Eastern Ethiopia;*Solanum tuberosum* L.; Tuber internal quality; Specific gravity.

1. Introduction

Potato global production has exceeded 376 million tonnes from over 19.3 million hectares (FAOSTAT, 2013). There is some estimate that the crop yield will have to double by 2050 to meet the demand of global food security. Importantly, potatoes are affordable, putting them within reach of the economically disadvantaged. Potato contains high protein-calorie ratio (17g protein: 1000 kcal) and yields more edible energy, protein and dry matter per unit area and time compared to cereals (Anderson et al., 2010). In Ethiopia, potato has been considered as a strategic crop to enhance food and nutrition security. In 2013/14, potato was produced on 179,159 hectares of land with the total 1,612,006 and average of 9.1 tonnes of yield in the country. In East Hararghe, potato is a co-staple food (ORARI, 2007) and export commodity. It is approximately grown by 52,710 farmers with a total area of 2,507.12 hectares and average yield of 19.3 t ha-1 (CSA, 2014).

In Ethiopia, research for potato variety development and other agronomic managements began in 1975. Starting the release of the first potato variety in 1987, 33 potato varieties were developed and registered until 2013 for production under different recommendation domains by research centers, Haramaya University and private companies (Baye and Gebremedhin, 2013; MoA, 2013). The National potato research effort has been in developing high yielding and late blight resistant varieties mainly for different kinds of traditional foods, but less emphasis was given to processing products such as French fries, chips and others. However, small scale potato chips processors are flourishing in cities and big towns (Elfnesh *et al.*, 2011). Potato chips and French fries are commonly found in hotels, restaurants, supermarkets and small shops. In addition, the country has potential to produce potatoes and supply it to large scale potato processing industries that might not be far from establishment. All cultivars are not suitable for the production of processed products (Kabira and Berga, 2006), therefore, it is necessary to evaluate the fitness of cultivarsfor the emerging economics of production until specific cultivarsare developed for specific end products.

Potato tubers quality often referred to the external and internal quality. The internal quality is determined by many traits, of which the most important are dry matter content, type and amount of starch, sugar and protein content (Van Eck, 2007). High tuber specific gravity, dry matter and starch content are important for processing by enhancing chip yield, crispness and reduces oil uptake in fried products (Johnson *et al.*, 2010; Freitas *et al.*, 2012). Potato cultivars are significantly different for tuber specific gravity and dry matter content and starch content is influenced by cultivar and/or growing location (Hassanpanah *et al.*, 2011; Kaur and Aggarwal, 2014; Ismail *et al.*, 2015). Significant influence of environment and genotypes on specific gravity and tuber dry matter content was also reported (Elfnesh *et al.*, 2011; Tefaye *et al.*, 2013; Ismail *et al.*, 2015). Therefore, it is necessary to evaluate potato cultivars for internal tuber quality traits across locations and over seasons. Potato thought to contribute to diabetes and obesity or weight gain due to the high carbohydrate content (Cordain, 2005; Mozaffarian *et al.*, 2011), therefore, cultivars are not only need to be evaluated for the fitness of different processing but also to be processed to healthy food. This forced breeders to evaluate, identify and recommend genotypes that processed and produce safe/healthy foods to outweigh the risks that are associated to potatoes.

There is close relationship among specific gravity, total solids and starch content and relationship has been developed by several workers among these traits (Johanson *et al.*, 1967; Fitzpatrick *et al.*, 1969;Willson and Lindsay, 1969; Verma *et al.*, 1972;Vakis, 1978; AOAC, 1980; Kleinkopf *et al.*, 1987; Dale and Mackay, 1994). Specific gravity conversion tables are available in other countries to be used by the potato processing industry (Houghland, 1966; Lulai and Orr, 1980; DEPI, 1995; USDA, 1997; Ezekiel *et al.*, 2003; Dinesh *et al.*, 2005). But these tables cannot be used in other countries since the relationship vary with the variety, location, season and the year of cultivation (Verma *et al.*, 1972). Therefore, it is necessary to evaluate potato cultivars across locations and seasons to determine their fitness for varied processing products as healthy food and to prepare conversion chart for specific gravity. In Ethiopia, limited reports are available for some of the released varieties regarding to potato tuber internal quality traits and processing aspects (Elfnesh *et al.*, 2011; Tefaye *et al.*, 2013). However, these studies did not include most of the cultivars under cultivation and conducted only for one cropping season. Moreover, conversion charts for specific gravity, dry matter and starch content has not been developed for cultivars in the country at large and in eastern Ethiopia in particular. Therefore, this research was conducted with the objectives of: i) evaluating potato cultivars for internal tuber quality traits, ii) studying the effect of growing locations and seasons on internal tuber quality traits; and iii) establishing the relationship among internal tuber quality traits and prepare conversion chart for specific gravity, dry matter and starch contents in eastern Ethiopia.

2. Materials and Methods

2.1. Description of the Study Sites

The field experiment was carried out at three locations namely; Haramaya, Hirna and Arberkete which are considered the representative mid and highland altitudes of potato growing areas of eastern Ethiopia. The experiment was conducted for two main cropping seasons (2012 and 2013) in all the three locations. In addition, at Haramaya, potato cultivarswere evaluated during 2014 main cropping season. This made the total of seven environments considering one location and one cropping season as one environment.

Haramaya University research farm is located at 2020 m.a.s.l., 9˚41"N latitude and 42˚03"E longitude. The area has a bimodal rainfall distribution with mean annual rainfall of 760 mm (Belay *et al.*, 1998). The long rainy season extends from June to October and accounts for about 45% of the total rainfall. The mean maximum temperature is 23.4^{0}C while the mean minimum annual temperature is 8.25^{0}C (Tekalign, 2011). The soil of the experimental site is a well-drained deep alluvial with a sub-soil stratified with loam and sandy loam. Hirna sub-station of Haramaya University is situated at a distance of about 134 km to the west of Haramaya. The site is located at 9 ^{o}12' North latitude, 41 ^{o}4'East longitude, and at an altitude of 1870 m.a.s.l. The area receives mean annual rainfall ranging from 990 to 1010 mm. The average temperature of the area is 24^o C (Tekalign, 2011). The soil of Hirna is vertisol (HURC, 1996). Arberekete field experiment was conducted on a farmer's field, which is located at a distance of about 171 km to the west of Haramaya. The site is located at 9 °14' North latitude, 41 °2'East longitude, and at an altitude of 2280 m.a.s.l. .

2.2. Experimental Materials

The experiment included 15 improved potato cultivars which are under production and two farmers' cultivars. These cultivars were developed and released for different regions of Ethiopia by five Research Centers and Haramaya University (Table 1).

2.3. Experimental Design and Procedures

The experiment was laid out as a randomized complete block design (RCBD) with three replications in each location and season. Each potato cultivar was assigned to one plot in each replication and six rows with 12 plants. The gross plot size was 16.2m² with 75 and 30cm between rows and within plant spacing, respectively. The spacing between plots and replications was maintained at 1.5m and 1m, respectively. For measuring the specific gravity and dry matter content, tubers were harvested from plants in the four middle rows, leaving the plants growing in the two border rows as well as those growing at both ends of each row to avoid border effects.

The experimental fields were cultivated by a tractor (Haramaya and Hirna) toadepthof25-30 cm andridges weremadeby hand.Medium sized (39-75g) and well sprouted tubers were planted at the sides of ridges. Planting was at the end of June and first week of July during the main growing season after the rain commenced and when the soil was moist enough to support emergence. The planting depth was maintained at 10cm. The whole recommended rate of phosphorus fertilizer (92 kg P_2O_5 ha^{-1})was applied at planting in the form of Diammonium Phosphate. Nitrogen fertilizer was applied at the rate of 75 kg N ha^{-1} in the form of urea in two splits, half rate after full emergence (two weeks after planting) and half rate at the initiation of tubers.

Table 7. Name, accession code, year of release, maintainer center of potato cultivars.

Cultivar	Accession code	Year of release	Breeding center	Recommended altitude (m.a.s.l.)
Araarsaa	CIP-90138.12	2006	Sinnana Research Center	2400-3350
Bedasa	AL-114	2001	Haramaya University	2400-3350
Belete	CIP-393371.58	2009	Holeta Research Center	1600-2800
Bete	Local cultivar			
Bubu	CIP-384321-3	2011	Haramaya University	1700-2000
Bulle	CIP-387224-25	2005	Hwassa Research Center	1700-2700
Chala	CIP-387412-2	2005	Haramaya University	1700-2000
Chirro	AL-111	1998	Haramaya University	2700-3200
Gabbisa	CIP-3870-96-11	2005	Haramaya University	1700-2000
Gera	KP-90134.2	2003	Sheno Research Center	2700-3200
Gorebela	CIP-382173.12	2002	Sheno Research Center	1700-2400
Gudanie	CIP-386423.13	2006	Holeta Research Center	1600-2800
Guasa	CIP-384321.9	2002	Adet Research Center	2000-2800
Jalenie	CIP-37792-5	2002	Holeta Research Center	1600-2800
Jarso	Local cultivar			
Mara Charre	CIP-389701-3	2005	Hwassa Research Center	1700-2700
Zemen	AL-105	2001	Haramaya University	1700-2000

Source: Plant Variety Release, Protection and Seed Quality Control Directorate, Crop Variety Register Issue No.16, pp.161-164 (MoA, 2013, June, Addis Abeba, Ethiopia).

2.4. Data Collection

Tuber dry matter content (%) was measuredfrom five fresh tubers in each plot. The randomly taken tubers were weighed at harvest, sliced and dried in oven at 75^{o}C until a constant weight was obtained and dry matter in percent was calculated according to Williams and Woodbury (1968) as follows.

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

Specific gravity of tubers was measured using weight in air and weight in water method. Five kg tubers of all shapes and sizes were randomly taken from each plot and washed with water then weighed first in air and then in water. The specific gravity of tubers was calculated using the following formula (Kleinkopf *et al*, 1987).

$$\text{Specific gravity}(gcm^{-3}) = \frac{Weight\ in\ air}{Weight\ in\ air - Weight\ in\ water}$$

Total starch content (g/100g) was estimated from specific gravity. Starch (%) =17.546 + 199.07 × (specific gravity-1.0988) (Talburt and Smith 1959 as cited by Yildrim and Tokuşoğlu, 2005) where specific gravity was determined as indicated above by the weight in air and weight in water method.

In addition, dry matter and starch content in percent were calculated from the measured specific gravity and dry matter of tubers using different methods established by different researchers and institutions of other countries. The calculation was made by placing the measured tubers specific gravity or dry matter of each cultivar in the equation and the measured specific gravity also used to read and obtain the corresponding dry matter and starch contents in Canada (DEPI, 1995) and USA (USDA, 1997) specific gravity conversion chart. These methods are as follows:

i) Dry matter (%) = -214.9206 + 218.1852 (specific gravity) (Kleinkopf *et al.*, 1987)

ii) Dry matter (%) = 3.33+211 (specific gravity-1) (Willson and Lindsay, 1969)

iii) Starch content (%)=17.565 + 199.07 (specific gravity – 1.0988) (Von Scheele equations cited by Hassel *et al.*, 1997)

iv) Starch content (%) = 17.55 + 0.891 * (tuber dry weight% – 24.182) (AOAC, 1980).

v) Both dry matter and starch content (%) estimated from Canada specific gravity conversion table (DEPI, 1995)

vi) Both dry matter and starch content (%) estimated from USA specific gravity conversion chart (USDA, 1997)

2.5. Data Analysis

Data collected for specific gravity, dry matter and starch content were subjected to i) analysis of variance for each location and season to test the presence of significant differences among cultivars in each location, ii) combined analysis of variance conducted for each location over cropping seasons/years, and iii) unbalanced general analysis of variance computed for seven environments considering the three seasons and locations. Homogeneity of error variances was tested using Bartlett's test for Haramaya site since the experiment was conducted for three cropping seasons while F-test was conducted for Hirna and Arberekete where cultivars were evaluated for two cropping seasons. After the homogeneity of error variances was observed in all locations across cropping seasons, combined analysis of variance was conducted for each location over cropping seasons. Similarly, Bartlett's test was conducted for seven environments and heterogeneity of the error variances was evident for specific gravity and starch content. Therefore, cultivars were compared for pooled means for each location over seasons and other analyses (regression and correlation) were made the same though the homogeneity of error variances was observed for dry matter content. Mean separation was employed following the significance of mean squares using Least Significant Differences (LSD) at 5% probability.

Linear regression analysis was used to establish the relationship among specific gravity, dry matter and starch content of which specific gravity was considered as independent variable and other two traits as dependent (response) variables. Linear regression analysis was conducted for each location and season as well as pooled mean values of each genotype at each location over seasons to understand the differences of the relationships among each location and season and each location over seasons. However, specific gravity conversion table was prepared on the regression equation computed in each location over seasons using pooled mean values of each genotype at each location over seasons. The specific gravity conversion for each location was presented in table and the computed regression was presented in graph for each location. Correlation analysis was conducted among the measured data and estimated values (using different methods and regression equation) for specific gravity, dry matter and starch content to test whether the recorded data were in agreement or in contrast to the established relationship among these traits.

The genetic distance of genotypes was estimated using Euclidean distance (ED) calculated from, i) the measured mean values of each trait for each cultivar in seven environments, ii) estimated tuber dry matter and starch contents of each cultivar using regression equation computed for each location and season, and iii) estimated tuber dry matter and starch contents of each cultivar using regression equation computed for each location over seasons after standardization (subtracting the mean value and dividing it by the standard deviation) as established by Sneath and Sokal, (1973) as follows:

$$EDjk = \sqrt{\sum_{i=1}^{n}(Xij - Xik)^{2}}^{\,2}$$

Where, EDjk = distance between genotypes j and k; xij and xik= tuber internal quality traits (specific gravity, dry matter and starch contents) mean values of the ith trait for cultivars j and k, respectively; and n= number of traits used to calculate the distance. The distance matrix from tuber internal quality traits was used to construct dendrograms based on the Unweighted Pair-group Method with Arithmetic means (UPGMA). The results of the cluster analysis were presented in the form of dendrogram.

3. Results

3.1. Analysis of Variance and Mean Performance of Cultivars

Potato cultivar showed significant differences for specific gravity, dry matter and starch content in all environments (at each location and growing season) (data not presented). The combined analysis of variance for each location over growing seasons revealed that tuber dry matter content was significantly influenced by genotype and the interaction of genotype x season at Haramaya while it was significantly influenced by genotype and season at Hirna and Arberkete (Table 2). Specific gravity and starch content showed significant variations due to genotype, growing season and interaction of genotype x season at Haramaya but only due to genotype and the interaction of genotype x season at Hirna and genotype and season at Arberkete. Mean squares from unbalanced combined analysis of variance over years and locations revealed that dry matter content was significantly influenced by genotype, season, location, interactions of genotype x location and location x season while starch content was significantly affected by all possible interactions (Table 3). Specific gravity was significantly influenced by genotype, season and interaction effect of genotype x season and genotype x location x season.

Table 2. Mean squares from combined analysis of variance in each location over years for specific gravity, dry matter and starch contents of 17 potato cultivars.

Location	Source of Variation	Dry matter content (%)	Specific gravity	Starch content (g/100g)
Haramaya	Replication (2)	6.058	0.0000116	0.5149
	Cultivar (16)	40.191**	0.000780**	30.716**
	Season	4.245	0.0001867**	7.871**
	Cultivar x Season (32)	2.269**	0.0000538**	2.1184**
	Error (100)	2.178	0.0000146	0.5744
	CV (%)	6	0.4	5.1
Hirna	Replication (2)	0.498	0.00000683	1.175
	Cultivar (16)	26.503**	0.00032602**	12.025*
	Season (1)	63.961**	0.00005775	5.13
	Cultivar x Season (16)	2.778	0.00008344*	4.847**
	Error (66)	1.696	0.00003203	1.481
	CV (%)	5.60	0.50	8.3
Arberkete	Replication (2)	2.821	0.0000823	1.745
	Cultivar (16)	21.139**	0.00052976**	19.579**
	Season (1)	3.491*	0.00028872*	4.743*
	Cultivar x Season (16)	1.78	0.0000662	2.108
	Error (66)	0.749	0.0000302	1.441
	CV (%)	3.5	0.50	10.3

** and **, significant at P<0.05 and P<0.01, respectively. Numbers in parenthesis represented degree of freedom.*

Belete followed by Gera, Bubu and Gorebela had highest specific gravity, dry matter and starch content across locations and years though the order of cultivars varied according to the rank differences across locations (Table 4). Belete produced tubers with >27% dry matter content at Hirna and Arberekete while Gera and Gorebela at Haramaya and Bubu at Arberekete produced tubers >26% dry matter content. Chirro and Chala also produced tubers with >25% dry matter content in all locations. None of the improved varieties produced tubers with <1.08 specific gravity and <14% starch content except Bedasa had <14% starch content at Haramaya and Hirna. On the other hand, the two farmers cultivars produced tubers with <1.07 specific gravity except Jarso at Hirna. The highest tubers dry matter content, specific gravity and starch content were observed at Haramaya (2014), Arberekete (2013) and Arberekete (2012), respectively.

Table 3. Mean squares from unbalanced combined analysis of variance over seven environments for tuber dry matter, specific gravity and starch contents of 17 potato cultivars

Source of variation	df	Dry matter content (%)	Specific gravity	Starch content (g/100g)
Replication	2	0.456	0.00005058	1.085
Cultivar	16	80.765**	0.00132426**	55.031**
Location	2	51.237**	0.00003343	4.260*
Season	2	8.196*	0.00013145*	4.964*
Cultivar x Location	32	2.788*	0.00005739	2.555*
Cultivar x Season	32	2.211	0.00008097*	2.669*
Location x Season	2	35.758**	0.00008907	7.062*
Cultivar x Location x Season	32	2.229	0.00006724*	2.815**
Error	236	1.782	0.00003885	1.291
CV (%)		5.5	0.57	7.65

** and **, significant at P<0.05 and P<0.01, respectively. Numbers in parenthesis represented degree of freedom.*

The dendrogram constructed using Unweighted Pair-group Method with Arithmetic means (UPGMA) clearly divided the cultivars into three clusters of which the first cluster consisted of 14 released varieties which was divided in to two sub-groups (Figure 1). The first sub-group consisted of eight varieties released between 2001 and 2006. The mean tuber specific gravity, dry matter and starch content of these varieties were either equal or less than the mean values of genotypes but most of these varieties performed higher than the mean of genotypes for dry matter and starch content at Haramaya and Arberkete, respectively. These varieties relatively perform better at these two locations for specific gravity. The second Sub-group consisted of six varieties which included the very old variety Chiro released in 1998 to recently (in 2011) released variety Bubu. This group had mean values higher than the overall mean values of genotypes for all traits in all locations, but they had much higher tuber dry matter content (≥25%) and specific gravity (≥1.09) at Haramaya. Belete was formed a solitary Cluster II with highest specific gravity ($\geq$1.096), dry matter (>27%) and starch content (>16.72%) except specific gravity of 1.095 and dry matter content of 26.63% at Haramaya. The third Cluster consisted of the two farmers cultivars which had <1.07, <20%, and <11% specific gravity, dry matter and starch content, respectively, in all locations, but these cultivars had 1.07 and 11% of specific gravity and starch content, respectively, at Hirna and 20% dry matter content at Arberkete for different seasons.

Table 4. Mean specific gravity, dry matter and starch contents of 17 potato cultivars over three locations and years (2012 to 2014).

Location	Haramaya			Hirna			Arberekete		
Cultivar	DM	SG	Starch	DM	SG	Starch	DM	SG	Starch
Araarsaa	24.4^{efg}	1.085^{efg}	14.79^{efg}	21.81^{f}	1.082^{efg}	14.19^{efg}	23.26^{g}	1.083^{ef}	15.27^{cd}
Bedasa	24.23^{fg}	1.081^{h}	13.9^{h}	22.63^{ef}	1.076^{gh}	13.28^{gh}	23.58^{fg}	1.082^{ef}	14.76^{d}
Belete	26.63^{a}	1.095^{a}	16.72^{a}	27.18^{a}	1.096^{a}	16.97^{a}	27.14^{a}	1.099^{a}	17.58^{a}
Bete	19.88^{h}	1.064^{i}	10.59^{i}	19.12^{g}	1.068^{i}	11.79^{i}	20.99^{h}	1.067^{g}	11.46^{e}
Bubu	25.79^{a-d}	1.09^{bc}	15.86^{bc}	25.35^{b}	1.089^{bcd}	15.57^{a-e}	26.82^{ab}	1.095^{ab}	17.23^{a}b
Bulle	24.84^{d-g}	1.087^{def}	15.11^{def}	22.33^{ef}	1.084^{def}	14.53^{d-g}	23.86^{efg}	1.085^{def}	15.21^{cd}
Chala	25.7^{a-e}	1.084^{fgh}	14.52^{fgh}	24.26^{bcd}	1.085^{c-f}	14.78^{c-f}	25.53^{cd}	1.089^{bcd}	15.52^{bcd}
Chirro	25.93^{a-d}	1.092^{ab}	16.23^{ab}	24.49^{bcd}	1.088^{b-e}	13.55^{fgh}	25.16^{cd}	1.089^{bcd}	15.62^{bcd}
Gabbisa	23.5^{g}	1.088^{cde}	15.3^{cde}	23.83^{cde}	1.087^{b-f}	15.15^{b-e}	24.57^{def}	1.081^{f}	14.79^{d}
Gera	26.27^{abc}	1.09^{bc}	15.87^{bc}	25.55^{b}	1.091^{abc}	15.94^{abc}	25.84^{bc}	1.091^{bc}	15.64^{bcd}
Gorebela	26.56^{ab}	1.092^{ab}	16.21^{ab}	25.13^{bc}	1.092^{ab}	16.27^{ab}	25.52^{cd}	1.091^{bcd}	15.95^{a-d}
Gudanie	25.25^{a-f}	1.089^{bcd}	15.56^{bcd}	24.75^{bc}	1.088^{b-e}	15.45^{b-e}	25.81^{c}	1.088^{cde}	16.65^{abc}
Guasa	25.59^{a-f}	1.092^{ab}	16.07^{ab}	23.77^{cde}	1.091^{abc}	15.97^{abc}	25.96^{bc}	1.088^{cde}	15.78^{bcd}
Jalenie	24.87^{d-g}	1.083^{gh}	14.37^{gh}	23.05^{def}	1.081^{fg}	14.21^{efg}	25.41^{cd}	1.09^{bcd}	15.33^{cd}
Jarso	19.08^{h}	1.061^{i}	10.00^{i}	19.27^{g}	1.072^{hi}	12.19^{hi}	20.11^{h}	1.061^{g}	10.32^{e}
Mara Charre	25.21^{b-f}	1.084^{efg}	14.69^{efg}	22.34^{ef}	1.084^{def}	14.59^{c-g}	23.65^{efg}	1.081^{f}	16.05^{a-d}
Zemen	25.17^{c-f}	1.087^{cde}	15.29^{cde}	23.66^{cde}	1.088^{b-e}	15.77^{a-d}	24.58^{de}	1.086^{c-f}	15.01^{cd}
Mean	24.64	1.085	14.77	23.44	1.085	14.72	24.576	1.085	15.19
LSD (5%)	2.39	0.0062	1.228	2.123	0.0092	1.984	1.4202	0.009	2.54
Year									
2012	24.36	1.083^{a}	14.32^{b}	22.65^{b}	1.08421	14.49	24.76^{a}	1.084^{b}	15.4^{a}
2013	24.62	1.086^{b}	14.96^{a}	24.23^{a}	1.08572	14.94	24.39^{b}	1.087^{a}	14.97^{b}
2014	24.94	1.086^{b}	15.03^{a}	--------	--------	-------	--------	--------	-------
LSD (5%)	NS	0.0015	0.298	0.515	NS	NS	0.344	0.0022	0.41

Means in each column with similar letter(s) are not significantly different each other. DM = dry matter content in percent, SG gcm^2= specific gravity, Starch = starch content g/100g of fresh tuber weight and LSD (5%) = least significant difference at 5% probability.

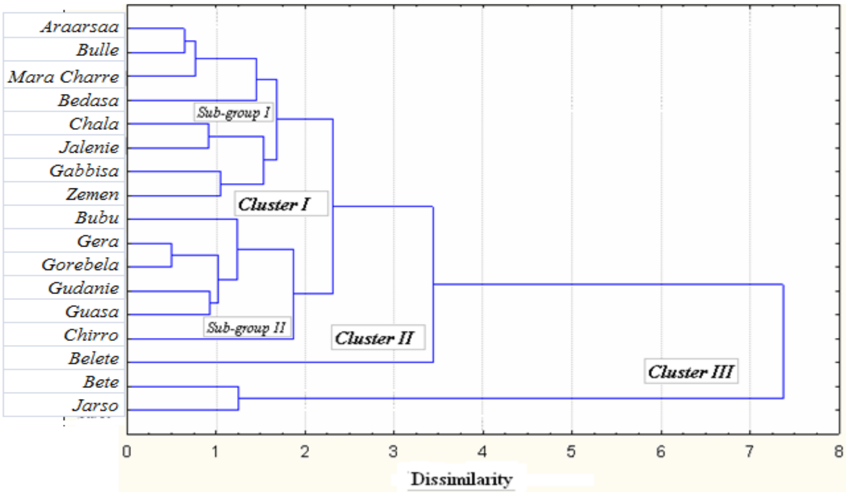

Figure 1.Dendrogram generated based on UPGMA clustering method depicting relationship among 17 potato cultivars based on tuber specific gravity, dry matter and starch contents over years at three locations.

3.2. Relationship among Internal Tuber Quality Traits and Conversion Chart

The regression equations for each location and cropping season and pooled means over years for each location are presented in Table 5. The highest coefficient of determination ($R^2 \geq 0.924$) and correlation ($r \geq 0.962$) were computed for the regression of specific gravity, starch and dry matter contents for all locations in each year. However, regression computed on the basis of pooled means over years for each location showed that both coefficient of determination and correlation values were ≥0.99, ≥0.96 and ≥0.97 for Haramaya, Hirna and Arberkete, respectively. The graphic presentation of regression computed on the basis of pooled means over years for Haramaya, Hirna and Arberkete are presented in Figure 2, 3 and 4, respectively.

Table 5. Regression equation, coefficient of determination (R^2) and correlation of separate years and pooled mean over years for specific gravity, dry matter and starch contents of 17 potato cultivars at three locations (2012 to 2014).

Location	Year	Regression equation	R^2	Correlation (r)
Haramaya	2012	DM=-171.2307+180.6728 x SG	0.937	0.968
		Starch=-199.7252+197.7151 x SG	0.999	0.999
	2013	DM=-242.9437+246.4027 x SG	0.976	0.988
		Starch=-202.1383+199.9253 x SG	0.999	0.999
	2014	DM=-260.2067+262.5102 x SG	0.986	0.993
		Starch=-203.0845+200.8024 x SG	0.999	0.999
Pooled means over three years		DM=-222.2202+227.5344 x SG	0.992	0.996
		Starch=-201.7442+199.5626 x SG	0.999	0.999
Hirna	2012	DM=-289.8848+288.2386 x SG	0.95	0.975
		Starch=-208.3541+205.5233 x SG	0.984	0.992
	2013	DM=-229.6209+233.7771 x SG	0.932	0.965
		Starch=-202.199+199.9677 x SG	0.999	1.00

Pooled means over two years		DM=-278.0942+277.9138 x SG	0.964	0.982
		Starch=-189.9193+188.6054 x SG	0.978	0.989
Arberekete	2012	DM=-154.9683+165.8926 x SG	0.974	0.987
		Starch=-176.3201+176.960 x SG	0.924	0.962
	2013	DM=-220.4252+225.2465 x SG	0.968	0.984
		Starch=-188.4728+187.126 x SG	0.959	0.979
Pooled means over two years		DM=-192.1592+199.745 x SG	0.982	0.991
		Starch=-192.189+191.5153 x SG	0.976	0.988

Correlation (r) = correlation coefficient, R^2 = coefficient of determination, DM = dry matter content, SG = specific gravity and Starch = starch content.

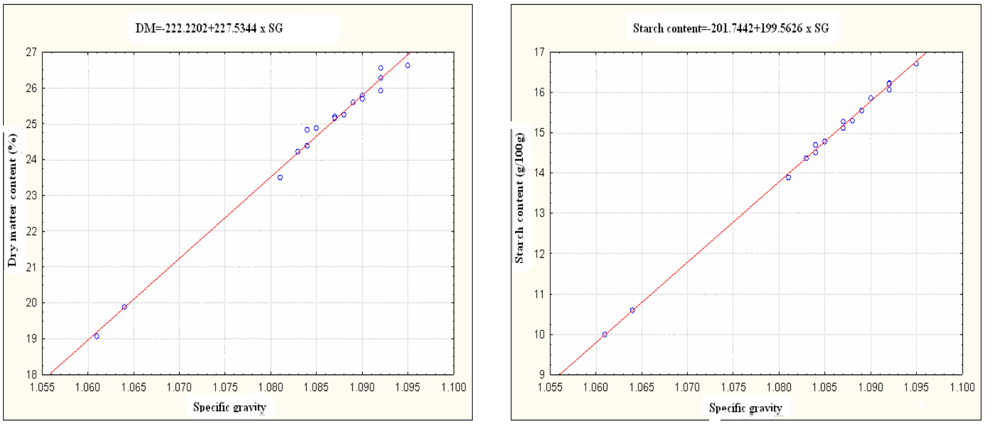

Figure 2. Linear regression of tuber specific gravity on dry matter (DM) and starch contents of 17 potato cultivars with equation of best-fit line on the basis three years mean values at Haramaya

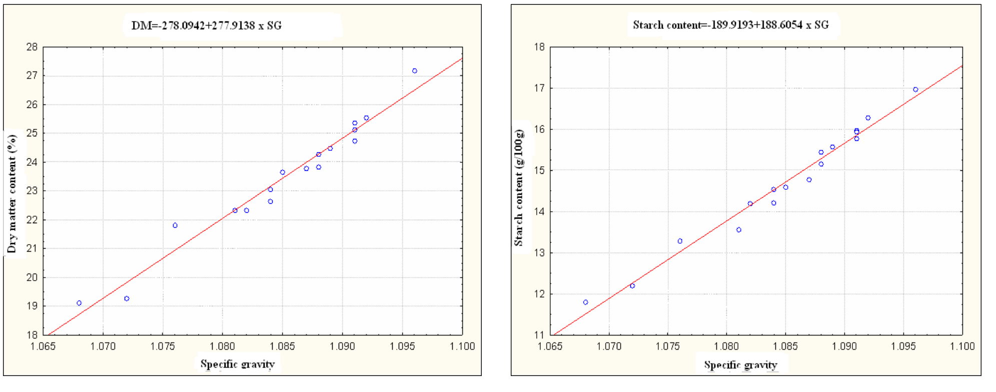

Figure 3. Linear regression of tuber specific gravity on dry matter (DM) and starch contents of 17 potato cultivars with equation of best-fit line on the basis two years mean values at Hirna

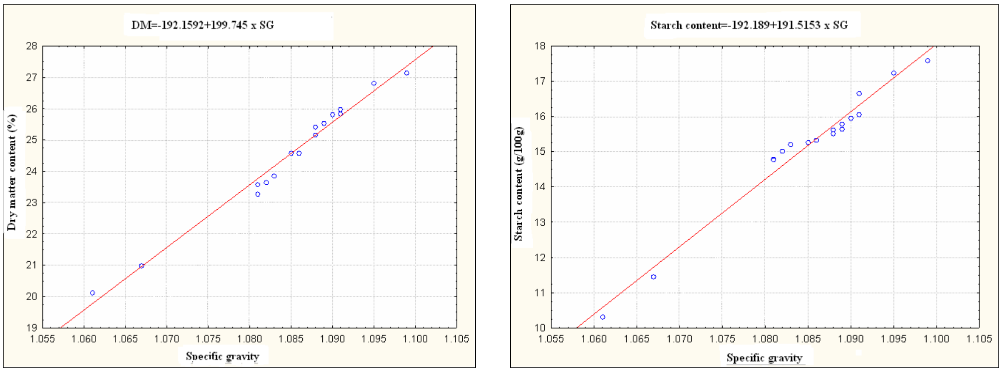

Figure 4. Linear regression of tuber specific gravity on dry matter (DM) and starch contents of 17 potato cultivars with equation of best-fit line on the basis two years mean values at Arberkete

Specific gravity conversion chart was prepared and presented in Table 6. The dry matter and starch contents were 18.97 and 9.79%, respectively for the lowest specific gravity of 1.06 at Haramaya, while the values were 16.49% (dry matter content) and 10% (starch content) at Hirna and 19.57 (dry matter content) and 10.82% (starch content) at Arberkete. Similarly, 28.09% (dry matter) and 17.79% (starch content) were computed for the highest specific gravity of 1.001 at Haramaya, while it was computed 27.64 (dry matter content) and 17.57% (starch content) at Hirna and 27.58 (dry matter content) and 18.50% (starch content) at Arberkete.

Table 6. Conversion of specific gravity to dry matter and starch content for three locations calculated from regression equation on the basis of pooled means over years data of 17 potato cultivars

Location	Haramaya		Hirna		Arberekete	
SG (gcm²)	DM (%)	Starch (%)	DM (%)	Starch (%)	DM (%)	Starch (%)
1.060	18.97	9.79	16.49	10.00	19.57	10.82
1.061	19.19	9.99	16.77	10.19	19.77	11.01
1.062-	19.42	10.19	17.05	10.38	19.97	11.20
1.063	19.65	10.39	17.33	10.57	20.17	11.39
1.064	19.88	10.59	17.61	10.76	20.37	11.58
1.065	20.10	10.79	17.88	10.95	20.57	11.77
1.066	20.33	10.99	18.16	11.13	20.77	11.97
1.067	20.56	11.19	18.44	11.32	20.97	12.16
1.068	20.79	11.39	18.72	11.51	21.17	12.35
1.069	21.01	11.59	19.00	11.70	21.37	12.54
1.070	21.24	11.79	19.27	11.89	21.57	12.73
1.071	21.47	11.99	19.55	12.08	21.77	12.92
1.072	21.70	12.19	19.83	12.27	21.97	13.12
1.073	21.92	12.39	20.11	12.45	22.17	13.31
1.074	22.15	12.59	20.39	12.64	22.37	13.50
1.075	22.38	12.79	20.66	12.83	22.57	13.69
1.076	22.61	12.99	20.94	13.02	22.77	13.88
1.077	22.83	13.18	21.22	13.21	22.97	14.07
1.078	23.06	13.38	21.50	13.40	23.17	14.26

1.079	23.29	13.58	21.77	13.59	23.37	14.46
1.080	23.52	13.78	22.05	13.77	23.57	14.65
1.081	23.74	13.98	22.33	13.96	23.77	14.84
1.082	23.97	14.18	22.61	14.15	23.96	15.03
1.083	24.20	14.38	22.89	14.34	24.16	15.22
1.084	24.43	14.58	23.16	14.53	24.36	15.41
1.085	24.65	14.78	23.44	14.72	24.56	15.61
1.086	24.88	14.98	23.72	14.91	24.76	15.80
1.087	25.11	15.18	24.00	15.09	24.96	15.99
1.088	25.34	15.38	24.28	15.28	25.16	16.18
1.089	25.56	15.58	24.55	15.47	25.36	16.37
1.090	25.79	15.78	24.83	15.66	25.56	16.56
1.091	26.02	15.98	25.11	15.85	25.76	16.75
1.092	26.25	16.18	25.39	16.04	25.96	16.95
1.093	26.47	16.38	25.67	16.23	26.16	17.14
1.094	26.70	16.58	25.94	16.42	26.36	17.33
1.095	26.93	16.78	26.22	16.60	26.56	17.52
1.096	27.16	16.98	26.50	16.79	26.76	17.71
1.097	27.39	17.18	26.78	16.98	26.96	17.90
1.098	27.61	17.38	27.06	17.17	27.16	18.09
1.099	27.84	17.58	27.33	17.36	27.36	18.29
1.100	28.07	17.77	27.61	17.55	27.56	18.48
1.1001	28.09	17.79	27.64	17.57	27.58	18.50

SG (gcm^2) = specific gravity, DM (%) = dry matter content and Starch (%) =starch content.

3.3. Correlation among the Observed and Estimated Internal Tuber Quality Traits

The correlation was highly significant among the observed (measured) and estimated (using different methods) tubers specific gravity, dry matter and starch contents. In most cases the correlation was perfect (r = 1.00) or near to perfect (r = 0.97 to 0.99) (Table 7). The measured tubers specific gravity showed perfect or near to perfect correlations with all calculated and estimated dry matter and starch contents except the correlations with measured dry matter content and estimated from regression equation and estimated starch content using AOAC (1980) method (r = 0.91 to 0.96). On the other hand, the observed dry matter content showed perfect or near to perfect correlations (r = 1.00 & r = 0.99) only with estimated dry matter and starch content using regression equation and AOAC (1980) methods, respectively. The measured dry matter content had correlation coefficient of r = 0.94 & r = 0.95 with estimated dry matter and starch contents from all other methods. As compared to measured dry matter content, the observed starch content had higher correlation coefficients ($r \geq 0.97$) with observed and estimated specific gravity, dry matter and starch contents except for the correlation with the estimated dry matter and starch content from regression equation and AOAC (1980), respectively.

Table 7. Correlation coefficient among the measured and estimated specific gravity, dry matter and starch contents computed for each location and cropping season (above diagonal) and pooled means of three locations over years (below diagonal) (2012-2014)

	OSG	ODM	OSTAR	CDM	CSTAR	WDM FSG	KlDM FSG	DEPI DMFSG	DEPI STAR	VSSTAR	USADDM	AOACST
OSG		0.91**	0.97**	0.96**	0.98**	1.00**	1.00**	1.00**	1.00**	1.00**	0.99**	0.91**
ODM	0.94**		0.93**	0.95**	0.91**	0.91**	0.91**	0.91**	0.91**	0.91**	0.92**	1.00**
OSTAR	0.98**	0.95**		0.95**	0.97**	0.97**	0.97**	0.97**	0.97**	0.97**	0.96**	0.93**
CDM	0.95**	0.99**	0.96**		0.96**	0.96**	0.96**	0.96**	0.96**	0.96**	0.96**	0.95**
CSTAR	0.97**	0.95**	0.99**	0.96**		0.98**	0.98**	0.98**	0.98**	0.98**	0.97**	0.91**
WDMFSG	1.00**	0.94**	0.98**	0.95**	0.97**		1.00**	1.00**	1.00**	1.00**	0.99**	0.91**
KlDMFSG	1.00**	0.94**	0.98**	0.95**	0.97**	1.00**		1.00**	1.00**	1.00**	0.99**	0.91**
DEPI DMFSG	1.00**	0.95**	0.99**	0.95**	0.97**	1.00**	1.00**		1.00**	1.00**	0.99**	0.91**
DEPI STAR	1.00**	0.94**	0.99**	0.95**	0.97**	1.00**	1.00**	1.00**		1.00**	0.99**	0.91**
VSSTAR	1.00**	0.94**	0.98**	0.95**	0.97**	1.00**	1.00**	1.00**	1.00**		0.99**	0.91**
USADDM	1.00**	0.94**	0.98**	0.95**	0.97**	1.00**	1.00**	1.00**	1.00**	1.00**		0.92**
AOACST	0.94**	1.00**	0.95**	0.99**	0.95**	0.94**	0.94**	0.95**	0.94**	0.94**	0.94**	

***, significant at P<0.01 probability. OSG = observed specific gravity, ODM = observed dry matter content, OSTAR = observed starch matter content, CDM = calculated dry matter content on the basis of regression equation, CSTAR = calculated starch content on the basis of regression equation, WDMFSG = estimated dry matter content from specific gravity using Willson and Lindsay (1969) method, KlDMFSG = estimated dry matter content from specific gravity using Kleinkopf et al. (1987) method, DEPI DMFSG = estimated dry matter content from specific gravity using specific conversion table of Department of Environment and Primary Industries of Canada (1995), DEPI STAR = estimated starch content from specific gravity using specific conversion table of Department of Environment and Primary Industries of Canada (1995), VSSTAR = estimated starch content from observed specific gravity using Von Scheele equations (cited by Hassel et al., 1997), USADDM = estimated dry matter content from observed specific gravity using specific gravity conversion chart of United States Agriculture Standard (USDA, 1997), AOACST = estimated starch content from observed dry matter content using official methods of analysis, Association of Official Analytical (AOAC, 1980).*

4. Discussion

The presence of wide variations among genotypes for tuber specific gravity, dry matter and starch contents indicated the genetic factor was important to influence the tuber internal quality traits. The observed differences are a good opportunity for the producers to select the varieties for production that fit the market demand. Many other researchers also reported the presence of significant differences among potato cultivars for these tuber quality traits (Elfnesh *et al.*, 2011; Hassanpanah *et al.*, 2011; Tesfaye *et al.*, 2013; Kaur and Aggarwal, 2014; Ismail *et al.*, 2015). These traits were also significantly influenced by growing season and location. The influence of growing location on starch content in dry matter was reported (Dorota *et al.*, 2011; Hassanpanah *et al.*, 2011; Kaur and Aggarwal, 2014). Specific gravity and tuber dry matter content are influenced by both the environment and cultivars (Elfnesh *et al.*, 2011; Ismail *et al.*, 2015). However, the interaction of cultivar x location x season was significantly influenced specific gravity and starch content indicating the unstable expression of these traits in different cultivars across locations and seasons. These quality traits are genetically controlled and also influenced with growing locations and seasons (Dorota *et al.*, 2011; Hassanpanah *et al.*, 2011; Kaur and Aggarwal, 2014). The result suggested the importance of testing potato cultivars across locations and seasons to identify wide adaptable varieties that could produce tubers with uniform specific gravity and starch content in all environments since it benefit producers, processors and consumers.

All improved cultivars produced tubers $\geq$1.08 and $\geq$23% specific gravity and dry matter content, respectively, in all locations and growing seasons except two varieties at two locations. On the other hand, the farmers' cultivars had tubers with low values of <1.07 and <20% specific gravity and dry matter content, respectively. Tesfaye *et al.* (2013) reported dry matter content ranged from 17.05 to 29.88% for 25 potato genotypes studied at three locations of northwestern Ethiopia. Elfnesh *et al.* (2011) also reported 20.33 to 27.33% and 1.078 to 1.110 gcm^{-3} dry matter content and specific gravity, respectively, for five improved potato varieties tested at three locations in eastern Ethiopia. Specific gravity values considered as low (< 1.077), intermediate (between 1.077 and 1.086) and high (>1.086) (Fitzpatrick *et al.*, 1969). Potato cultivars with a dry matter content of 20% or higher are the most preferred for processed products (Kirkman, 2007). Kabira and Berga, (2006) suggested a dry matter content of 20 to 24% are ideal for making French fries while those with up to 24% for preparing crisps. They suggested also, potato tubers should have a specific gravity value of more than 1.080 and those with less than 1.070 are generally unacceptable for processing. This suggested the evaluated potato varieties were suitable for processing but farmers' cultivars were not suitable for processing to French fries and chips.

The eight cultivars (Ararsa, Bule,Marachere, Bedasa,Challa, Jalenine, Gabisa and Zemen) included in thefirst Sub-group of Cluster I had with mean tuber starch content of 14.56 to 15.24% whereas the six cultivars (Bubu, Gera, Gorbella, Gudenine, Gusa and Chiro) in the second Sub-group had a mean starch content $\geq$15.46%. Belete formed solitary Cluster II had highest mean values up 17.58% of tuber starch content while farmers cultivars in Cluster III had lowest mean values of <12%. Tesfaye *et al.* (2013)

reported starch content ranged between 10.44 and 18.51% for 25 potato genotypes that Betete had the highest tuber starch content. Starch is of special importance for the nutritional value ranges between 15-20% (Schafer-Pregl *et al.*, 1998), important role in the cooking quality (Binner *et al.*, 2000), starch production in starch processing industries (Liu *et al.,* 2003) and healthy food processing and consumption in relation to moderating blood glucose levels. Esendal (1990) suggested three groups: the highest starch content (>19.0%, mashing), high starch content (between 16.0 and 19.0%, roasting), intermediate starch content (between 13.0 and 15.9%, cooking or roasting), and low starch content (up to 12.0%, boiling). In general, potato varieties with a starch content of 13% and above are the most preferred for processed products (Kirkman, 2007). All improved varieties could be grouped under intermediate starch content fit for processed products either for cooking or roasting while Belete with high starch content for roasting and farmers' cultivars with low starch content for boiling.

Starch concentration represents the dry matter content of potatoes (Hogy and Fangmeier, 2009). Since starch content has direct influence on technological quality, especially with regard to the texture of the processed products. High dry matter content increases chip yield, crispy consistency, and reduces oil absorption during cooking (Rommens *et al.*, 2010). However, tubers with very high dry matter content produces too hard and dry French fries and the crisps will be too brittle. Potatoes with a dry matter content of 20 to 24% are preferred for French fries 22 to 24 for chips and >21% are preferred for flakes production (NIVAA, 2002). In this regard, the eight potato varieties under Cluster I, Sub-group I might serve for all purpose (French fries, chips and flakes) while the six varieties in same cluster in Sub-group II might be used for flakes production. Varieties in Sub-group II and more likely Belete may not serve for both French fries and chips because the products may have a higher chance to be too hard, dry and brittle due to tubers high dry matter content. The high tuber starch content of these varieties may result cell separation, reduced cohesiveness and softening during cooking (Binner *et al.*, 2000) and may not preferred by diabetic patients. In this regard the two farmers' cultivars may be preferred as producing healthy food due to their low starch content. Potato due to its high starch content mainly carbohydrate thought to contribute to some health problems such as diabetes and weight gain (Cordain, 2005; Mozaffarian *et al.*, 2011). Studies showed variability among potato genotypes for glycemic index values (Henry *et al.*, 2005; Parada and Aguilera, 2009). The waxy potatoes are with high moisture and low starch content and had medium glycemic index and the floury potatoes are high in starch and had high glycemic index (Henry *et al.*, 2005). Glycemic index is a measure of foods ability to affect human blood sugar levels. Foods with low glycemic index values are considered healthy food choices since they have the innate property of moderating blood glucose levels, while foods with a high values are considered to be the opposite (Jenkins *et al.*, 1981).

The relationship of specific gravity with tuber dry matter and starch contents was linear with high coefficient of determination and high positive correlation. The relationship was differing from location to location and season to season in the same location. However, the measured specific gravity showed perfect or near to perfect correlations with the estimated tuber dry matter and starch contents from regression equation. The relationship between specific gravity dry matter and starch contents of potatoes has been developed by several workers and association (Johanson *et al*., 1967; Fitzpatrick *et al*., 1969;Willson and Lindsay, 1969; Verma *et al*., 1972;Vakis, 1978; AOAC, 1980; Kleinkopf *et al*., 1987; Dale and Mackay, 1994; Hassel *et al*., 1997). The relationship among internal tuber quality traits has been found to vary with the variety, location, season and the year of cultivation (Verma *et al*., 1972). On the other hand, the correlation of measured and estimated dry matter content with specific gravity and tuber starch content had lower coefficient values. This suggested measuring specific gravity and estimating starch content is preferred than estimating starch content from measured dry matter content. Tekalign and Hammaes (2005) reported the positive and significant correlation of tuber dry matter content and specific gravity and suggested specific gravity as a true indicator of the amount of tuber dry mater.

Specific gravity of potatoes is commonly used by the potato processing industry as a tool for quick estimation of dry matter content. The preparation specific conversion chart needs to test genetically different potato genotypes at different locations and seasons. Johanson *et al*. (1967) suggested the importance of testing varieties for a few years under local conditions and to select wide adaptable varieties with the same specific gravity when grown across environments. Many authors reported the significant influence of growing season and location other than genotype on specific gravity and the two traits to be converted (Dorota *et al*., 2011; Elfnesh *et al*., 2011; Hassanpanah *et al*., 2011; Kaur and Aggarwal, 2014; Ismail *et al*., 2015). The prepared conversion chart for specific gravity was: i) the result of testing considerable number of potato cultivars at representative potato growing areas of eastern Ethiopia for a couple of years, ii) it was observed positive and highly significant correlations of the measured specific gravity with the measured dry matter and starch contents, iii) most importantly, it was observed perfect or near to perfect correlations of the measured specific gravity with the estimated dry matter and starch contents using regression equation, and iv) it was also observed perfect or near to perfect correlations of the measured specific gravity with the estimated dry matter and starch contents with several methods. These could allow recommending the importance of measuring specific gravity and using the prepared specific gravity conversion chart as reliable indicator of tuber quality traits of the tested cultivars and other potato genotypes in eastern Ethiopia.

5. Summary and Conclusion

The research results suggested the importance of evaluating cultivars for internal tuber quality traits (specific gravity, dry matter and starch content) across representative locations of growing region over seasons. This is because, it is difficult to predict the tuber quality of potato cultivars by testing them in one location over seasons due to the observed significant influence of cultivar x location x season interaction on these traits. This also suggested the important of identifying wide adaptable (stable) cultivars that produce tubers with uniform specific gravity and starch content throughout the production areas of the region that benefit producers, processors and end consumers. Though, the varieties were developed for high tuber yield, all the improved varieties produced tubers above the minimum requirements to fit different processing products (French fries, chips flakes etc.). However, some varieties produced tubers with high dry matter and starch content that might not be preferred for French fries and chips, because the products may have a higher chance to be too hard, dry and brittle. These cultivars also might contributed to diabetes and weight gain due to the highest starch content of tubers. The farmers' potato cultivars might be preferred to be used for boiling to produce healthy foods though not be used for French fries and chips processing due to the low starch and high moisture contents of tubers.

The research also suggested that measuring specific gravity of tubers is most appropriate to determine the quality of tubers. The prepared specific gravity conversion chart can be used as indicator of tuber dry matter and starch contents of potato genotypes and thereby to determine the internal quality of tubers for processing in eastern Ethiopia. From the research results it is possible to make conclusion and recommendation such as follow: i) it is necessary to develop wide adaptable cultivars in the country that produce tubers with the same specific gravity through evaluation of genotypes across major potato growing regions of the country, ii) use of specific gravity than dry matter content as good indicator of internal quality of tubers for processing, iii) it is necessary to prepare specific gravity conversion chart in the country at least for major potato growing regions of representative locations to be used by processors, other consumers and researchers, and iv) it is necessary to evaluate the cultivars further for other physical tuber quality and quality of processed products to identify which cultivar(s) fit to which processing to produce healthy food. These could not be accomplished with separate efforts of researchers at different research centers rather it will be successful with the coordinated joint efforts of potato researchers in the country.

6. Acknowledgements

The author thanks Haramaya University and National Potato Improvement Project for the financial support, Holeta, Adet and Sinna Research Centers for providing planting materials, and technical staff of Horticulture Program at Haramaya University for their careful management of the experiment and data recording. The author also thanks Prof. Nigussie Dechassa and Dr. Mengistu Urge for their unreserved support for the success of the experiment particularly at the time when the researcher faced difficulties and Ms. Simret Burga, Mr. Habtamu G/selassie and Mr. Birhanu Blate who involved in the first year research activities of the experiment.

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