

A photograph of a person's hands harvesting carrots in a garden. The person is wearing a blue shirt and green rubber boots. They are holding a bunch of harvested carrots in their left hand and pulling a carrot from the soil with their right hand. The garden is filled with green leafy vegetables and other carrots growing in the soil. The background is a blurred green field.

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## ARTICLES

- Popularization of dorper sheep breed for enhancing production and productivity of local sheep: In the case Enda-Mekoni Woreda Southern Tigray, Ethiopia** 1861  
Temesgen Tesfay, Hagos Kidane, Tesfay Atsebha, Teshale Teklu, Solomon Wayu, Adehanome Baraki, Yekalo Teklay, Zebrhe Teklay, Haileselassie Amare and Zelealem Tesfay
- Response of African Nightshade (*Solanum* sp.) to cassava peel-based manure in the humid forest zone of Cameroon** 1866  
Nkengafac Jetro Njukeng, Francis Ajebesone Ngome, Ives Bruno Mousseni Efombagn and Carine Nono Temegne
- Isolation, identification and molecular characterization of *Rhizobium* species from *Sesbania bispinosa* cultivated in Bangladesh** 1874  
Nusrat Nahar, Ridwan Bin Rashid, Anowara Begum and Humaira Akhter
- Perceptions of risk and risk management strategies in family agroindustries** 1881  
Cristian Rogério Foguesatto and João Armando Dessimon Machado
- Improving grain legume yields using local Evate rock phosphate in Gùrué District, Mozambique** 1889  
António Rocha, Ricardo Maria, Unasse S. Waite, Uatema A. Cassimo, Kim Falinski and Russell Yost
- Technical efficiency of smallholder barley farmers: The case of Welmera district, Central Oromia, Ethiopia** 1897  
Wudineh Getahun and Endrias Geta
- Influence of *Bradyrhizobia* inoculation on growth, nodulation and yield performance of cowpea varieties** 1906  
Tarekegn Yoseph, Bekele Baraso and Tewodros Ayalew
- Quality of planting systems in varieties of sugarcane** 1914  
André Ferreira Damasceno, Carlos Eduardo Angeli Furlani, Cristiano Zerbato, Rafael Henrique de Freitas Noronha and Remo Marini Zoia

<b>Further studies on Bovine Ixodide Ticks in and around Bedelle, Southwest Ethiopia</b>	<b>1922</b>
Hunde Aboma, Assefa Kebede and Mukarim Abdurahaman	
<b>Effects of genotype on yield and yield component of soybean (Glycine max (L) Merrill)</b>	<b>1930</b>
Liberatus Dominick Lyimo, Musa Rashid Tamba and Richard Raphael Madege	
<b>Agronomic efficiency of inoculant based on Azospirillum brasilense associated with nitrogen fertilization at maize</b>	<b>1940</b>
Vandeir Francisco Guimarães, Marcelo Andreotti and Jeferson Klein	

*Full Length Research Paper*

# Popularization of dorper sheep breed for enhancing production and productivity of local sheep: In the case Enda-Mekoni Woreda Southern Tigray, Ethiopia

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Ethiopia has endowed sheep breed with poor productivity in terms of meat yield. Therefore demonstration of Dorper breed sheep was conducted at Enda-Mekoni Woreda with the objective of improving production and productivity of local sheep. Two pure male Dorper sheep were introduced to Enda-Mehoni Woreda for community based breeding strategies. Data such as live Body weight and farmer perception were collected. Data was analyzed using descriptive statistics and Ranking methods. At the beginning, community had negative perception and not willing to cross their sheep with Dorper sheep. However, Farmers were given higher scored (49) to pure Dorper sheep on selection criteria for, male breeding stock. As a result, it's off spring (50% local and Dorper) was scored higher (97) on productivity and reproductive performance. In the present study, body weight of male crossed sheep was recorded 40 kg which is 18.7 kg higher body weight gaining as compared to local sheep with similar age. Moreover, crossed ewe had 8.5 kg superior on body weight gain compared to similar age of local sheep. Farmers notified that, pure Dorper and its crossed sheep had superior traits on loin, sternum, shoulder, body conformation, puberty age and marketable weight. The popularized Dorper sheep and its crossed had increased body weight growth as well as reproductive performance while crossed with local sheep. Therefore, it can be conclude that Droper sheep is adaptable to highland Tigray region as well as preferable to upgrade local sheep productivity.

**Key Words:** Breed, body weight, crossed, dorper, local, sheep.

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## INTRODUCTION

Ethiopia owns 55.0 million cattle, 27.3 million sheep, 28.2 million goats, 1.1 million camels, 51.3 million poultry and 5 million beehives (CSA, 2014). The livestock sub-sector is also already as a major contributor to the overall economy. The livestock sector contributes 19% of GDP, and 16 to 19 % of the foreign exchange earnings of the country (Ministry of Agriculture (MOA), 2012). Though,

Ethiopia has the largest livestock population, the contribution of these sector to livelihood improvement has remained very limited. The current production level of meat is low (4%) when compared to other regions of the world. Low growth rates of livestock result in relatively small carcasses (average carcass weights of cattle, sheep and goats estimated to be 129, 13.2 and 11.8 kg,

respectively (FAO, 2006). In Ethiopia, the per capita meat availability is estimated at 9.94 kg per person per annum which is much lower than that of the per capita consumption levels (32.3 kg per person/annum), in the developing countries (MoARD, 2007). The government's Growth and Transformation Program (GTP), launched in 2010 to 2011, has established annual export goals of 111,000 metric tons of meat and 2,000,000 live animals by 2015 which has led to an increment of, nearly four-fold from 2011 (MoARD, 2007). Moreover, sheep production in Ethiopia is based on indigenous breeds except Awassi-Menz cross breeds that contribute less than 1% of the population. Despite low level of productivity due to several technical (genotype, feeding and animal health), institutional, environmental and infrastructural constraints (Tibbo, 2006), indigenous sheep breeds have great potential in contributing more to the livelihoods of the people in low-input, small-holder crop livestock and pastoral production systems (Kosgey and Okeyo, 2007).

In Tigray 3.6 million cattle, 1.2 million sheep, 3.04 million goats, 4.3 million poultry and 213 thousand beehives population were found (CSA, 2011). Livestock use important economic functions as household savings, assets that provide interest-free credit, and as insurance to mitigate risk. According to Alemayehu and Tikabo (2010), findings showed that yearly age of sheep body weight was noted as  $17.90 \pm 2.59$  and  $19.66 \pm 2.78$  kg for female and male sheep, respectively. This breed was found to be relatively less as compared to other exotic sheep breeds like Dorper sheep that weigh around 40 kg (Snyman et al., 2010). The Dorper is a non-selective feeder, with both browsing and grazing behavior. The maternal influence is a desert sheep that has thrived and reproduced under harsh conditions for centuries. As a whole, this problem government has a strategy to improve the livelihood of rural society using livestock farming system. Therefore considering vital importance of sheep production under small holder farm system, operational research technology dissemination project was initiated to popularize Dorper sheep breed for enhancing livelihood of safety net households in south zone of Tigray region.

## MATERIALS AND METHODS

### Area description

The study was carried out in Enda-Mekoni Woreda and the Woreda lie from 1800 to 3925 m.a.s.l which was found in 662 Km from Addis Ababa and 120 km from the Regional capital, Mekelle. Topography of the area can be classified as very steep 65%, steep 12%, gentle 15% and valley 8%. The total land area of the Woreda is 62,184 ha. The study area has the altitude ranges from 2850 m.a.s.l (meter above sea level) and is predominantly classified as

high land 65%, Woina-Dega 30% and kola 5% agro ecology.

Based on the existing digital data, mean annual rainfall is 650 to 950 mm. *Belg* (small rains) and *Keremti* (long rainy season) are the two cropping seasons. Farmers depend on *Keremti* season for crop production. The dominant soil type in the Woreda plains is clay soil, loam and sandy soil in the medium and high altitude areas. The mean annual temperature of the woreda is between 12°C and 18°C.

### Beneficiary selection and selection procedure

Two pure Dorper breed sheep were brought with financial support of operational research project, distributed to farmers on the objective of community based breeding strategies. Two farmers research group each contained 15 members which were established at Enda-Mekoni Woreda and the group take responsibility for the sheep as breeding service for the community. In line with this, the economic status and experience of the households on their previous practices to adopt technologies, availability of enough feed and space, living standard and initiations were included as selection criteria.

### Data collection and analysis

Linear body measurements such as heart girth (HG), body length (BL) and height at wither (HAW) were collected. The animals were measured in their standing position under field conditions using plastic measuring tape and measuring stick and at the same time, the body weight was taken using a 50 kg size spring balance early in the morning before allowing the animals for grazing. Male and female animals were measured separately. Body weight measurements were taken at 12 months age of local and crossed Dorper sheep. 30 (16 male, 14 female) experienced farmers were collected at a perception data using group discussion. Perception data were collected through participatory rural appraisal (PRA) tools. The data were analyzed through mean body weight and descriptive statistics.

The following linear body measurements were taken as per the procedures of ESGPIP (2009).

- Body length: the distance from the base of the tails to the base of neck (first thoracic vertebrae).
- Heart girth: the circumferential measure taken around the chest just behind the front legs and withers.
- Wither height: the distance from the surface of a platform on which the animal stands the wither.

## RESULTS AND DISCUSSION

### Phenotypic characterization of crossed sheep (50% local highland X Dorper sheep)

The crossed sheep (50% local highland and Dorper) has both sex pattern coat description with black head, course fiber type, course wool hair type, long and straight hair type. In line with this, both sexes has short and

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**Table 1.** Mean comparison of body measurements of the different sheep breeds of Tigray.

Breed	Mean body measurements					
	Height at Withers		Girth Circumference		Body length	
	Male	Female	Male	Female	Male	Female
Crossed(50% local X Dorper sheep)	63	55.5	81	72	50	47.5
Begait	65.26 <sup>a</sup>	65.7 <sup>a</sup>	72.34 <sup>a</sup>	75.1 <sup>a</sup>	53.95 <sup>a</sup>	54.87 <sup>a</sup>
Abergelle	58.8 <sup>b</sup>	60.56 <sup>b</sup>	69.1 <sup>b</sup>	74.8 <sup>a</sup>	49.7 <sup>b</sup>	52.17 <sup>b</sup>
Ille	53.81 <sup>c</sup>	55.9 <sup>c</sup>	66.9 <sup>ab</sup>	73.0 <sup>a</sup>	48.63 <sup>b</sup>	52.06 <sup>ab</sup>
Common highland sheep	56.67 <sup>bc</sup>	58.88 <sup>b</sup>	69.62 <sup>b</sup>	74.57 <sup>a</sup>	46.88 <sup>b</sup>	48.58 <sup>c</sup>

**Table 2.** Body weight gain of crossed sheep.

Age	Sex	Body Weight (kg)		Difference in body weight (kg)
		Crossed (50% Local and Dorper)	Local	
1 year	M	40	21.3	18.7
1year	F	29	20.5	8.5

compacted body shape, straight profile face and lateral ears orientation with 5 and 10.5 cm ear length in male and female, respectively. In addition to this, male crossed sheep has short length horn but in female crossed sheep does not exist. Moreover, both sexes have long thin tailed and early puberty age at six month. The first lambing and mating age at female and male were 9 and 7 month age, respectively. Both sexes crossed sheep has medium udder and testicle size.

In the absence of weighing scale, a number of body linear measurements such as heart girth values can be used to estimate the weight of an animal (Nsoson et al, 2003; Vargas et al., 2007; Elizabeth et al., 1997). For example, studies have shown that heart girth can explain 86 to 91% of the body weight in sheep (Thys and Harouin, 1991). Mean body measurements of different sheep breeds are given in Table 1. Crossed sheep in male and female sex had comparable height at withers of 63 and 55.5 cm with begait sheep which is the biggest sheep in Tigray. Moreover, girth circumference of crossed sheep was higher than other Tigray sheep breeds especially male crossed sheep which had 81 cm girth circumference but female crossed breed had comparable girth circumference. Male crossed sheep had comparable body length with male begait breed but higher than other breed. However, female crossed sheep has lower body length (47.5 cm) than other female sheep breed in Tigray. Similarly, according to Alemayehu and Tikabo (2010) reported the height at withers, Girth circumference and body length of highland sheep of Tigray for male  $58.10 \pm 3.20$ ,  $62.20 \pm 3.52$  and  $47.94 \pm 3.12$  cm, respectively. However, according to the authors report, the height at withers ( $58.18 \pm 3.21$ cm), Girth circumference( $65.03 \pm 4.43$  cm) and body length ( $49.59 \pm$

3.11 cm) of highland sheep of Tigray was comparable with Dorper crossed sheep.

### Body weight gain

Body weight of male crossed sheep (50% local and Dorper) at yearling age was 40 kg in the study area but similar age of local sheep had 21.3 kg live body weight (Table 2). This implies that crossed breed sheep had superiority of 18.7 kg higher live body weight gaining compared to local breed at similar age. Moreover, the weight of female crossed sheep at yearling age was 29 kg while similar age of local sheep is 20.5 which is 8.5 kg live body lower than crossed sheep. According to Alemayehu and Tikabo (2010), findings showed that yearly age of sheep body weight was noted as  $17.90 \pm 2.59$  and  $19.66 \pm 2.78$  kg for female and male sheep, respectively. Similarly Berhe (2010) reported that, yearling weight of Ethiopian shoat was 22 kg and to compare with the current study, the minimum and maximum average matured weights of sheep were also reported as  $21.6 \pm 9.3$  and  $41.5 \pm 2.0$  kg, respectively (according to Abebe, 2010) in Ethiopia.

### Perception of the community on Dorper sheep

During group discussion farmers explained that at the beginning, the community was not interested to breed their sheep with Dorper sheep. This was due to Dorper sheep has short tail, short leg, black color and huge body conformation. The community thought that Dorper sheep as donkey, dog and pig and they also decided not to slaughter and eat. Selling their attitude was link with other

**Table 3.** Farmer selection criteria on male breeding stock.

Traits	Farmer score on male breeding stock	
	Local	Dorper
Loin	-	10
Sternum	-	10
Shoulder	-	10
Body conformation	-	10
Tail length and width	10	-
Ear length	5	5
Body color	8	2
Height	8	2
Total score	31	49
Percentage (%)	38.7	61.3

**Table 4.** Farmer's perception crossed sheep performance.

Traits	Farmer scored	
	Crossed (50% Local and Dorper)	Local
Birth weight	10	-
Weaning weight	10	-
Feed efficiency	10	-
Body conformation	10	-
Loin area	10	-
Shoulder area	10	-
Sternum area	10	-
Height	-	10
Ear length	5	5
Body color	2	8
Tail length	1	9
Puberty age	10	-
Tolerant for feed and water shortage	-	10
Resistance to external parasites and diseases	-	10
Marketable weight	10	-
Total Scored	97	52
Percentage (%)	65	35

cultural norms and religion. Later on, the community was interested to breed their sheep with Dorper sheep and later saw the off-spring of Dorper sheep.

Farmers reported that traits to be consider when selecting breeding stock is given in Table 3. They were given higher scored (49) to pure Dorper sheep on a criteria use, in the selection for male breeding stock. Farmers also notified that, pure Dorper sheep had superior traits on loin, sternum, shoulder and body conformation when compared. However, farmers observed that local sheep had superior traits on body color, height, tail length and width. In line with this local sheep has as superior as Dorper sheep on ear length. All the respondents agreed that Dorper sheep was preferable to

have male breeding stock as compared to local sheep.

#### **Farmer's perception on crossed sheep traits**

Farmers were given higher scored (65%) for crossed sheep (50% local and Dorper), on the productivity and reproductive performance when compared to pure local sheep (Table 4). The respondents reported that, crossed sheep had superior traits on birth weight, weaning weight, feed efficiency, body conformation (a wide, straight back, smooth shoulder, fullness through the heart area, a good spring of ribs and a long, well balanced, with adequate skeletal size), loin area, shoulder area, sternum area,

puberty age and marketable weight but the breed is a heavy eater and non-selective. Hence the sheep require more supplementation feeding during feed shortage and cannot be maintained at a high stocking rate on limited grazing area.

Farmers in study area preferred red or white body color with fat tailed sheep for holiday scarification, than short and less fat tailed sheep. Moreover, crossed sheep had early marketable age (6 month) with high body weight and conformation than local sheep. However, local highland sheep has superior traits on tolerance of feed, water, parasite and disease than crossed sheep. In line with this, farmers reported that crossed female sheep had early first age at puberty (9 month age) which is 4 month early than local female similar age. Generally, the community had interest to breed their sheep with Dorper sheep after they seen the off-spring of Dorper sheep.

## Conclusion

Popularization of Dorper breed has improved productivity of local breed by 50% in yearling weight. Beside this, farmers were perceived positively on Dorper crossed breed in terms of body weight gain, early puberty age and marketable weight. Therefore, popularization of pure Dorper sheep breed in large scale is very crucial for enhancing income of small scale farmers.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Response of African Nightshade (*Solanum* sp.) to cassava peel-based manure in the humid forest zone of Cameroon

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Studies were conducted at Ekona in the humid forest agro-ecological zone of Cameroon to evaluate the effects of cassava peel-based manures on the growth and yield of African Nightshade. The first experiment consisted of evaluating the composted cassava peel and poultry manure applied at the rates of 0, 5, 10 and 20 t/ha and 150 kg/ha NPK (20:10:10) fertilizer. In the second experiment, a dried and grind mixture of cassava peel and poultry manure were applied at rates of 0, 5, 10 and 20 t/ha and 150 kg/ha NPK (20:10:10) fertilizer. Treatments were laid out in a randomized complete design and replicated three times. Growth and yield data were collected and subjected to analysis of variance (ANOVA). Significant treatments means were separated using students T test at  $p \leq 0.05$ . The different manures prepared were rich in macro nutrients particularly N (1-1.6%), P (0.7-1.4%) and K (1-1.7%). The experimental results showed that the applications of cassava peel-based manures significantly ( $p < 0.05$ ) influenced number of leaves, branches and biomass yield of African Nightshade. The use of cassava peel-based organic manure was very comparable to the use of inorganic fertilizer (NPK). Thus, cassava peel-based manure is promising for the enhancement of African Nightshade production, which could reduce the cost of fertilizer use and limit environmental pollution in Cameroon.

**Key words:** Biomass yield, cassava peel, African Nightshade production, poultry manure.

## INTRODUCTION

There is increasing attention towards the use of organic wastes for soil fertility enhancement to boost food production without necessarily destroying the environment. This is because organic manures contain

both macro and micro nutrients (Wang et al., 2016) and also improve soil structure, aeration, soil moisture holding capacity and water infiltration.

An increase in production and utilization of cassava has

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brought with it the release of huge quantities of wastes to the environment. Cassava peels are the major waste produced during cassava processing. This waste can be valorized as animal feed, used for the production of biodiesel as well as composted for other uses like mushroom substrate production and soil amendments (Iren et al., 2015; Sangodoyin and Amori, 2013). Studies by Iren et al. (2015) showed the nutrient composition of cassava peel to be: Organic carbon (12.57%), total nitrogen (1.47%), total phosphorus (0.79%), total potassium (0.11%), total calcium (1.89%), total magnesium (0.81%) and total sodium (0.012%). Based on this data, cassava peels are a potential alternative fertilizer source for the farmers which can be sold or used by the farmers for crop production. This will go a long way to improve the farmers' income, thus reducing poverty.

Consumption of traditional leafy vegetables in most of the developing countries is increasing due to their good adaptability to harsh climatic conditions and tolerance to pests and diseases, and also their nutritional and medicinal values (Prasad et al., 2008). One of the most commonly consumed traditional leafy vegetables in Cameroon is the African Nightshade (Kahane et al., 2005). African Nightshade grows in various soil types, but are best adapted to soils of high fertility; especially those rich in nitrogen, phosphorus and organic matter.

Some studies have shown that composting cassava peels eliminated the problem of waste disposal and increased the manure value of the materials (Adediran et al., 2003; Akanbi et al., 2007). Poultry manure is known to contain nutrient elements which can support crop production and enhance the physical and chemical properties of the soil (John et al., 2011). Although compost is increasingly becoming a suitable substitute for inorganic fertilizers to improve crop productivity, the information on the ideal cultural practices that enhance the quality of composts is scanty. It is a proven fact that the effectiveness of compost depends primarily on source and type of organic material, method of composting and compost maturity. Mature compost (finished compost) provides a stabilized form of organic matter. In effect, bio-fertilizers and soil conditioners have far greater values than just their macronutrient contents. These materials have a much greater residual effect on soil tilth and fertility than most chemical fertilizers because of the slow-release character of their nitrogen and phosphorus components. Compost application improves the development of root systems, increases the diversity of root fungal flora, promotes the growth of plants, reduces the incidence of soil-borne diseases, and depresses the propagation of pathogens (Nitta, 1994). Thus, the aim of this study was to evaluate the effects of cassava peel-based manures on the growth and yield of African Nightshade. This study had as specific objectives to assess the mineral composition of cassava peel compost prepared following different approaches, and to

determine their effects on growth and yield of African Nightshade.

## MATERIALS AND METHODS

### Composting

Cassava peels were collected from water fufu (a local staple food from cassava) producers in Muea and Ekona areas of the South West Region of Cameroon. Poultry manure was bought from a poultry farm in Mamu Village and *Pueraria phaseoloides* harvested around the IRAD (Institute of Agricultural Research for Development) Ekona experimental fields. Cassava peel compost was prepared using two different recipes: Firstly cassava peel and poultry manure in the ratio of 3:1 (dry weight) and secondly using cassava peel, poultry manure and weed (*P. phaseoloides*) in the ratio of 3:1:1 (dry weight). These materials were combined in the said ratios and piled in heaps covering a surface area of 1 m<sup>2</sup> and height of 1.5 m. The weed was chopped into small pieces before usage. Two heaps were prepared using each of the recipes above with different turning frequencies:

1. Compost 1 (CP 1): Cassava peel and poultry manure (ratio 3:1) turned every two weeks;
2. Compost 2 (CP 2): Cassava peel and poultry manure (ratio 3:1) turned once a month;
3. Compost 3 (CP 3): Cassava peel, *P. phaseoloides* grass and poultry manure (ratio 3:1:1) turned every two weeks;
4. Compost 4 (CP 4): Cassava peel, *P. phaseoloides* grass and poultry manure (ratio 3:1:1) turned once a month;
5. Compost 5 (CP 5): Cassava peel only turned once a month;
6. Compost 6 (CP 6): Cassava peel and poultry manure (ratio 3:1) dried and grind without composting.

Cassava peel was combined with other local fertilizer sources such as poultry manure and *P. phaseoloides* to compare the nutritional value of CP 5 (cassava peel only) to that of cassava peel combinations with other fertilizers.

During the composting process, the temperatures in the different heaps were monitored every two weeks using a field thermometer. Watering was done twice for each heap during turning. The compost was matured by the end of the 12<sup>th</sup> week and the temperature dropped and remained unchanged with the compost having no peculiar smell.

Dry cassava peel manure (CP 6) was prepared by drying cassava peels and poultry manure and mixing them in the ratio 3:1 for cassava peel and poultry manure respectively. This mixture was grind into fine particles using a shredder.

The different manures (fertilizers) obtained using the different procedures were analysed for their macro nutrient composition (nitrogen, phosphorus, potassium, calcium and magnesium as well as the pH) following standard procedures. Based on the results of the analysis and considering the N levels, the two best manures (fertilizers) were used to grow African Nightshade. The fertilizer rates were 0, 5, 10 and 20 t/ha for the manure and 150 kg/ha NPK (20:10:10) with treatment 1 (T1) = control, T2 = 5 t/ha, T3 = 10 t/ha, T4 = 20 t/ha and T5 = NPK (20:10:10) at 150 kg/ha.

### Study area

Field experiments were carried out at IRAD Ekona. The Ekona site belongs to the humid forest zone with unimodal rainfall regime. This area is characterized by volcanic soils (andosol) and precipitation of 3,076 mm per year. The average air temperature varies from 19 to 23°C. It is governed by a "cameroonian" climate (very hot and

**Table 1.** Physico-chemical properties of the soil of the experimental site.

M	OC	N	C/N	P	pH (H <sub>2</sub> O)	pH (KCl)	CEC	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>+</sup>	Ca <sup>2+</sup>	Al+H
	%			mg/kg	(1:2.5)				cmol/kg			
10.99	2.56	0.74	4.0	52	6.70	5.77	16.11	0.09	0.66	3.37	3.40	0.03

M, Moisture 105°C; OC, organic carbon; N, total N; P, phosphorus (Bray 2); CEC, Cation exchange capacity.

**Table 2.** Chemical composition of the different cassava peel-based manures.

Code	pH(H <sub>2</sub> O)	pH(KCl)	% N	% P	% K	% Ca	% Mg
CP 1	8.39	7.87	1.36	0.70	1.33	3.07	0.22
CP 2	8.57	7.93	1.04	0.86	1.46	2.71	0.23
CP 3	8.06	7.53	1.28	1.01	1.37	2.60	0.23
CP 4	8.42	7.82	1.07	0.72	1.10	2.01	0.22
CP 5	8.45	7.75	0.91	0.41	1.64	3.07	0.24
CP 6	7.77	7.65	1.63	1.43	1.73	3.66	0.26

humid), a variant of the equatorial climate (Temegne et al., 2015a).

### Soil sampling and analysis

A composite sample of the top soil (0-15 cm depth) was collected from the experimental site with an auger before bed preparation following the transect method described by Okalebo et al. (2002). About 200 g of the samples were analysed for physical and chemical properties in the IRAD soil laboratory of Nkolbisson (Cameroon). The soil sample was air-dried and ground to pass through a 2 mm sieve. For carbon (C) and nitrogen (N) analysis, the soil was further fine ground to pass through a 0.5 mm sieve. Soil pH in water, was determined in a 1:2.5 (w/v) soil: water suspension. Organic C was determined by chromic acid digestion and spectrophotometric analysis (Heanes, 1984). Total N determined from a wet acid digest and analysed by colorimetric analysis (Anderson and Ingram, 1993). P was extracted using Bray extractant and the resulting extract analysed using the molybdate blue procedure described by Murphy and Riley (1962). Exchangeable cations Ca, Mg, K and Na were extracted using the ammonium acetate (NH<sub>4</sub>OAC, pH: 7) and determined by flame atomic absorption spectrophotometry. Cation exchange capacity (CEC) was determined using ammonium acetate. Results of the soil physico-chemical properties are presented in Table 1.

### Experimental layout

The experimental design was a randomized complete block design with three replicates. A land area of 10 m × 10 m was ploughed and harrowed and mapped out into plots (blocks) and sub-plots (treatments). Each sub-plot measured 1.5 m × 2 m with 1.5 m alley between plots and 0.5 m alley within each sub-plot. Thus each treatment measured 2 × 1.5 m.

To determine the moisture content in the compost, 100 g of the compost was oven dried at 80°C to constant weight and the amount of absorbed moisture contained in the compost determined as the difference between fresh weight and dry weight. Equivalent weights of 5, 10 and 20 t/ha of manure on dry weight basis were applied to designated plots on the field two weeks before planting (0, 1.5, 3 and 6 kg/subplot representing 0, 5, 10 and 20 t/ha). NPK (20:10:10)

at 150 kg/ha (45 g/subplot) was applied to the designated plots three weeks after planting.

Seedlings of African Nightshade were planted at a spacing of 20×20 cm on slightly raised beds. Weeding and watering were done regularly. At five weeks after planting, five plants were tagged per plot and sampled for the following growth parameters: Plant height, leaf area, number of leaves and branches. Each plot was ratooned (at 10 cm above the soil level) for marketable yields assessments at the 6th and 10th weeks. The ratooned plants were allowed to regenerate, and four weeks later, the marketable yields were again re-assessed.

### Data analysis

Data collected was subjected to an analysis of variance (ANOVA) and means separated with student t test at  $P < 0.05$  using the JMP 5 SAS software (SAS, 2002).

## RESULTS AND DISCUSSION

### Nutrient content of the prepared manures

The chemical composition of the different compost prepared is presented in Table 2. All pH values of the prepared composts were greater than 7, so they were basic. CP 2 presented the highest pH value while the lowest was obtained for CP 6 which is the dry fertilizer prepared by grinding a mixture of dry cassava peels and dry poultry manure in the ratio of 3:1. However, the difference was not significant. The pH obtained for these prepared compost manures was generally within the range of 7.5 to 8.5 recommended by USDA-NRCS (2000).

The highest level of nitrogen (1.63%) obtained for CP 6 dry manure compared to the other manures could be attributed to the fact that most of its nitrogen was not lost

**Table 3.** Mean African Nightshade growth parameters as influenced by cassava and poultry manure-based compost at five weeks after planting.

Experiment	Manure	Plant height (cm)	No. leaves	No. branches	Leaf length (cm)	Leaf width (cm)	Plant with flowers
1	T1	24.5 <sup>a</sup>	8.3 <sup>a</sup>	0.9 <sup>a</sup>	7.0 <sup>a</sup>	5.4 <sup>ab</sup>	2.7 <sup>a</sup>
	T2	24.9 <sup>a</sup>	9.7 <sup>a</sup>	1.5 <sup>a</sup>	7.9 <sup>a</sup>	6.0 <sup>a</sup>	4.7 <sup>a</sup>
	T3	23.7 <sup>a</sup>	9.9 <sup>a</sup>	1.3 <sup>a</sup>	7.2 <sup>a</sup>	5.1 <sup>b</sup>	4.3 <sup>a</sup>
	T4	24.5 <sup>a</sup>	10.2 <sup>a</sup>	1.3 <sup>a</sup>	7.0 <sup>a</sup>	5.0 <sup>b</sup>	3.7 <sup>a</sup>
	T5	23.0 <sup>a</sup>	10.7 <sup>a</sup>	1.5 <sup>a</sup>	7.7 <sup>a</sup>	5.2 <sup>b</sup>	3.5 <sup>a</sup>
<b>Means</b>		<b>24.12<sup>A</sup></b>	<b>9.76<sup>A</sup></b>	<b>1.3<sup>A</sup></b>	<b>7.4<sup>A</sup></b>	<b>5.3<sup>A</sup></b>	<b>3.8<sup>A</sup></b>
2	T1	22.3 <sup>b</sup>	10.1 <sup>a</sup>	0.9 <sup>b</sup>	7.5 <sup>a</sup>	4.9 <sup>b</sup>	5.0 <sup>a</sup>
	T2	25.9 <sup>ab</sup>	9.8 <sup>a</sup>	1.3 <sup>b</sup>	7.6 <sup>a</sup>	4.8 <sup>b</sup>	5.0 <sup>a</sup>
	T3	24.4 <sup>ab</sup>	12.9 <sup>a</sup>	2.5 <sup>a</sup>	7.8 <sup>a</sup>	4.9 <sup>ab</sup>	5.0 <sup>a</sup>
	T4	25.6 <sup>ab</sup>	10.7 <sup>a</sup>	1.5 <sup>ab</sup>	7.9 <sup>a</sup>	5.2 <sup>ab</sup>	3.3 <sup>a</sup>
	T5	26.5 <sup>a</sup>	9.9 <sup>a</sup>	1.1 <sup>b</sup>	8.9 <sup>a</sup>	6.2 <sup>a</sup>	5.0 <sup>a</sup>
<b>Means</b>		<b>24.94<sup>A</sup></b>	<b>10.68<sup>A</sup></b>	<b>1.5<sup>A</sup></b>	<b>7.9<sup>A</sup></b>	<b>5.2<sup>A</sup></b>	<b>4.7<sup>A</sup></b>

For each experiment, means with same letter in a column are not significantly different ( $p < 0.05$ ).

during drying compared to the N loss during composting. This is in agreement with other studies which confirm nitrogen lost during composting (Liang et al., 2006). The lowest level of N in CP 5 is logical because no additional nitrogen source was added to the cassava peels during composting. The fact that only cassava peels were composted for CP 5 production could account for its lowest phosphorus level. The K, Ca and Mg levels for CP 5 were among the highest suggesting that cassava peels are quite rich in these elements. Comparing the values obtained from composted cassava peels and literature values of un-composted cassava peels showed that some values like N, P and Mg decreased during composting while K and Ca values increased. This increase was in agreement with the study of Iren et al. (2015).

Comparing compost 1 and 2, then 3 and 4 which had the same compositions but varied in the frequency of turning, there were no clear trends or significant differences between most chemical elements in the various composts. Although for nitrogen, there were lower N concentrations for composts that were turned once in a month (CP 2 and CP 4). This showed that more nitrogen was lost with less turning. This disagrees with the results of De Guardia et al. (2008), Körner and Stegmann (2003) and Liang et al. (2004) who found higher ammonia emissions with higher aeration rates. This difference could result from the reaction of the different nitrifying bacteria with aeration and the nature of the compost material (De Guardia et al., 2009). The results of this study therefore suggest that both frequencies of turning could be used with little effects on the properties of the finished compost.

It is worth noting that the composition of N, P and Mg obtained in this study was lower than the values obtained

by Iren et al. (2015) who composted cassava peels and poultry manure in the ratio of 1:1. The pH (H<sub>2</sub>O), K and Ca levels were higher in this study than in the study by Iren et al. (2015). This difference could be attributed to differences in the proportions of the different components since the composting period was 12 weeks for both studies.

### Influence of the different amendments on African Nightshade growth

Based on the N levels of the various manures, CP 1 (cassava + poultry manure at 3:1 and turned every two weeks) and CP 6 (dried and ground cassava peels and poultry manure at 3:1 without composting) were used to grow African Nightshade at different dosages known respectively as Experiments 1 and 2.

### Growth parameters at five weeks after transplanting

As shown in Table 3, there were no significant differences in mean plant height, number of leaves, number of branches, leaf length and number of plant with flowers for the various treatments in Experiment 1. Leaf width however presented a significant difference with T2 having the highest mean leaf width. The plot treated with inorganic fertilizer had the lowest mean plant height. This could be attributed to the fact that the plants had not taken up most of the fertilizer applied since it was applied three weeks after transplanting and this measurement was done just two weeks after fertilizer application. Although plant height was lowest, T5 presented the highest number of leaves showing that most nutrients

**Table 4.** Mean African Nightshade growth parameters as influenced by cassava and poultry manure-based compost manure (Experiment 1) at the sixth week.

Experiment	Manure	Plant height (cm)	No. leaves	No. branches	Leaf length (cm)	Leaf width (cm)
1	T1	35.2 <sup>a</sup>	30.9 <sup>a</sup>	5.6 <sup>a</sup>	8.0 <sup>a</sup>	6.5 <sup>a</sup>
	T2	30.4 <sup>a</sup>	26.1 <sup>ab</sup>	3.7 <sup>c</sup>	8.4 <sup>a</sup>	6.6 <sup>a</sup>
	T3	33.5 <sup>a</sup>	27.6 <sup>ab</sup>	4.9 <sup>abc</sup>	8.8 <sup>a</sup>	6.9 <sup>a</sup>
	T4	27.1 <sup>a</sup>	22.6 <sup>b</sup>	4.1 <sup>bc</sup>	7.6 <sup>a</sup>	6.1 <sup>a</sup>
	T5	28.2 <sup>a</sup>	29.4 <sup>ab</sup>	5.1 <sup>ab</sup>	7.4 <sup>a</sup>	5.5 <sup>a</sup>
<b>Means</b>		<b>30.9<sup>A</sup></b>	<b>27.3<sup>A</sup></b>	<b>4.7<sup>A</sup></b>	<b>8.0<sup>B</sup></b>	<b>6.3<sup>B</sup></b>
2	T1	30.1 <sup>a</sup>	28.4 <sup>a</sup>	4.6 <sup>a</sup>	9.5 <sup>a</sup>	7.4 <sup>a</sup>
	T2	30.0 <sup>a</sup>	28.6 <sup>a</sup>	4.3 <sup>a</sup>	9.2 <sup>a</sup>	7.5 <sup>a</sup>
	T3	33.3 <sup>a</sup>	29.0 <sup>a</sup>	4.0 <sup>a</sup>	8.6 <sup>a</sup>	6.3 <sup>a</sup>
	T4	27.7 <sup>a</sup>	23.8 <sup>a</sup>	3.3 <sup>a</sup>	9.7 <sup>a</sup>	7.6 <sup>a</sup>
	T5	23.5 <sup>a</sup>	28.9 <sup>a</sup>	4.2 <sup>a</sup>	11.4 <sup>a</sup>	9.1 <sup>a</sup>
<b>Means</b>		<b>28.9<sup>A</sup></b>	<b>27.7<sup>A</sup></b>	<b>4.1<sup>A</sup></b>	<b>9.7<sup>A</sup></b>	<b>7.6<sup>A</sup></b>

For each experiment, means with same letter in a column are not significantly different ( $p < 0.05$ ).

were used for leaf formation at the expense of height. The control plot presented the lowest number of leaves, branches, leaf length and number of plants with flowers. This could be attributed to lack of adequate nutrients for growth and development. There was a significant difference between the different amendments with regards to the average leaf width. In most parameters, the organic amendments were very comparable to the inorganic fertilizer.

Considering results of the experiment carried out using ground dry cassava peels and poultry manure (Experiment 2), there were significant differences in the growth parameters at the fifth week after transplanting (Table 3), except for the average number of leaves, leaf length and number of plants with flowers. For plant height, leaf length and width, the plot treated with inorganic fertilizer (T5) presented the highest values while the plants in the control plot presented the lowest average plant height, number of branches and leaf length.

The experiment did not influence the growth parameters of African Nightshade (Table 3).

### Growth parameters at six weeks after transplanting

At the sixth week after planting, in Experiment 1, there was no significant difference in most of the growth parameters with respect to the different amendments (Table 4). However, there were significant differences observed in the average number of leaves per plant and number of branches with the control treatment having the highest values. This suggests that at this time, during the experiment, the plots that were amended loose more nutrients to microbes during manure breakdown, which

was not the case with the control.

In Experiment 2, growth parameters at the sixth week showed that there were no significant differences between the various treatments. For plant height and number of leaves, plants treated with organic manure at 10 t/ha (T3) presented the highest values (Table 4). The highest average number of branches (4.6) and flowers per plant (5) observed for the control treatment could be attributed to nutrient deficiency especially nitrogen. This is similar to the study of Wolf (1999) who explained that plants suffering from nitrogen deficiency mature earlier and their vegetative growth stage is shortened.

In this study, the various treatments did not influence the plant height, number of leaves and number of branches of African Nightshade at six weeks after planting (Table 4). However, the leaf length and the leaf width were significantly higher in Experiment 2 than Experiment 1. This suggested that composting would have resulted in loss of nutrients during the decomposition process and grinding of dried cassava peels conserved most nutrients.

Plant height increased with maturity, with maximum plant height of 35 cm recorded in this study. *Solanum nigrum* plant height of between 30.48 and 60.96 cm were reported by Millspaugh (1974) in his study and the results of the present study fall within this range. Edmonds and Chweya (1997) recorded 70 cm as the maximum height of *S. nigrum* in their study. Bvenura and Afolayan (2013) recorded a maximum height of 90.33 cm. These values are however higher than the values obtained in the present study. This could be attributed to the fact that records for plant height for this study ended at six weeks while the previous studies were much longer. Comparing results obtained from different parts of the world, different agro-ecological conditions presumably produce different

**Table 5.** Mean African Nightshade fresh weight as influenced by cassava and poultry manure-based compost at six and ten weeks after planting.

Experiment	Manure	Fresh weight (kg) at 6 weeks	Dry weight (kg) at 6 weeks	Fresh weight (kg) at 10 weeks	Dry weight (kg) at 10 weeks	Total fresh weight (kg)
1	T1	0.18 <sup>a</sup>	0.09 <sup>a</sup>	0.11 <sup>a</sup>	0.01 <sup>a</sup>	0.29
	T2	0.23 <sup>a</sup>	0.09 <sup>a</sup>	0.21 <sup>a</sup>	0.02 <sup>a</sup>	0.44
	T3	0.24 <sup>a</sup>	0.09 <sup>a</sup>	0.10 <sup>a</sup>	0.01 <sup>a</sup>	0.34
	T4	0.20 <sup>a</sup>	0.12 <sup>a</sup>	0.11 <sup>a</sup>	0.01 <sup>a</sup>	0.31
	T5	0.19 <sup>a</sup>	0.08 <sup>a</sup>	0.20 <sup>a</sup>	0.02 <sup>a</sup>	0.39
<b>Means</b>		<b>0.21<sup>A</sup></b>	<b>0.09<sup>A</sup></b>	<b>0.15<sup>A</sup></b>	<b>0.02<sup>A</sup></b>	<b>0.35<sup>A</sup></b>
2	T1	0.19 <sup>a</sup>	0.10 <sup>a</sup>	0.08 <sup>a</sup>	0.01 <sup>b</sup>	0.23
	T2	0.23 <sup>a</sup>	0.09 <sup>b</sup>	0.09 <sup>a</sup>	0.01 <sup>b</sup>	0.32
	T3	0.23 <sup>a</sup>	0.094 <sup>ab</sup>	0.09 <sup>a</sup>	0.02 <sup>b</sup>	0.32
	T4	0.22 <sup>a</sup>	0.09 <sup>b</sup>	0.09 <sup>a</sup>	0.02 <sup>b</sup>	0.31
	T5	0.25 <sup>a</sup>	0.094 <sup>ab</sup>	0.19 <sup>a</sup>	0.12 <sup>a</sup>	0.44
<b>Means</b>		<b>0.22<sup>A</sup></b>	<b>0.09<sup>A</sup></b>	<b>0.11<sup>A</sup></b>	<b>0.04<sup>A</sup></b>	<b>0.32<sup>A</sup></b>

For each experiment, means with same letter in a column are not significantly different ( $p < 0.05$ ).

plant heights of the same plant. Nitrogen generally stimulates vegetative growth (Zhang et al., 2010) meaning the formation of more buds and a subsequent increase in the number of leaves. From this study, it is obvious that as the height increased due to the uptake of N in its nitrate form, there was a general increase in vegetative growth as indicated by the increase in number of leaves with time.

### Marketable yield

The marketable yields for the five plants sampled per plot at the 6<sup>th</sup> and 10<sup>th</sup> week after transplanting for experiment are presented in Table 5. This vegetable is usually marketed and consumed fresh, thus the total fresh weight for both harvests was calculated. The results showed that the control plot presented the lowest yields while the plot treated with the lowest quantity of manure (T2) presented the highest total fresh weight, but the difference was not statistically significant. This showed the beneficial effects of amendment in African Nightshade yields similar to the observation of Ondieki et al. (2011). The overall fresh weight for the compost amended plots was very similar to that of the plot treated with inorganic fertilizer. In addition, the work of Ngome et al. (2013), Mary and Nithiya (2015) and Temegne et al. (2015a, b) showed that the chemical composition of the soil affect the growth and plant yield.

In Experiment 2, yield data collected at the 6<sup>th</sup> and 10<sup>th</sup> week after transplanting showed no significant difference in the fresh weight of plants from the various amended plots. However, the plot treated with inorganic fertilizer (T5) had the highest fresh weight while the control treatment had the lowest fresh weight (Table 5). As

concerns the dry weight, there were significant differences between treatments with the control treatment having the highest weight at the 6<sup>th</sup> week and the plants treated with inorganic fertilizer (T5) having the highest dry weight at the 10<sup>th</sup> week. The highest dry weight obtained for the control at the 6<sup>th</sup> week suggested that the application of soil amendments increased plant water uptake which is lost during drying. The total fresh weight was highest for the plot amended with inorganic fertilizer. However, the values were very close to those obtained in organic manure amended plots. The control plot presented the lowest total fresh yield. This showed that amending the plots improved yield and this is in agreement with the results of Ondieki et al. (2011) who showed that compost manure improved yield of African Nightshade species. Tarla and Fontem (2010) obtained similar results on huckleberry (*Solanum scabbrum*) with poultry manure.

The mean fresh weight was better at 6<sup>th</sup> than 10<sup>th</sup> week after planting for both experiments. In fact, flowering took place 5 weeks after transplanting and most plant resources were mobilized for biomass production which would justify the higher fresh weight obtained at the 6<sup>th</sup> week (Table 5). On the other hand, at 10 weeks after planting, the roots of the plant are depleted, the plant supply is reduced, and the leaves of the base quickly become senescent and fall. This last observation could justify the low fresh weight at the 10<sup>th</sup> week.

### Conclusion

Based on the N levels of the various manures, CP 1 (cassava + poultry manure at 3:1 and turned every two weeks) and CP 6 (dried and ground cassava peels and

poultry manure at 3:1 without composting) were the best formulations. Using CP 6 gave better results than CP 1 for African Nightshade growth at six weeks after transplanting. The cassava peels based-manures prepared in this study were very comparable to other organic manures. The effects of the used organic fertilizers on African Nightshade growth and yield were very similar to that of inorganic fertilizer. This study has demonstrated that the use of cassava peels in the preparation of compost or as dry manure is promising in improving African Nightshade fresh yields. T2 (5 t/ha), T3 (10 t/ha) and T4 (20 t/ha) were the best compost doses. The results of this study conclude that very high dosages of the organic manure are not very necessary since they do not improve yield or growth significantly. T2 can therefore be recommended for the cultivation of African Nightshade in the South-West Region of Cameroon. Thus using the organic fertilizers for crop production would be more economical and will also be very useful in the development of sustainable food production systems. This approach (use of organic fertilizer) will go a long way to promote environmental safety for waste will be converted to wealth. The cumulative agronomic and economic value of some organic materials applied to agricultural soils could be more than five times greater in the post-application period than the value realized during the year of application. Thus it is suggested for this study that, more significant effects of the amendments could be observed following studies on residual effects.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Isolation, identification and molecular characterization of *Rhizobium* species from *Sesbania bispinosa* cultivated in Bangladesh

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The investigation was carried out to study the characteristics of rhizobial strains isolated from the leguminous plant, *Sesbania bispinosa* growing in different regions of Bangladesh. Forty-four isolates were studied for biochemical and molecular characteristics. Isolates were able to utilize different carbohydrates. All isolates showed complete resistance to cloxacillin and penicillin G that results in increased survivability of rhizobial populations in antibiotic stressed conditions. Isolates were able to form nodule in the plant infection test. The majority of the strains showed positive results for *nodC* and *nifH* gene amplification which are the typical characteristics of *Rhizobium* species. Genetic relatedness was assessed by comparing the sequences of 16S rRNA. Two distinct clusters were seen in the dendrogram constructed by the Complete Linkage method. The isolates R7, R8, R17, R33 and R3 were distinct from the 20 reference strains. The first cluster was phylogenetically distinct from the reference strains and might have evolved from a distinct lineage. Isolate R4 was placed adjacent to *Rhizobium cnuense*. So, the findings may represent new species of *Rhizobium*. This study helps to identify an ideal strain of *Rhizobium* from *S. bispinosa* that can be function as biofertilizer when released in the soil and contribute to sustainable agricultural practices by improving yields.

**Key words:** *Rhizobium*, *Sesbania bispinosa*, nodulation gene, nitrogen fixation gene, sequencing.

## INTRODUCTION

Nitrogen is one of the most abundant nutrient supplement for plant growth and is not directly available to plants because of its strong triple covalent bond. Increasing global population demands large food production which can be achieved by using plant beneficial microorganisms. Rhizobia are plant growth promoting bacteria that plays a key role in enhancing nitrogen fixation ability of leguminous plants by converting

nitrogen to ammonia and provide organic nitrogenous compound to the plants. Rhizobia form nodules on root to fix nitrogen where nod factor and flavonoids initiate nodulation (Oldroyd, 2013). Nodulins are *nod* gene protein that are associated with Nod factor assembly and *nod* gene is the determinant of host range (Perret et al., 2000). The *nifH* gene is selected as a nitrogen fixation marker. Rhizobia take up carbon sources derived for

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plant photosynthesis. Rhizobial isolates harbour plasmid. Mulligan et al. (1985) demonstrated that nodulation genes, the regulatory genes, and the nitrogen fixing genes are located on large symbiotic plasmid. Antibiotics accumulation in soils after excretion from feces and urine was reported by Ji et al. (2012). These antibiotics are lethal to susceptible rhizobial population and decreased rhizobial persistence in the soil and ultimately total nitrogen fixation. Cole and Elkan (1973, 1979) demonstrated that antibiotic resistance is a plasmid-born character. Cole et al. (1973) and Frioni et al. (2001) reported that greatest difference of antibiotics tolerance was found between fast and slow growing rhizobia. The antibiotic-resistant *Rhizobium* occupies large number of nodules in legumes (Belachew, 2010; Gemell et al., 1993). *Sesbania* is an important green manure that fixes large quantities of N<sub>2</sub>. In the wet season, *Sesbania bispinosa* grew rapidly, exhibiting high level of nitrogen accumulation in soil Ladha et al. (1992) and Ndoye et al. (1988). *S. bispinosa* has not been much investigated regarding the rhizobial infection in Bangladesh. Carbohydrate utilization test is used to characterize rhizobia. The utilization of different carbohydrates serve as a diagnostic tool in the differentiation of *Rhizobium* species (El-Idrissi et al., 1996; Graham et al., 1991). Molecular methods including polymerase chain reaction (PCR) and 16S rRNA sequencing are more reliable for identification of bacterial isolates.

The aim of study was to characterize forty-four *Rhizobium* species isolated from *S. bispinosa* and also to identify the antibiotic resistant *Rhizobium* which has increased survivability and can be used as biofertilizer to increase productivity.

## MATERIALS AND METHODS

### Isolation of *Rhizobium* strains

Pink and healthy root nodules of *S. bispinosa* were washed in water. It was then transferred to 3% H<sub>2</sub>O<sub>2</sub> solution and soaked for 2-3 min. To remove the traces of H<sub>2</sub>O<sub>2</sub> solution, nodules were rinsed 5-6 times in sterile distilled water. Nodules were washed in 70% ethanol and then sterile glass rod was used to crush the nodule. Nodule suspension was streaked on yeast-mannitol agar (YMA) plates and incubated at 30°C for 24 h (Agrawal et al., 2012). Well isolated typical single colonies were restreaked on freshly prepared YMA plates in order to obtain pure cultures and colony characteristics were observed.

### Biochemical testing for identification

All isolates were processed for oxidase test as described by Kovaks (1956); catalase and nitrate reduction test as determined by Graham and Parker (1964). Urea agar slants were used to determine the urease activity that was incubated for 7 days at 30°C (Christensen, 1946). Isolates were also tested for indole production as described by Lowe (1962), gelatin liquefaction and motility test as mentioned by Arora (2003), and ONPG (O-nitro phenyl--β-D-galactoside) test as described by Cappuccino (2007). For

**Table 1.** Antibiotic used in the susceptibility testing of *Rhizobium* isolates.

Name of antibiotic	Concentration (µg)
Kanamycin	30
Gentamicin	10
Cephalexin	30
Streptomycin	10
Cloxacillin	5
Penicillin G	10
Polymixin B	300
Erythromycin	15
Nalidixic acid	30
Rifampicin	5

carbohydrate utilization tests, the basal medium used was that of Bishop et al. (1976) and for carbohydrate utilization test, different carbohydrates were substituted for mannitol, and KNO<sub>3</sub> (0.6 g/liter) was used as the nitrogen source.

### Determination of the antibiotic susceptibility

Bacterial suspension was prepared on nutrient broth and lawn on Muller-Hinton agar plates by using sterile cotton swab. Antibiotic discs were placed aseptically and plates were then incubated at 30°C for 24 h. The diameter was measured in millimeters after incubation. Name of the antibiotic and their concentration are given in Table 1.

### Plant infection test

Plant infection test was performed to confirm whether the isolates were able to form nodule and fix atmospheric nitrogen in modified Jensen's Agar medium. Seed was surface sterilized by covering with 3% hydrogen peroxide solution and left for 2 min. After washing three times in sterile water, surface sterilized seeds of *S. bispinosa* were spread onto surface of water agar plates. Plates were inverted and left in dark until germination occurred for 2 days to 1 week. Germinated seeds were aseptically transferred to big size test tubes containing modified Jensen's agar medium and roots were pointing downward with the contact of the agar. When leaves appeared, 1 ml of rhizobial suspension was added at their base. Plants were grown in light for approximately 8 weeks.

### Detection of *nifH* and *nodC* genes

Amplification was performed in a 12.5µL reaction volume for each specimen containing 2.5 µL (100 ng/µL) of template DNA. The reaction volume was prepared by mixing the following reagents: 7 µL of DEPC treated water, 1 µL of 20 mM MgCl<sub>2</sub>, 0.2 µL of 10 mM dNTP, 0.625 µL of 10 mM forward and 0.625 µL of 10 mM reverse primer, 0.05 µL of Taq polymerase and 2.5 µL of template DNA. Mastermix was transferred to each PCR tube and then the corresponding DNA samples (2.5 µL) were added to each tube. For the amplification of the *nifH* gene, the PCR contents were subjected to initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 45 s. Annealing, elongation and final extension phase were maintained at 63°C for 45 s, 72°C for 45 s and 72°C for 7 min, respectively. An identical regime was

**Table 2.** Biochemical characteristics of isolates.

Test	Results
Oxidase	Positive
Catalase	Positive
Urease production	Positive
Nitrate reduction	Positive
Motility	Positive
ONPG (O-nitro phenyl-- $\beta$ -D-galactoside)	Positive
Indole production	Negative
Gelatin liquefaction	Negative

maintained for *nodC* detection except the fact that the annealing was maintained at 54°C. The nucleotide sequences of the primers were as follows: *nifH* (forward 5'-CGTTTTACGGCAAGGGCGGTATCGGCA-3' and reverse 5'-TCCTCCAGCTCCTCCATGGTGATCGG-3') (Perret and Broughton, 1998) and *nodC* (forward 5'-GCCATAGTGGCAACCGTCGT-3' and reverse 5'-CTCGCCGCTGCAAGT-3') (Jacob et al., 1985).

#### Phylogenetic analysis

For the amplification of the 16S rRNA gene, the PCR contents were subjected to initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 45 s. Annealing, elongation and final extension phase were maintained at 46°C for 45 s, 72°C for 1.5 min and 72°C for 8 min, respectively. PCR was conducted using 16S rRNA primers (Uni\_euF:5'-AGAGTTTGATCCTGGCTCAG-3' and Uni\_euR:5'-TACCTTGTTTACGACTT-3') (Singh et al., 2013). The chromatogram sequences were inspected with Chromas 2.3 (Technelysium, Australia), and contigs was prepared using SeqMan II (DNASTAR, Madison, Wis.). The dendrogram was constructed using the online software Phylogeny.fr ([http://www.phylogeny.fr/simple\\_phylogeny.cgi](http://www.phylogeny.fr/simple_phylogeny.cgi)).

## RESULTS AND DISCUSSION

Isolates were initially studied for morphological and cultural characteristics. The colonies were circular, convex, gummy with entire margin and no pigmentation on YMA agar plates after 24 h incubation. The isolates were positive for catalase, oxidase, motility, ONPG (O-nitro phenyl-- $\beta$ -D-galactoside) tests and negative for indole production and gelatin liquefaction tests (Table 2). These findings were in close agreement with Naz et al. (2009). Isolates also showed positive result for nitrate reductase and urease test. Same test results were found by Sadowsky et al. (1983). They were helpful in presumptive identification of the genus of the isolates. It was also found that isolates were able to grow before 24 h incubation. So, it is concluded that our representative isolates were fast grower.

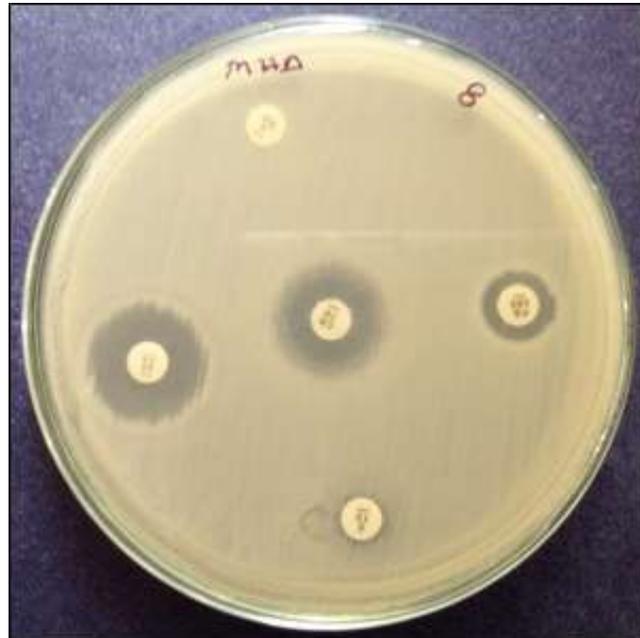
Carbohydrate utilization capacity deduced that isolates were able to utilize different carbohydrates. Isolates were unable to utilize sucrose, succinate and lactose (Table 3). Same tests results were also observed by Sadowsky et al. (1983). Glenn and Dilworth (1981) reported that

**Table 3.** Sugar fermentation tests of the isolates.

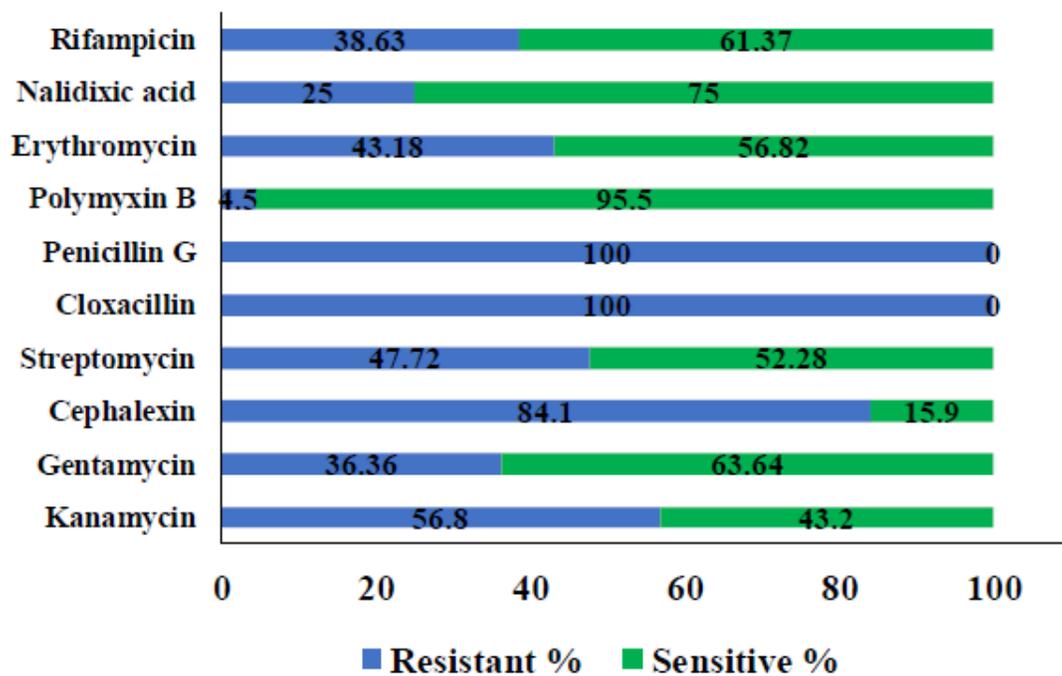
Carbohydrate	Results
Arabinose	Positive
Sucrose	Negative
Fructose	Positive
Glucose	Positive
Mannitol	Positive
Mannose	Positive
Rhamnose	Positive
Succinate	Negative
Xylose	Positive
Lactose	Negative

catabolic enzymes and disaccharides uptake systems were absent in slow growing rhizobia. That is why fast grower rhizobia utilize a wide variety of carbohydrate unlike slow grower rhizobia (Fred et al., 1932; Graham and Parker, 1964). These results also confirmed that majority of our isolates were fast grower.

Antimicrobial resistance has emerged over last few decades due to abuse of these drugs. *Rhizobium* was examined for their genetic basis for resistance to antibiotics (Figure 1). All isolates were resistant to penicillin G and cloxacillin and 84.1% isolates were resistant to cephalexin. The resistant isolates might have beta-lactamase enzyme that conferred the resistance property to these isolates. *Rhizobium laguerreae* resistance to ampicillin, penicillin antibiotics was reported by Saidi et al. (2014). Twenty-five (56.8%) isolates were resistant to kanamycin, 36.4% isolates were resistant to gentamicin and 47.72% isolates were resistant to streptomycin. Streptomycin, kanamycin and gentamicin are aminoglycoside antibiotics and conferred resistance by interrupting protein synthesis. Nineteen (43.2%) isolates were resistant to erythromycin. Since erythromycin molecules are large, they are unable to pass through the outer cell membrane. Prasuna et al. (2014) found a strain that was resistant to many antibiotics (chloramphenicol, erythromycin, kanamycin, neomycin and penicillin G) and these results were in close agreement with our findings. About 38.6, 25 and 4.5% of isolates were resistant to rifampicin, nalidixic acid and polymixin B, respectively (Figure 2). Mihaylova et al. (2014) reported that *Rhizobium* strains were sensitive to gentamicin and polymyxin B. So, these isolates showed higher antibiotic resistance towards beta-lactamase antibiotics. Antibiotic resistant *Rhizobium* increases rhizobial survivability in the soil (Naamala et al., 2016). So that, these antibiotics resistant *Rhizobium* can survive antibiotic stressed conditions and help to increase soil productivity. Presence of *nod* gene was confirmed by plant infection test. Twenty strains were studied for plant infection test and positive results were obtained for



**Figure 1.** Antibiotic susceptibility test of *Rhizobium* species by disc diffusion test.

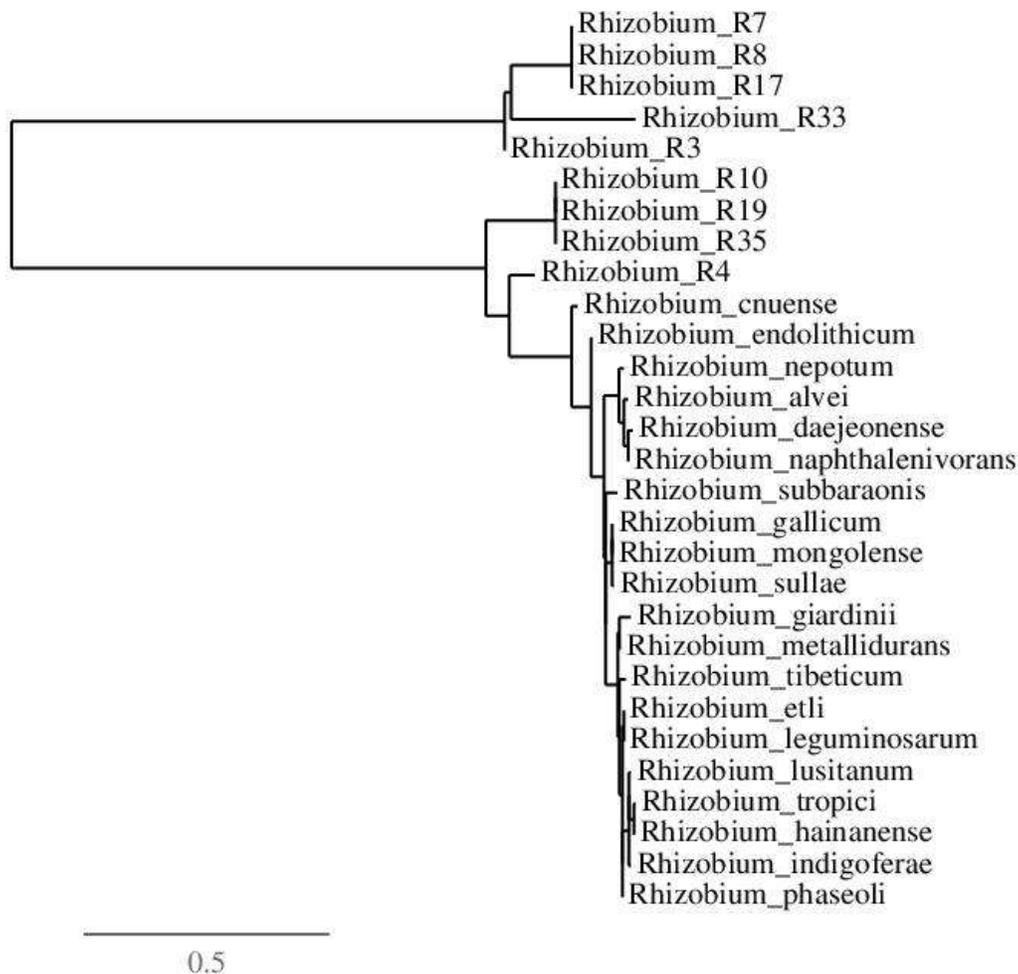


**Figure 2.** Antibiotic susceptibility patterns of *Rhizobium* species.

isolates R3, R5, R7, R8, R10, R12, R17, R18, R24, R32, R35 and R41.

Nitrogenase enzyme, encoded by *nif* gene, reduced nitrogen to ammonia and occurred in only nitrogen fixing

organisms (Singh et al., 2013). The majority of the strains gave PCR amplified product with an approximate length of 781 bp for the *nifH* gene. The *nodC* gene with the help of Nod factors initiated the root nodule development in



**Figure 3.** Dendrogram constructed by the complete linkage method base on 16S rRNA sequences of *Rhizobium* species.

leguminous plants (Barny et al., 1996). When DNA was amplified with *nodC* specific primer, an amplicon of 500 bp was observed. Not all the strains harboured both *nifH* and *nodC* genes. The absence of the amplicon product was probably due to some nucleotide mismatches in *nifH* and *nodC* gene which agreed with the result of Laguerre et al. (2001). Some strains (R1, R14, R15, R16, R30, R31, R37 and R39) showed positive result in *nodC* gene amplification but failed to form nodule in the plant infection test. We concluded that pot experiment might have failed to form nodule for certain limitations such as temperature, humidity etc. which were controlled in the laboratory experiment.

The 16S rRNA sequence of nine isolates were compared with 20 species from the NCBI data base. The sequences were aligned and dendrogram was constructed (Figure 3). In the 16S rRNA sequencing, two distinct clusters were seen. In the first cluster, the isolates R7, R8, R17, R33, R3 were grouped together. On the other hand, the isolates R10, R19, R35 and R4 were

clustered together with the 20 reference strains. So, first cluster was phylogenetically distinct from the reference strains and might have evolved from a distinct lineage. Based on the 16S rRNA sequences, the isolates R7, R8 and R17 were indistinguishable from each other. A similar conclusion was applicable for the isolates R10, R19, R35. It could also be inferred that the isolates R10, R19 and R35 exhibited a convincing degree of dissimilarity as compared to the reference strains and hence branched off to form a different cladogroup. The isolate R4 was placed adjacent to *Rhizobium cnuense*. It is concluded that the findings may represent new species of *Rhizobium*. Menna et al. (2006) also observed new rhizobial isolates based on the sequencing of 16S rRNA.

### Conclusion

Most of the isolates found in this study were fast grower and were able to utilize different carbohydrates. Antibiotic

susceptibility test showed that isolates were resistant to antibiotics that might help them to survive in antibiotic stress environment. Isolates harbouring *nifH* and *nodC* genes might be useful to increase soil fertility. The sequencing results revealed the genetic variability of the isolates. The goal of the present study was to raise the worldwide knowledge of the biodiversity of soil rhizosphere and identified the usefulness of rhizobial population so that isolates can be used as biofertilizers. To identify the genetic diversity of *Rhizobium* populations, molecular characterization such as fingerprinting techniques are needed. Different environmental stress conditions such as temperature, pH, heavy metals and pesticidal effects needs to be checked prior to the release of *Rhizobium* among the field populations so that it can survive under the adverse environmental condition and improve soil productivity.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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*Full Length Research Paper*

# Perceptions of risk and risk management strategies in family agroindustries

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The importance of risk perception and risk management has been addressed by studies of agriculture and livestock activities; however, there have been few studies in other contexts, such as family agroindustries. This study provides information on risk perception and risk management in family agroindustries in the state of Rio Grande do Sul, Brazil. The analysis involves 72 family agroindustries in 43 counties, and the respondents were the individual decision makers of those organizational units. 28 sources of risks and 28 risk management strategies were analyzed. The results suggested that the country's current economic situation, inflation/deflation, changes in product prices and the elimination/reduction of government support are the most important sources of risk. The main types of risk management included updating to new technologies, the use of technical support, maintaining/increasing market liquidity for products, and the commercialization of products without mediators. Two sources of risk (low-qualified staff and lack of motivation) and four risk management strategies (commercialization of products without mediators, acquiring certification, improving production practices and maintaining relationships with customers) were identified in the present study, but not found in the literature reviewed.

**Key words:** Agroindustries, food production, risk, risk perception, risk management.

## INTRODUCTION

The consolidation and strengthening of Brazilian family farming, especially in the 1990s, resulted in more support for the development of public policies aimed at these agricultural establishments (Schneider and Gazolla, 2015). Due to their limited technology and financial resources (as compared to high tech and specialized agriculture that produces commodities, many family farmers do not obtain satisfactory economic results, and

need to diversify their activities and products (Nichele and Waquil, 2011). In this regard, agroindustries have emerged as an alternative to family farms (Gazolla et al., 2012; Schneider and Ferrari, 2015).

Family agroindustries are organizational units that play a key role in the composition of the income of families that perform agriculture and livestock activities. These organizations are described by Pelegri and Gazolla

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**Table 1.** Studies of risk perception and risk management strategies.

Authors	Study/country	Year
Wilson et al.	Dairy farmers/USA	1988
Meuwissen et al.	Livestock farmers/Netherlands	2001
Flaten et al.	Dairy farmers/Norway	2005
Akcaoz et al.	Dairy farmers/Turkey	2009
Borges and Machado	Producers of agricultural commodities/Brazil	2012
Zhou et al.	Dairy farmers/China	2012
Finger and Waquil	Rice producers/Brazil	2013
Gebreegiabher and Tadesse	Smallholder dairy farmers/Ethiopia	2014
Khan et al.	Dairy farmers/India	2014
Hayran and Gül	Dairy farmers/Turkey	2015

(2008) as undertaking activities that involve the processing of agricultural and livestock products (value is added to the product). Dairy products, sweets, fruit jellies, preserves, pasta, biscuits, pork and beef sausages are examples of family agroindustry products. With the commercialization of these products, these agroindustries can stimulate income generation and improve the social status of family farmers (Gazolla et al., 2012; Schneider and Ferrari, 2015). They are exposed to many risks that may trigger negative results.

The word "risk" comes from the Old Italian word "risicare", which means "to dare" (Bernstein, 1996). In general, the concept of risk concerns a possible future event and can be understood as a potential loss affecting the desired results in a given personal or organizational activity (Nelson, 1997; Ayala-Cruz, 2016). Risks are present everywhere and are characterized as adverse results associated with a given action (Hardaker, 2000; Huirne, 2003), and risk perception varies according to the dimension and consequences of the risk (van Winsen et al., 2014).

Studies about risks have emerged in several areas, such as physics, biology, engineering and social sciences (Micic, 2016). A number of studies in recent decades have assessed risk perceptions and risk management in agricultural and livestock activities (Flaten et al., 2005; Akcaoz et al., 2009; Borges and Machado, 2012; Finger and Waquil, 2013; Gebreegiabher and Tadesse, 2014; Khan et al., 2014). These studies are important because they show it is necessary to perceive risks to develop strategies to reduce or eliminate them.

Risk management deserves attention due to its importance in organizational management (Borges and Machado, 2012). Risk management strategies involve the use of specific methods (Akcaoz et al., 2009) and are generally considered as one of the factors that determines the financial success or failure of an organization. Management measures are, therefore, essential because although there are several sources of risk, there are also countless mechanisms/strategies with which to manage them (Borges and Machado, 2012).

The aim of this research was to analyze perceptions of risk and risk management strategies based on the perception of decision makers in family agroindustries in the state of Rio Grande do Sul, Brazil. Although, several studies have addressed risk perception and risk management strategies, none of those identified focused on family agroindustries. In addition to attempting to identify the main sources of risk and risk management strategies, the importance of conducting interviews with these decision makers prior to the administration of the questionnaire was assessed.

## METHODOLOGY

### Study description

The study population undertakes agroindustrial activities in the state of Rio Grande do Sul, a state that has 497 municipalities. The selection of respondents was based on a non-probabilistic sampling method because the respondents were not accessible to be randomly sampled. However, when non-probabilistic sampling is properly done, it can obtain satisfactory results faster, and at lower costs, than the probabilistic method (Curwin and Slater, 2008).

Two procedures were used in development of the questionnaire. At first, based on a bibliographic review of studies of risk perceptions and risk management (Table 1), a preliminary questionnaire was developed for pre-testing its suitability for meeting the objective of the survey. Pre-tests were conducted with the individuals responsible for decision making of family agroindustries and this data was not used in the present study.

The second procedure consisted of semi-structured interviews with the decision makers of family agroindustries, recorded by note taking. This step aimed to identify sources of risk and risk management strategies not considered in the analyzed literature, to identify other variables based on the respondents' views. The interview was based on two questions: i) what would you consider a source of risk? ii) what would you consider as a risk management strategy? Seven decision makers of family agroindustries were interviewed. The size of this sample was based on saturation of answers.

### Sample description and characterization

After construction of the questionnaire, it was used with respondents

**Table 2.** Descriptive statistics of the decision makers.

Variable	Average	Minimum	Maximum	Std <sup>c</sup>
Age (years)	42.56	21	78	12.66
Farm area (ha-own)	13.53	0	68	13.17
Farm area (ha-leased)	1.93	0	27	4.8
Total farm area (ha)	15.47	0.5	68	13.27
Variable	Relative frequency (%)			
Schooling	Incomplete elementary education			18
	Elementary education			7
	Incomplete secondary education			4
	Secondary education			39
	Incomplete higher education			9.7
	Higher education			19.3
	Postgraduate studies			2.8
Marital status	Single			19.44
	Married			79.16
	Separated			1.4

respondents from April to July 2015. The participants were 72 family agroindustries located in 43 counties. These agroindustries produce items such as baked goods, pasta, juices, sweets, wines, dairy products and sausages. The respondents were the decision makers in the agroindustries, the managers of family agroindustries.

The respondents were identified with the aid of the Federação dos Trabalhadores na Agricultura Familiar da Região Sul (FETRAF/SUL) and Empresa de Assistência Técnica e Extensão Rural (EMATER/RS) in the counties of Ajuricaba and Ijuí. The 72 questionnaires were administered as follows: at the producer fairs often held weekly in the municipalities (Ajuricaba) (3); at the 6th Feira de Negócios das Indústrias de Ijuí (FENII) (12); during visits to family agroindustries (13) and by e-mail through Google Docs (44), (in total, 246 emails were sent with the questionnaire, and 47 were completed and returned, of these, three were excluded due to inappropriate or incomplete responses).

#### Data processing and analysis

Based on the sources of risk and risk management strategies, as well as the variables identified by semi-structured interviews, the participants indicated their perceptions using a Likert scale (where 1 = not relevant and 5 = very relevant). The data was analyzed using descriptive statistical measures and factor analysis. Factor analysis is a multivariate technique and aims to consolidate the information contained in a series of original variables into a smaller number of variables (factors), (Hair et al., 2006). The Bartlett's test of sphericity (which analyzes the hypothesis that the variables are not correlated in the population) and Kaiser-Meyer-Olkin (KMO) test were used to evaluate the factor analysis. It is considered an acceptable condition when  $KMO > 0.5$  (Bezerra and Corrar, 2006).

The variation between variables in each factor was expressed by latent root criterion (Eigenvalue  $> 1$ ) (Hair et al., 2006). The factorial matrices were rotated using the orthogonal method (Varimax). Varimax method maximizes the variance of squared loadings of a factor.

The factorial analysis also takes into account the communalities. Communalities values represent the proportion of the variance for each variable included in the analysis that is explained by the components extracted. Usually, is acceptable, a variable with communalities  $\geq 0.5$  (Hair et al. 2006). In interpreting factorial

coefficients, significance were considered when presenting value  $\geq |0.30|$  according to Flaten et al. (2005). These factorial coefficients are in bold in Tables 4 and 6.

## RESULTS AND DISCUSSION

### General characteristics of respondents

The main characteristics of the respondents are shown in Table 2. Age and farm area were examined by average, minimum, maximum and standard deviation. Also, the relative frequency was used to examine schooling and marital status.

According to the results, farmers are average 42.56 years old and the average of total farms size is 15.47 ha. In addition, most farmers (79.16%) are married and 19.3% have concluded the higher education. On the other hand, many respondents have a low education level (7% have concluded elementary education and 18% have elementary education incomplete).

### Perception of risk sources

In total, 28 sources of risks were considered and presented to the respondents. Table 3 shows the ranking, mean, mode and standard deviation of these risks, which are classified in descending order, considering the mean values.

The source of risk with the highest mean was the country's economic situation followed by inflation/deflation (in 2015, the inflation rate was higher than 10%). One possible reason to explain these results is the concern of these respondents about the economic and political scenario in Brazil and in the study region. On the other

**Table 3.** Risk perception (ranking).

Risks	Rank	Mean	Mode <sup>d</sup>	Std <sup>e</sup>
Country's economic situation	1	4.05	5	1.18
Inflation/deflation	2	3.84	5	1.21
Changes in product prices	3	3.83	5	1.26
Elimination/Reduction of governmental support	4	3.81	5	1.38
Difficulties faced in finding skilled labor <sup>a</sup>	5	3.81	5	1.36
Changes in governmental policies	6	3.66	5	1.25
Changes in prices of inputs <sup>a</sup>	7	3.63	4	1.30
Poor hygiene <sup>a</sup>	8	3.59	5	1.63
Health and safety of manager/staff	9	3.54	5	1.46
Death of manager/staff	10	3.47	5	1.69
Emergence of new technologies	11	3.44	5	1.37
Development of new production techniques	12	3.41	5	1.40
Lack of raw material <sup>a</sup>	13	3.40	5	1.42
Low-qualified staff <sup>c</sup>	14	3.38	5	1.54
Non-occurrence of family succession	15	3.37	5	1.55
Excess supply	16	3.36	5	1.41
Changes in interest rates	17	3.36	4	1.39
Lack of technical support	18	3.34	5	1.43
Market competition	19	3.34	4	1.38
Climate changes	20	3.30	3	1.32
Indebtedness	21	3.27	5	1.38
World economic situation	22	3.16	3	1.35
Lack of motivation <sup>b,c</sup>	23	3.15	3	1.42
Occupational accidents	24	2.98	1	1.52
Robbery/theft	25	2.76	1	1.52
Fires	26	2.56	1	1.43
Family conflicts	27	2.54	1	1.39
Division of labor between the participants	28	2.43	3	1.16

<sup>a</sup>Variables with one missing value were replaced with the mean of that variable for all the cases;

<sup>b</sup>Variables with two missing values were replaced with the mean of that variable for all the cases;

<sup>c</sup>Source of risks not found in the literature reviewed. They were detected in the interviews; <sup>d</sup>Value that appears most often in the set data; <sup>e</sup>Standard deviation.

hand, the third highest mean was changes in product prices, or the risk of financial fluctuations that might result in losses. This risk had the third highest mean in the studies conducted by Flaten et al. (2005) and Meuwissen et al. (2001), and the second highest mean in the study of Borges and Machado (2012), also conducted in Rio Grande do Sul (geographic micro-region of Vacaria). The elimination/reduction of governmental support was the fourth largest mean, possibly also due to the economic crisis in Brazil. This risk also ranked among the top four risks in the study by Gebreegziabher and Tadesse (2014). However, it appears in a middle ranking position in the study by Meuwissen et al. (2001). These results may demonstrate the importance of government actions targeted at some productive activities in countries characterized as emerging economies.

The health and safety of manager and staff variable was in the upper part of the table, however Flaten et al.

(2005) found this source of risk in a middle ranking position, and Gebreegziabher and Tadesse (2014) found it in the last ranking position. These differences are addressed by Patrick et al. (1985), Renn (1998) and Finger and Waquil (2013), who reported that the perception of risk differs considerably between groups of individuals and cultures.

Sources of risks not found in the literature studied but identified during the interviews were: low-qualified staff and lack of motivation. These risks were not placed in the first few positions, but respectively in the 14th and the 23rd positions. Low-qualified staff and lack of motivation may have a negative impact on agroindustrial production, affecting the production system and thus economic results.

Following the descriptive analysis of the sources of risks, a factorial analysis was performed, as shown in Table 4. The value obtained for Bartlett's sphericity test

**Table 4.** Factor analysis of the sources of risks– rotation matrix (Varimax).

Sources of risks	Factors									Commonality
	1	2	3	4	5	6	7	8	9	
Changes in interest rates	<b>0.81</b>	0.01	0.09	-0.10	0.01	-0.03	0.19	0.01	0.01	0.723
World economic situation	<b>0.77</b>	0.08	0.03	-0.01	0.13	0.09	-0.06	-0.01	0.12	0.654
Country's economic situation	<b>0.64</b>	-0.04	-0.18	<b>0.37</b>	<b>0.39</b>	-0.01	0.23	0.09	-0.02	0.803
Inflation/Deflation	<b>0.62</b>	0.02	0.02	0.21	<b>0.39</b>	-0.08	0.24	0.05	0.03	0.734
Lack of technical support	<b>0.55</b>	<b>0.41</b>	0.01	0.06	0.29	0.26	0.16	0.01	-0.21	0.720
Lack of raw material	<b>0.53</b>	0.16	0.17	0.07	-0.01	0.02	-0.13	<b>0.55</b>	0.18	0.707
Changes in product prices	<b>0.52</b>	0.14	-0.06	<b>0.34</b>	-0.01	<b>0.31</b>	0.22	0.07	0.02	0.628
Poor hygiene	-0.03	<b>0.78</b>	-0.01	0.06	-0.24	0.01	0.20	0.26	0.02	0.793
Health and safety of manager/staff	-0.11	<b>0.77</b>	0.20	-0.04	0.16	0.15	-0.06	-0.13	0.14	0.754
Death of manager/staff	0.26	<b>0.63</b>	<b>0.37</b>	0.11	0.14	-0.10	-0.08	-0.03	0.12	0.678
Robbery/Theft	0.03	<b>0.58</b>	-0.05	0.26	0.10	-0.12	0.02	0.04	0.28	0.592
Low-qualified staff	<b>0.43</b>	<b>0.57</b>	-0.08	0.11	-0.15	-0.13	0.17	0.22	-0.16	0.693
Non-occurrence of family succession	0.18	<b>0.57</b>	0.29	-0.13	0.07	0.13	0.18	-0.16	-0.16	0.580
Occupational accidents	-0.10	0.06	<b>0.87</b>	-0.01	-0.03	0.14	0.03	0.02	0.01	0.811
Fires	-0.01	0.27	<b>0.83</b>	-0.01	0.06	0.14	-0.03	0.21	0.01	0.842
Excess supply	0.11	0.03	0.03	<b>0.80</b>	0.12	-0.07	-0.08	<b>0.33</b>	0.12	0.821
Market competition	0.06	0.14	-0.26	<b>0.65</b>	0.05	<b>0.33</b>	0.05	0.08	0.23	0.699
Family conflicts	0.03	0.13	<b>0.50</b>	<b>0.58</b>	0.03	-0.01	0.29	-0.14	-0.02	0.771
Indebtedness	<b>0.37</b>	<b>0.36</b>	0.18	<b>0.42</b>	0.28	-0.19	-0.08	-0.19	-0.22	0.690
Changes in governmental policies	0.18	0.01	0.06	0.01	<b>0.83</b>	0.04	0.07	0.14	0.18	0.796
Elimination/Reduction of governmental support	0.17	0.1	-0.01	0.17	<b>0.77</b>	0.05	0.11	0.13	0.01	0.771
Development of new production techniques	0.03	-0.03	0.11	0.03	0.06	<b>0.88</b>	-0.01	0.05	0.08	0.814
Emergence of new technologies	0.05	0.04	0.15	0.03	0.01	<b>0.79</b>	0.14	0.05	-0.11	0.704
Difficulties faced in finding skilled labor	0.19	0.10	-0.10	0.02	0.24	0.00	<b>0.71</b>	0.10	-0.07	0.646
Division of labor between the participants	0.18	0.08	0.19	0.01	0.01	0.21	<b>0.68</b>	0.02	0.25	0.651
Lack of motivation	0.03	0.13	<b>0.50</b>	<b>0.58</b>	0.03	-0.01	<b>0.36</b>	-0.14	-0.02	0.771
Changes in prices of inputs	-0.09	-0.13	0.10	0.23	<b>0.35</b>	0.04	0.21	<b>0.66</b>	0.05	0.708
Climate changes	0.08	0.08	0.01	0.10	0.12	-0.01	0.09	0.03	<b>0.83</b>	0.740
Cumulative percentage of variance	23.34	34.03	42.59	49.41	54.89	59.37	63.54	67.42	71.13	-

was 1014.763 ( $p$ -value < 0.05), indicating that variables were uncorrelated. The result of the KMO test was 0.602, validating the adequacy of the present sample. Regarding commonality, no variables were excluded. Nine factors with Eigenvalues higher than 1 were identified and they explain more than 71% of variance.

The factors were defined and named considering the variables with greater loads (Hair et al., 2006). The nomenclatures created are as follows: (1) external environment, (2) internal environment, (3) casual losses, (4) market, (5) institutional, (6) innovation, (7) personal relationships, (8) financial planning and (9) climate changes. Borges and Machado (2012) also found that external environment was the factor with the highest value of variance explanation. Similarly, family issues (variables that compose the factor internal environment) were also factors with values that explained a significant amount of variance in the researches by Meuwissen et al. (2001) and Borges and Machado (2012).

Considering the cumulative percentage of variance, the factors extracted are shown in descending order, based on the most explained variance. The total variance explanation of these factors is 71.13%. In this regard, the sum of the three factors with higher variance explanation is 42.59%.

### Risk management strategies

Following the analysis of risk perception sources, the same methodology was used to analyze risk management strategies. In total, 28 risk management strategies were considered and presented to the respondents. Table 5 shows the ranking, mean, mode and standard deviation of these strategies which are classified in descending order based on the mean values.

Risk management based on updating with new technologies was highest in the ranking, followed by the

**Table 5.** Risk management strategies (ranking).

Strategies	Rank	Mean	Mode <sup>d</sup>	Std <sup>e</sup>
Updating with new technologies	1	4.33	5	1.01
Use of technical support	2	4.23	5	1.05
Maintaining/Increasing market liquidity of the end product	3	4.20	5	0.90
Commercialization of products without mediators <sup>a,c</sup>	4	4.19	5	1.09
Maintaining reserves of resources (in general)	5	4.16	5	1.08
Acquiring certification <sup>b,c</sup>	6	4.07	5	1.19
Cooperative practices	7	4.04	5	1.19
Negotiating loans	8	4.02	5	1.37
Improving production practices <sup>c</sup>	9	3.98	5	1.09
Obtaining information about new production techniques	10	3.93	5	1.19
Obtaining accounting information	11	3.93	5	1.30
Selecting companies with low levels of risks	12	3.90	5	1.11
Obtaining information about new regulations	13	3.88	5	1.19
Relationships with several organizations	14	3.87	5	1.28
Getting loans (funding)	15	3.84	5	1.21
Performing external activities (outside the agroindustry)	16	3.83	5	1.33
Diversifying production	17	3.80	5	1.24
Maintaining reserve credit	18	3.77	5	1.28
Personal insurance	19	3.75	5	1.32
Maintaining flexible costs	20	3.73	5	1.11
Family succession plan	21	3.73	4	1.17
Maintaining relationships with customers <sup>c</sup>	22	3.66	5	1.39
Spreading sales throughout the year	23	3.65	5	1.16
Negotiating through contracts	24	3.60	4	1.26
Property insurance	25	3.47	4	1.29
Being informed about government actions	26	3.47	3	1.16
Equipment insurance	27	3.26	4	1.29
Workers compensation program	28	3.09	3	1.25

<sup>a</sup>Variables with one missing value were replaced with the mean of that variable for all the cases; <sup>b</sup>Variables with two missing values were replaced with the mean of that variable for all the cases; <sup>c</sup>Risk management strategies not found in the literature reviewed. They were detected in the interviews. <sup>d</sup> Value that appears most often in the set data. <sup>e</sup>Standard deviation.

use of technical support. These results demonstrate that decision makers attach importance to the advice of experts and possible innovations that might contribute to family agroindustries. However, according to research carried out by Flaten et al. (2005), Borges and Machado (2012) and Gebreegziabher and Tadesse (2014), these management strategies appear in middle ranking position.

The third highest mean is concerned with maintaining/increasing the market liquidity of the product to be marketed. This strategy suggests that agroindustry products are easily marketed and hence converted into money. For Borges and Machado (2012), this strategy appeared in a middle ranking position. However, in the study by Flaten et al. (2005), strategies targeting liquidity was in the highest position.

The strategies identified in the interviews were: the commercialization of products without mediators, acquiring certification and maintaining relationships with clients. These appeared respectively in the 4th, 6th, 9th and

22nd positions. Commercialization without mediators means selling directly to the final consumer, maximizing the possible financial return. Acquiring certification increases the credibility of the agroindustry product, and contributes to its commercialization in other regions or fairs (Schneider and Ferrari, 2015), in wholesale and retail sales. Improving production practices is associated with taking courses, graduating from education and other similar activities that contribute to the development of the family agroindustry. Maintaining relationships with customers is about interacting with consumers, in order to increase the levels of confidence between consumer and agroindustry.

Most strategies obtained a value of five for the mode. This indicates that many respondents considered them very important in risk mitigation. Based on this assumption, the role of management processes should be stressed, and the decision made by an individual regarding the appropriate combination of actions or

**Table 6.** Factor analysis of risk management strategies– Rotation matrix (Varimax).

Risk management strategies	Factors								Commonality
	1	2	3	4	5	6	7	8	
Acquiring certification	<b>0.75</b>	0.22	0.14	0.04	0.24	0.13	0.14	0.03	0.734
Maintaining appropriate market liquidity of the end product	<b>0.68</b>	0.18	0.11	0.07	-0.07	0.13	0.12	0.21	0.604
Selecting companies with low levels of risks	<b>0.67</b>	-0.03	0.11	<b>0.36</b>	0.18	0.16	-0.04	0.03	0.668
Maintaining reserve credit	<b>0.65</b>	0.23	-0.05	0.22	0.18	-0.11	0.09	0.27	0.672
Maintaining flexible costs	<b>0.63</b>	<b>0.83</b>	-0.02	0.01	-0.02	0.01	0.03	-0.07	0.715
Improving production practices	0.08	<b>0.83</b>	0.03	0.17	0.07	-0.01	0.03	0.03	0.740
Distributing sales throughout the year	0.12	<b>0.64</b>	0.02	-0.04	0.06	0.15	-0.09	<b>0.46</b>	0.687
Being informed on government's actions	<b>0.41</b>	<b>0.59</b>	0.05	0.01	0.10	0.10	0.05	0.01	0.556
Negotiating loans	0.22	-0.10	<b>0.81</b>	0.01	-0.01	0.02	0.07	-0.01	0.734
Workers compensation program	-0.05	<b>0.32</b>	<b>0.72</b>	0.16	0.02	0.10	0.05	-0.08	0.675
Performing external activities (outside the agroindustry)	-0.01	-0.25	<b>0.58</b>	<b>0.42</b>	-0.08	0.26	0.12	-0.08	0.685
Obtaining accounting information	0.12	0.16	<b>0.56</b>	-0.09	0.26	0.13	0.10	<b>0.54</b>	0.765
Getting loans (funding)	0.24	-0.08	<b>0.47</b>	<b>0.36</b>	-0.02	0.17	<b>0.43</b>	-0.14	0.667
Use of technical support	0.28	0.12	<b>0.42</b>	<b>0.32</b>	0.23	-0.05	0.24	<b>0.45</b>	0.709
Cooperative practices	0.12	0.04	0.13	<b>0.77</b>	0.10	0.07	0.13	0.08	0.669
Maintaining reserves of resources (general)	0.20	0.21	0.09	<b>0.63</b>	-0.05	0.13	0.09	0.14	0.603
Obtaining information on new production techniques	0.16	-0.01	0.03	-0.06	<b>0.76</b>	-0.02	0.10	0.18	0.665
Obtaining information on new regulations	0.07	0.04	0.13	<b>0.38</b>	<b>0.71</b>	0.26	0.09	-0.16	0.787
Family succession plan	-0.22	0.22	-0.10	0.27	-0.09	<b>0.75</b>	-0.03	0.11	0.840
Commercialization without mediators	0.28	-0.07	0.14	-0.07	0.07	<b>0.68</b>	0.27	0.08	0.730
Updating with new technologies	0.21	0.07	<b>0.38</b>	0.21	0.16	<b>0.62</b>	0.01	0.02	0.654
Maintaining relationships with customers	0.17	0.20	<b>-0.39</b>	-0.10	0.03	<b>0.59</b>	0.12	0.20	0.697
Negotiating through contracts	0.41	-0.03	0.16	0.07	<b>0.41</b>	<b>0.57</b>	0.18	-0.24	0.806
Equipment insurance	0.18	<b>0.42</b>	-0.06	-0.15	-0.13	0.02	<b>0.72</b>	0.13	0.796
Property insurance	-0.08	-0.09	0.12	0.14	0.03	-0.01	<b>0.70</b>	0.03	0.665
Relationships with several organizations	0.17	-0.07	<b>0.30</b>	0.27	<b>0.36</b>	<b>0.32</b>	<b>0.42</b>	-0.12	0.704
Personal insurance	0.23	-0.01	0.16	0.23	0.15	0.23	<b>0.69</b>	0.02	0.756
Diversifying production	0.20	-0.03	-0.20	0.15	0.01	0.01	0.02	<b>0.79</b>	0.744
Cumulative percentage of variance	26.59	38.75	46.06	52.07	57.21	61.94	66.39	70.45	-

practices depends on the relative analysis of the expected return, considering risk perception. Once risk management strategies are developed to address the perceived risks, decision makers can use them as tools and methods of risk management in the organization (Ghadim and Pannell, 1999).

Table 6 shows the factorial analysis of risk management strategies. This analysis obtained a value of 968.020 (p-value < 0.05), for Bartlett's test of sphericity, indicating that the variables were uncorrelated. The KMO test results also validated sampling adequacy, with a value of 0.746, validating the adequacy of the present sample. Regarding commonality, no variables were excluded. Eight factors with eigenvalues higher than 1 were identified and they explain more than 70% of variance.

The factors obtained were named as follows: (1) financial management, (2) marketing tactics, (3) strategic planning, (4) flexibility, (5) innovation, (6) personal relationship, (7) security and (8) diversification. The factors were named considering the variables with

greater loads, which had a great impact on the development of the nomenclature of each factor (Hair et al., 2006). Financial management had the highest variance explanation value. Similar results were found in the study by Borges and Machado (2012), where variables related to financial management comprised the two factors that explained most of the variance (respectively, consulting and price control).

The factors extracted are shown in descending order (from those with higher variance explanation values to those with lower variance explanation values) and the total variance explanation of these factors is 70.45%. The sum of the three factors with higher variance explanation is 46.06%.

## Conclusion

The present study aimed to assess risk perception and risk management strategies in family agroindustries

through the administration of questionnaires to decision makers of these organizational units. Based on a literature review and the methodological procedures performed, 28 sources of risks and 28 risk management strategies were analyzed. The results revealed that the country's current economic situation, inflation/deflation, changes in product prices and the elimination/reduction of government support were perceived as the most important sources of risks. Updating with new technologies, the use of technical support, maintain/increase market liquidity of product and commercialization of products without mediators were considered the main risk management strategies. Besides, sources of risk and risk management strategies were grouped, respectively, in nine and eight factors.

These results may contribute to the development of management tools for the decision makers of family agroindustries. Such information can also be valuable for institutions, providing guidance to experts in technical extension services and technical assistance to producers, such as the EMATER. These data may support the creation of public policies aimed at strengthening family agroindustries. In addition, the importance of conducting interviews with these decision makers prior to the administration of the questionnaire was demonstrated. Contact with the respondents is important in studies on perceptions and it is can result in the identification of "new" sources of risks and risk management strategies not detected in the literature analyzed.

## CONFLICT OF INTEREST

The authors have not declared any conflict of interest

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## Full Length Research Paper

# Improving grain legume yields using local Evate rock phosphate in Gùrué District, Mozambique

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**Acid, infertile reddish-brown soils characterize large amounts of central Mozambique. Few of these soils are in food production representing a missed opportunity for agricultural productivity and a missed alternative to improve the food security of the country. Low levels of soil nutrients such as calcium, phosphorus, and potassium limit crop growth. Local agricultural amendments for acid, infertile soils such as limestone and rock phosphate exist but are unexploited. An experiment was conducted to assess the feasibility of using local Evate rock phosphate (40.7% total P<sub>2</sub>O<sub>5</sub>) as a corrective to supply phosphorus. The rock phosphate was applied at rates of 20, 40, 80 and 160 kg total P ha<sup>-1</sup>. Comparison triple super phosphate was also added at four P levels (0, 10, 20 and 40 kg P ha<sup>-1</sup>). A long growth cycle crop of pigeon pea (*Cajanus cajan* L., Mill sp. variety "ICAEP00020") with a growth cycle of 190 days was used to assess effectiveness of the local rock phosphate. A pigeon pea grain yield of 1000 kg grain ha<sup>-1</sup> was possible with an application of 80 kg ha<sup>-1</sup> of total P added as Evate rock phosphate. By comparison 20 kg P ha<sup>-1</sup> as TSP was needed to reach a maximum yield of pigeon pea grain. This ratio suggests that Evate rock phosphate was 25% as effective as TSP on a total P basis. This research suggests that the Evate rock phosphate can be an effective amendment that can enable or enhance food grain production on the acid, infertile upland soils of Central Mozambique. Whether for direct application for acid-tolerant crops or acid soils or processed into soluble fertilizer phosphate, the existence of such a valuable resource provides a great opportunity for improved local food crop production.**

**Key words:** Rock phosphate, pigeon pea, acid soils, food grains, food security.

## INTRODUCTION

In Sub-Saharan Africa (SSA), phosphorus has long been identified as the major limiting nutrient in the vast majority of soils (Bationo et al., 1997). Such soils constitute up to

55% of the agricultural land in SSA (Bationo et al., 1986). SSA contains numerous rock phosphate deposits, and some are sufficiently reactive for direct application

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(Buresh et al., 1997) and some are not. Direct application of indigenous rock phosphates has been viewed as an attractive option for building soil phosphorus (P) fertility because it potentially involves lower production costs and capital investments than the production of water-soluble P fertilizers from indigenous rock phosphate sources (Hammond et al., 1986; Rajan et al., 1996).

Food security has been the focus of recent agricultural projects including the Legume Innovation Laboratory, which is conducting farmer decision-making research regarding grain legume crops. A consistent limitation to food security has been the extremely low fertility and acidity of the highly weathered soils of the country (Maria and Yost, 2006). Recent studies indicate that such conditions are wide-spread in the central, potentially highly productive region of Mozambique. Soil management of nutrient poor acid soils has been highly successful in regions with acid soils (Fageria et al., 2013) and multiple management alternatives are possible.

In Mozambique, fertilizers such as superphosphates are exceedingly expensive, of low quality, and seldom available in local markets. Recent research has explored the possible use of indigenous agro-minerals such as rock phosphates, as substitutes for expensive, imported fertilizers. According to Zavale et al. (2005), Mozambique can increase the production of all of crops by using its enormous potential of natural resources, improving agricultural infrastructure, and increasing household adoption of improved crop varieties and other new agricultural technologies.

### Local food grain - pigeon pea

One of the crops of growing popularity among farmers in the acid soil region of Mozambique is pigeon pea (*Cajanus cajan*, L., Millsp.) (ICRISAT, Malawi). The crop is moderately tolerant of the acid soil conditions characteristics of the region and also is well-known for drought resistance due to deep rooting. This crop is tolerant of the acid soil conditions that are favorable for the reaction and dissolution of rock phosphates and it has a long duration growth cycle also favorable for the slowly dissolving rock phosphate. The locally preferred cultivars of pigeonpea, for example, range in maturity from 170 to 190 days (O. Madzonga, ICRISAT/Malawi, personal communication, 2016; C. Malita (IIAM/Nampula), personal communication, 2016). Tolerance to soil acidity by pigeonpea is not well characterized, but several researchers have documented that the plant roots exude organic acids that dissolve and solubilize otherwise insoluble phosphates (Otani et al., 1996). These researchers report that pigeon pea exudes some 10-fold more malonic acid than do groundnut, cowpea or rice. Adugyamfi et al. (1990) report that pigeon pea tolerates low P conditions better than soybean. For these reasons, *C. cajan* may become a useful rotation crop in food

production systems in this zone of Mozambique.

### Local deposits of rock phosphate

Manhiça (1991) characterized Mozambican phosphate deposits as primarily deposits of apatite of two types: (1) Monte Muande-Monte Fema near Tete Province, and (2) The Evate deposits in Nampula Province. The first was original crystalline limestones of Pre-Cambrian replaced and metasomatized together with injection of apatite-carbonate, apatite-magnetite, and apatite-silicate. The second was carbonatites with low contents of apatite dispersed or in hydrothermal veins. The Evate deposit was discovered by the geophysical investigations for graphite by a Russian team in 1983. The Evate deposit was initially quantified at 155,413,000 tons of apatite ore with an average content of 9.32% P<sub>2</sub>O<sub>5</sub>. The analytical methodology used by the Russian Team was unknown, however. In preparation for this research a sample of the Evate rock phosphate was submitted to the International Fertilizer Development Center (IFDC) Laboratory in Alabama, USA, and the results are given in Table 1.

Yager (2014) reported that the corporation Vale S. A. of Brazil was engaged in a prefeasibility study on a new phosphate mine at the Evate deposit. Depending on the results of the study, predictions were that the mine could produce 2 million metric tons year<sup>-1</sup> of Evate phosphate rock.

The objective of our study was to assess the potential of using Evate rock phosphate of Mozambique to supply P for food grain legumes in the acid, infertile soils of Central Mozambique. Specifically, the study is aimed at determining the amount of locally available Evate rock phosphate in comparison with the imported, expensive triple super phosphate needed to achieve maximum yield and biomass of pigeon pea in a reddish-brown soil of the summit topographic position in Mepuagiu community of Gùrué District, Zambézia Province, Mozambique.

## MATERIALS AND METHODS

### Study area

Mozambique researchers have been using FAO, USDA Soil Taxonomy and others to classify and attribute names to soils that they worked on. In this study, the soils of the study area are named by its color due to lack of sufficient soil data for an accurate soil classification.

The experiment was conducted in Mepuagiu community, Gùrué district of Mozambique. Gùrué district is about 5,606 km<sup>2</sup> with a population of nearly 303,000. Gùrué district is located at an elevation of 734 m and -15°27'48.8 (south latitude), 36°58'54.0 (east longitude). The district has wet/dry climate. Summer daily maxima temperatures range from 30 to 34°C. Winters have temperatures in the range of 17 and 20°C. Typical of tropical climates, winter is usually referred to as the dry season, while summer is called the rainy season. Average annual rainfall is 1900 mm. The climate in Gùrué has much more rainfall than the rest of

**Table 1.** Analytical results of Evate rock phosphate sample (International Fertilizer Development Center, Muscle Shoals, Alabama. Analyzed November 9, 2015).

Chemical	Results	Analyst	Method
Total P <sub>2</sub> O <sub>5</sub> (%)	40.7	CSG	HNO <sub>3</sub> /HClO <sub>4</sub> - Molybdovanadate color method - visible spec
Citric Acid Sol. P <sub>2</sub> O <sub>5</sub> (%)	3.75	CSG	SSSAP 1957 21 :183-188
Formic acid Sol. P <sub>2</sub> O <sub>5</sub> (%)	2.12	CSG	ZPDB 1953 62:262-264
NAC Sol. P <sub>2</sub> O <sub>5</sub> (% 1 <sup>st</sup> ext.)	1.46	CSG	AOAC - Molybdovanadate color method - visible spec
NAC Sol. P <sub>2</sub> O <sub>5</sub> (% 2 <sup>nd</sup> ext.)	0.95	CSG	AOAC - Molybdovanadate color method - visible spec
Cd (ppm)	0.52	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Co (ppm)	7	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Cr (ppm)	9.8	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Cu (ppm)	14	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Mn (%)	0.1	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Mo (ppm)	2.2	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Ni (ppm)	4.7	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Pb (ppm)	14	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP
Zn (ppm)	22	CSG	AFPC- HNO <sub>3</sub> /HCL- ICP

**Table 2.** Local soil names, topographic location and farmer reported suitable crops along a soil catena at Mepuagia.

Soil types	Characteristics	Catena location	Predominant crops
Ehava	Sandy reddish- brownish	High elevation, back slope	Cassava, pineapple, sorghum and fava beans
Epuu	Blackish soil, less moist, less clay content	High elevation	Maize, common bean, pigeonpea and butter beans
Ekotchokwa	Reddish soil	High elevation	Cassava, sorghum, pineapple and fava bean
N'tchokwa	Blackish, high moist, high level of clay particles	Low elevation (river streams)	Rice, beans, maize and sugar cane

**Table 3.** Selected chemical and physical properties of soil of Mepuagia community, Gùrué district, Mozambique.

Depth Cm	pH	Soil C g kg <sup>-1</sup>	Ca <sup>2+</sup> cmol <sub>c</sub> kg <sup>-1</sup>	Mg <sup>2+</sup> cmol <sub>c</sub> kg <sup>-1</sup>	K <sup>+</sup>	Sand	Silt %	Clay	ECEC cmol <sub>c</sub> kg <sup>-1</sup>	ECEC/clay cmol <sub>c</sub> kg <sup>-1</sup> % <sup>-1</sup>
0-15	4.9-6.5	10.0-37.0	1.8-17.6	0.85-2.6	0.09-0.62	33-63	7.3-30.5	23.4-45.4	3.44-8.57	7.58-35.5
15-30	5.3-6.3	7.5-33.7	3.1-10.3	1.1-2.0	0.11-0.50	37-61	5.3-24.5	34.4-45.4	5.04-12.9	10.1-50.8

Soil pH was obtained from a 1:1 soil: water ratio, Cations Ca, Mg, were measured in a neutral salt extraction (1 M KCl). Potassium was measured in a sodium bicarbonate extractant used for phosphorus. Effective CEC was obtained by summing the cations plus the KCl-extractable acidity.

the province due to the orographic effect of the mountains that surround the town. The average maximum temperature is 24 to 25°C and average rainfall per day is 57 to 165 mm (<https://www.worldweatheronline.com/gurue-weather-averages/zambezia/mz.aspx>).

## Soils

Soils of the summit topographic position “Ekotchokwa” (Table 2) with the minimum values of soil properties (Table 3) were selected for the study. These soils are, according to local farmer knowledge, not capable of producing the important local food crops, including

common bean (*Phaseolus vulgaris* L.).

Soils from six sites in the Mepuagia community were analyzed and selected chemical and physical properties are summarized in Table 3. The reddish-brown soils are located in the summit topographic position and are characterized by the minimum values in all soil properties (Table 3) and low pH, low soil Ca and Mg that suggest a highly weathered soil. The low levels of cations, especially the low Ca<sup>2+</sup> and Mg<sup>2+</sup> and low effective cation exchange capacity (ECEC), confirms the low activity of the clays and the status as being highly weathered and indicating a very low capacity of the soils to retain nutrient cations, a condition typical of highly weathered soils of the tropics. There is a wide range in soil C contents, but generally it is very low as well such that soil C may not

**Table 4.** Selected soil chemical properties of experimental plots, Mepuaguiya community, Gùrué district, Mozambique. Depth 0-15 cm.

Parameter	pH	Bicarbonate P	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	KCl-extractable acidity (largely Al <sup>3+</sup> )
		mg kg <sup>-1</sup>			cmol <sub>c</sub> kg <sup>-1</sup>	
Median	5.03	15	0.62	0.53	0.40	0.40
Range	4.56 - 5.66	10 - 47	0.35 - 1.93	0.27 - 1.5	0.04 - 0.85	0.04 - 0.85

be contributing to soil nutrient content nor retention capacity. The variation in soil texture observed in field is reflected in the range of both soil sand and clay contents.

Soils from the experimental site (Table 4) represent the acid, more highly weathered range of soils reported in Table 3.

### Rock phosphate analysis

Table 1 provides the analytical results of the Evate rock phosphate according to reference methodology of the International Fertilizer Development Center, a worldwide authority and reference on rock phosphate deposits and characteristics.

### On-farm experiment

An on-farm rotation experiment was established to compare the availability of P supplied by Evate rock phosphate with that of the expensive, imported triple super phosphate (TSP). The experiment was a randomized complete block design with 3 replicates and 8 treatments per replication. An experimental plot consisted of 6 rows; 6 m long and 3 m wide. Furrows were opened for each line of 6 m long per plot using a hoe. Seeds were planted 0.5 m between each row and 0.4 m within the row. Seeds were placed on the left side of the furrow and fertilizer on the right side and then covered with soil. The Evate rock phosphate was applied at 20, 40, 80 and 160 kg total P ha<sup>-1</sup> and the TSP was applied at 10, 20 and 40 kg total P ha<sup>-1</sup>. Twenty kilogram of N was applied per hectare as urea and 40 kg of K<sub>2</sub>O ha<sup>-1</sup> was applied as potassium chloride as blanket applications of these nutrients for all treatments. Planting took place on 20 January 2016 and the first weeding was done on 5 February 2016. Harvest took place 27 August, 2016 after 220 days of growth.

### Crop selection

The pigeon pea variety selected for the first crop in this rotation experiment (ICAEP00020) has a relatively long growth cycle of 190 days. Local farmers in Central Mozambique and Malawi are accustomed to using varieties of even longer growth cycles of 240 to 270 days (Dr. O. Madzonga, ICRISAT/Malawi, personal communication 2016). ICRISAT/Malawi has introduced varieties of medium duration (160 to 190 days) in the Gurue district, but adoption seems slow. Local practice is to seed at very low plant populations such as 1 m apart (Malawi recommendations are to space hills at 90 cm x 90 cm). For this experiment a much closer spacing of rows 50 cm apart with hills placed at 40 cm apart in the row was chosen. This plant population still seems too low to obtain maximum grain yield.

### Data collection

#### Crop measurements

Non-destructive and destructive measurements of six selected

plants were taken and recorded at approximately two week intervals throughout the growth cycle. These measurements included plant height, stem circumference and plant population. At harvest pigeon pea pods were collected from the four central rows, which comprised a harvest area of 12 m<sup>2</sup>. Pods were weighed and air-dried for final weight and yield calculation. Above-ground biomass was also collected, weighted, and sub-samples taken for dry weight calculation.

#### Soil measurements

Soil samples were taken from each experimental plot after harvest to assess suspected gradients of soil pH. Samples of each plot were composites of 3 sub-samples taken from the plot harvest area. Soils were analyzed for soil water pH (1:1 ratio), 0.5 M sodium bicarbonate, soil calcium, magnesium and KCl-extractable aluminum determinations were also made. Effective cation exchange capacity was calculated by summing the cations Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and KCl-extractable Al. The initial survey samples from six sites are listed in Table 3, while data from the experimental site are listed in Table 4.

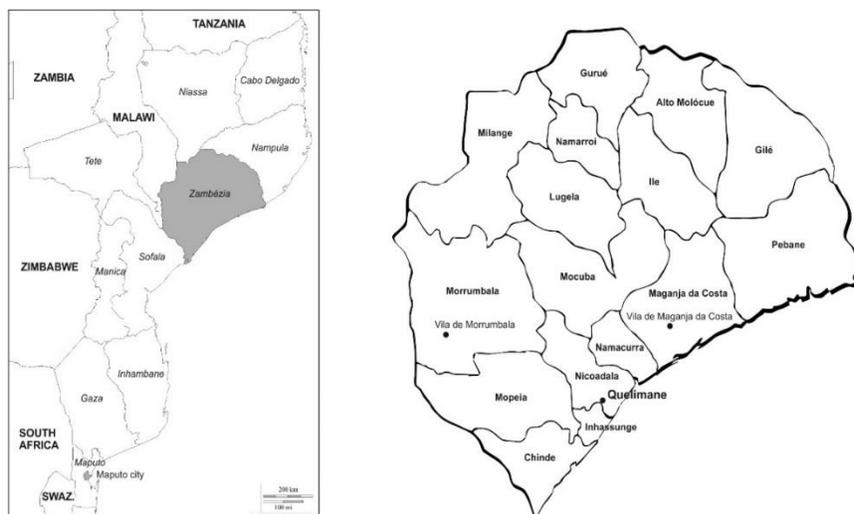
#### Statistical analysis

A randomized complete block ANOVA was calculated on yields and above ground biomass yields using both Statistix® v. 10 statistical analysis software and JMP (SAS, 2017). The results were plotted using Sigmaplot® v. 12.5 graphics software. Plots of the relationship between grain, biomass, plant height and stem circumference and total amounts of applied P either in soluble TSP form or in the form of rock phosphate were developed. These plots served to quantitatively compare pigeon pea response to the Evate rock phosphate in relation to that of TSP and permitted a comparison of relative solubility and availability of the P in the Evate phosphate.

Because substantial variation in soil pH was observed among the experimental plots (Table 4), contour plots of soil pH, grain and biomass yields were prepared using the Surfer® version 12.8 software. Individual plot yields and corresponding mean soil measurements for the plot provided the basis for the contours. Other plots of yields in relation to soil measurements were developed to explore the effect of soil acidity on pigeon pea growth and response.

## RESULTS AND DISCUSSION

Pigeon pea yielded as much as 1200 kg grain ha<sup>-1</sup> (Figure 1). These yields were similar to or greater than average pigeon pea grain yields of the Gùrué District (2014) of about 800 to 1000 kg ha<sup>-1</sup> (ICRISAT/Malawi, Dr. O. Madzonga, personal communication 2016). As expected, yields were greatest with the imported, highly soluble TSP fertilizer. Grain yields where the local rock



**Figure 1.** Location of the Gùrué District, Zambézia Province, Mozambique.

phosphate was applied were higher than expected with yields reaching almost  $1200 \text{ kg ha}^{-1}$ . A closer comparison shows that maximum yields occurred with TSP rates of 20 and  $80 \text{ kg P ha}^{-1}$  as Evate rock phosphate. Pigeon pea yields where rock phosphate was applied were surprisingly high given the low solubility of the material (3.75%) and were almost similar to yields where the soluble TSP fertilizer was applied. This relatively high effectiveness of Evate rock phosphate may be related to several factors that may have led to the relatively high availability of the Evate rock phosphate:

1. The pigeon pea growth cycle is long and in this case the crop was harvested after 220 days of growth allowing a long time for rock phosphate dissolution.
2. Pigeon pea is known for the exudation of large amounts of the organic acid, malonic acid that solubilizes otherwise unavailable forms of soil phosphorus (Otani et al., 1996).
3. Soils of the experimental site are both acidic (ranging in soil pH from 4.5 to 5.7) and low in exchangeable bases such as Ca (ranging from 0.4 to  $2 \text{ cmol}_c \text{ kg}^{-1}$ ), which are well known factors that enhance rock phosphate dissolution (Diarra et al., 2004)

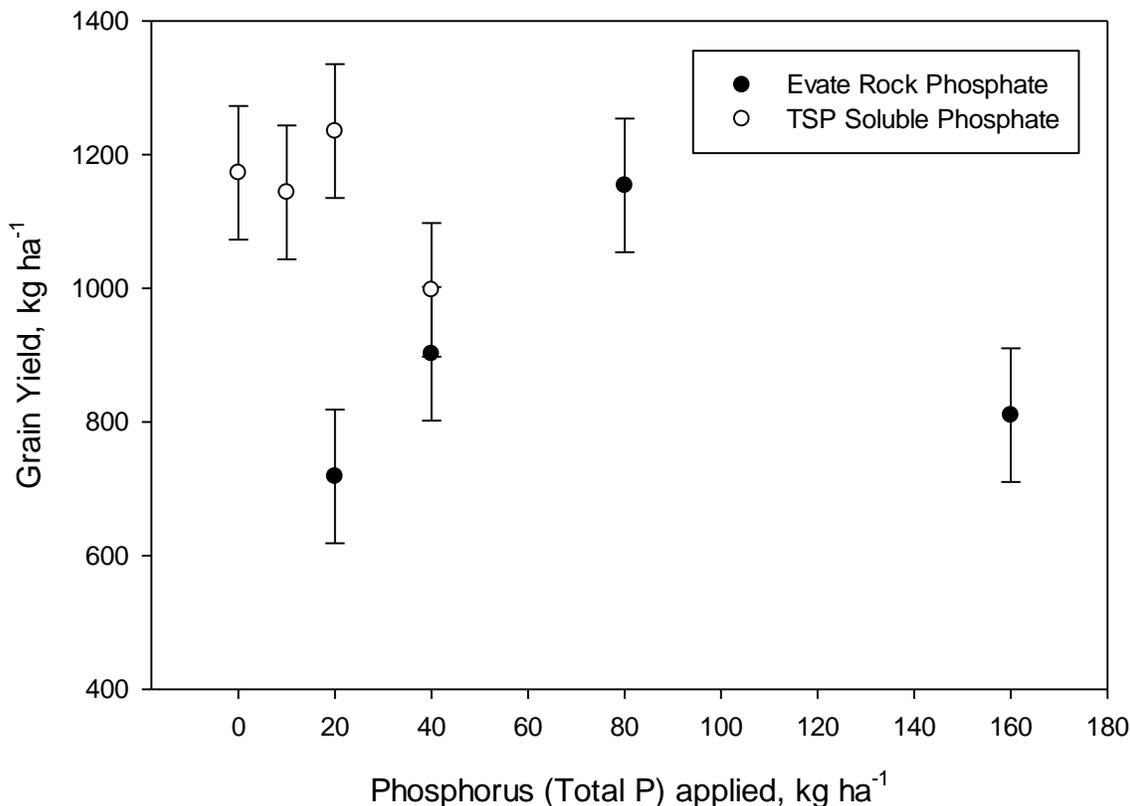
This result indicates that in these conditions, Evate rock phosphate was an effective source of the nutrient phosphorus.

Plant height and plant population were measured at given intervals but are not reported here. Field observations of pigeon pea growth during the experiment revealed zones of superior growth and plant height unrelated to treatment. In addition, grain yields on the check treatment (no P added) plot were surprisingly high (Figure 2) leading to inquiry into the experimental plot preparation. A comparison of soil measurements from

individual plots indicated a large variation in soil pH as in levels of calcium, magnesium, and other nutrients (Figure 3). This analysis revealed that two of the three replications of the check plot were on plots with abnormally high soil pH. These differences were not apparent when soil was sampled by compositing by blocks, replicates, or across the entire site. In addition, variation in three variables, soil pH, soil  $\text{Ca}^{2+}$  and soil  $\text{Mg}^{2+}$  seem closely related. This variation appears to have resulted from the preparation of the experiment whereby all plant residue from the experimental area was gathered and burned in the plot center, clearly a time efficient alternative, but not optimal for the experimental objectives. A further analysis suggests that, in fact, the pigeon pea did respond to the gradient on soil pH induced by burning of plant residue from clearing (Figure 4). These results do suggest that while pigeon pea is known for its tolerance to soil acidity, it did respond to the reduction in soil acidity and less toxic extractable Al as well as increased levels of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .

Soil measurements on a per plot basis (Table 4) indicate an unfortunately wide range in pH, Ca, and Mg as mentioned previously. The levels of soil pH are clearly in the acidic range and suggest that acid tolerant plants such as pigeon pea, cassava, cowpea, and certain varieties of peanut would likely be most successful on such soils. The levels of soil P were variable, but generally low relative to typical plant requirements. Levels of soil Ca and Mg were particularly low indicating very low cation retention capacity of the soils. With such low levels sufficiency of Ca might be limiting for certain legumes (Fageria et al., 2013).

An analysis of pigeon pea aboveground biomass in relation to source and rate of applied phosphate revealed essentially the same pattern of response as did grain yield results (Figure 5). The biomass result also indicates



**Figure 2.** Grain yields of pigeon pea (*Cajanus cajan*) as influenced by rate and source of phosphate. The bars represent standard errors of treatment means.

that the Evate rock phosphate effectively supplied nutrients to this pigeon pea crop and in this acid, infertile soil.

## Conclusions

A pigeon pea grain yield of 1000 kg grain ha<sup>-1</sup> was obtained with an application of 80 kg ha<sup>-1</sup> of total P added as Evate rock phosphate. By comparison 20 kg P ha<sup>-1</sup> as TSP was needed to reach a maximum yield of pigeon pea grain. If this relationship was used to estimate relative effectiveness it is 20/80 or 25%. This research suggests that the Evate rock phosphate can be an effective amendment that can enable food grain production on the acid, infertile upland soils of Central Mozambique.

Evate rock phosphate, in this combination of soils and crops was an effective source of the often missing nutrient P for food grain production in Mozambique. This rock phosphate has an unusually high level of P (40.7% P<sub>2</sub>O<sub>5</sub>), however solubility is low (3.5 citric acid solubility and 0.95 neutral ammonium acetate solubility). These values are high in total P and low in solubility compared with data from Smallberger et al. (2010). The solubility of

this rock phosphate can be improved by increased surface area with fine grinding, use on acid soils, and with crops of either long duration and / or that acidify their rhizosphere. Use of this potentially very important local fertilizer resource needs to be tested in other conditions and cropping systems whereby the dissolution might be initiated and largely carried out during the pigeon pea cropping period. Other research shows that subsequent crops also benefit from the P released during the pigeon pea phase of the rotation. Additional field experimentation using this rock phosphate are needed to quantify and assess its potential role in increased food crop productivity in Central Mozambique.

## CONFLICTS OF INTEREST

The authors have not declared any conflict of interest.

## ACKNOWLEDGMENTS

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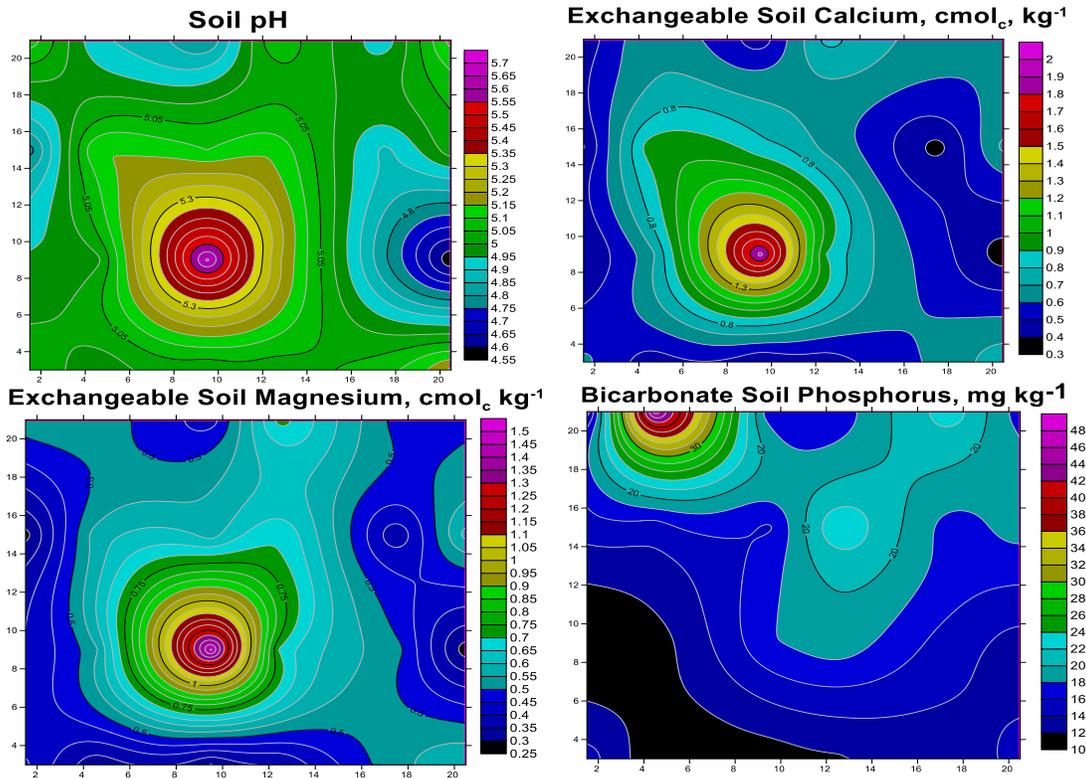


Figure 3. Variation in soil pH, exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup>, and P after pigeon pea harvest.

Mepuaguia, Pigeon Pea Grain as affected by soil pH

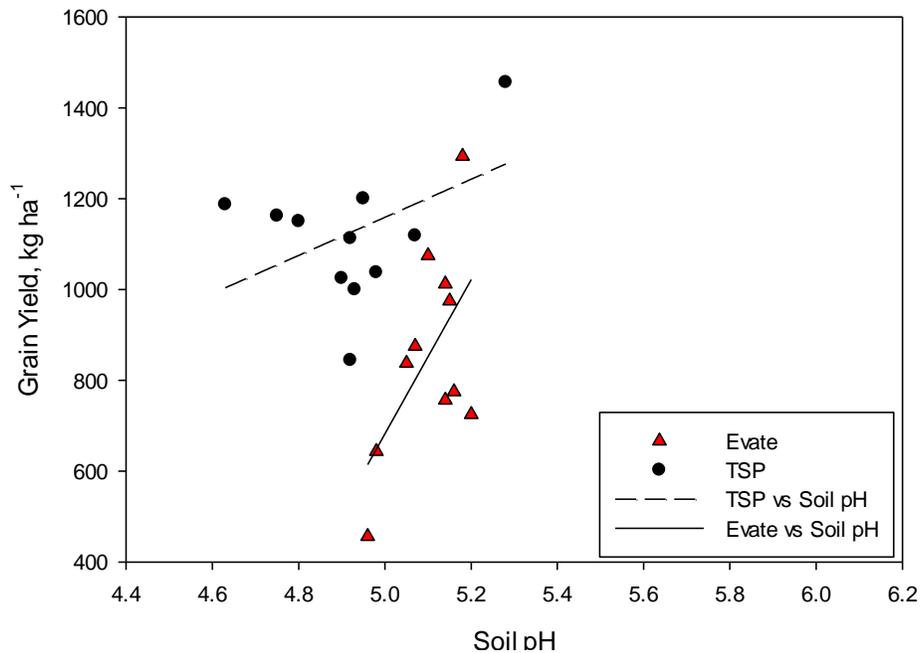
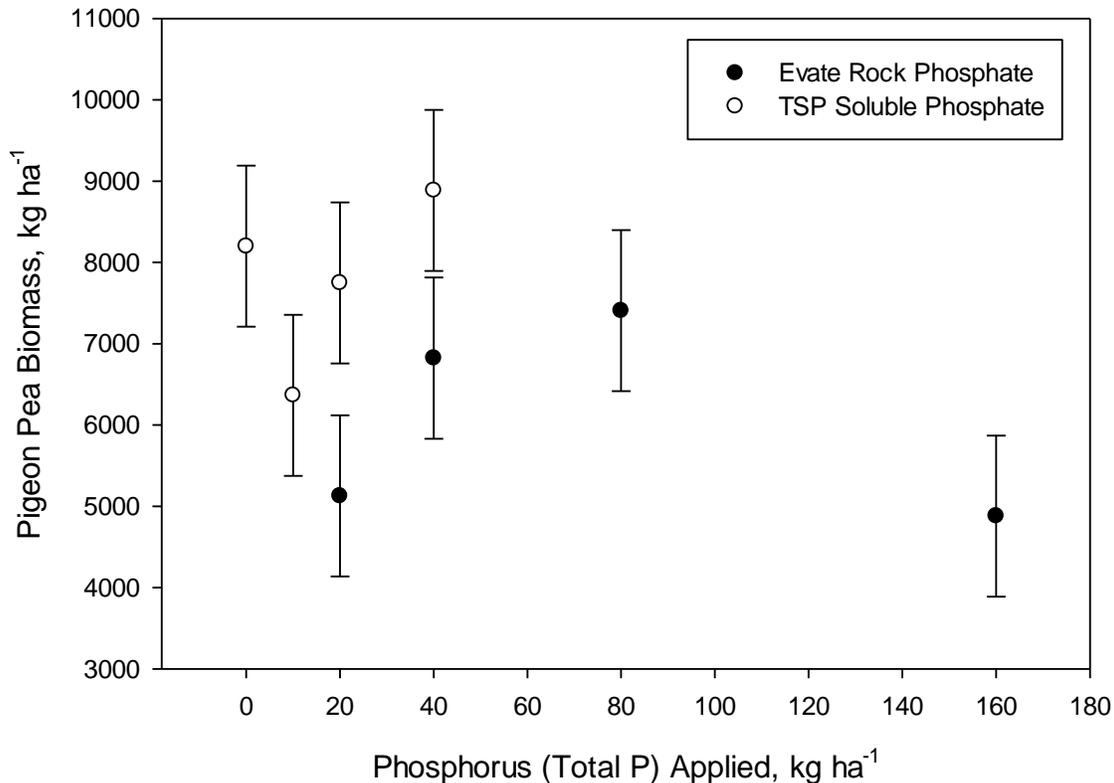


Figure 4. Relationship between grain yield and soil pH of all treatments.



**Figure 5.** Pigeon pea above ground biomass in relation to source and amount of applied P.

'Farmer Decision Making Strategies for Improved Soil Fertility Management in Maize-Bean Production Systems. The authors are especially grateful to Dr. Andrew Lenssen who proofread the whole document and provided useful comments, in spite of his health condition. Also their special thanks to Dr. O. Madzonga, ICRISAT/Malawi for sharing results and data with them. The views expressed in this paper are the authors' and do not necessarily reflect the views of USAID or the authors' institutions.

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*Full Length Research Paper*

# Technical efficiency of smallholder barley farmers: The case of Welmera district, Central Oromia, Ethiopia

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Crop production and productivity are not only inevitably affected by level of adoption of improved technologies and external factors but also the technical efficiencies of producers. The main objectives of this study are to estimate technical efficiency of sample barley farmers, assess determinant factors and compute yield loss due to inefficiency. Plot level data from 180 barley growers were collected through three stage systematic random sampling procedures. A one-step maximum likelihood was used to estimate stochastic frontier translog production function to determine the level of technical efficiency and its determinant factors. The estimated value of technical efficiency ranges from 0.11 to 0.99 with an average of 0.53 allowing inefficiency gap of 0.47 indicating the opportunity to increase barley output by 47% by using the same inputs mix and existing technology. The study found sex, age and education level of the household head, distance to all weather roads, credit service, group membership, extension contact, training, plot fragmentation, tenure status, and investment in fertilizers significantly impact technical efficiency. The result suggests the need to involve female headed households into extension and trainings, increase the education level of households through informal and formal literacy, inspire household membership into farmers' groups and enable them to share best practices from model and more experienced farmers, inspire barley producers to invest on fertilizers and strengthen rural micro finance institutions to provide credit at some reasonable costs.

**Key words:** Barley farms, translog production function, technical efficiency, Welmera.

## INTRODUCTION

Ethiopia is currently following productivity-led agricultural transformation playing an active role in its economic transformation and making agriculture the main driver of growth. According to ATA (2014) the transformation of the agriculture is central to Ethiopia to reach middle-

income country position by 2025. Research and development in this vital sector, therefore, helps to increase growth in agriculture and total factor productivity by increasing crop production and productivity through the development of best-fit new technologies, and

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increased adoption and utilization (Nicostrato and Mark, 2015). In its endeavor, Ethiopia completed its first Growth and Transformation Plan (GTP I) from 2010/2011 to 2014/2015 with some success and has already started its second phase five years Growth and Transformation Plan (GTP II) (FDRE, 2015). This strategic plan focuses on smallholder agriculture intensification through improved access to modern inputs like improved seed varieties, fertilizers and farming practices, and transform traditional subsistence to market oriented commercial agriculture.

The Ethiopian government has given more emphasis to barley research and development to boost production and productivity to food security. Research on barley improvement started in 1955, since then several new technologies, information and knowledge have been produced (Begna et al., 2014). According to MoA (2014), about 36 food and 16 malt barley varieties were released by the national research system and disseminated to the beneficiaries, and reported as they are under production. There is also evidence of barley technology adoption (Tiruneh et al., 2015; Yu and Nin-Pratt, 2014; Beshir et al., 2012). In an effect, barley showed steady growth in production and productivity over recent years. For example yields increased from 1.17 metric tons per hectare to 1.87 metric tons per hectare; and total production grew from 1.0 million tons in 2005 to about 1.9 million tons in 2014 (CSA, 2005, 2014). However, this productivity of barley is not as expected as compared to the yield of 3.5 metric tons per hectare obtained by progressive farmers (Begna et al., 2014) and 5 metric tons per hectare under on-station (Fekadu et al., 2011), indicating a huge potential of increasing productivity.

Crop yields and productivity are not only inevitably affected by weather conditions, quality of seeds and varieties, input prices, amount of fertilizers and farming practices but also the efficiency of production (Tiruneh and Geta, 2016; Debebe et al., 2015; Alemu et al., 2014; Ahmed et al., 2013; Yami et al., 2013). The efficiency of agricultural production reflects the effectiveness and describes the quality of managing the farm. Some argued that technology adoption and area expansion improve production and productivity of crops (Kamruzzaman and Mohammad, 2008; Haji, 2006). However, the first option requires a huge financing for technology generation and dissemination, the second option is hardly possible since land is limited and overly subdivided into small units which resulted in very fragmented production systems.

Certainly, production and productivity cannot only be increased by adoption and area expansion but also by enhancing the efficiency level of farmers to attain the maximum possible level of output from inputs at the disposal of the farmers and available technology. This last option requires identifying the level of efficiency and factors influencing inefficiencies (Tiruneh and Geta, 2016; Alemu et al., 2014; Gebregziabher et al., 2012; Asefa, 2012; Alene et al., 2006).

Thus, improving crop production and productivity

among smallholder producers through efficiency requires a good knowledge of the current efficiency level characteristic in the area as well as factors determining efficiency levels. There have been small empirical studies conducted to estimate level of efficiencies and identify its determining factors for major crops including barley (Wassie, 2014; Alene and Zeller, 2005) and reported a significant level of inefficiencies, and no studies computed and highlighted the loss of outputs due to inefficiency. To the best of the authors' knowledge there are no similar studies started in the study area. Moreover, it is important to update the information based on current inputs and technologies. Therefore, this study was carried out to estimate technical efficiency of smallholder barley production, identify variables affecting technical efficiency and compute frontier output and amount lost by an average efficient producer.

## METHODOLOGY

### Study area

The study was conducted in Welmera district of Addis Ababa Zuria Special zone of Oromia, Regional State in Ethiopia. Welmera district is one of the eight administrative units of the Addis Ababa Zuria Special zone of Oromia Regional State. Geographically, the district is located between 8°50'-9°15'N latitude and 38°25'-38°45'E longitude, and the altitude from 2060 to 3380 m above sea level. The area is chosen based on its potential to barley production.

### Sampling procedure and sample size

A three-stage sampling technique was employed. In the first stage, study district was purposively selected based on the extent of barley production. In the second stage, six *kebeles* (note that *Kebele* is the lowest administrative unit in the Ethiopian condition) were selected from the selected district based on the discussion with district level agricultural extension experts. Finally, from a list of barley growers obtained from extension offices at each *Kebele* level, 180 sample households were selected using systematic random sampling.

### Data source and collection

The enumerators were recruited and trained to facilitate the job of primary data collection. The process was supervised by the researcher and the secondary data were extracted from information documented at various levels (Agricultural Office, Holetta Agricultural Research Center (HARC) and Cooperative Offices) and from published and unpublished sources. Detailed information on households' socioeconomic and demographic characteristics, farm characteristics, inputs utilization, output produced, institutional and policy related variables were collected from selected farm households. The survey was conducted from July to August of 2013.

### Data analysis

To achieve the study's objectives, both descriptive and inferential statistics were used. Descriptive statistics like means, standard

deviations, percentages and frequency counts were used in describing socioeconomic characteristics of households, inputs, output variables, frequency distribution efficiency levels and responses on the constraints of barley production. A stochastic frontier analysis with one step approach was employed in which both technical efficiency and its determinant factors were analyzed simultaneously using the econometric software, FRONTIER 4.1 computer programme.

**Analytical framework**

In this study, the stochastic frontier analysis approach was adopted to measure the technical efficiency of barley farms. The model was independently proposed by Aigner et al. (1977) and Meeusen and Broeck (1977). The merits for this approach over Data Envelopment Analysis (DEA) (non-parametric) is that it accounts for a composite error term (one for statistical noise and another for technical inefficiency effects) in the specification and estimation of the frontier production function. For a number of reasons, the stochastic frontier analysis (econometric) approach has generally been preferred in the empirical application of stochastic production function model in the developing countries' agriculture like Ethiopia. This might be due to, first, the assumption that all deviations from the frontier arise from inefficiency as postulated by DEA is hard to accept, given the inherent variability of smallholder agricultural production due to external factors like pests and weather conditions. Second, most farms are very small and operated by family labor and hence farm records kept rarely. The available data on barley production are most likely subject to measurement errors. Therefore, the stochastic frontier production required for estimating plot level efficiency is specified as:

$$Y_i = \exp(X_i\beta + V_i - U_i) \tag{1}$$

where  $Y_i$  denotes the output for the  $i^{th}$  sample farm,  $X_i$  represents a  $(1 \times K)$  vector whose values are functions of inputs and explanatory variables for the  $i^{th}$  farm,  $\beta$  is a  $(K \times 1)$  vector of unknown production parameters to be estimated,  $V_i$ s are assumed to be independent and identically distributed random errors which have normal distribution with mean zero and unknown variables,  $\sigma_v^2$ , that is  $V_i \sim N(0, \sigma_v^2)$  and  $U_i$ s are non-negative unobservable associated with the technical inefficiency of production such that for a given technology and levels of inputs, the observed output falls short of its potential output ( $U_i \sim N(0, \sigma_u^2)$ ) or it is a one-sided error term ( $U \geq 0$ ) efficiency component that represents the technical inefficiency of the farm. In short,  $U_i$  estimates the shortfall in output  $Y_i$  of barley from its maximum value given by the stochastic frontier function.

In other words, the basis of a frontier function can be illustrated with a farm using  $n$  inputs for barley ( $X_1, X_2, \dots, X_n$ ) to produce output  $Y$  of barley. Efficient transformation of inputs into output is characterized by the production function  $f(X_i)$ , which shows the maximum output obtainable from various input vectors. The stochastic frontier production function assumes the presence of technical inefficiency of production. Hence, the function is defined as:

$$Y_i = f(X_i, \beta) + \varepsilon_i ; \forall i = 1, 2, \dots, n=230 \tag{2}$$

The stochastic frontier analysis has been used in many studies. For example, Yami et al. (2013), Beshir et al. (2012), Jaime and Salazar (2011), Tan et al. (2010), and Daniel et al. (2008) used this approach and the approach specifies technical efficiency as the ratio of the observed output to the frontier output, that means the

technical efficiency of an individual farmer or farm is defined as the ratio of observed output and the corresponding frontier output, given the state of available technology, and presented as follows:

$$TE = \frac{F(X_i; \beta) \cdot \exp(v_i - u_i)}{F(X_i; \beta) \cdot \exp(v_i)} = \exp(-u_i) \tag{3}$$

where  $F(X_i; \beta) \cdot \exp(v_i - u_i)$  is the observed output ( $Y$ ) and  $F(X_i; \beta) \cdot \exp(v_i)$  is the frontier output ( $Y'$ ). Pursuing Battese and Coelli (1995), the error term ( $v_i$ ) permits random variations in output due to factors outside the control of the farmer like weather and diseases as well as measurement error in the output variable, and is assumed to be identically, independently and normally distributed with mean zero and constant variance ( $\sigma_v^2$ ), that is,  $v_i \sim N(0, \sigma_v^2)$ .

The  $u_i$  is the inefficiency component of the error term and a one-sided non-negative ( $u > 0$ ) random variable, is assumed to be independently distributed as truncations at  $\mu$  of the normal distribution and variance ( $\sigma_u^2$ ), that is,  $u_i \sim N(\mu_i, \sigma_u^2)$ , but if  $u_i = 0$ , the assumed distribution is half-normal. The technical inefficiency model suggested by Battese and Coelli (1995) exemplified by:

$$\mu_i = Z_i \delta_i \tag{4}$$

where  $Z_i$  is a  $(1 \times M)$  vector of exogenous explanatory variables associated with the technical inefficiency effects in the  $i^{th}$  time period,  $\delta_i$  is an  $(M \times 1)$  vector of unknown parameter to be estimated.

As mentioned earlier, this study employed the single stage maximum likelihood estimation method in estimating the technical efficiency levels and its determinants simultaneously. This estimation procedure guarantees that the assumption of independent distribution of the inefficiency error term is not violated. The maximum likelihood estimation of the stochastic frontier model yields the estimate for beta ( $\beta$ ), sigma squared ( $\sigma^2$ ) and gamma ( $\gamma$ ), and are variance parameters;  $\gamma$  measures the total variation of observed output from its frontier output. The study used the parameterization following Battese and Coelli (1995) and given as,  $\sigma^2 = \sigma_v^2 + \sigma_u^2$  and  $\gamma = \sigma_u^2 / (\sigma_v^2 + \sigma_u^2)$ , where the gamma lies between zero and one ( $0 \leq \gamma \leq 1$ ). If the value is very close to zero, then the deviations are as a result of random factors and/or if the value is very close to 1, then the deviations are as a result of inefficiency factors from the frontier.

**Model specification**

Following Aigner et al. (1977), the translog production function has been used recently by many studies to estimate technical inefficiency (Tiruneh and Geta, 2016; Geta et al., 2013; Yami et al., 2013; Beshir et al., 2012). Moreover, the uses of different functional forms have a distinct but small impact on estimated efficiency (Kopp and Smith, 1980). Therefore, the translog production function stated in Equation 6 is used for the study mainly for its flexibility for which it places no restriction unlike the Cobb-Douglas production function.

$$\ln Y_i = \beta_0 + \sum_{i=1}^5 \beta_i \ln X_i + (v_i - u_i) \tag{Cobb-Douglas} \tag{5}$$

$$\ln Y_i = \beta_0 + \sum_{i=1}^5 \beta_i \ln X_i + \frac{1}{2} \sum_{i=1}^5 \sum_{j=1}^5 \beta_j (\ln X_i)(\ln X_j) + (v_i - u_i) \tag{6}$$

where  $i=1, 2, \dots, n=230$ , and  $X$ = vector of five input variables.

**Table 1.** Selected farm households from each *Kebele* (Own Sampling Design).

<i>Kebele</i>	Total households	Sample households
Burkusami Gebeya Robi	672	37
Telecho Gebriel	540	30
Bekekana Kore Odo	503	28
Welmera Chokie	664	36
Wajitu Harbu	452	25
Geresu Sida	446	24
Total	3277	180

Based on the aforementioned model, a stochastic frontier model for barley farmers is given by:

$$\begin{aligned} \ln(\text{output})_i = & \beta_0 + \beta_1 \ln(\text{Area})_i + \beta_2 \ln(\text{Fert})_i + \beta_3 \ln(\text{Oxndays})_i + \\ & \beta_4 \ln(\text{seed})_i + \beta_5 \ln(\text{clab})_i + 1/2 \beta_{11} \ln(\text{Area})^2 + 1/2 \beta_{22} \ln(\text{Fert})^2 + 1/2 \\ & \beta_{33} \ln(\text{Oxndays})^2 + 1/2 \beta_{44} \ln(\text{seed})^2 + 1/2 \beta_{55} \ln(\text{clab})^2 + \beta_{12} \ln(\text{Area}) \\ & \ln(\text{Fert}) + \beta_{13} \ln(\text{Area}) \ln(\text{Oxndays}) + \beta_{14} \ln(\text{Area}) \ln(\text{seed}) + \\ & \beta_{15} \ln(\text{Area}) \ln(\text{clab}) + \beta_{23} \ln(\text{Fert}) \ln(\text{Oxndays}) + \beta_{24} \ln(\text{Fert}) \ln(\text{seed}) + \\ & \beta_{25} \ln(\text{Fert}) \ln(\text{clab}) + \beta_{34} \ln(\text{Oxndays}) \ln(\text{seed}) + \\ & \beta_{35} \ln(\text{Oxndays}) \ln(\text{clab}) + \beta_{45} \ln(\text{seed}) \ln(\text{clab}) + v_i - u_i \end{aligned} \quad (7)$$

where *output* represents total yield of the  $i^{\text{th}}$  plot in kilo gram (kg); *Area* represents operational area of barley of the  $i^{\text{th}}$  plot in hectare (ha); *Fert* represents the total amount of inorganic fertilizers used per plot in kg; *Oxndays* represents the amount of oxen days used for plowing from land preparation to planting, *Seed* represents the amount of seed used per plot in kg; *clab* represents the total cost of labour and herbicide ( because herbicide was used instead of hand weeding) in Birr (Birr is the Ethiopian currency), and *ln* represents Natural logarithm.

The specification of inefficiency model for barley individual producer at a plot level is given as:

$$\mu_i = \delta_0 + \sum_{j=1}^{15} \delta_j Z_{ji} \quad (8)$$

$$\begin{aligned} \mu_i = & \delta_0 + \delta_1 \text{Sex} + \delta_2 \text{Age} + \delta_3 \text{Educ} + \delta_4 \text{Fsize} + \delta_5 \text{Proxwroad} + \\ & \delta_6 \text{Acredit} + \delta_7 \text{Livestock} + \delta_8 \text{Offrmy} + \delta_9 \text{Gpmship} + \delta_{10} \text{Ext} + \delta_{11} \text{Train} \\ & + \delta_{12} \text{Frmsize} + \delta_{13} \text{Frgmnt} + \delta_{14} \text{Tenurstatus} + \delta_{15} \text{Costfert} \end{aligned} \quad (9)$$

### Status of barley production

Ethiopia is home for the great diversity of barley in terms of morphological types, genetic races, disease resistant lines, and endemic morph types (Abteu et al., 2015; Fekadu et al., 2011; Bonman et al., 2005). Barley has been produced in Ethiopia since ancient times. It is one of the most important staple food crops both as food and malt. However, Ethiopia produces mostly food barley, with its share estimated to be 90% (Alemu et al., 2014).

Barley is cropped both in the main season (Meher) using June to September rains and off-season (belg) using March to June rains. However, the major barley production is in the main season and the off-season season is irregular throughout Ethiopia characterized by a little grain production. Farming of crops under rain fed condition is the main agricultural production activity. Barley grain is used to prepare various type of food, and local and industrial beverages. There are four major growing areas of barley, namely, Oromia, Amhara, Southern Nations and Tigray regions which account for about 99.5% of the total annual barley grain production (MoA, 2014). Nationally, barley ranked fifth preceded by tef, maize, sorghum and wheat, and again ranked fifth in Oromia region

preceded by tef, maize, wheat and sorghum (CSA, 2012) and finally ranked second in the study area following wheat in area of production (Tiruneh and Geta, 2016).

The national agricultural research and extension system generated barley technologies and disseminated to large number of farmers during the last more than four decades to enhance barley productivity. However, the productivity level is still low which could be marked mainly by inefficiencies in using the existing technologies. Therefore, knowing the technical efficiency level and its determinant factors need to be analyzed.

## RESULTS AND DISCUSSION

### Estimation of productivity parameters and determinant factors

The summary of descriptive statistics of variables used in the econometric models and the results of hypothesis testing are presented in the Appendices 1 and 2.

The coefficients of barley area, amount of fertilizers, oxen days and seed used were positive and significant implying that an increase to some optimum level in these inputs would increase barley output. The coefficients of the square of barley area, interaction of barley area with fertilizer, oxen days, seed and cost of labor were positive and significant implying that an increase in these inputs would increase barley yield while the square of fertilizers, oxen days and cost of fertilizers were negative at 1% significant level.

About 15 socioeconomic of the household heads, institutional factors and plot level characteristics were assumed to affect level of technical efficiency of barley farmers in the study area. The simultaneously estimated maximum likelihood results show that 11 variables (sex, age and education of the household head, and distance to all weather roads, access to credit, group membership, extension contact, training, farm fragmentation, tenure status and investment on fertilizers) were found to affect inefficiency of barley productivity significantly.

The coefficient of sex had a positive effect on technical efficiency of barley farmers at 1% level of significance. Indicating that male headed households operating more efficiently than their female counterparts. This might be due to the fact that men had more resource endowments (for example land, training, improved inputs) and physical

**Table 2.** Summary of socioeconomic variables used in the efficiency model (Own Survey Data, 2013).

Variable	Description of variable	Mean (SD), %
Sex	Sex of the household head (1= male, 0=female)	89.4
Age	Age of the household head in Years	43.9 (11.3)
Educ	Stands for formal education attained by the household head in years	3.8 (3.7)
Fsize	Stands for Family size in Labor force unit	3.55 (1.5)
Proxroad	Stands for Proximity with all weather road in walking minute from the residence	20 (22)
Acredit	Stands for credit accessed in Ethiopian Birr	926.4 (1704.20)
Livestock	Livestock holding in TLU	7.83 (4.17)
Offrmy	Stands for off-farm income earned in Ethiopian Birr	3961.8 (9018.5)
Gpmship	Stands for group membership (1= if the household belongs to more than one group, 0= otherwise)	32.2
Ext	Stands for extension contact in number of days	7 (7)
Train	Stands for training attended by the family member in number of days	1 (1)
Frmsize	Stands for farm size (operational farm land) in ha	2.5 (1.52)
Frgmnt	Stands for fragmentation (number of barley plots) the household had	1.27 (0.5)
Tenurstatus	Stands for tenure status (1= if the household used own farm, 0= otherwise)	86
Costfert	Stands for proportion of cost of fertilizers (cost of fertilizers/total variable cost)	0.36 (0.12)

fitness to some of the agricultural operations. Daniel et al. (2008) and Kibaara and Kavoi (2012) found similar results. While Yami et al. (2013) reported opposite results for waterlogged area wheat farmers in Ethiopia. The variable age was negative and statistically significant at 1% significance level. This means that elder farmers may take benefit of their experiences to use inputs more efficiently to their barley production. Chiona et al. (2014), Mazumder and Gupta (2013), Dlamini et al. (2012) and Asogwa et al., (2012) reported similar results. While Yami et al. (2013), Simonyan et al. (2011), and Jaime and Salazar (2011) reported opposite results. Education was negative and statistically significant at 5% significant level. This means that more years of schooling will improve technical efficiency in barley production. Education enables farmers to better access to, understand and interpret agricultural information to adopt technologies and use them more efficiently. Tiruneh and Geta (2016), Geta et al. (2013), Yami et al. (2013), and Asogwa et al. (2012) reported similar results.

The result shows that distance to all weather roads affected technical efficiency of barley farmers positively and significantly at 1% significance level against the priori expectation, indicating that farmers living far from all-weather roads operate efficiently than the roadside residents. This might need further investigation by including more study areas. The findings of this study show that access to credit is found to affect technical efficiency positively and significantly at 1% significance level by easing financial constraints on inputs purchase. This means that access to credit would have the potential to improve technical efficiency of barley farmers in the study area. Being membership to more than one farmers' group (because by default, a head farmer is a member of one for five development team) affects technical

efficiency positively and significantly at 1% significance level. Implying that membership in more than one farmers' group will improve technical efficiency through better access to agricultural information and sharing of best practices among members. Daniel et al. (2008) and Kariuki et al. (2008) reported similar results. The coefficients of frequency of extension contact and training were also found negative and significant at 1% level, indicating that having more frequent extension contact and participating in training activities could improve technical efficiency of farmers as extension agents provide information on technologies to farmers. Mango et al. (2015) and Obare et al. (2010) found similar results.

The coefficient of farm fragmentation was found to be negative and significant at 10% level, indicating that farmers having more plots of barley were more efficient. Tiruneh and Geta (2016), Yami et al. (2013) and Tan et al. (2010) reported similar findings. The coefficient of tenure status was negative and statistically significant at less than 1% level, indicating that farmers with own plots of barley are more efficient than renters and/or share croppers. Kariuki et al. (2008) reported similar results. Cost of fertilizers or investment on fertilizers was found to be negatively affecting technical inefficiency of barley farmers at less than 1% significance level, indicating investing more in fertilizers will improve barley productivity. Giannakas et al. (2001) reported similar results.

### Estimation of technical efficiency, spatial distribution and yield gap

The core feature of stochastic production frontier is the ability to estimate individual farm specific efficiency level.

**Table 3.** Maximum likelihood estimates of inefficiency effects model.

Variable	Coefficient	t-value
Constant ( $\beta_0$ )	0.381	0.295
Ln (Area)[A]	0.273***	2.90
Ln (Fertilizer)[F]	0.40***	4.257
Ln (Oxen)[O]	0.851***	-9.05
Ln (Seed)[S]	1.323***	-14.065
Ln (Costlabor)[C]	-0.137	-0.146
Ln (A) <sup>2</sup>	1.078***	-14.696
Ln (F) <sup>2</sup>	-0.334***	-4.557
Ln (O) <sup>2</sup>	-0.345***	-4.097
Ln (S) <sup>2</sup>	-0.441***	-6.01
Ln (C) <sup>2</sup>	-0.075	-1.028
Ln(A)Ln(F)	0.077	0.87
Ln(A)Ln(O)	1.285***	14.468
Ln(A)Ln(S)	0.208**	2.34
Ln(A)Ln(C)	0.313***	3.53
Ln(F)Ln(O)	0.239	0.269
Ln(F)Ln(S)	0.082	0.093
Ln(F)Ln(C)	-0.053	-0.622
Ln(O)Ln(S)	0.174	0.196
Ln(O)Ln(C)	-0.156*	-1.76
Ln(S)Ln(C)	0.05	0.056
<b>Inefficiency model</b>		
Constant ( $\delta_0$ )	-0.044	-0.764
Sex	-0.396***	-10.51
Age	-0.794***	-3.078
Education	-0.542**	-2.172
Family size	-0.24	-0.465
Distance TAWRs	-0.171***	-3.86
Credit	-0.002***	-2.93
Livestock	-0.05	-0.15
Off-farm income	0.002	1.307
Membership	-0.609***	2.79
Extension contact	-0.94***	-8.39
Training	-0.273*	-1.956
Farm size	-0.282	-0.12
Fragmentation	-0.675*	-1.86
Tenure status	-0.144***	-8.536
Cost of fertilizer	-0.135***	-9.31
$\sigma_s^2$	0.153***	5.203
$\gamma$	0.795***	3.458

\*\*\*, \*\*\*, \* show significant at 10, 5 and 1%, respectively.

This study used data collected from 230 barley farms of 180 households. The inefficiency model variance,  $\gamma$  was estimated to be 0.795 and statistically significant at 1% level, implying about 79.5% variation in barley output among farms was due to technical inefficiencies while about 20.5% came from external factors (Table 3). Table

4 presents the spatial distribution of technical efficiencies and average yield gap due to inefficiency. The result shows an enormous gap in technical efficiency between farmers. The majority (56.9%) of the farmers score a technical efficiency levels between 0.41 and 0.70 and about 28.7% score below 0.4 levels. The average

**Table 4.** Spatial distribution of technical efficiency and yield gap of barley producers (Own Survey Data, 2013).

Range of technical efficiency	Frequency	Percent
0.11-0.4	66	28.7
0.41-0.7	131	56.9
≥ 0.71	33	14.4
Total	230	100
Minimum TE score (%)		11
Maximum TE score (%)		99
Mean TE score (%)		53
Observed output (kg/ha)		1800
Frontier output (kg/ha)		3396
Output lost due to inefficiency (kg/ha)		1596

technical efficiency level of barley farms in the study area was estimated to be 0.53 ranging from 0.11 to 0.99, which shows a substantial technical inefficiency existing and there is a huge room to increase barley output by about 47% through adopting best practicing farmers' practices using the same combination of inputs and existing technology. As mentioned earlier, the average yield was 1800 kg/ha with a mean technical efficiency score of 53% and the frontier yield was computed to be 3396 kg/ha if an average farmer had used the existing inputs and technologies efficiently. Therefore, on average, 1596 kg/ha of barley yield was lost due to inefficiency effects. According to FDRE (2015), the government of Ethiopia has set a strategy to raise the production and productivity of non-stalk cereals like barley from 2.1 to 3.1 ton/ha during its GTP II plan period by using mainly improved agricultural technologies. However, the results of this study suggest that it is possible to increase the productivity of barley by using the existing inputs and technology through improving the technical efficiency of farmers via determinant factors.

## CONCLUSION AND SUGGESTIONS

The main objectives of this study were to assess the technical efficiency of barley smallholder farmers, sources of technical efficiencies and compute yield loss from inefficiency effects in Welmera district of Oromia region. The study used translog stochastic frontier function with a one-step approach to achieve its objectives. The results showed that a significant variation in technical efficiency scores ranging from 0.11 to 0.99 with an average of 0.53, implying that there is a wider room for increasing barley production by about 0.47, to operate on full technical efficiency frontier level by simply adopting best practices of model farmers. Concerning the spatial distribution of farm level technical efficiency scores, the majority (56.9%) of the farmers score a technical efficiency levels between 0.41 and 0.70 and

about 28.7% score below 0.4 levels.

Among the strategy variables considered in an inefficiency effects model, sex, age and education level of the household head, distance to all weather roads, credit service, group membership, extension contact, training, plot fragmentation, tenure status and investment in fertilizers were identified to have higher influence on technical efficiency in barley production in the study area.

In order to improve the efficiency level of barley farmers, the result suggests the need to involve female headed households into extension and trainings, increase the education level of households through informal and formal literacy, inspire household membership into farmers' groups and enable them to share best practices from model and more experienced farmers, inspire barley producers to invest on fertilizers and strengthen rural micro finance institutions to provide credit at a reasonable costs.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## Appendices

**Appendix 1.** Summary of variables used in the econometric models (Own Survey Data, 2013).

Input variable	Units	Minimum	Average	Maximum	Std. Deviation
<b>Continuous</b>					
Yield	Ton/ha	0.6	1.8	3.8	0.7
Area	ha	0.06	0.55	2.75	0.41
Seed	Kg/ha	105.7	129.17	156.5	13.4
Fertilizer	Kg/ha	30.77	134.46	491.67	70.9
Oxen-days	days/ha	14.25	18.09	21.85	1.95
Cost of labor	birr/ha	750	1282.90	1838.70	238.50
Age of HHH	years	24	43.9	78	11.3
Education	years	0	3.8	12	3.74
Family size	LFU	1	3.55	8.57	1.5
Distance to all WRs	minute	1	20	120	22
Credit	birr	0	926.40	10000	1704.20
Live stock	TLU	1.04	7.83	27..3	4.17
Off-farm income	Birr	0	3961.80	94600	9018.50
Extension contact in days	Number	0	7	42	7
Trainings in days	Number	0	1	4	1
Farm size	Ha	038	2.5	9.13	1.52
Fragmentation	number	1	1.27	4	0.5
Cost of fertilizer	proportion	0.11	0.36	0.72	0.12
<b>Discrete</b>					
	<b>Labels</b>			<b>Frequency</b>	<b>Percent</b>
Sex of HHH	Female=0			19	10.6
	Male=1			161	89.4
	Total			180	100
Membership	1, if the household belongs to >1 FG			58	32.2
	0 otherwise			122	67.8
	Total			180	100
Tenure status	Own=1			198	86
	Rented=0			32	14
	Total			230	100

**Appendix 2.** Summary of tested hypotheses (Own Survey Data, 2013).

Hypothesis	L(H <sub>0</sub> )	LR( $\lambda$ ) statistics	critical $\chi^2$ value	DF	Decision
1. H <sub>0</sub> : $\beta_{ij} = 0$	-104.22	142.62	18.3	10	H <sub>0</sub> rejected
2. H <sub>0</sub> : $\gamma = 0$	-77.24	88.68	2.7*	1	H <sub>0</sub> rejected
3. H <sub>0</sub> : $\delta_1 = \dots = \delta_{15} = 0$	-53.54	41.28	24.99	15	H <sub>0</sub> rejected

\*Shows it was taken from Table 1 of Kodde and Palm (1986).

## Full Length Research Paper

# Influence of *Bradyrhizobia* inoculation on growth, nodulation and yield performance of cowpea varieties

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Cowpea (*Vigna unguiculata* L. Walp) has a major role in daily diet of the rural community and poor urban population, serving as a source of energy, protein and minerals, in developing countries. Its straw used for animal feed, and the crop also improves soil fertility by fixing the atmospheric nitrogen. However, its productivity is constrained by lack of improved varieties and poor agronomic practices. A field experiment was conducted at Ziway, central rift valley of Ethiopia, to evaluate the response of cowpea varieties to *Bradyrhizobia* inoculation. The treatment consisted of four *Bradyrhizobia* strains (control, GN-100, GN-102 and MB-140) and five cowpea varieties (Bole, Black eye-bean, TVU1977.0D1, Assebot and White Wonder). The experiment was carried out using a randomized complete block design with three replications. The results revealed marked varietal differences in plant growth, nodulation, yield and yield components. Of the five cowpea varieties studied, Black eye-bean generally showed superior performance in most measured parameters. *Bradyrhizobium* inoculation significantly ( $p \leq 0.05$ ) increased plant growth, nodulation, yield and yield components. The interaction effect of variety and *Bradyrhizobium* caused significant variations in the number of nodules, number of seeds, hundred seed weight and seed yield. The highest grain yield was recorded from Black eye-bean variety (3.08 t/ha) and *Bradyrhizobium* strain GN-102 (3.11 t/ha) inoculation. It could, thus, be deduced that the use of strain GN-102 and variety Black-eye bean markedly increases the productivity of the crop in the region.

**Key words:** *Bradyrhizobia*, cowpea, nodulation, growth, yield components.

## INTRODUCTION

Grain legumes play a vital role in the lives of millions of people in developing countries to achieve food and nutritional security. They complement staple low-protein cereal crops as a source of protein and minerals (Gharti et al., 2014). Legume crops are also valued for their ability to fix atmospheric nitrogen into the soil and play an

important role as a rotation crop with cereals and vegetable crops (Jensen et al., 2012; Biswas and Gresshoff, 2014; Stagnari et al., 2017). Legumes also serve as a feed crop in many farming systems and are also grown to supplement farmers' incomes (Muli and Saha, 2002; Voisin et al., 2013). The important and

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diverse role played by food legumes in the farming systems and in diets of poor people, makes them ideal crops for achieving the developmental goals to reduce poverty and hunger, improving human health and nutrition, and enhancing ecosystem resilience.

Cowpea (*Vigna unguiculata* L. Walp) being a legume is an important source of food, income and livestock feed and forms a major component of tropical farming systems because of its ability to improve the fertility status of marginal lands through nitrogen fixation (Timko and Singh, 2008). It has considerable adaptation to high temperatures and drought compared to other crop species, making it suitable for cultivation in semiarid areas (Hall, 2004; Tekle, 2014).

Despite its role in improving soil fertility and serving as a food security crop, little efforts are made to know the response of cowpea varieties to *Bradyrhizobia* inoculation in Ethiopia. Furthermore, *Bradyrhizobia* inoculation of cowpea is not a common agronomic practice among smallholder farmers. Thus, the development of new cultural practice, *Bradyrhizobium* inoculation as N source, which enhance yield and mineral nutrition of grain is imperative to achieve food and nutritional security in the country. Therefore, the aim of this study was to evaluate the effect of *Bradyrhizobium* inoculation on plant growth, nodulation and yield of cowpea varieties grown at Ziway, central rift valley of Ethiopia.

## MATERIALS AND METHODS

### Description of study site

The experiment was conducted at Ziway, in the Agricultural Research Station of Hawassa University, in 2012 cropping season. The site is located on latitudes of 8°00' N and longitudes of 38° 45' E, with an elevation of 1645 m above sea level. Average rainfall during the growing season of five months was 763.0 mm, which is more or less similar to the five years average rain fall (760.9 mm). The mean maximum and minimum temperatures were 27 and 12.7°C, respectively. The area is characterized by bimodal rainfall pattern with a short rainy season from February to April and a long rainy season from June to September with a peak in August (NMA, 2012).

### Source of planting material and *Bradyrhizobium* strains

Seeds of *Vigna unguiculata* (L) Walp varieties (Bole, Black eye bean, TVU1977.0D1, Assebot and White Wonder) were obtained from the Melkassa Agriculture Research Center, Melkassa, Ethiopia. The varieties were chosen based on their high grain yield and acceptability by farmers. Strains of *Bradyrhizobial* sp. (GN-100, GN-102, MB-140) were obtained from NUFU-Hawassa University collaborative project (NUFUPRO-2007/10144) authenticated collections and which have been biochemically characterized (Negash, 2010; Zikie, 2010).

### Experimental design and treatments

Seeds were planted in a factorial randomized complete block design which had a total of 20 treatment combinations with three

replicate plots for each treatment. The treatments consisted of four *Bradyrhizobia* inoculation (control, strains: GN-100, GN-102 and MB-140) and five cowpea varieties (Bole, Black eye-bean, TVU1977.0D1, Assebot and White Wonder).

### Cultural practices

Seed inoculation was done under shade in the field to reduce the bacterial cell death. Inoculated seeds were allowed to air-dry for a few minutes before planting. Planting was done using a spacing of 40 cm between rows and 20 cm between plants. Each experimental plot measures 2.2 m × 3.0 m (6.6 m<sup>2</sup>). Two seeds were sown in each hole for both inoculated and uninoculated treatments. To avoid cross contamination, the uninoculated seeds were always planted first, followed by inoculated treatment. Soil ridges were made to separate inoculated and uninoculated treatments from each other in order to prevent cross contamination through rainwater movement. After sowing, the seeds were immediately covered with moist soil to avoid rhizobial cell death from desiccation. Weeding was done manually by hoe at two weeks after seedling emergence, and three weeks from the first weeding. To avoid cross contamination, weeding was done in the uninoculated plots first.

### Data collection

#### Plant height

Five plants from the central rows of each plot were randomly selected for measuring plant height. Then the average values of these plants were recorded as plant height of the crop.

#### Nodule number/plant

Nodulation assessment was undertaken at mid (50%) flowering stage by carefully uprooting five plants randomly from each plot. The plants were separated into shoot and roots. The adhering soil was carefully washed from the roots over a metal sieve. The nodules from each plant were picked and spread on the sieve to drain water from their surface. Nodules were counted and their average was taken for plots as nodule number/plant. Then after, the nodules were oven-dried at 70°C for 48 h for nodule dry weight determination.

#### Yield and yield components

Yield and yield components at harvesting time for the determination of yield components such as number of pods/plant, number of seeds/pod, hundred seed weight and grain yield, ten randomly picked plants were used. Seed weight was determined by randomly taking 100 seeds of the ten sample plants and weighing it with sensitive balance after oven drying to constant weight.

#### Soil sampling and analysis

Before planting, 20 soil samples were randomly taken from the experimental field at a depth of 0 to 30 cm using an auger and the samples were mixed thoroughly to produce one representative composite sample of 1 kg. The composite soil sample was air-dried and ground to pass through 2 and 0.5 mm (for total N) sieves. Soil analysis was done following standard laboratory procedures as outlined by Sahlemedhin and Taye (2000). Soil pH was measured in the supernatant suspension of a 1: 2.5 soil to water ratio using a

standard glass electrode pH meter (Rhoades, 1982). The Walkley and Black (1934) method was used to determine the organic carbon (%). Total N was determined using Kjeldahl method as described by Bremner and Mulvaney (1982). Available P ( $\text{mg kg}^{-1}$ ) was determined by employing the Olsen et al. (1954) method using ascorbic acid as the reducing agent. The soil particle size distribution was determined using the Bouyoucos hydrometer method (Bouyoucos, 1951).

### Statistical analysis

The collected data was subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System software (SAS, 2002) version 9.0. Mean separation was done using Least Significant Difference (LSD) test at 5% probability level.

## RESULTS AND DISCUSSION

### Physicochemical properties of the soil before planting

The result of soil analysis (Table 1) indicated that texture of the soil was clay loam and pH of the soil was alkaline in reaction with a pH value of 8.2. Different crops have different pH requirements. Accordingly, the optimum pH range for cowpea is 6.8 to 8.5 (Onyibe et al., 2006). Therefore, the pH of experimental soil is suitable for cowpea. According to Jackson (1958) and Herrera (2005), soil organic carbon content was rated as very low (< 2%), low (2 to 4%), medium (4 to 10%), high (10 to 20%) and very high (>20%) and thus the soil at the experimental site has low organic carbon content 2.65%. According to Olsen et al. (1954), P content ( $\text{mg kg}^{-1}$ ) <5 is very low, 5 to 15 is low, 15 to 25 is medium and >25 is high. Therefore, the available P in soil at the experimental site (22.00 mg/kg) was medium range. Total nitrogen in the experimental soil was 0.13%. Total N is rated as very low (<0.1), low (0.1 - 0.15), medium (0.15 - 0.25) and high (>0.25) (Havlin et al., 1999). Hence, total N of the soil of the experimental field was in the low range. This shows that the soil requires external application of N fertilizer if satisfactory level of crop production is to be achieved and also most Ethiopia soils, similar to the agricultural soils in other tropical countries, are reported to be generally low in N content (Asgelil, 2000). In general for soils low in mineral nutrients, effective *Bradyrhizobia* strains which able to contribute N to the soil can substantially contribute to the replenishment of soil N for improved crop yield.

### Effect of cowpea variety and *Bradyrhizobia* inoculation on growth and nodulation

#### *Plant height (cm)*

As indicated in Table 2, varieties showed significant

differences ( $P < 0.001$ ) in plant height. However, the response of plant height to *Bradyrhizobia* inoculation was not significant ( $P > 0.05$ ). Regarding variety effect, the highest value for plant height was recorded with Black eye-bean variety (68.1 cm) followed by var. Wonderer. Whereas, the lowest value of plant height was recorded from Assebot variety (31.0 cm) which was statistically at par with varieties Bole and TUV. The observed difference in plant height among cowpea varieties might be attributed to inherent genotypic difference (Magani and Kuchinda, 2009; Nwofia et al., 2015). This is in agreement with findings of Karikari et al. (2015) who reported variation on plant height among cowpea cultivars.

#### *Shoot dry weight (g/plant)*

Shoot dry weight was significantly ( $P < 0.001$ ) affected by cowpea variety and *Bradyrhizobia* inoculation (Table 2). The highest (31.64 g/plant) and lowest (22.04 g/plant) shoot dry weight was recorded from vars. Bole and Wonderer, respectively. This is in line with finding of Singh et al. (2011) that some varieties have the ability to out yield the other varieties and exhibit superior plant growth. Addo-Quaye et al. (2011) also found that cowpea varieties have different capacities for dry matter accumulation. The highest (31.2 g/plant) and lowest shoot dry weight (21.46 g/plant) was recorded from *Bradyrhizobium* strain GN-102 inoculation and the control treatment, respectively. The increased shoot dry weight due to inoculation can be attributed to the effectiveness of the *Bradyrhizobia* inoculants. A similar result was reported by Salih (2002) who observed increased shoot dry weight in soybean plants inoculated with *Bradyrhizobium* strains.

#### *Nodule number per plant*

Nodule number was significantly ( $P < 0.001$ ) affected by variety, *Bradyrhizobium* inoculation and their interaction (Table 2 and Figure 1A). Regarding the main effect, the highest and lowest nodule number was recorded from Black eye-bean (15) and Wonderer (10), respectively. The marked variation in nodule number per plant among the varieties could be attributed to difference in the genetic makeup of the individual varieties (Ayodele and Oso, 2014). Likewise, the maximum (15.6) and minimum (5.1) values of this parameter was obtained from *Bradyrhizobium* strain GN-102 and the control treatment, respectively. The enhancing effects of inoculation on nodule number per plant was also supported by the finding of Manish et al. (2011), which showed rhizobial inoculation increased number of root nodules as compared with uninoculated treatments. Thus, the selection of highly effective and competitive strain as

**Table 1.** Selected physicochemical properties of the soil before planting.

Particle size distribution (%)			Texture	pH (1:2.5 H <sub>2</sub> O)	Av. P (mg/kg)	Total N (%)	OC (%)
Clay	Silt	Sand					
28	28	44	Sandy loam	8.20	22.00	0.13	2.65

**Table 2.** Effect of *Bradyrhizobium* strains on growth and nodulation performance of cowpea varieties.

Treatments	Plant height (cm)	Shoot dry weight (g)	Nodule number per plant	Nodule dry weight (g)
<b>Variety</b>				
Bole	32.5 <sup>c</sup>	31.6 <sup>a</sup>	13.6 <sup>b</sup>	0.14 <sup>a</sup>
Black eye-bean	68.1 <sup>a</sup>	28.6 <sup>ab</sup>	15.0 <sup>a</sup>	0.14 <sup>a</sup>
TUV1977.0D1	33.7 <sup>c</sup>	23.7 <sup>d</sup>	9.9 <sup>d</sup>	0.12 <sup>bc</sup>
Assebot	31.0 <sup>c</sup>	26.2 <sup>bc</sup>	11.6 <sup>c</sup>	0.13 <sup>ab</sup>
Wonderer	46.6 <sup>b</sup>	22.0 <sup>d</sup>	10.0 <sup>d</sup>	0.10 <sup>c</sup>
LSD (5%)	10.3	3.7	1.1	0.02
<b><i>Bradyrhizobium</i></b>				
Control	39.0	21.5 <sup>c</sup>	5.1 <sup>d</sup>	0.09 <sup>c</sup>
GN-100	42.9	28.2 <sup>ab</sup>	14.4 <sup>b</sup>	0.14 <sup>ab</sup>
GN-102	44.5	31.2 <sup>a</sup>	15.6 <sup>a</sup>	0.16 <sup>a</sup>
MB-140	43.1	25.0 <sup>b</sup>	13.0 <sup>c</sup>	0.13 <sup>c</sup>
LSD (5%)	NS	3.3	1.0	0.02
CV%	29.4	17.0	11.3	22.5.
<b><i>F-value</i></b>				
Variety (V)	18.9 <sup>***</sup>	8.7 <sup>***</sup>	182.4 <sup>***</sup>	11.4 <sup>***</sup>
<i>Bradyrhizobium</i> (Br)	0.5NS	13.0 <sup>***</sup>	32.8 <sup>***</sup>	4.1 <sup>**</sup>
V * Br	0.5NS	1.7NS	4.5 <sup>***</sup>	0.53 <sup>NS</sup>

Means with the same letter(s) within a column are not significantly different at  $p < 0.05$ .

inoculum is very important to increase nodulation and hence the amount of N fixed and yields of the crop. Similar results were obtained by Sharma and Kumawat (2011), who reported increased nodulation by inoculating soybean varieties with *Bradyrhizobium* strains. Less number of nodule also reported in soybean and chick pea without *Bradyrhizobium* inoculation (Elkoca et al., 2008).

#### **Nodule dry weight/plant**

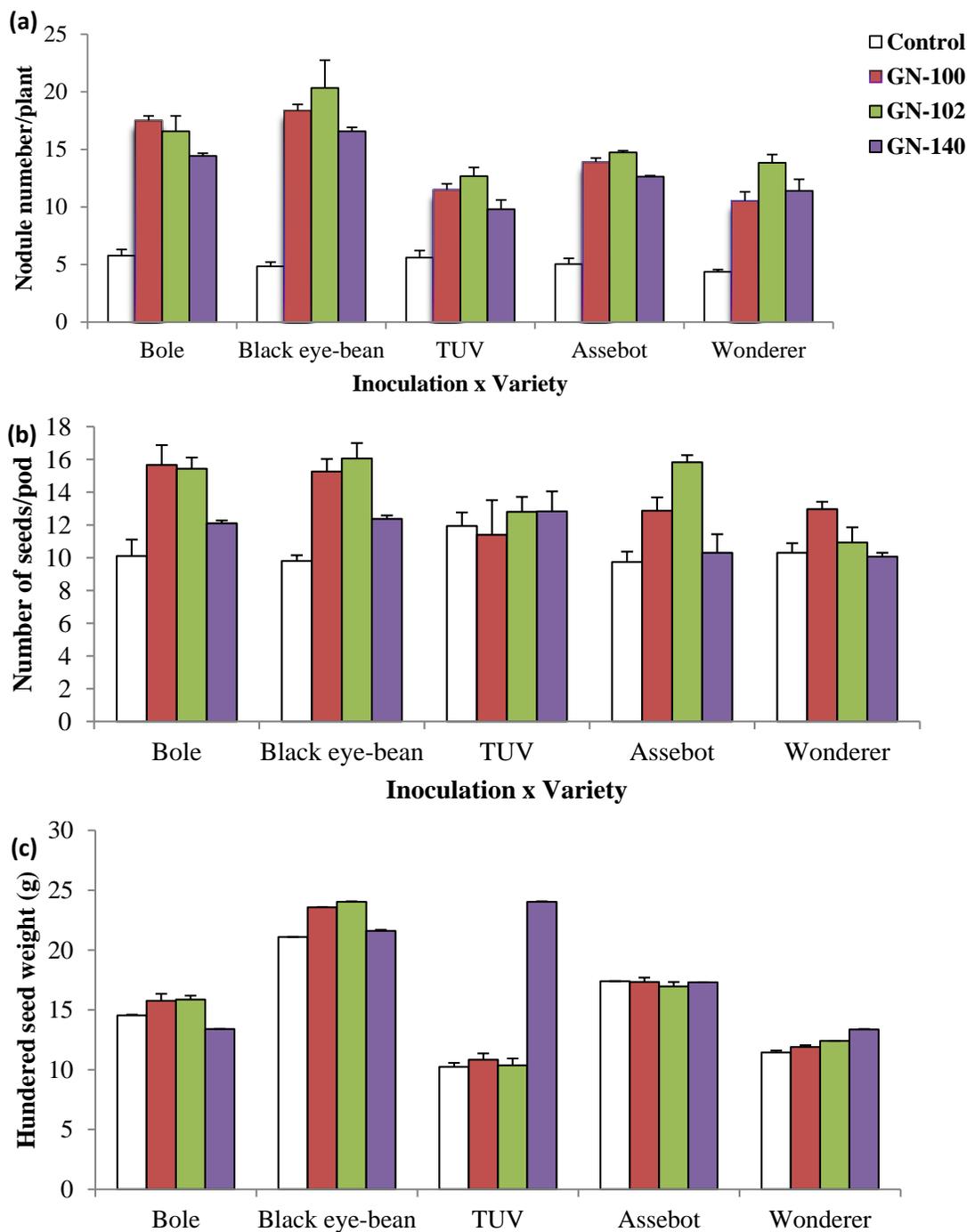
Nodule dry weight was significantly ( $P < 0.001$ ) affected by cowpea variety and *Bradyrhizobium* inoculation (Table 2). The highest nodule dry weight (0.14 g/plant) was recorded from vars. Bole and Black eye-bean, while the lowest (0.10 g/plant) nodule dry weight was recorded from vars. Wonderer reflecting inherent genetic differences among the cultivars for nodule dry weight. Similar results have been reported by Karadavut and Özdemir (2001) where the effects of cultivars were statistically significant on the nodule dry weight. On the other hand, maximum nodule dry weight (0.16 g/plant) was recorded from *Bradyrhizobium* strain GN-102

followed by strain GN-100, while the minimum nodule dry weight (0.09 g/plant) was recorded from the control treatment. The difference between the nodule dry weight obtained from inoculated plants and uninoculated plants may be attributed to the size of the nodules. Inoculated plants formed bigger nodules than uninoculated plants due to the effectiveness of the introduced rhizobia strain to initiate nodulation with cowpea roots which agrees with the report of Chiamaka (2014). A similar promoting effect of seed inoculation on dry weight of nodules per plant has also been reported by Bejandi et al. (2011) and Nyoki and Ndakidemi (2014).

#### **Effect of varieties and *Bradyrhizobium* inoculation on yield and yield components**

##### ***Number of pods/plant***

The means of statistical analysis indicates that pods/plant was significantly affected by the treatments (Table 3). Maximum pods/plant (40.3) was achieved by var. Bole, while minimum pods per plant (30.0) was observed in



**Figure 1.** The interactive effect of *Bradyrhizobia* inoculation x variety on: (A) Nodule number/plant; (B) Number of seeds/pod and (C) Hundreded seed weight (g). Vertical lines on bars represent S.E of the statistical means.

var. Wonderer, but, the differences in pods/plant among Wonderer, Assebot, TUV1977.0D1 and Black eye-bean varieties were observed to be statistically at par. The mean values for inoculation also shows significant effect for pods/plant. Maximum pods/plant (40.5) was obtained with *Bradyrhizobium* strain GN-102 inoculated plants,

while less pods/plant (22.6) was recorded for the control treatment. This increased pod number with applied inoculants could be associated with enhanced growth and higher assimilate accumulation which resulted from better N nourishment due to symbiotic N fixation. The result is in agreement with the work of Malik et al. (2006)

**Table 3.** Effect of *Bradyrhizobium* strains on yield and yield components of cowpea varieties.

Treatments	Number of pods per plant	Number of seeds per pods	Hundred seed weight (g)	Grain yield (t/ha)
<b>Variety</b>				
Bole	40.3 <sup>a</sup>	13.3 <sup>a</sup>	14.9 <sup>c</sup>	2.67 <sup>b</sup>
Black eye-bean	33.9 <sup>b</sup>	13.4 <sup>a</sup>	22.8 <sup>a</sup>	3.08 <sup>a</sup>
TUV1977.0D1	31.8 <sup>b</sup>	12.2 <sup>ab</sup>	13.9 <sup>d</sup>	2.73 <sup>b</sup>
Assebot	31.6 <sup>b</sup>	12.2 <sup>ab</sup>	17.3 <sup>b</sup>	2.68 <sup>b</sup>
Wonderer	30.0 <sup>b</sup>	11.1 <sup>b</sup>	12.3 <sup>e</sup>	2.38 <sup>c</sup>
LSD (5%)	6.1	1.3	0.4	0.3
<b><i>Bradyrhizobium</i></b>				
Control	22.6 <sup>c</sup>	10.4 <sup>c</sup>	14.9 <sup>c</sup>	2.34 <sup>c</sup>
GN-100	32.2 <sup>b</sup>	13.6 <sup>a</sup>	15.9 <sup>b</sup>	2.76 <sup>b</sup>
GN-102	40.5 <sup>a</sup>	14.2 <sup>a</sup>	15.9 <sup>b</sup>	3.11 <sup>a</sup>
MB-140	34.8 <sup>b</sup>	11.5 <sup>b</sup>	17.9 <sup>a</sup>	2.60 <sup>b</sup>
LSD (5%)	5.4	1.2	0.3	0.2
CV%	21.9	12.6	2.9	11.6
<b>F-value</b>				
Variety (V)	10.6 <sup>***</sup>	19.6 <sup>***</sup>	107.3 <sup>***</sup>	7.36 <sup>***</sup>
<i>Bradyrhizobium</i> (Br)	3.6 <sup>*</sup>	4.5 <sup>**</sup>	867.6 <sup>***</sup>	15.8 <sup>***</sup>
V* Br	0.9 <sup>NS</sup>	2.9 <sup>**</sup>	141.8 <sup>***</sup>	0.82 <sup>NS</sup>

Means with the same letter(s) within a column are not significantly different at  $p < 0.05$ .

and Dereje (2007) which showed increased number of pods per plant with *B. japonicum* inoculation in soybean. Similarly, Argaw (2014) also obtained similar result in soybean, in that number of pods in soybean increased due to *Bradyrhizobium* inoculation. Hoque and Haq (1994) reported that seed inoculation increased number of pods per plant of lentil in Bangladesh. Contrastingly, Yamur and Engin (2004) reported that inoculation did not affect the number of pod per plant.

#### Number of seeds/pod

Number of seeds/pod was markedly affected by variety, *Bradyrhizobium* inoculation and their interaction (Table 3 and Figure 1B). Regarding main effect, the highest and lowest seeds/pod was recorded from Black eye-bean (13.4) and Wonderer (11.1), respectively (Table 3). The differences among Bole, Black eye-bean, TUV and Assebot were statistically at par. The report is in agreement with the findings of Dugje et al. (2009) and Singh et al. (2011), that different cowpea varieties have different genetic makeup as such they have different number of seeds. Similarly, number of seeds/pod was significantly increased with *Bradyrhizobium* inoculation where the highest (14.2 and 13.6) and the lowest (10.4) values of this parameter were obtained from *Bradyrhizobium* strains GN-102 and GN-100 and the control treatment, respectively. This significant difference of number of seeds/pod among inoculants and varieties were in line with the finding of Muhammad (2002) where

inoculation with *Bradyrhizobium*, not only increased the shoot growth, but also the seeds/pod.

#### Hundred seed weight

Hundred seed weight was significantly ( $P < 0.001$ ) affected by variety, inoculation and their interaction (Table 3 and Figure 1C). Regarding main effect, the heavier and lighter seed weight was recorded due to Black eye-bean (22.8 g) and Wonderer (12.3 g), respectively. The significant difference in hundred seed weight among the varieties may be due to the difference in translocation and partitioning efficiency of assimilates from source to sink (El Naim and Jabereldar, 2010). Likewise, hundred seed weight was significantly increased with *Bradyrhizobium* inoculation where the maximum (17.9 g) and the minimum (14.9 g) values of this parameter were obtained from *Bradyrhizobium* strain MB-140 and the control treatment, respectively. Similar, findings were reported by Ali et al. (2004) where inoculation brought a significant effect on seed weight, of chickpea. Kazemi et al. (2005) reported that soybean seed inoculation with bradyrhizobia significantly increased seed weight.

#### Grain yield

Inoculation of *Bradyrhizobia* strains significantly affected the grain yield/ha (Table 3). Among the varieties significantly highest grain yield (3.08 t/ha) was recorded

for var. Black eye-bean followed by var. Bole and the lowest grain yield (2.38) was obtained from var. Wonderer. The significant variation in grain yield among the varieties is largely due to differences in inherent yielding potential of the varieties. Similarly, Haruna and Usman (2013) observed a significant variation in grain yield of some improved varieties of cowpea at the same location and attributed it to genetic makeup of the varieties examined. The highest grain yield (3.11 t/ha) was recorded from *Bradyrhizobium* strain GN-102 inoculation followed by strain GN-100 and MB-140, whereas the lowest grain yield (2.34 t/ha) was recorded from the control treatment. The increase in grain yield due to *Bradyrhizobia* inoculation may be attributed to the effectiveness of the inoculant in fixing N thereby meeting the nutrient requirement of the plant (Nyoki and Ndakidemi, 2013). Ulzen et al. (2016) also observed a significant increase in grain yield of cowpea after inoculation with *Bradyrhizobium* inoculant.

### Interaction effects of *Bradyrhizobium* inoculation and variety

Among the parameters tested under this study, number of nodules/plant, number of seeds/pod and hundred seed weight were significantly affected by the interaction effect of *Bradyrhizobium* inoculation and variety (Figure 1A to C). Inoculating cowpea seed with *Bradyrhizobia* strains were observed to be associated with increment in most of these parameters. Generally, the maximum values were obtained from *Bradyrhizobia* inoculation when compared to uninoculated control.

### Conclusion

The results obtained in this study have shown that *bradyrhizobia* inoculation can improve plant growth, nodulation and grain yield of cowpea varieties grown in the low-N soils of Ethiopia. The higher plant growth and increased nodulation in *Rhizobium*-inoculated plants translated into increased grain yield. Based on the findings of the current study the use of cow pea variety Black-eye bean with *Bradyrhizobium* strain GN-102 could be recommended for enhanced growth and yield performance of cowpea.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## Quality of planting systems in varieties of sugarcane

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**This study aimed to assess the operational quality of mechanized planting and semi-mechanized sugarcane for RB835054 and SP813250 varieties. The sugarcane has significant importance in Brazil, as it is a strong presence of culture in the economic field, with sugar and ethanol production. The mechanization of agricultural operations has been implemented in order to increase operational performance and reduce cost, thus presenting some possible advantages as the semi-mechanized plantation system. The experimental design was a completely randomized design. There were qualitative differences as regards the number of gems, damage to gems, parallelism and failure of deposition seedlings. The planting system, semi mechanized has better indices for the variables: total gems, viable gems and failures. For furrow depth, the values are similar between the two systems. Mechanized planting system showed better quality for variable row spacing. Among the varieties, there were similar values for almost all variables, but for failures in the deposition of seedlings, SP813250 variety obtained unsatisfactory results as compared to RB835054 in mechanized planting system.**

**Key words:** Mechanization, quality control, *Saccharum officinarum*, varietal efficiency.

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### INTRODUCTION

Sugarcane (*Saccharum* spp.) is between socioeconomic highlights (Neto et al., 2006) and agribusiness in Brazil, which is the largest producer (Ripoli and Ripoli, 2010) and exporter of alcohol and cane sugar (Silva et al., 2008; Duarte Junior et al., 2008).

Planting is the operation that involves the greatest knowledge of soil-plant-atmosphere interface, which provide conditions for culture, fundamental role of soil tillage operations (Tavares et al., 2010), contributes positively to the increase in productivity and influence in a

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positive way, the longevity of sugarcane (Barros and Milan, 2010; Braunack and McGarry, 2006), and faults in this context, according to Ferreira et al. (2008), may represent years of impaired productivity.

With the importance of this operation, Chaddad et al. (2010) reported that the mechanization of the plantation system was consolidated as a technique regarding the renewal and expansion of sugarcane plantations. In cultivation of sugarcane, mechanization of operations has been implemented due to the lack and cost of labor (Ripoli and Ripoli, 2010) and so it has been important due to several factors, leading to reduced costs, as compared to semi-mechanized planting system, replacing most of the labor-work in the operation due to increased operation of mechanized sets (Voltarelli et al., 2013) and maintaining the competitiveness of the sector, even in times of crisis (Ramos et al., 2015).

According to Ripoli and Ripoli (2010), semi-mechanized planting system is still the most used, in which the furrow operation and the groove cover is held mechanically, while the distribution of seedling is done in a manual way. In the mechanized planting system, sugarcane is harvested mechanically, being chopped into billets that are directed to the hopper planter whose function is to deposit them in the open groove immediately before and immediately closed after the operation (Ferreira et al., 2008).

In order to analyze the behavior and quality in mechanized farming operations, the aid quality control tools is a reality, because it indicates that the decrease in variability of the results is the quality of the operation, resulting in closer to the limits results specified (Milan and Fernandes, 2002; Toledo et al., 2008). The Statistical Process Control (CEP) aims to analyze the operation point to point and quickly identify changes in parameters correcting problems before they take place in several nonconforming items (Silva et al., 2008).

As the planting operation has great impact on the development of culture, and this has been mechanized gradually changing the entire scope of work at this stage, through this work, it is meant to denote the quality of different cropping systems, mechanical and semi-mechanized, the sugarcane crop in different varieties by the Statistical Process Control.

## MATERIALS AND METHODS

The study was conducted in the 2014 harvest in farm situated in the municipality of Monte Alto, São Paulo, in a commercial area of a sugarcane plant, with geodetic coordinates latitude of 21°16' S and longitude 48°24' W, with average altitude of 640 m. The relief is predominantly soft and wavy, with a mean slope in the 8.8% range, with exposure facing the east and west. The climate is the mesothermal type with dry winter and average rainfall of 1,400 mm. The soil found in the area is sandy loam Paleudalf. The average water content in the soil was 16% at planting and determined its composition and characteristics through the removal of 20 single

samples of equal volume and depth (Table 1).

The experimental design was completely randomized design, analyzed in a 2x2 factorial design, with four treatments and 20 replicates per treatment, distributed in two planting systems (semi-mechanized and mechanized) and two varieties of sugarcane (RB835054 and SP813250). The dimensions of the sample plots were 10 by 3 m, being distributed at random, totaling 80 installments.

The control chart was the tool used as a quality indicator of the mechanized operations of sugarcane planting systems for the variable depth of grooves, row spacing, the total number of gems, number of viable gems per meter and failure deposition seedlings. The parameters evaluated and taken as quality indicators were chosen by the technical staff of the plant (Table 2). The semi mechanized planting process comprises the steps of:

1. Mechanized furrow operation with fertilizer using 4x2 tractor TDA 133 kW of engine power at 2000 rpm and 2 lines trencher (1.5 m spacing) which operated at an average speed of 6 km/h,
2. Followed by the distribution of seedlings cut manually using a bucket truck with loading and unloading seedlings aided by a loader tractor 4x2 with claw 73 kW engine at 1800 rpm,
3. And mechanized groove mating with insecticide with a tractor of 73 kW engine, hedger 2 line (1.5 m spacing) reservoir tank 300 L capacity which operated with an average speed of 8 km/h. Mechanized planting process consisted of:

1. A sugarcane harvester 251 kW of engine power, mats, ready for harvest seedlings with parts protected by rubber, transhipments trucks with a capacity of 7000 kg, 4x2 tractor TDA power 133 kW engine at 2000 rpm,
2. And a planter of 2 lines equipped planting with tank for manure with 1250 kg capacity, storage tanks with 600 L tank and for storing the chopped plants with capacity of 6,000 kg.

The furrow depth is obtained by removal of the excess layer of soil that is deposited between the lines, placing an iron rod on the groove surface, with the aid of graduated rule positioned perpendicularly to the rod, measure the depth of sugarcane that are deeper in the groove.

Monitoring of the quantities of total and viable buds was held, with 30 m from the carrier, marking in each plot eight straight grooves and casting a jig by chance in each groove. The seedlings were cut at the ends of the template with the aid of a knife and then the number of viable and non-viable bud was counted. The percentage of viable gems was obtained by calculating the difference between the total number of gems and the total amount of viable gems.

For evaluation of failure deposition seedlings, which can be classified as voids which are in sugar cane planting rows, 30 m from the carrier was determined, if disassociating five plots per treatment, 10 m useful, measuring the distance between the seedlings deposited with the aid of graduated measuring tape. Faults above 30 cm are not acceptable.

Data analysis consisted of descriptive statistics to understand the behavior of data, analysis of variance and statistical process control through the Minitab program. The tool of individual control charts and moving range shows the range of values of each point, with the average indicated by a center line and control limits, higher (UCL) and lower (LCL), calculated based on the standard deviation of variables, to check if the results have variation sorting operation with or without quality, or if the operation is being influenced by special causes.

As in all variables, according to the Anderson-Darling test, the data were not normally distributed, the adjustment models were used, as listed in Table 3, in order to meet the standards

**Table 1.** Particle size analysis and texture average area.

Soil	Depth (m)	Clay (g kg <sup>-1</sup> )	Silt (g kg <sup>-1</sup> )	Sand (g kg <sup>-1</sup> )	Texture
Sandy loam	0.00 - 0.20	150	60	790	Sandy
	0.20 – 0.40	230	50	720	
	0.40 – 0.60	270	40	690	
	0.60 – 0.80	297	33	670	
	0.80 – 1.00	301	31	668	

**Table 2.** Quality standards established by the power plant.

Variables	Standard
Billets per meter	6 - 7
Gems por meter	15 - 18
Maximum infeasible gems (%)	13%
Billets cover	5 - 8 cm
Furrow depth	26 - 30 cm
Length of billets	40 - 45 cm
Cutting height	4 - 6 cm
Number of failures (>50 cm)	Proposed by Stolf (1986)
High	<10%
Medium	21 - 35%
Low	>50%

**Table 3.** Fit model of data in each variable analyzed.

Variable	Model
Row spacing	$Y' = Y^2$
Furrow depth	$Y' = Y^2$
Total gems	$Y' = \log_e Y$
Viable gems	$Y' = (Y)^{1/2}$
Failures	$Y' = \log_e Y$

parameters for subsequent tests applying the range of experimental data.

## RESULTS AND DISCUSSION

According to the parameters descriptive analysis found in [Table 4](#), the average are close to the median and pre-established for the plant quality control standards, though the distributions of data were asymmetric for all variables at Anderson Darling test. The variable row spacing shows dispersion values (standard deviation and coefficient of variation) classified as low, except for the variable failures.

The asymmetry coefficient for variable spacing and depth values indicated negative nature, indicating a slight

asymmetry in the left elongation curve and concentration data to the right of distribution. The remaining variables obtained moderate positive values having a concentration data distribution to the left and an elongation of the distribution curve to the right. The kurtosis coefficient showed all variables with positive values and leptokurtic distributions due to stretching or thinning of the data available curve (Montgomery, 2009). The results for the variable spacing between grooves and deep grooves corroborated with Marques and Pinto (2013) and Roque et al. (2010), with values from 1.40 to 1.50 and 0.25 to 0.40 m, respectively, for spacing and depth of the grooves.

According to the results obtained by analysis of variance and mean test, according to [Table 5](#), the variables spacing and groove depth did not differ from their average for both planting systems and for the varieties, also, there was no interaction between the different factors corroborating Khedkar and Kamble (2008), where two planters of sugarcane (a full reed and other chopped cane) in Akola, India, finding no change to the spacing and depth of the grooves. For the total gems indicators and viable buds ([Table 6](#)), there was no difference in mean values for the planting and varieties used systems. For the variable failures in the deposition of seedlings, there was significant interaction between the factors cited, resulting in an increase of 8% failure on

**Table 4.** Descriptive statistics for the variables analyzed.

Variable	X	A	$\sigma$	M	Cs	Ck	CV(%)	AD
Grooves spacing (m)	1.48	0.20	0.046	1.50	-0.03	0.10	3.07	2.717 <sup>A</sup>
Depth of grooves (m)	0.30	0.18	0.035	0.30	-0.68	1.21	11.60	2.716 <sup>A</sup>
Total gems (un m <sup>-1</sup> )	16.3	22.0	3.047	16.0	1.69	6.67	18.74	2.499 <sup>A</sup>
Viable gems (un m <sup>-1</sup> )	15.6	18.0	2.737	16.0	0.94	3.42	17.59	1.534 <sup>A</sup>
Failures (%)	25.7	64.0	13.80	22.5	1.02	0.75	53.73	2.048 <sup>A</sup>

X- Average, A- full range,  $\sigma$ - standard deviation, M- median, Cs- asymmetry coefficient, Ck- coefficient of kurtosis, CV (%) - coefficient of variation, AD- Anderson-Darling normality test (A: asymmetrical distribution).

**Table 5.** Analysis of variance and means test for spacing and depth of grooves.

Factors	Row spacing (m)	Depth of grooves (m)
<b>Planting (P)</b>		
Mechanized	1.49 <sup>a</sup>	0.30 <sup>a</sup>
Semi-mechanized	1.47 <sup>a</sup>	0.31 <sup>a</sup>
<b>Variety (V)</b>		
SP813250	1.47 <sup>a</sup>	0.30 <sup>a</sup>
RB835054	1.49 <sup>a</sup>	0.30 <sup>a</sup>
<b>F-test</b>		
P	4.044 <sup>ns</sup>	0.905 <sup>ns</sup>
V	2.379 <sup>ns</sup>	0.036 <sup>ns</sup>
P x V	2.077 <sup>ns</sup>	0.680 <sup>ns</sup>
CV (%)	3.07	11.70

In each column, for each factor, means followed by the same letters do not differ by Tukey test at 5% probability. ns: Not significant, by F-test. CV(%): coefficient of variation.

the mechanized planting system, because in the interaction between the factors planting systems and varieties for the variable failures deposition seedlings, there was split of the data as indicated in [Table 7](#).

There were significant differences among varieties for planting system showing sensitivity to mechanization of variety SP813250 among the 6M's quality factors, machine stands out (components and active organs of the machine) as compared to labor (operating level and experience). In mechanized planting, a larger number of stalks per hectare must be used as compared to semi-mechanized, to obtain a homogeneous distribution of wheels per meter of furrow (Ripoli et al., 2006).

The non-transformed data were subjected to statistical process control (CEP) with the aid of individual control charts tool and mobile range for the variables. According to [Figure 1](#), there was a **stable** performance for the indicator row spacing, where all the points were within the limits specified by the plant, with the exception of semi-mechanized planting system for RB835054 variety that met points with upper limit control, indicating a low quality

for process and influence of special causes. In the mechanized system, both varieties showed stability data. The mobile range cards have greater stability in the mechanized system for variety SP813250. For groove depth ([Figure 2](#)), there was a higher quality of furrow operation, semi-mechanized operation of the mechanized system, explained by Ripoli et al. (2006) by the greater complexity of machine operation because of its dimensions. There was instability in mechanized planting process using SP813250 for the variables total and viable gems, was shown in [Figures 3 and 4](#), because of this greater sensitivity to mechanization, as shown in [Tables 6 and 7](#), within the 6M's, with principle of quality of the process operations (Montgomery, 2009).

For failures in the deposition of seedlings ([Figure 5](#)), the mechanized planting process is stable, but there are larger amplitudes, especially for the variety SP813250. In the semi-mechanized, planting is checked with smaller amplitude, but with the presence of an "outlier" for variety RB835054 featuring semi-mechanized operation as unstable process.

**Table 6.** Analysis of variance and mean test for total gems, viables gems and failures deposition seedlings.

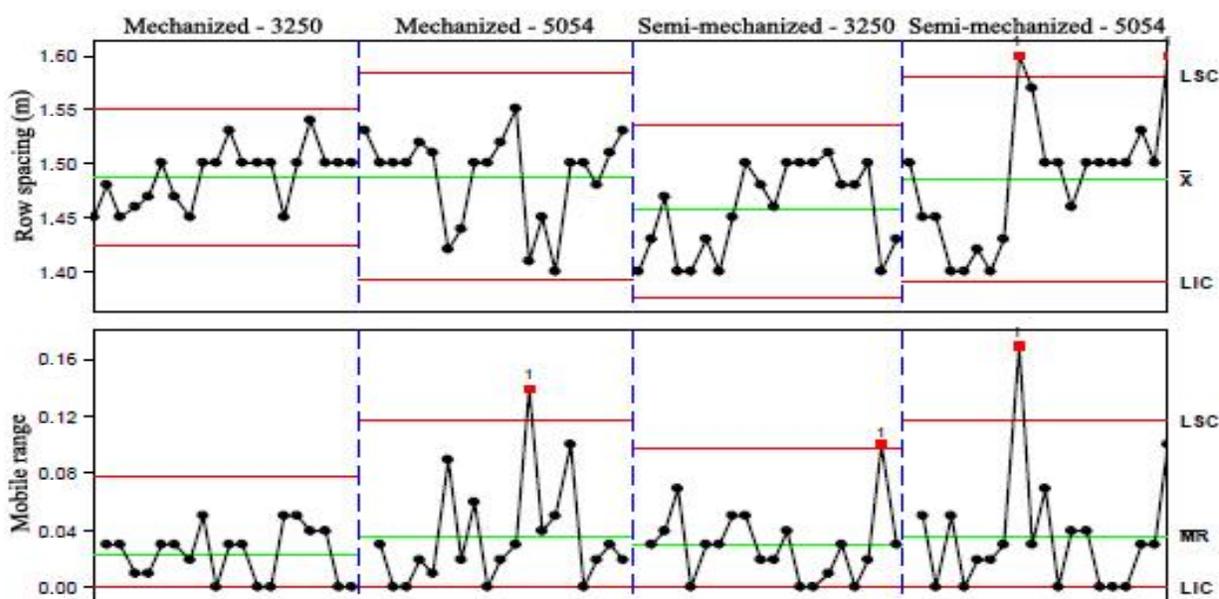
Factors	Total gems m <sup>-1</sup>	Viable gems m <sup>-1</sup>	Failures (%)
<b>Planting (P)</b>			
Mechanized	16.8 <sup>a</sup>	15.9 <sup>a</sup>	29.7 <sup>a</sup>
Semi-mechanized	15.8 <sup>a</sup>	15.3 <sup>a</sup>	21.7 <sup>b</sup>
<b>Variety (V)</b>			
SP813250	16.0 <sup>a</sup>	15.2 <sup>a</sup>	26.4 <sup>a</sup>
RB835054	16.6 <sup>a</sup>	15.9 <sup>a</sup>	25.0 <sup>a</sup>
<b>F-test</b>			
P	2.363 <sup>ns</sup>	0.887 <sup>ns</sup>	8.739*
V	0.744 <sup>ns</sup>	1.222 <sup>ns</sup>	0.260 <sup>ns</sup>
P x V	3.375 <sup>ns</sup>	1.222 <sup>ns</sup>	18.410*
<b>CV (%)</b>	18.74	17.59	53.73

In each column, for each factor, means followed by the same letters do not differ by Tukey test at 5% probability. ns: Not significant, by F-test. CV (%): coefficient of variation.

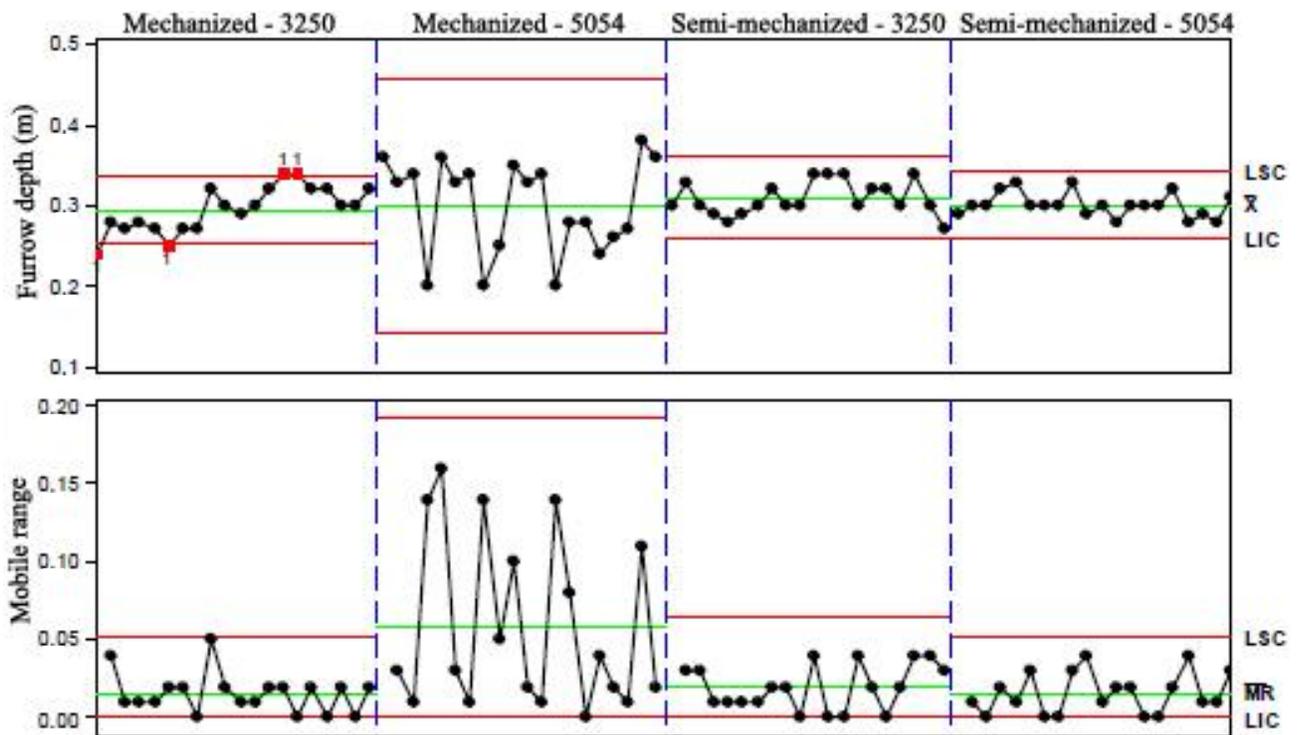
**Table 7.** Split of the interaction between the factors planting and varieties for the variable failures deposition seedlings.

Variety (V)	Planting	
	Mechanized	Semi-mechanized
SP813250	36.2 <sup>Aa</sup>	16.6 <sup>Bb</sup>
RB835054	23.2 <sup>Ab</sup>	26.8 <sup>Aa</sup>

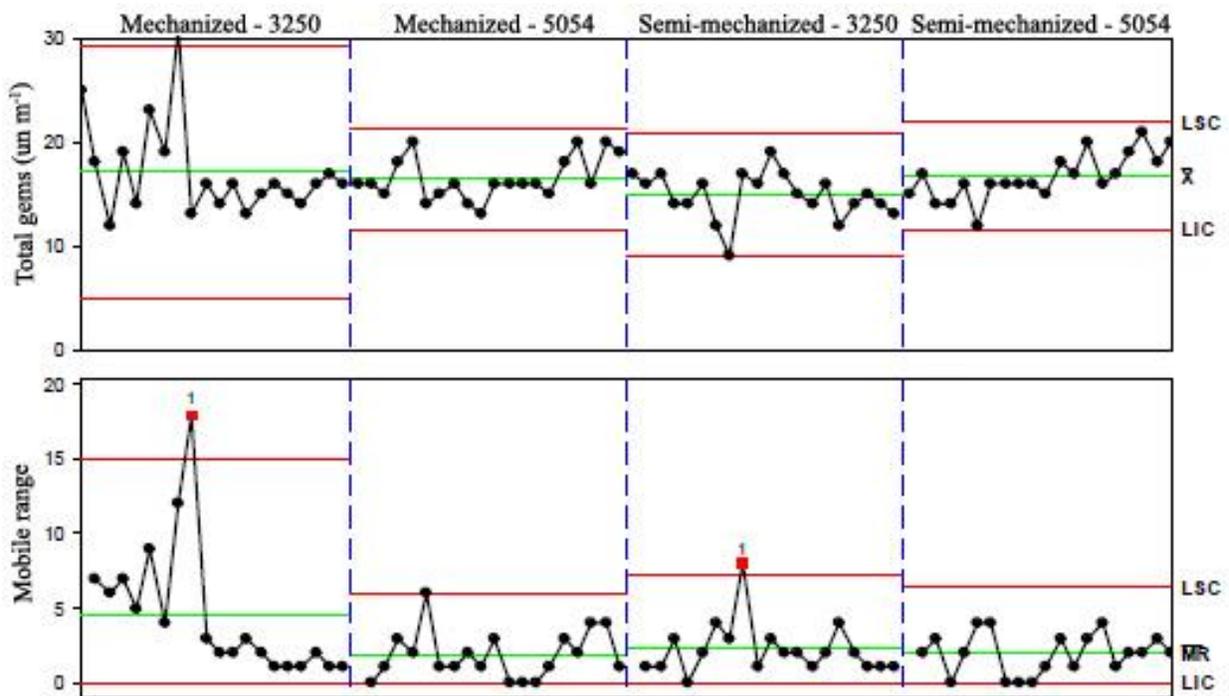
Means followed by the same uppercase and lowercase in rows and columns do not differ by Tukey test at 5% probability.



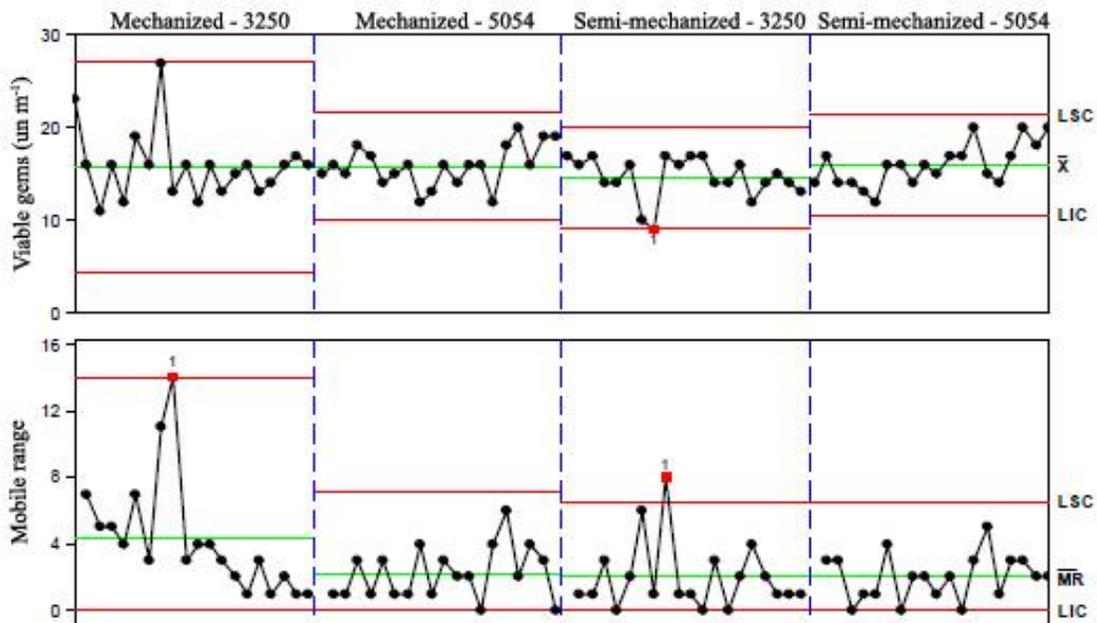
**Figure 1.** Control charts of individual values and mobile range for row spacing. UCL: upper control limit. LCL: Lower control limit. X: average of the individual values. MR: average mobile range.



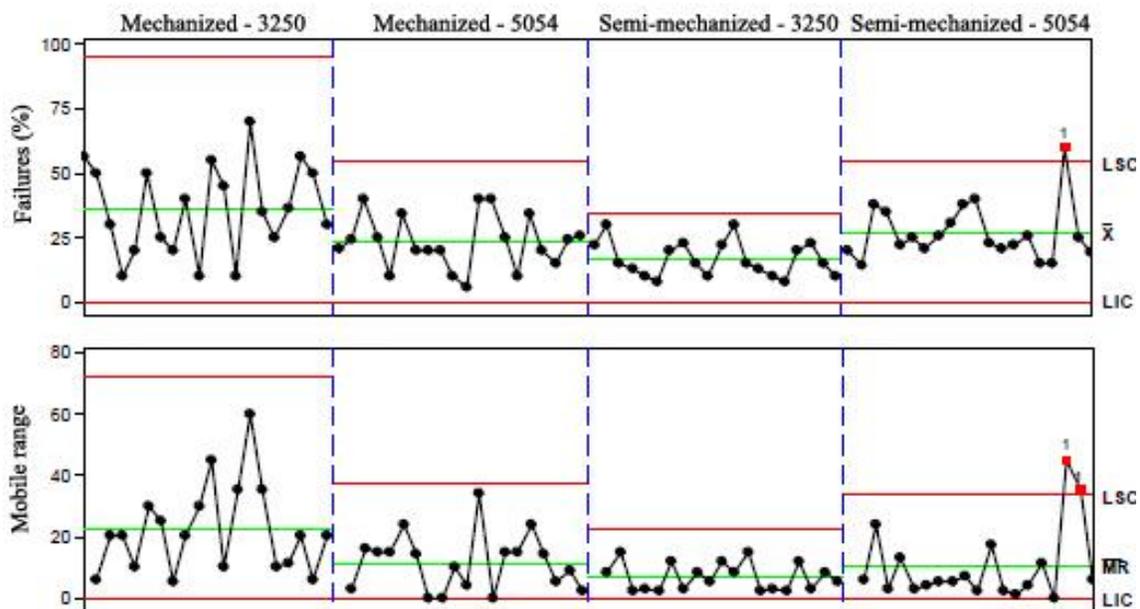
**Figure 2.** Control charts of individual values and mobile range for furrow depth. UCL: upper control limit. LCL: lower control limit. X: average of the individual values. MR: average mobile range.



**Figure 3.** Control charts of individual values and mobile range for total gems. UCL: upper control limit. LCL: Lower control limit. X: average of the individual values. MR: average mobile range.



**Figure 4.** Control charts of individual values and mobile range for viable gems. UCL: upper control limit. LCL: lower control limit. X: average of the individual values. MR: average mobile range.



**Figure 5.** Control charts of individual values and mobile range for failures in the deposition of seedlings. UCL: upper control limit. LCL: Lower control limit. X: average of the individual values. MR: average mobile range.

## Conclusions

The semi-mechanized plantation system had advantages over mechanical when the quality planting operation is

evaluated, specifically for the variables: total gems, gems viable and failures. For furrow depth, the values for the semi-mechanized planting system are shown with great quality, very close to the values required by the power

plant.

For the spacing between rows, in semi-mechanized planting system, some points were outside the control limits in the charts of individual values and are classified as non-acceptable, while the mechanized plantation system was indicated as high quality, showing more advantage. Among the varieties, the later variety (SP813250) in mechanized planting system, showed lower values qualitatively in almost all variables as compared to early variety.

## Conflicts of Interests

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Further studies on Bovine Ixodide Ticks in and around Bedelle, Southwest Ethiopia

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Considering the economic impact of various ticks species on livestock, the present study was conducted for epidemiological characterization of common ticks infesting Ethiopian cattle between November 2013 and March 2014 period at various locations of Bedelle region. A total of 384 cattle were examined on random basis throughout the five months, out of which 231 cattle were found to be infested with ticks (60.12%). On the basis of morphological studies, a total of 2108 ticks belonging to three genera (that is, *Amblyomma*, *Boophilus* and *Rhipicephalus*) were collected during the study period. The species of ticks encountered in this study comprise of *Amblyomma coherence* (32.97%), *Amblyomma gemma* (4.6%), *Amblyomma lepidum* (3.7%), *Amblyomma variegatum* (3.98%), *Rhipicephalus (Boophilus) decoloratus* (31.87%) and *Rhipicephalus evertsi evertsi* (22.87%). Furthermore, the present study showed a significant difference in the prevalence of tick infestation among the different age groups with higher prevalence in cattle with age >6 years (79.07%), whereas no statistically significant association was observed among breed, sex groups and different localities of the studied region ( $P > 0.05$ ). Additionally, the results indicated that the favorable predilection sites of *Amblyomma* species were axilla, scrotum, udder, and belly/groin, while adult *R. evertsi-evertsi* had a strong predilection for tail as well as ano-vulva areas. Further studies on factors affecting tick burden and tick control strategies are recommended.

**Key words:** Bedelle, cattle, prevalence, tick species.

## INTRODUCTION

Ethiopia is well known for its cattle population. Moreover, the general epizootiological factors make it a favorable hub for various parasitic diseases which are a global problem and considered as a major obstacle in the health and product performance of livestock. Additionally, cattle play a significant role in the socio-economic life of the people of Ethiopia. In addition to the products of meat

and milk, cattle provide draught power for cultivation of the agricultural lands of many peasants. Skins and hides are also important components of the livestock sector in generating foreign export earnings.

In Ethiopia, ectoparasites in ruminant cause serious economic losses to small holder farmers, the tanning industry and country as a while through mortality of

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animals, decreased production, downgrading and rejection of skin and hide (Tiki and Addis, 2011). From the ectoparasites, ticks are ranked as the most economically important of livestock in tropics including sub-Saharan Africa (Jongejan and Ulienberg, 2004). Ticks are small, wingless ectoparasitic arachnid arthropods that are cosmopolitan and prevalent in warmer climates (Olwoch et al., 2009).

Ectoparasites, mainly ticks, play an important role in all species of domestic animals and pose a wide range of health problems that confront the productivity of animal, birds and reptiles worldwide (Rajput et al., 2006).

Moreover, ticks either cause direct losses through tick worry, blood loss, damage to hides and udders, toxin production and body weight loss (Stachurski et al., 1993; Kaufman et al., 2006; Marufu, 2008) or indirectly through transmission of bacterial, viral and protozoan infections, predisposing for secondary disease condition such as screw-worm myiasis and dermatophytosis (Mtshali et al., 2004; Soulsby, 2006; Kaufman et al., 2006; Marufu, 2008) reduction in milk yield and stunted growth (FAO, 2004). More significantly, ticks transmit diseases from infected cattle to healthy ones. Ticks transmit a greater variety of pathogenic micro-organisms than any other arthropod vector group, and are among the most important vectors of diseases affecting animals (Jongejan and Ulienberg, 2004). A single female engorged tick imposes a daily loss of 0.5 to 2 ml of blood, 8.9 ml of milk and 1 g of body weight (Minjauw and McLeod, 2003; Soulsby, 2006).

Annual worldwide losses due to tick infestation and diseases transmitted by ticks have been estimated to be 18 billion US\$ (de Castro, 1997). Furthermore, the costs associated with maintaining chemical control of ticks in tropical and subtropical regions of the world have been estimated at US\$25.00 per head of cattle per year (Pegram, 2001).

According to Walker et al. (2003) ticks which are considered to be most important to health of domestic animal in Africa comprise about seven genera. Among these genera the main tick genera found in Ethiopia includes *Ambylomma*, sub genus *Rhipicephalus* (*Boophilus*), *Haemaphysalis*, *Hyalomma* and *Rhipicephalus*. The genus *Ambylomma* and *Rhipicephalus* are predominating in many parts of country, *Hyalomma* and sub genus *Rhipicephalus* (*Boophilus*) also have significant role (Solomon et al., 2001).

Due to economic and veterinary importance of ticks, their control and transmission of tick born diseases remain challenge for the cattle industry of the world and it is a priority for many countries in tropical and subtropical regions (Lodos et al., 2000).

However, it is essential to have up-to-date information on the importance of ticks in various ecological zones as this provides an option to develop cost effective ecologically sound tick control strategy. Therefore, the

present study was undertaken to know the prevalence of ticks in relation to the different age groups, sex, breeds, and localities of the animals, sites of their attachment and identification of ticks up to species level.

## MATERIALS AND METHODS

### Study area

The study was conducted in Southwestern Ethiopia, Ilu Ababora zone, Bedelle district of Oromia regional state, which is 480 km from Addis capital city of the country. Bedelle is located at elevation of 2060 m above sea level and 8-9°N latitude and 36-37°E longitude. The area receives annual range of rainfall from 1800 to 2050 mm and mean annual temperature varies from 20 to 25°C from an October to January and decline to level of 15 to 25°C during the rest of the months (CSA, 2010). The district has resident of 36,945 people, 107,446 heads of cattle (21,061 cows, 15,633 oxen, 10,810 bulls, and 10,562 calves), 23,607 heads of sheep, 24,192 heads of goats, 8,134 equine and 48,400 heads of poultry. The total area of Bedelle Woreda is 74,600.01 km<sup>2</sup>, from this 29,449.25 km<sup>2</sup> is used for cultivation, 1,960 km<sup>2</sup> for forest, 1,440 km<sup>2</sup> for grassland and 41,750.76 km<sup>2</sup> is used for others (BWAB, 2006).

### Study design and study population

A cross sectional study was conducted from November 2013 to March 2014 on cattle brought to Bedelle Veterinary Clinic. Weather condition was Bega which typically occurs between October and January, and is characterised by generally dry weather and followed by short rainy season, known as the Belg, which runs from February to May. Both local and cross breeds of cattle with all age groups in both male and female animals coming to Bedelle Veterinary clinic were examined for presence of adult tick.

### Sampling technique

For the collection of data a random sampling technique (Thrusfield, 2007) was applied to gathered the data on tick prevalence from ten districts of the Bedelle. All the information about the date, host, age, and locality were entered on the label of each container.

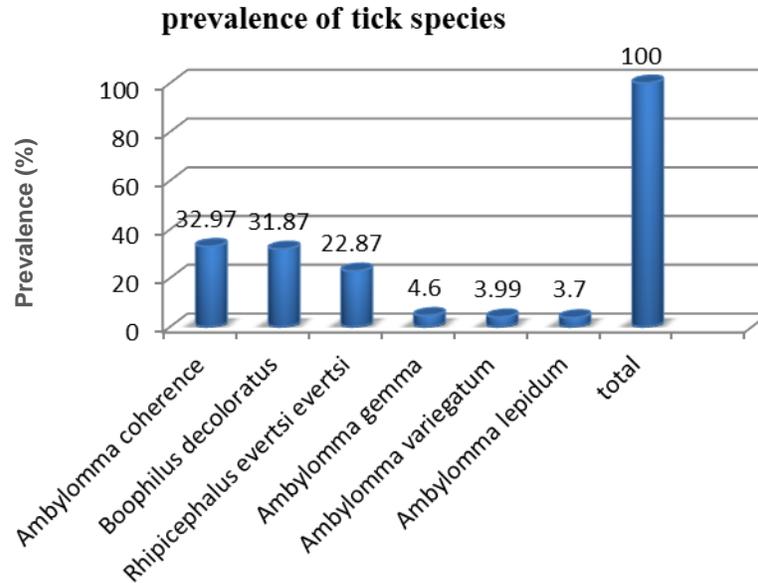
The sample size was decided based on the formula described by Thrusfield (2005) with 95% confidence interval at 5% desired absolute precision and assuming the expected prevalence of 50%.

$$N = 1.96^2 (P_{exp}) (1 - P_{exp}) / D^2$$

where N=sample size required, P<sub>exp</sub>=expected prevalence (50%), and D=desired level of precision (5%); therefore, the sample size was 384 cattle.

### Specimen collection

Before collection of ticks, animals were restrained properly and their whole body was thoroughly inspected visually for the presence of tick. Tick specimens were collected following the procedure of Ica et al. (2007). Ticks were gently plucked up from the body of the host by hand manipulation or with the aid of blunt pointed forceps without damaging their mouth parts. The specimens were kept in separate plastic containers with ventilated cap according to the sites of attachment. These samples were transported to the



**Figure 1.** Prevalence of tick species in the study area.

laboratory of Bedelle Regional Veterinary Laboratory for identification.

#### Mounting and identification of ticks

Following the methods of Soulsby (2006), the tick specimens were mounted and identified using standard morphological tick identification keys (Walker et al., 2003) under stereomicroscope.

#### Data analysis and management

The data recorded was entered into Microsoft excel database system and statistical analysis was done by STATA statistical software (STATA corp, 2005). The prevalence rate was calculated by dividing the proportion of cattle infested with tick by the total number of animal examined and multiplied 100. The risk factors for infestation by tick are investigated using percent values and Pearson Chi-square ( $\chi^2$ ). A P-value less than 0.05 at 95% confidence intervals was considered for significance.

## RESULTS

### Prevalence of tick infestations

Out of the total 384 animals examined, 231 (60.12%) were found to be infested with at least a single and/or different species of ticks. From the total of 2108 ticks collected, two genera, one sub genus and six species were identified. Of which *Amblyomma* accounts for 954 (45.25%), sub genus *Rhipicephalus* (*Boophilus*) 672 (31.87%) and *Rhipicephalus* 482 (22.86%). *Amblyomma coherence* (32.97%), *Amblyomma gemma* (4.6%), *Amblyomma lepidum* (3.7%), *Amblyomma variegatum* (3.98%), *Rhipicephalus* (*Boophilus*) *decoloratus* (31.87%)

and *Rhipicephalus evertsi evertsi* (22.87%). *A. coherence* was the highest species collected and *A. lepidum* is the least species encountered (Figure 1).

### Abundance of ticks

Tick were collected from 10 parts of animal namely axilla, anovulval, back/flank, dewlap/neck, head, groin/belly, under tail, leg, udder and scrotum. Different species of ticks found to prefer different predilection sites where *Amblyomma* found most predominately in the axilla, groin/belly, and udder, scrotum whereas, sun genus *Rhipicephalus* (*Boophilus*) found abundantly around dewlap, back and head but also found on the rest of the body and *Rhipicephalus* found predominating around the smooth skin (under tail, anovulval) areas of examined animals (Table 1).

During the study, the collected ticks were identified as male and female. The proportion of male ticks was found higher than its counterpart except for *R. (Boophilus) decoloratus* (Table 2).

### Prevalence of tick infestations in relation to age groups, sex, breed, and localities

Among breed and between sex and breed group of animals examined, infestation was found to be statistically insignificant ( $P > 0.05$ ), whereas infestation was found significant between age groups ( $P < 0.05$ ). Infestation shows no significant association among different localities of animal origin in Bedele district ( $P > 0.05$ ) (Table 3).

**Table 1.** Distribution of tick species in different body parts of cattle.

Attachment site	<i>A. coherence</i>	<i>A. gemma</i>	<i>A. lepidum</i>	<i>A. variegatum</i>	<i>R. (B) decoloratus</i>	<i>R. evertsi evertsi</i>	Overall (%)
Scrotum	187	27	13	-	72	-	299 (14.18)
Udder	243	18	14	-	21	-	296 (14.04)
Dewlap/Neck	45	17	13	-	305	-	380 (18.03)
Belly/Groin	108	17	15	49	33	-	222 (10.53)
Axilla	81	18	12	25	36	-	172 (8.16)
Anovulva	31	-	11	-	36	21	99 (4.7)
Under tail	-	-	-	-	-	461	461 (21.87)
Head	-	-	-	-	62	-	62 (2.9)
Back/Flank	-	-	-	-	81	-	81 (3.8)
Leg	-	-	-	10	26	-	36 (1.7)
Total	695	97	78	84	672	482	2108 (100)

**Table 2.** Sex ratio of Major tick species in the study area.

Tick species	Male	Female	Sex ratio (M:F)	Total
<i>Ambylomma coherence</i>	419	276	1.58:1	695
<i>Ambylomma gemma</i>	76	21	3.62:1	97
<i>Ambylomma lepidum</i>	45	33	1.36:1	78
<i>Ambylomma variegatum</i>	54	30	1.8:1	84
<i>Rhipi(Boophilus) decoloratus</i>	173	499	0.34:1	672
<i>Rhipicephalus evertsi evertsi</i>	307	175	1.75:1	482
Total	1074	1034	1.06:1	2108

## DISCUSSION

From the total 384 examined for the presence of tick species, 231 infested with one or more tick species yielding an overall prevalence of 60.12% and this finding is in agreement with the findings of Wasihun and Doda (2013) with prevalence of 61% in Southern Nations, Nationalities, and Peoples' Region (SNNPR), Ethiopia. However, it is different from the findings of Nigatu and Teshome (2012) who reported an overall prevalence of 89.4% in Amhara regional state, this difference may be due agroecology of the study conducted. From the total of 2108 ticks collected two genera, one sub genus and six species were identified. The distribution and abundance of tick species infesting cattle in Ethiopia vary greatly from one area to another area (Yitbarek, 2004; Abera et al., 2010; Abunna et al., 2012; Fanos et al., 2012; Ayana et al., 2013). The distribution limits of ticks are determined by a complex interaction of factors such as climate, host density, host susceptibility and grazing habits (Minjauw and de Castro, 2000). The study was limited to adult ticks for the reason that they are more visible, relatively easier to collect and believed to be the most important ectoparasite stage in causing reduced productivity in cattle (Regassa, 2001).

Accordingly, from the total of 2108 ticks collected three genera and six species were identified, *A. coherence* was found to be the most abundant tick species (32.97%) in the present study. The present study agrees with the result of Fanos et al. (2012) in Mizan Teferi that *A. coherence* was the abundant tick species and Yitbarek (2004) in Jimma was found to be the most prevalent in the area with a prevalence of 50.5 and 83.1%, respectively. In the same study conducted on ruminants showed that species of ticks encountered comprise of *A. coherence* (44.1%) which is the highest of all species encountered which make agreement with the current study (Abera et al., 2010), but decreasing now. This report disagrees with result in Asella with prevalence of *A. coherence* 11.9% (Tesema and Gashaw, 2010). *A. coherence* is the most prevalent and abundant tick on cattle (Pergam et al., 1981). According to de Castro (1994), *A. coherence* is the most abundant tick species infesting cattle in Southwestern Ethiopia (Yitbarek, 2004; Fanos et al., 2012). It has been recorded in areas between 1,750 and 2,500 m above sea level receiving annual rainfall ranging between 750 and 1,500 mm (Sileshi et al., 2007). This tick prefers high altitudes and moist climate and is absent in the drier regions. The occurrence of this tick in western Ethiopia has also been

**Table 3.** Tick burden within group of sex, breed, age and localities.

Risk factors	Total number of examined animal	Number of infested animal	P-value	$\chi^2$
<b>Sex</b>				
Male	126	82(65.07%)	0.169	1.896
Female	258	149(18.99%)		
<b>Breed</b>				
Local	374	224(81.75%)	0.523	0.455
Cross	10	7(70%)		
<b>Localities</b>				
Kebele 01	82	46 (56.09%)	0.263	10.022
Kebele 02	82	42 (51.12%)		
Bitamute	29	17 (58.62%)		
Ilke kerero	36	26 (72.22%)		
Lalistu	29	21 (72.41%)		
Oddoo	50	33 (66%)		
Secho	19	13 (68.42%)		
Siddisa	38	20 (52.63%)		
Urgessa	19	13 (68.42%)		
<b>Age</b>				
<1	82	17 (20.73%)	0.000	72.039
1-3	87	54 (62.07%)		
3-6	172	172 (73.26%)		
>6	43	34 (79.07%)		

\*P>0.05 for sex, breed and localities, whereas P<0.05 for age group, Loca= zebu breed. Cross= Zebu with Holstein Friesian.

recorded by other workers (Ali and de Castro, 1993; Sileshi et al., 2001). This abundance variation is possibly due to the difference in ecological, climatic, animal breed, farming practice, etc., in study areas.

This study has also revealed *R. (Boophilus) decoloratus* was the second most abundant tick species in the region (31.87%). As studied by other authors in Assosa, higher abundance of *R. (Boophilus) decoloratus* was reported with prevalence of 45% (Fantahun and Mohammed, 2012) which is in agreement with this result. Similar result was also reported in Haramaya (Kassa and Ayalew, 2012) with the result of 31.54% and in Southern Nations, Nationalities, and Peoples' Region (SNNPR), Ethiopia reported that the prevalence of *R. (Boophilus) decoloratus* was 30% which the highest from the tick species collected from the study area (Wasihun and Doda, 2013). The basic reason for this difference could be possibly accounted to the weather; environment and agro-ecology of the study area that might not be conducive to its reproduction and survival.

*R. evertsi evertsi* was the third abundant (22.8%) tick species in the present study and the result of the current research was in line with Tesema and Gashaw (2010) in

Assela, Husen (2009) in Bako and Wasihun and Doda (2013) in Humbo districts of SNNPR with prevalences of 22, 21.5 and 25%, respectively. This finding disagrees with result of study conducted in Mizan with prevalence of 3.6% (Fanos et al., 2012) and in Haramaya with prevalence of 5.5% (Bedasso et al., 2014). Pegram et al. (1981) described its wide distribution throughout the Ethiopian faunal region. This tick species is reported by different other authors result such as in Bahir Dar (Mesele, 1989) and in Holeta (Tiki and Addis, 2011), Morel (1980) confirmed that the native distribution of *Rhipicephalus evertsi evertsi* in Ethiopia seems to be connected with middle height dry savannas, bamboo and steppes in association with ruminant. This tick is the most abundant species with no marked seasonality (Yehulashet et al., 1995; Tesema and Gashaw, 2010). This tick species shows no apparent preference for particular altitude, rainfall zones or seasons (Morel, 1980).

*A. gemma* was the fourth tick species in this study with the prevalence of (4.60%). This result make agreement with report from Mizan (Fanos et al., 2012) with prevalence of 8.3% and disagreements with the results in

Borena Pastoral area by Ayana et al. (2013) with prevalence of 23.64% and in Somali region (Rahmeto et al., 2010) with 19.1%. Morel (1980) regards this species as not abundant in Ethiopia. Most of the previous studies conducted in the country revealed, as the distribution of *A. gemma* was clearly associated with dry types of vegetation or semi-arid rangelands and in lowland areas (Morel, 1980; Rahmeto et al., 2010; Ayana et al., 2013). Morel (1980) stated that *A. gemma* widely distributed in woodland, bush land, wooded and grassland in arid and semiarid area between altitude 500 to 1750 m above sea level and receiving 350 to 750 mm annual rain fall. The abundance of *A. gemma* as compared to other species may also perhaps be due to their host seeking and invasion properties, behavior and their adaptation for wide varied environment. Latif and Walker (2004) reported that tick populations in any particular environment, with a particular type of animals and management system; the average size fluctuation of tick populations depends partly upon the properties of ticks themselves; it might be host seeking.

*A. lepidum* is the least species of tick encountered during this study period (3.7%). This result agrees with the result reported in Northwest Ethiopia with prevalence of 1.95% (Moges et al., 2012) and in Borena Pastoral area (Ayana et al., 2013) with prevalence of 1.72%. In the present study, the prevalence of *A. lepidum* was very low in comparison to the reports of Sileshi et al (2001) who had reported this species of tick to be common in northeast of Ethiopia. This may be associated with the differences in agro ecological factors. Pegram et al. (1981) found *A. lepidum* throughout the 500 to 2000 m altitude zone and the 250 to 1000 mm rainfall zone and rare in wetter areas. However, the present study area agroecology was with altitude of 2060 m.a.s.l and receive 1800 to 2050 mm rain fall.

Ticks are known to be distributed in different parts of the host body. In this study, ticks were collected from different part of animal and rate of tick infestation differ from site to site. The predilection site mentioned in the result of this study was similar to with those reported by other authors (Okello-Onen *et al.*, 1999). *Amblyomma* species found on scrotum, udder, belly/groun, dewlap and anovulval areas, whereas *R. (Boophilus) decoloratus* species were found around neck/dewlap, udder, head, leg and scrotum. *R. evertsi evertsi* showed high preference to the anogenital region of the body, this is similar with report of Tiki and Addis (2011) and Bedasso et al. (2014). The predilection sites found in this study were also in line with those reported by Siyoum (2001) and Behailu (2004) in their study conducted in North Wollo zone and Asella, respectively. Factors such as host density, interaction between tick species, time and season (Kettle, 1995) and inaccessibility for grooming determine the attachment site of ticks (Chandler and Read, 1994). Information on predilection sites of ticks is helpful in spraying individual animals since it gives a clue

as to which part of the body requires more attention (Pegram et al., 1981).

The sex ratio of all tick species identified during this study periods were skewed towards male except (Table 3) for *R. (Boophilus) decoloratus*, this condition is due to the small size of males of *R. (Boophilus) decoloratus* make it difficult to see and get missed during collection. The high number of male tick of other species may be due to the fact that substantial proportion of females may be engorged in few days and fall on the ground in short period of time as compared to males. Therefore, this study was in agreement with the finding of Mekonnen et al. (2001) who also suggested that engorged females may be removed by self-grooming of the host, because of the large size (Solomon et al., 2001; Abebaw and Tamiru, 2010).

The prevalence of infestation was relatively similar ( $P=0.523$ ,  $\chi^2=0.512$ ) in two breeds (Local, Zebu, Cross, Zebu and Holstein Friesian) of cattle. It was 81.75% ( $n=224$ ) in local breeds and 70% ( $n=7$ ) in cross breed cattle. The relative higher prevalence of infestation in local breed cattle compared to the cross breed may be due to management practice. Most of the time cross breed and exotic animals got attention and treated more frequently than indigenous ones. This result is in agreement with the result of in and around Mekele (Yacob et al., 2008; Hilina et al., 2012), and Mulualem (2009), in and around Debre Zeit. My finding is in disagreement with that of Tamiru and Abebaw (2010) in and around Asella town, and Esihak (2011) in Adami Tulu. This variation may be due to the indigenous Ethiopian cattle breeds which were different from breed to breed.

There is statistically significant difference ( $p=0.000$ ) in infestation rate among different age groups of cattle. The old and adults are more susceptible than calves due to the fact that the calves are not often driven with adult age groups into grazing and watering points. This practice naturally reduces the chance of exposure of calves to ticks. This result agrees with that of Yismashewa (2005), in Decha woreda, Southern Ethiopia and Esihak (2011) in Adami Tulu. Similarly, Seyoum (2001) also found that the number of ticks attached to animals increases with their age. Since host seeking activity involves awaiting hosts in an environment, there is greater chance of attaching on larger animals than calves because of body surface area. Calves are less attractive to ticks than cows because they are protected by some form of innate, age related resistance (Okello-Onen et al., 1999).

The result of the present study revealed that the prevalence is getting decreased as compared to the previous, for example the studies conducted in Bedelle, the same with present study in 2010 and 2012 reported that the prevalence of *A. coherence* were 41.1 and 35.25% on ruminants and small ruminant, respectively (Abera et al., 2010; Abunna et al., 2012). This is probably due to an increase in the level of awareness of the

farmers on how to reduce the tick infestation of their cattle, improvement in the management of their animals and increase of veterinarians' number per district regular treatment with acaricides.

The attention given to the infestation had not been sufficient and lack of available information on tick species makes it difficult to determine their impact that there were little attempts made to control the infestation of tick. However, the attentions given to the infestation were not sufficient and the lack of available information on tick species and the demerits behind tick infestation aggravates the infestation of the livestock population in the area by ticks. Acaricide application is the main method of tick control in the region, these suggested that strategic deepening programs using acaricides should be applied to control tick population on animals. In general, the distribution limits of ticks are not fixed but are determined by a complex interaction of factors such as climate, host susceptibility and grazing habits.

Therefore, further studies on the distribution of tick species and their epidemiology are necessary for the continuous understanding of improved control strategies.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Effects of genotype on yield and yield component of soybean (*Glycine max* (L) Merrill)

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In 2013, the multi-location trial was implemented to evaluate the new soybean genotypes for their agronomic performance against the local check. The experiment was conducted in three locations namely Ilonga, Kibaha, and Mlingano in each location a triplicated trial involving six genotypes of soybeans were implemented. The effects of genotype, location and genotype x environment interaction under combined analysis on agronomic yield, and soybean yield were found significant at  $P < 0.05$ . The highest mean yield was found from TGX 1954-1F and TGX 1908-8F in all locations. Correlations coefficient for seed yield revealed a positive and significant association with all agronomic yield except 100 seed weight in all locations. The phenotypic coefficient of variation and genotypic coefficient of variation estimates were significantly high for pods per plants (49.49/27.04), while crude protein had the lowest values (1.45/0.98). The finding also revealed that the differences between phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV) were significantly lower for crude protein (0.45), followed by pod length (1.45) and 100 seed weight (2.6). The result suggests that the environment had less effect on the expression of these traits. Therefore, selection based on these traits might increase soybeans performance in all locations. The findings have demonstrated the stability of traits in different locations which is a useful information in soybean breeding programs. TGX 194-1F and TGX 1908-8F were genotypes with high crude protein content, and revealed stable performance across the three environments. TGX 1987-10F, TGX 1987-20F and TGX 1910-14F had better performance compared to Bossier.

**Key words:** Soybeans, genotype, yield component, yield.

## INTRODUCTION

Soybean (*Glycine max* (L) Merrill) is a legume produced worldwide, and its production has increased from 17

million metric tonnes in 1960 to 230 million metric tonnes in 2008 (Hartman et al., 2011).

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Soybeans cultivation has been increasing every year due to population demand, it is estimated that 6% of world's arable land is under soybean production (Aditya et al., 2011). The crop enjoys global acceptability because it grows well in a wider range of agro-ecological zones ranging from tropical and subtropical to temperate climate (Malik et al., 2007).

Apart from ecological adaptability, soybean is of choice by many farmers due to its high nutritional qualities including protein 35%, oil 19%, carbohydrate 35%, minerals 5% and vitamins (Bueno et al., 2013, Dixit et al., 2011, Popovic et al., 2013). The demand for cheap oil and protein is increasing annually to match the growing world population (Hartman et al., 2011). These nutritional values from soybeans are important to human being especially to resource-poor families, who cannot afford expensive sources of protein from meat, fish, and eggs (Aditya et al., 2011, Taira, 1990).

In addition, the soybean hull contains approximately 65% dietary fibre and offers a good source of fibre when used in various food applications (Shogren *et al.*, 1981). Soybean is also environmentally friendly in a sense that it serves in soil conservation by reducing soil erosion. Not only that but also whether under monoculture or intercropped, Soybean plays a central role replenishing soil fertility due to its legume bacterial symbiotic relationship (Bekele and Alemahu, 2011; Di Mauro et al., 2014; Gibson, 2015; Singh and Shivakumar, 2010).

In Tanzania, soybean is grown by smallholder farmers, and production varies between regions (Malema, 2005). High production has been recorded in Southern highland regions of Mbeya, Iringa and Ruvuma with an average of 900,000 kg per year (Wilson, 2015), whereas the lowest annual production (230,000 kg) have been reported in Eastern zone regions of Dar-es-salaam, Coast, Tanga, and Morogoro. Global soybean production in 2015/16 is currently forecast at 314 million tonnes (Hallam et al., 2013).

In Tanzania, soybean production is still far below the world average (Malema, 2005). The low production in Tanzania can partly be due to low yielding genotype and unfavourable environmental factors particularly erratic rains and diseases. In the eastern agro-ecological zones, the loss of genetic diversity like *3H/1* and *Bossier* soybean varieties which were previously adapted (Malema, 2005) and the outbreak of the disease (Oerke, 2006, Tukamuhabwa et al., 2012) could be an important constraint to soybean production in the region.

Breeding for new high yielding soybean genotypes that can withstand harsh climate, diseases resistant across wide agro-ecological zones of Tanzania has been a priority research agenda over years. Development of new genotypes has involved the introduction of proven high yield varieties from other research centres around the world. Alternatively breeding for preferred traits using locally available genetic resources has been an ongoing process. Recently in Tanzania, the agricultural research

institute (ARI) Ilonga has introduced new soybean varieties from IITA research centre based in Malawi.

Together with these, the breeding program at the station has developed three soybean lines which are in different stages of evaluation before they can be declared new varieties.

It is one of the procedure that both newly developed and introduced genotypes have to be evaluated for their agronomic performance against the existing local check before they can be considered new varieties for commercial production or further improvement. The two varieties from IITA and the three soybean lines from Ilonga have never been evaluated.

The objective of this study, therefore, was to evaluate the new soybean genotypes for their agronomic performance. The study specifically aimed at determining the differences between genotypes in terms of their yield and yield components. Secondly, the study estimated the genetic parameters based on eleven characters of soybean genotypes. Lastly, for each genotype, the study established the relationships between yield and yield components. Results from this study are important as a basis for a successful future breeding program and increasing soybean yield in the country.

## MATERIALS AND METHOD

### Location and experimental design

Three locations within eastern agro-ecological zones were used as experimental sites. The names of the locations are Ilonga (06° 7'S 37° 38' E, 506 m.a.s.l), Mlingano (05° 9' S 38° 54' E, 183 m.a.s.l), and Kibaha (06° 46' S 30° 55' E, 162 m.a.s.l). The research was implemented during 2013 growing season from March to July. Average rainfall, maximum, minimum and mean temperature is presented in Table 1.

Six genotypes (Table 2) were evaluated for grain yield in three growing environments varying mainly in their monthly rainfall averages (Table 1). A variety called *Bossier*, a released was used as a local check as it has been grown in eastern agro-ecological zone since 1978 (Malema, 2005).

The experiment was laid out in a randomised complete block design with three replications in each location. The plot size was 2.5 m x 2 m while the spacing used was 50 cm x 10 cm between and within the rows respectively. Each treatment was sown in five rows per plot. Data were collected from the net area of 1.5 m x 1.8 m of each plot excluding two border rows. The harvested net plot area was 2.7 m<sup>2</sup>.

All agricultural practices recommended for soybeans production were applied during the course of experimentation in all 3 locations. Before maturity, all the agronomical yield components traits (days to 50% flowering, days to 95% maturity, plant height, number of pods per plant, number of seeds per plant) were recorded and at maturity, ten plants were randomly collected from each sub-plot to measure quantitative traits for example, seed weight per plant (g). Seed yield (t ha<sup>-1</sup>) was calculated based on the plot area.

### Data analysis

STATISTICA version 10 was used to compute Analysis of variance (ANOVA) for bean yield, yield components, and crude protein

**Table 1.** Monthly meteorological data of the test locations during the 2013 growing season.

Location	Variable		Month				
			March	April	May	June	July
Mlingano	RF	-	111.90	137.20	123.40	23.50	25.50
	Temp	Max	33.20	31.50	29.80	28.90	29.30
	-	Min	24.30	23.90	22.40	20.80	20.30
	-	Mean	28.80	27.70	26.10	24.90	24.80
Ilonga	RF		292.10	132.50	117.70	1.00	9.00
	Temp	Max	32.40	30.60	29.20	28.20	28.50
	-	Min	22.60	21.90	20.30	16.90	16.50
	-	Mean	27.50	26.30	24.80	22.60	22.50
Kibaha	RF		282.30	140.50	36.60	3.20	1.60
	Temp	Max	33.30	31.20	29.90	29.60	29.50
	-	Min	32.30	30.40	29.60	21.10	19.80
	-	Mean	32.80	30.80	29.80	25.40	24.70

Source: National meteorological agency, Eastern zone, SUA branch; RF = Rainfall (mm), Temp = Temperature (°C), Max =Maximum temperature; Min = Minimum temperature.

**Table 2.** List of 6 soybean genotypes evaluated in three growing environments (Mlingano, Kibaha, and Ilonga).

Entry	Genotype	Source	Status
1	TGX 1987-10F	IITA-Malawi	Improved
2	TGX 1987-20F	IITA-Malawi	Improved
3	TGX 1954-1F	ARI- Ilonga	Line
4	TGX 1908-8F	ARI- Ilonga	Line
5	TGX 1910-14F	ARI-Ilonga	Line
6	BOSSIER (Local check)	ARI- Ilonga	Improved

content, data were subjected to ANOVA separately for each location and over combined locations. The statistical model applied for this ANOVA are:

**Single location**

Single location analysis was carried out as described by Gomez and Gomez (1984) for randomised complete block design (RCBD).

$$Y_{ij} = \mu + B_i + G_j + \epsilon_{ij} \tag{1}$$

Combined location analysis

$$Y_{ijk} = \mu + B_i + G_j + L_k + GL_{jk} + \epsilon_{ijk} \tag{2}$$

Where,  $Y_{ijk}$  = observed value of genotype j in block i of location k,  $\mu$ =grand mean,  $B_i$ =block effect,  $G_j$ =effect of genotype,  $L_k$ = Location effect,  $GL_{jk}$  = the interaction effect of genotype j with location k,  $\epsilon_{ijk}$  = error (residual) effect of genotype j in block i of environment k. Means among each character were compared by least significant difference (LSD) test at 5% levels of significance.

The combined component of variance and correlation coefficient was calculated as described by Al-Jibouri et al. (1958). The observed mean squares obtained in the combined ANOVA was

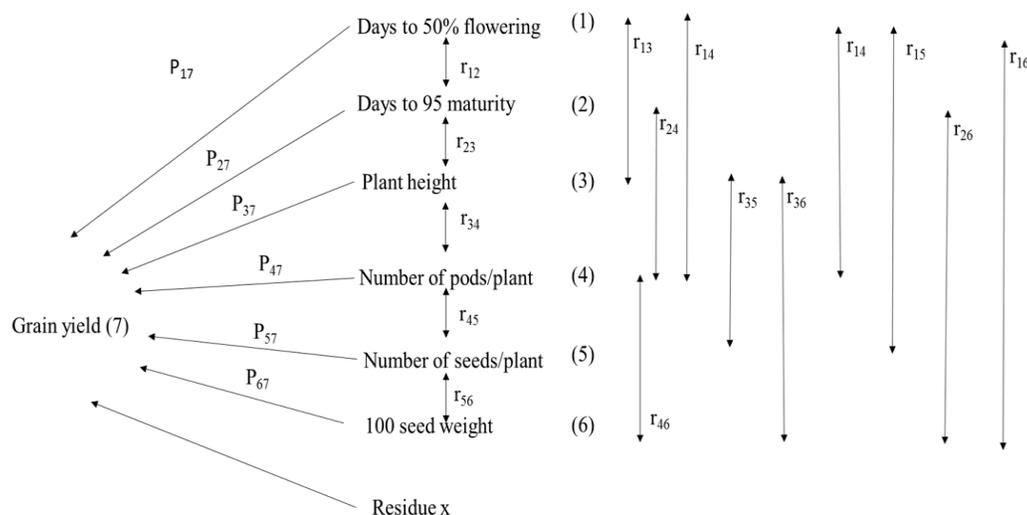
used to separate out the effects of genotype, environments, and their interaction. Path coefficient analysis (Dewey and Lu, 1959) was used to determine direct and indirect effects of days to 50% flowering, days to 95% maturity, plant height, the number of pods per plant, the number of seeds per plant, 100 seeds weight and grain yield (Figure 1).

The double arrow lines represent the correlation between variables ( $r_{ij}$ ), while the single arrow lines indicate the direct effects of yield component to the soybean yield as measured by path coefficient ( $P_{ij}$ ) (Figure 1). The path coefficient in the present study was calculated based on following equations:

$$\begin{aligned} r17 &= P17+r12P27+r13P37+r14P47+r15P57+r16P67 \\ r27 &= r12P17+P27+r23P37+r24P47+r25P57+r26P67 \\ r37 &= r13P17+r23P27+P37+r34P47+r35P57+r36P67 \\ r47 &= r14P17+r24P27+r34P37+P47+r45P57+r46P67 \\ r57 &= r15P17+r25P27+r35P37+r45P47+P57+r56P67 \\ r67 &= r16P17+r26P27+r36P37+r46P47+r56P57+P67 \end{aligned}$$

The residual factor (PX7) was computed as follows:

$$\begin{aligned} 1 &= P2X5+P217+P227+P237+P247+P257+P267+2P17r12P27+2P1 \\ &7r13P37+2P17r14P47+2P17+r15P57+2P17r16P67+2P27r23P37+ \\ &2P27r24P47+2P27r25P57+2P27r26P67+2P37r34P47+2P37r35P5 \\ &7+2P37r36P67+2P47r45P57+2P47r46P67+2P57r56P67 \end{aligned}$$



**Figure.1** Path coefficient analysis of 7 yield components: double arrow lines represent the correlation between variables ( $r_{ij}$ ), while the single arrow lines indicate the direct effects of yield component to the soybean yield as measured by path coefficient ( $P_{ij}$ ).

In the path model:

$R_{ij}$  = simple correlation coefficients for measuring the mutual association of two variables

$P_{ij}$  = path coefficient for measuring direct influence between variables to yield

$R_{ij}P_{ij}$  = indirect effects of variables upon another through the other variable

$P_x$  = the residue effect in the path analysis model computed as  $1 - P_{2 \times 7}$  i and j = (1, 2, 3, ..., 7).

## RESULT AND DISCUSSION

### Effects of genotypes

The result in Table 3 show that the effects of genotypes on yield and yield component was significant ( $P < 0.05$ ) confirming the previous studies (De Bruin and Pedersen, 2009; Liu et al., 2005; Norsworthy and Shipe, 2005).

In this study, the genotypes TGX 1954-1F and TGX 1908-8F outperformed the local check in all the three locations with the average mean performance of 611.69 and 609.93 kg/ha respectively, while Bossier had the lowest (260.46kg/ha) yield in all locations. Alongside TGX 1987-10F, TGX 1987-20F and TGX 1910-14F yield performance were significantly high than the control (Bossier) in all locations. The low yielding ability of Bossier variety was previously reported by Bonato et al. (2006). The mean performance of the genotypes across the location revealed that TGX 1908-8F had the highest number of seed per plant (66.11), followed by TGX1954-1F (52.00) and Bossier showed the lowest (41.22). TGX1954-1F and TGX 1908-8F had the largest number of pods per plant with 58.11 and 53.11 respectively, and Bossier revealed the least value (31.66).

Similarly, the genotype TGX1954-1F and TGX 1908-8F had the highest plant height with 34 and 33cm respectively while Bossier recorded the least (27.05cm). High yields attained by TGX 1954-1F and TGX 1908-8F genotypes could be explained by the high performance of agronomic variables such as the number of pods per plant and number of seeds per plant which featured high in these genotypes compared to others (Table 3).

### Effects of environment

The agronomic yield performance and yield across the 3 locations are presented in Table 4. It was established from this study that, yield and yield components varied significantly ( $P < 0.05$ ) with location. The mean yield was significantly high at Ilonga (728kg/ha) and the lowest was recorded at Kibaha. High yield at Ilonga could be attributed to relatively adequate rainfall during the growing month of March and 2°C lower average temperatures which mimics closer to the highland agro-ecosystem where there is a cooler environment suitable for soybeans as also reported by other authors (Liu et al., 2008; Ragsdale et al., 2011). Number of pods per plant, pod length, number of seed per plant, plant height and 100 seed weight were significantly high at Ilonga compared to other sites. These agronomic performance attributed to high yield performances recorded at Ilonga site (Table 4). The seed yield performance across the three locations showed that the performance of all genotypes are consistent under varying agro-ecological zones.

However, moderate yield performances to all genotypes recorded at Kibaha might be due to low precipitation (36.6mm) during critical period of pod set.

**Table 3.** Effect of genotype on yield and yield components.

Genotype	50% flowering (days)	95% maturity (days)	Plant height (cm)	No. of pods/plant	Pod length (cm)	No. of seeds/plant	100 seeds (g)	Yield (kg/h)	Crude protein (%)
TGX 1987-10F	40.55 <sup>d</sup>	73.88 <sup>d</sup>	33.58 <sup>a</sup>	40.68 <sup>b</sup>	3.21 <sup>b</sup>	53.44 <sup>b</sup>	12.15 <sup>de</sup>	496.99 <sup>bc</sup>	40.26 <sup>b</sup>
TGX 1987-20F	41.88 <sup>c</sup>	73.33 <sup>e</sup>	28.72 <sup>b</sup>	29.22 <sup>c</sup>	3.09 <sup>b</sup>	53.78 <sup>b</sup>	11.21 <sup>e</sup>	559.27 <sup>ab</sup>	40.46 <sup>b</sup>
TGX 1954-1F	43.11 <sup>b</sup>	86.88 <sup>a</sup>	34.00 <sup>a</sup>	58.11 <sup>a</sup>	3.13 <sup>b</sup>	52.00 <sup>b</sup>	13.01 <sup>cd</sup>	611.69 <sup>a</sup>	37.12 <sup>b</sup>
TGX 1910-14F	37.77 <sup>f</sup>	72.55 <sup>f</sup>	27.00 <sup>b</sup>	33.22 <sup>bc</sup>	3.68 <sup>a</sup>	61.44 <sup>a</sup>	14.44 <sup>a</sup>	519.68 <sup>b</sup>	39.81 <sup>b</sup>
TGX 1908-8F	45.33 <sup>a</sup>	86.44 <sup>b</sup>	33.00 <sup>a</sup>	53.11 <sup>a</sup>	3.18 <sup>b</sup>	66.11 <sup>a</sup>	13.38 <sup>bc</sup>	609.93 <sup>a</sup>	40.21 <sup>b</sup>
BOSSIER	39.77 <sup>e</sup>	54.77 <sup>c</sup>	27.05 <sup>b</sup>	25.89 <sup>c</sup>	2.49 <sup>a</sup>	30.33 <sup>c</sup>	14.34 <sup>ab</sup>	260.46 <sup>c</sup>	23.65 <sup>a</sup>
Overall mean	41.40	78.97	30.55	41.00	3.32	54.66	13.08	538.82	38.92
S.E (±)	0.26	0.25	1.32	4.75	0.09	2.96	0.62	48.05	0.15
CV	1.16	0.49	7.65	20.37	4.52	9.5	7.94	15.92	0.65

Means with the same superscript letter(s) in the same column are not statistically different.

**Table 4.** Effects of location on yield and yield components of soybean.

Location	50% flowering (days)	95% maturity (day)	Plant height (cm)	No. of pods/plant	Pod length (cm)	No. of seed/plant	100 seeds wt (g)	Yield (kg/ha)	Crude protein (%)
Ilonga	42.56 <sup>a</sup>	81.56 <sup>a</sup>	36.50 <sup>a</sup>	49.60 <sup>a</sup>	3.39 <sup>a</sup>	68.80 <sup>a</sup>	15.28 <sup>a</sup>	728.00 <sup>a</sup>	38.92 <sup>a</sup>
Kibaha	41.67 <sup>b</sup>	77.50 <sup>b</sup>	28.53 <sup>b</sup>	48.22 <sup>a</sup>	3.20 <sup>b</sup>	50.56 <sup>b</sup>	12.14 <sup>b</sup>	343.08 <sup>c</sup>	38.94 <sup>a</sup>
Mlingano	38.00 <sup>c</sup>	77.88 <sup>b</sup>	26.61 <sup>b</sup>	25.17 <sup>b</sup>	3.12 <sup>b</sup>	44.67 <sup>c</sup>	11.84 <sup>b</sup>	545.76 <sup>b</sup>	38.89 <sup>a</sup>
Overall mean	40.74	78.95	30.55	40.99	3.24	54.67	13.08	538.95	38.92
S.E (±)	0.27	0.22	1.34	4.82	0.09	2.99	0.59	49.50	0.14
CV	1.16	0.49	7.64	20.37	4.52	9.50	7.94	15.92	0.65

Mean with the same superscript letter(s) in the same column are not statistically different following Least Square Difference comparison at 5% level.

The released variety (Bossier) had poor performance across all locations (Table 5). These genotypes showed strong stability and promising stock for future soybean breeding programmes.

#### Combined effects of genotype and environment

The interaction of genotype x location computed from this study is presented in Table 5. The

genotype by environment interaction resulted in significant differences in yield and yield components of soybean. The combination involving the genotypes with Ilonga resulted into the higher performance of soybean in all parameters while the combination of genotype with Mlingano had the poorest performance. This implies that, all genotypes were better adapted at Ilonga than Mlingano where the control check was the poorest performer. The poor performance at Mlingano and

Kibaha could be associated with their ecological condition as they are located more at lower altitude with relatively higher temperatures than Ilonga. Adaptability of soybean to high altitude location has been reported by many authors (Liu et al., 2005; Liu et al., 2008; Ragsdale et al., 2011). However, of the all the genotypes tested, TGX 1954-1F combined well with all the three locations (Table 5) implying that it can well be used as a potential variety for all the three locations.

**Table 5.** Combined effects of genotypes and environment on the mean square values of yield and yield components of soybean.

Source of variation	Days 50% flowering	Days 95% maturity	Plant height (cm)	No. of pods/plant	Pod length (cm)	100seed weight (g)	Yield (kg/ha)	Crude protein (%)
ILONGA*G1	41.67b	76.67b	39.00e	52.67fg	3.43b	14.32bc	774.77ef	40.18b
ILONGA*G2	43.67b	75.00b	37.33de	27.67bcd	3.27b	13.04bc	748.01ef	40.43b
ILONGA*G3	44.33b	89.67b	37.67de	73.67i	3.40b	13.92bc	897.87f	37.13b
ILONGA*G4	40.00b	75.33b	35.00cde	39.33cdef	4.27b	17.56c	663.11def	40.24b
ILONGA*G5	46.00b	88.67b	39.33e	77.00i	3.53b	15.70bc	860.93f	40.20b
ILONGA*G6	39.67b	84.00b	30.67bcde	27.33bcd	3.97b	17.15c	420.32bcd	35.35b
KIBAHA*G1	40.00b	72.00b	31.83bcde	39.67def	3.20b	11.00bc	252.60b	40.38b
KIBAHA*G2	40.00b	73.00b	26.83bcd	36.67bcde	3.00b	10.23b	385.23bcd	40.61b
KIBAHA*G3	43.00b	85.00b	33.00bcde	70.00hi	3.00b	13.27bc	421.36bcd	37.19b
KIBAHA*G4	36.33b	70.00b	22.33b	36.33bcde	3.5b	12.67bc	319.03bc	39.56b
KIBAHA*G5	46.00b	84.67b	31.00bcde	56.33gh	3.00b	13.47bc	319.35bc	40.28b
KIBAHA*G6	41.67b	80.33b	26.17bcd	50.33efg	3.5b	12.20bc	360.95bc	35.61b
MLINGANO*G1	40.00b	73.00b	29.67bcde	29.67bcd	3.00b	11.13bc	463.59bcd	40.23b
MLINGANO*G2	42.00b	72.00b	22.00b	23.33b	3.00b	10.37b	544.56cde	40.35b
MLINGANO*G3	42.00b	86.00b	31.33bcde	30.67bcd	3.00b	11.83bc	515.84bcde	37.06b
MLINGANO*G4	37.00b	72.33b	23.67bc	24.00bc	3.27b	13.10bc	576.91cde	39.62b
MLINGANO*G5	44.00b	86.00b	28.67bcde	26.00bcd	3.00b	10.97bc	649.50def	40.15b
MLINGANO*G6	0.00a	0.0000a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

G1= TGX 1987-10F, G2= TGX 1987-20F, G3= TGX 1954-1F, G4= TGX 1908-8F, G5= TGX 1910-14F, G6= Bossier: Mean with the same supescript letter(s) in the same column are not statistically different following Least Square Difference comparison at 5% level.

### Genotypic coefficients of variation

The estimates of the genotypic coefficient of variation (GCV), the phenotypic coefficient of variation (PCV), broad sense heritability and genetic advance in percent of the mean for eleven traits of soybean are presented in Table 6. Significant differences were recorded for all agronomic traits under study. Indicating that all accessions are promising for breeding programs. The PCV and GCV estimates were significantly high for pods per plants (49.49/27.04) followed by yield (39.16/32.81), seed per plant (35.36/22.53), plant height (20.56/15.76), initial plant per plot

(20.56/15.76), 100 seed weight (17.95/15.36), and pod length (11.14/9.69).

The lowest PCV/GCV estimate was revealed in crude protein (1.45/0.98). The finding also revealed that the differences between PCV and GCV were significantly lower for crude protein (0.45), followed by pod length (1.45), 100 seed weight (2.6) and 50% flowering day (3.2). Indicating that the environment had less effects on the expression of these traits, thus can be useful in soybean screening programs. (Aditya et al., 2011) also reported significant lower differences between PCV and GCV in 50% flowering and 100 seed weight.

### Correlation analysis

The correlation coefficient of 7 agronomical traits are shown in Table 7. The findings revealed that all the agronomic characters studied showed strong positive correlation with grain yield except plant height and 100seed weight at Mlingano and Kibaha (Table 7).

Days to 50% flowering, number of pods per plant and number of seeds per plant showed positive and strong correlation with grain yield. Indicating that these traits are important in determining quantitative traits such yield in soybean. Several authors (Abady et al., 2013;

**Table 6.** Estimation of genetic parameters for eleven characters of soybean genotypes.

Characters	GCV	PCV	hb2 (%)	EGA	GAM (%)
Initial plants /plot	15.76	20.56	72.60	9.17	12.70
50% flowering (days)	2.26	5.46	17.40	1.02	2.47
95% maturity (days)	2.00	6.44	9.60	1.29	1.64
Plant height	15.76	20.56	58.78	9.73	31.91
Pods per plant	27.04	49.49	29.87	12.48	30.45
Pod length	9.69	11.14	75.81	0.74	22.29
Seeds per plant	22.53	35.36	40.60	20.72	37.90
100 seeds wt (gm)	15.36	17.95	73.30	4.54	34.72
Yield (Kg/ha)	32.81	39.16	70.18	390.91	72.55
Crude protein (%)	0.98	1.45	46.42	0.69	1.78

The genotypic coefficient of variation (GCV), the Phenotypic coefficient of variation (PCV), Broad sense heritability (hb2), Expected genetic advance (EGA) and Genetic advance as percent of the mean (GAM).

**Table 7.** Correlation coefficients between characters computed from six genotypes of soybean grown in different locations, the upper: value Ilonga: middle: Kibaha and lower: Mlingano.

Characters	(7)	(6)	(5)	(4)	(3)	(2)	(1)
(1) DF	0.7067 **	-0.5185 *	0.6206 **	0.6681 **	0.6282 **	0.5037 *	1.000
	0.1680	0.3179	0.3510	0.6622 **	0.6263 **	0.8905 ***	1.000
	0.2466	-0.7190***	-0.3440	0.3448	0.4061	0.6018**	1.000
(2) DM	0.2264	0.0129	0.1944	0.6606 **	0.0447	-	-
	0.4099	0.4615	0.2103	0.8033 ***	0.5322 *	-	-
	0.2702	-0.0415	-0.1828	0.2120	0.6189**	-	-
(3) PH	0.6751 **	-0.4174	0.6958 **	0.5818 *	-	-	-
	-0.0187	0.1852	-0.0416	0.4278	-	-	-
	-0.0441	-0.2183	-0.1287	0.7077**	-	-	-
(4) NPP	0.6277 **	-0.0758	0.7652 ***	-	-	-	-
	0.2597	0.4648	-0.0875	-	-	-	-
	0.1652	0.3316	0.0508	-	-	-	-
(5) NSP	0.7783 ***	-0.2889	-	-	-	-	-
	0.4209	-0.2038	-	-	-	-	-
	0.4349	0.2515	-	-	-	-	-
(6) SW	-0.4442	-	-	-	-	-	-
	-0.0548	-	-	-	-	-	-
	0.0539	-	-	-	-	-	-
(7) GY	1.000	-	-	-	-	-	-
	1.000	-	-	-	-	-	-
	1.000	-	-	-	-	-	-

\*, \*\*, \*\*\*: Significant at P=0.05, P= 0.01 and P=0.001 probability levels, respectively DF= days to 50% flowering, DM=days to 95% maturity, PH=Plant height, NPP=Number of pods per plant, NSP=Number of seeds per plant, SW=100 seeds weight, GY= Grain yield.

Aditya et al., 2011; Malik et al., 2007; Ngalamu et al., 2013) reported similar results on the importance of the same yield components in determining grain yield in soybeans, hence selection based on these traits could improve soybean yields. 100 seed weight showed

negative correlation yield, similar result was revealed by Malik et al. (2007) and Srinives and Giragulvattanaporn (1986).

Path coefficient analysis presented in Table 8 and Figure 1 showed that all the yield components studied had

**Table 8.** Path coefficients for soybean grain yield influencing factors at Ilonga, Kibaha, and Mlingano.

S/N	Effect	Ilonga	Kibaha	Mlingano
1	<b>Correlation of days to 50% flowering on with yield, <math>r_{17}</math></b>	<b>0.707**</b>	<b>0.168</b>	<b>0.247</b>
	Direct effect of days to 50% flowering, $P_{17}$	0.279	-1.587	0.622
	Indirect effect via days to 95% maturity, $r_{12}P_{27}$	0.001	1.549	0.194
	Indirect effect via plant height, $r_{13}P_{37}$	0.070	0.065	-0.275
	Indierct effect via number of pods per plant, $r_{14}P_{47}$	-0.021	0.031	0.162
	Indierect effect via number of seeds per plant, $r_{15}P_{57}$	0.323	0.198	-0.171
	Indierct effect via 100 seed weight, $r_{16}P_{67}$	0.055	-0.089	-0.285
	<b>Total</b>	<b>0.707</b>	<b>0.167</b>	<b>0.247</b>
2	<b>Correlation of days to 95% maturity with yield, <math>r_{27}</math></b>	<b>0.226</b>	<b>0.409</b>	<b>0.270</b>
	Direct effect of days to 95% maturity, $P_{27}$	0.001	1.741	0.323
	Indirect effect via days to 50% flowering, $r_{21}P_{17}$	0.141	-1.412	0.374
	Indierct effect via plant height, $r_{23}P_{37}$	0.005	0.055	-0.419
	Indirect effect via number of pods per plant, $r_{24}P_{47}$	-0.021	0.038	0.099
	Indirect effect via number of seeds per plant, $r_{25}P_{57}$	0.101	0.116	-0.091
	Indirect effect via 100 seed weight, $r_{26}P_{67}$	-0.001	-0.129	-0.017
	<b>Total</b>	<b>0.226</b>	<b>0.409</b>	<b>0.270</b>
3	<b>Correlation of plant height with yield, <math>r_{37}</math></b>	<b>0.675**</b>	<b>-0.019</b>	<b>-0.044</b>
	Direct effect of plant height, $P_{37}$	0.112	0.104	-0.677
	Indirect effect via days to 50% flowering, $r_{31}P_{17}$	0.175	-0.993	0.253
	Indirect effect via days to 95% maturity, $r_{32}P_{27}$	0.000	0.926	0.199
	Indirect effect via number of pods per plant, $r_{34}P_{47}$	-0.018	0.020	0.331
	Indirect effect via number of seeds per plant, $r_{35}P_{57}$	0.362	-0.024	-0.064
	Indirect effect via 100 seed weight, $r_{36}P_{67}$	0.044	-0.052	-0.087
	<b>Total</b>	<b>0.675</b>	<b>-0.019</b>	<b>-0.045</b>
4	<b>Correlation of number of pods per plant with yield, <math>r_{47}</math></b>	<b>0.627**</b>	<b>0.260</b>	<b>0.165</b>
	Direct effect of number of pods per plant, $P_{47}$	-0.031	0.047	0.468
	Indirect effect via days to 50% flowering, $r_{41}P_{17}$	0.186	-1.051	0.215
	Indirect effect via days to 95% maturity, $r_{42}P_{27}$	0.001	1.398	0.068
	Indirect effect via plant height, $r_{43}P_{37}$	0.065	0.045	-0.479
	Indirect effect via number of seeds per plant, $r_{45}P_{57}$	0.398	-0.049	0.025
	Indirect effect via 100 seed weight, $r_{46}P_{67}$	0.008	-0.130	-0.132
	<b>Total</b>	<b>0.627</b>	<b>0.260</b>	<b>0.165</b>
5	<b>Correlation of number of seeds/plant with yield, <math>r_{57}</math></b>	<b>0.778***</b>	<b>0.421</b>	<b>0.435</b>
	Direct effect of number of seeds per plant, $P_{57}$	0.520	0.563	0.497
	Indirect effect via days to 50% flowering, $r_{51}P_{17}$	0.173	-0.557	-0.214
	Indirect effect via days to 95% maturity, $r_{52}P_{27}$	0.000	0.366	-0.059
	Indirect effect via plant height, $r_{53}P_{37}$	0.079	-0.004	0.086
	Indirect effect via number of pods per plant, $r_{54}P_{47}$	-0.024	-0.004	0.025
	Indirect effect via 100 seed weight, $r_{56}P_{67}$	0.030	0.057	0.100
	<b>Total</b>	<b>0.778</b>	<b>0.421</b>	<b>0.435</b>
6	<b>Correlation of 100 seed weight with yield, <math>r_{67}</math></b>	<b>-0.444</b>	<b>-0.055</b>	<b>-0.054</b>
	Direct effect of 100 seed weight, $P_{67}$	-0.105	-0.280	0.397
	Indirect effect via days to 50% flowering, $r_{61}P_{17}$	-0.145	-0.505	-0.447
	Indirect effect via days to 95% maturity, $r_{62}P_{27}$	0.000	0.804	-0.014
	Indirect effect via plant height, $r_{63}P_{37}$	-0.047	0.019	0.148
	Indirect effect via number of pods per plant, $r_{64}P_{47}$	0.002	0.022	-0.155
	Indirect effect via number of seeds per plant, $r_{65}P_{57}$	-0.150	-0.115	0.125
	<b>Total</b>	<b>0.445</b>	<b>-0.055</b>	<b>0.054</b>

positive direct effects on yield in all locations except 100 seed weight which had negative direct effects on yield in all locations. Similar results were also reported by (Sharma et al., 1983).

However, these are contrary to the result of Malik et al. (2007) and Srinives and Giragulvattanaporn (1986) who revealed that days to maturity and days to 50% flowering had negative direct effect to yield. This inconsistency in results might be due to the effect of abiotic factors. Several reports (Arshad et al., 2006; Malik et al., 2007; Srinives and Giragulvattanaporn, 1986) documented that correlation coefficient for seed yield revealed a significant association with plant height.

Contrary to the findings of this study, plant height showed positive direct effect on yield at Ilonga ( $r=0.675$ ), while at Kibaha and Mlingano revealed negative direct effect on yield ( $r= -0.019$  and  $-0.044$ ) respectively. This inconsistency might be due to significant low plant height recorded at Kibaha (28.5) and Mlingano (26.61) (Table 4) which might also affected the seed yield performance. The reasons for low yield performance of genotypes at Kibaha and Mlingano could also be attributed to low precipitation recorded during the study period (Table 8).

Based on the present findings, days to 50% flowering, days to 95% maturity, plant height, number of pods per plant, and number of seed per plant showed a positive and significant correlation across the locations studied. These traits suggested being effective selection criterion in soybean improvement programmes.

## Conclusion

The results from the present study therefore conclude that genotype and location interaction had a high positive correlation with all agronomic yield expect 100 seed weight. The LSD mean separation picked all genotypes as the high adaptable and good yielder across the all three locations as compared to the check.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Agronomic efficiency of inoculant based on *Azospirillum brasilense* associated with nitrogen fertilization at maize

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The objective was to evaluate the agronomic efficiency in the field of the inoculant Fluid and Turfoso containing the bacterium *Azospirillum brasilense*, strains AbV5 and AbV6 applied by treatment of seeds in the corn crop associated with nitrogen fertilization in different localities. Four experiments were carried out in different sites involving two in Paraná and one each from Mato Grosso do sul and Santa Catarina. The design was randomized blocks with 6 treatments: T1- 0 kg ha<sup>-1</sup> of nitrogen (N), without inoculation (control); T2 - 80 kg ha<sup>-1</sup> of N, without inoculation; T3 - 160 kg ha<sup>-1</sup> of N, without inoculation; T4 - 80 kg ha<sup>-1</sup> of N + seed inoculation AzoTotal® 'Liquid'; T5 - 80 kg ha<sup>-1</sup> of N + seed inoculation Nitro 1000 Gramines 'Liquid'; T6 - 80 kg ha<sup>-1</sup> of N + seed inoculation Nitro 1000 'Peat' grasses. The evaluations were composed of nutrient content in leaves and grains, and at the end of the cycle length and ear diameter, number of rows of grains and grains per row, mass of one thousand grains and productivity. The results showed that the inoculation of the seeds, regardless of the physical nature of the inoculant, was efficient for the maize crop, reducing the use of mineral nitrogen in all evaluated sites. It is concluded that the use of inoculation with *A. brasilense* regardless of the physical nature of the inoculant reduces the need for nitrogen fertilization of the corn crop by 50%, without reducing the final yield of the crop.

**Key words:** *Zea mays*, biological nitrogen fixation, productivity, *Azospirillum brasilense*, sustainable agriculture.

## INTRODUCTION

The corn crop (*Zea mays* L.) is the most cultivated cereal in Brazil and in the world. National production in the 2015/2016 harvest was 83.33 million tons of grain

(Conab, 2016; USDA, 2016). In the world scenario it has great social, economic and cultural relevance, being relevant for human consumption and mainly for animal

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consumption.

In relation to mineral nutrition corn is one of the most demanding into fertilizer, mainly the nitrogenous one (Carvalho et al., 2013), since this material needs high amount of nitrogen (Moda et al., 2014). In this study, the use of nitrogen fertilizers was considered to be an important determinant of the nutrient availability of the nutrient in the crop (Ferreira et al., 2007). Therefore, nitrogen fertilization is necessary because of the insufficient amount that the soil provides for adequate plant growth. This situation is particularly important for the maize crop, since, among the nutrients that influence its productivity, the N is one of the most absorbed during the development cycle of the plants.

The use of nitrogen fertilizers in maize crop presents high costs and high environmental impact (Garcia et al., 2013). In addition, there is a low efficiency of the current available sources, which are based on the 50% mark (Costa et al., 2003). The quest for maximizing the use of N is a challenge. One of the ways to increase yield and minimize the production costs is linked to plants that have a more developed root system (Costa et al., 2015), able to better exploit the soil and increase nutrient and water absorption. In this way, better development conditions are provided to the plant, resulting in increased productivity (Basso et al., 1994).

An alternative to reduce the use of mineral fertilizers is the use of seed inoculation by diazotrophic bacteria (Novakowski et al., 2011; Quadros et al., 2014; Inagaki et al., 2015), widely used in leguminous crops.

Among the diazotrophic bacteria, the *Azospirillum* genus can colonize plant roots and shoots (Pedrinho et al., 2010). This is a promising alternative, since these microorganisms increase the availability of N to the plant, by breaking the triple bond of the atmospheric N<sub>2</sub> and making it available to the plants. This practice can provide up to 50% of the total N to the plant (Piccinin et al., 2013). In addition to the above, this specie is capable of producing plant growth promoting substances (Santi et al., 2013), with the most important being indole acetic acid, an auxin (Radwan et al., 2005; Perrig et al. 2007), resulting in a higher root development (Rodrigues et al., 2014) and thus increasing the area of root system exploration and nutrient absorption (Ferreira et al., 2013).

The use of diazotrophic bacteria as an alternative to increase the availability of nitrogen to crops may be a less costly and ecologically viable option, since it contributes to the reduction of atmospheric CO<sub>2</sub> up to 0.309 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> demonstrated in *Brachiaria* (Hungria et al., 2016). In light of this finding, several studies have been conducted to verify the potential of *Azospirillum* spp. (Dartora et al., 2013; Repke et al., 2013; Guimarães et al., 2014; Quadros et al., 2014).

In this line, inoculation of the seeds with *A. brasilense* increases foliar area and shoot dry matter by 12% on the foliar area and dry issue of aerial part (Marini et al., 2015). Costa et al. (2015), showed an increase in plant

height, dry stem and root mass, chlorophyll content, a thousand-grain mass, and final crop yield of the second crop. This increase was 29%.

The objective of the present work was to evaluate the agronomic efficiency in the field of Fluid and Turfoso inoculant containing the bacterium *Azospirillum brasilense*, strains AbV5 and AbV6 applied by treatment of seeds in maize crop associated with nitrogen fertilization in different localities.

## MATERIALS AND METHODS

The study was carried out by conducting experiments in four sites with different edaphoclimatic conditions in the 2013/2014 harvest. Four experiments were carried out in different sites involving two in Paraná and one each from Mato Grosso do sul and Santa Catarina., all in Brazil (Table 1).

The areas where the experiments were developed were being cultivated with annual and perennial crops under no-tillage system for at least five years. Therefore, in order to characterize it initially, that is to say, before sowing of the maize, samples were taken in twenty profiles of tradition for the collection of the soil with deformed structure, realized with a screw thread in the depth of 0 to 0.20 m, whose chemical and physical characteristics are presented in Table 2.

The count of diazotrophic microorganisms to determine the population of bacteria in cell numbers per mL was performed by estimating the Most Probable Number (MPN) using the MacCrady table in NFB (*Azospirillum* spp.) where the Semi-solid medium was used for bacterial growth according to methodology (Döbereiner et al., 1995). The results of the counts of diazotrophic microorganisms by the estimation of MPN in soils of the experimental areas were as follows: Site 1: 2x10<sup>6</sup> g<sup>-1</sup> soil; site 2: 1.1x10<sup>7</sup> g<sup>-1</sup> soil; site 3: 1.8x10<sup>6</sup> g<sup>-1</sup> soil and site 4: 4.5 x10<sup>6</sup> g<sup>-1</sup> soil.

The inoculant 'Nitro 1000 Gramines liquid' has the following characteristics: 2.0x10<sup>8</sup> CFU mL<sup>-1</sup> (Colony Forming Units) of *A. brasilense* strains AbV5 and AbV6; Physical nature: Fluid; Density: 1.0 g / mL; Target Culture: Maize (*Z. mays* L.); Dosage tested 100 mL for 25 kg of seed; Lot: 001/2013; Manufacture: 10/31/13. The inoculant 'Nitro 1000 Gramines Peaty' has the following characteristics: Guarantee: 2.0x10<sup>8</sup> CFU g<sup>-1</sup> of *A. brasilense* strains AbV5 and AbV6; Physical nature: Solid; Density: 1.0 g / mL; Target Culture: Maize (*Zea mays* L.); Dosage tested: 100 g for 25 kg of seed; Lot: 001/2013; Manufacture: 10/31/13.

The AzoTotal® inoculant was used as reference (standard inoculant) in the four experiments. The inoculant presents 2.0x10<sup>8</sup> CFU mL<sup>-1</sup> of *A. brasilense*, strains AbV5 and AbV6; Physical nature: Liquid; Density: 1.0 g / mL. The dosage used in the test 100 mL for 25 kg of seed: Lot: 1101213; Manufactured: 09/13/13.

All the inoculants used were submitted to laboratory tests of concentration, purity and characterization. The analyzes followed official methods, according to Normative Instruction number 30, dated November 12, 2010 (MAPA). AzoTotal® Inoculant presented 2.15x10<sup>8</sup> CFU mL<sup>-1</sup>; Nitro 1000 Grasses 'Liquid' presented 2.33x10<sup>8</sup> CFU mL<sup>-1</sup> and Nitro 1000 Grasses 'Turfa' presented 2.12x10<sup>8</sup> CFU g<sup>-1</sup>.

The four experiments used Pioneer® 30F53 YH hybrid simple and were conducted in a randomized block design with six treatments and four replicates. The treatments were: T1 - 0 kg ha<sup>-1</sup> of nitrogen (N), without inoculation (control); T2 - 80 kg ha<sup>-1</sup> of N, without inoculation; T3 - 160 kg ha<sup>-1</sup> of N, without inoculation; T4 - 80 kg ha<sup>-1</sup> of N + seed inoculation with standard AzoTotal® 'Liquid' inoculant at the dose of 100 mL 25 kg<sup>-1</sup> of seeds; T5 - 80 kg ha<sup>-1</sup> of N + seed inoculation with inoculant Nitro 1000 Gramines 'Liquid' at the dose of 100 mL 25 kg<sup>-1</sup> of seeds; T6 - 80 kg ha<sup>-1</sup> of N + seed

**Table 1.** Geographic site, type of soil and climate of the places where the experiments were carried out.

Site	Coordinates	Level	Soil Type	Weather
Site 1 – PR	24°40'S 54°16'W	248	RED LATOSOL Eutrophic	Humid Subtropical Mesothermic
Site 2 – PR	24°43'S 53°46'W	565	RED LATOSOL Dystrophic	Humid Subtropical Mesothermic
Site 3 – SC	27°16'S 50°30'W	1000	CAMBISOL Acric	Mild weather
Site 4 – MS	20°18'S 52°39'W	357	RED LATOSOL Dystrophic	Tropical Umid

**Table 2.** Chemical and physical characterization of the soils before the implantation of the experiments.

L	Chemical characteristics											Granulometry			
	pH	V	P	MO	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Al <sup>3+</sup>	H+Al	SB	CTC	Argila	Silte	Areia	
	CaCl <sub>2</sub>	- % -	mg dm <sup>-3</sup>	g dm <sup>-3</sup>	-----cmol <sub>c</sub> dm <sup>-3</sup> -----									-----g kg <sup>-1</sup> -----	
1	5.07	62.16	21.12	28.71	6.11	1.32	0.29	0.10	4.70	7.72	12.42	559	359	83	
2	5.20	61.43	16.70	24.94	6.62	2.92	0.35	0.00	6.21	9.89	16.10	720	170	110	
3	6.30	79.80	10.70	44.23	8.35	4.11	0.10	0.00	3.18	12.56	15.74	550	363	87	
4	5.5	62	17	25.00	26.00	18.00	3.00	0.00	29.00	47.00	76.00	700	250	50	

L: sites. (P,K, Micronutrients) Mehlich<sup>-1</sup>; (Al, Ca, Mg); KCl 1 mol L<sup>-1</sup>; (H+Al) pH SMP (7,5); (pH); CaCl<sub>2</sub> 0,01 mol L<sup>-1</sup>.

**Table 3.** N, P and K content in the cultural remains, at the time of the implantation of the experiments in the four sites.

Site	Haystack	N	P	K
	t ha <sup>-1</sup>	----- kg ha <sup>-1</sup> -----		
Site 1 – PR	8.0	105	17	75
Site 2 – PR	7.5	98	16	69
Site 3 – SC	13.0	171	28	117
Site 4 - MS	12.0	160	28	110

inoculation with inoculant Nitro 1000 'Peat' grasses at the dose of 100 g 25 kg<sup>-1</sup> seeds.

Nitrogen fertilization was performed in two applications. At the time of sowing, 30 kg ha<sup>-1</sup> of N was applied in all treatments, with the exception of T1 (control). In the V6 stage of maize 50 kg ha<sup>-1</sup> of N were applied in treatments with 80 kg ha<sup>-1</sup> of N (T2, T4, T5 and T6). For T3 treatment, 80 kg ha<sup>-1</sup> of N at the V4 stage and 50 kg ha<sup>-1</sup> of N at the V8 stage were applied. Urea nitrogen (45% N) was used as the source of both the sowing and coverage.

Prior to corn sowing, the areas were desiccated with glyphosate herbicide at a dose of 4 L of p.c. ha<sup>-1</sup>. At this moment the cultural remains were collected for analysis of N, P and K in the samples (Table 3).

The experiments were implanted with the following sowing dates: Site 1 (10/10/2013); Site 2 (11/05/2013); Site 3 (10/25/2013) and Site 4 (12/9/2013). The seed were treated with (trichloromethylthio) cyclohex-4-ene<sup>-1</sup>,2-dicarboxy fungicide at a dose of 0.2 kg 100 kg<sup>-1</sup>

of seeds, as well as with insecticide imidacloprid + Thiodicarb at the dose of 0.2 L 100 kg<sup>-1</sup> of seeds.

Inoculation of the seeds was realized in high density plastic bags, where the inoculants were directly deposited according to the treatments. Then they were agitated for approximately two minutes to standardize the distribution of the inoculant in the seeds. Sixty minutes after inoculation, the sowing was done, being the standard procedure for the four experiments.

Phosphorus fertilization (80 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>) and potassium (60 kg ha<sup>-1</sup> of K<sub>2</sub>O) were applied to the sowing furrow using the mechanized fertilizer sowing machine (Embrapa, 2012).

Sowing of the experiments was carried out with the aid of a manual seeder (matracas), with five seeds per meter being distributed in the sowing furrow, reaching a final population of 70000 ha<sup>-1</sup> plants. Each experimental plot consisted of 6 lines of 0.70 m spacing, 6 m long and 4.2 m wide, totaling 25.2 m<sup>2</sup> per plot, spaced apart by 1 m, and total area of 604.80 m<sup>2</sup>. To obtain the useful area of the plots the outer lateral lines and 1.0 m of the ends of the lines of each plot were disregarded.

During the conduction of the experiments, the control of weeds, pests and diseases were carried out according to the needs of the crop (Embrapa, 2012).

When the plants were in the full flowering stage, they were collected the middle third of 10 leaves opposite and immediately below the main spike, per plot. After harvesting the cobs and threshing of the grains, samples of grains corresponding to each experimental plot were taken. These were dried at 65°C in a forced air circulation oven until mass reached constant. Afterwards, they were ground and analyzed for N, P and K content (Malavolta et al., 1997).

Foliar and grain samples were ground and subjected to sulfur

**Table 4.** Nitrogen, phosphorus and potassium contents in foliar tissue and grain of plants of hybrid corn Pioner® 30F53 YH in Place 1, from October 2013 to February 2014.

Treatments	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	----- (g kg <sup>-1</sup> ) -----					
Control	21.00 <sup>b1</sup>	3.05 <sup>b</sup>	24.07 <sup>ns</sup>	9.40 <sup>b</sup>	1.57 <sup>ns</sup>	3.08 <sup>ab</sup>
80 kg ha <sup>-1</sup> N	26.47 <sup>ab</sup>	3.70 <sup>ab</sup>	23.66	10.94 <sup>ab</sup>	1.39	3.43 <sup>ab</sup>
160 kg ha <sup>-1</sup> N	27.13 <sup>a</sup>	4.13 <sup>a</sup>	22.17	11.81 <sup>ab</sup>	1.24	3.65 <sup>a</sup>
Inoculum Pattern <sup>2</sup>	26.03 <sup>ab</sup>	3.54 <sup>ab</sup>	23.26	11.16 <sup>ab</sup>	1.26	2.32 <sup>b</sup>
Inoculum Liquid <sup>3</sup>	26.25 <sup>ab</sup>	3.97 <sup>a</sup>	26.36	9.62 <sup>ab</sup>	1.26	3.11 <sup>ab</sup>
Inoculum Peaty <sup>4</sup>	27.03 <sup>a</sup>	3.92 <sup>a</sup>	24.62	12.03 <sup>a</sup>	1.12	3.62 <sup>a</sup>
Average	25.58	3.72	24.02	10.83	1.31	3.20
C.V. (%)	13.76	12.49	10.60	13.97	24.08	15.81

<sup>1</sup>Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. ns not significant at 5% error; <sup>2</sup>80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup>80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup>80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Peat'.

digestion according to the methodology of Embrapa (2009).

The experiments harvest was performed on the following dates: Site 1 (05/02/2014); Site 2 (3/26/2014); Site 3 (03/24/2014) and Site 4 (04/09/2014). The components of the production were determined by sampling ten ears per useful plot. The evaluations were length of cobs (CE, expressed in cm), ear diameter (DE, in mm), number of rows per cobs (NFGE), number of grains per row in the cobs (NGF) and mass of thousand grains (MMG, in grams).

For the determination of grain yield, all the cobs of the useful plot were milled and the grains weighed. The results were expressed in kg ha<sup>-1</sup>, correcting the values for 13% moisture in the wet basis.

The data, after tabulation, were submitted to analysis of variance by the Fisher-Snedecor test (test F) and the means of the treatments were compared by Duncan's test ( $P \leq 0.05$ ). The analyses were carried out using the GENES computer program of the Federal University of Viçosa (UFV) (Cruz, 2013).

## RESULTS

The results obtained Site 1 showed that nutrient contents in foliar tissue and maize grains were significantly influenced by treatments with the exception of foliar K content and P content in the grains. The N and P contents in the foliar and K tissues in the grains were superior in the treatment with 160 kg ha<sup>-1</sup> N, without inoculation; however, they presented significant difference only of the control, without addition of N and inoculation. For the N content in the grains, the highest levels were obtained in plants whose seeds were treated with turfous inoculant; however, this treatment only differed statistically from the control (Table 4).

For the production variables, there was no significant effect on the length of spikes (CE), number of rows per spike (NFGE) and number of grains per cobs (NGF) (Table 5). Regarding the cob diameter (DE) and mass of a thousand grains (MMG), the treatment was 160 kg ha<sup>-1</sup> of N, which was statistically higher to the control. When the productivity was observed, the agronomic efficiency

of *A. brasilense* was verified through seed inoculation, since the treatments that received inoculation with *A. brasilense* + 80 kg ha<sup>-1</sup> N, were equal to the treatment with 160 kg ha<sup>-1</sup> N (Table 5).

For Site 2, there were no significant effects of treatments on P and K accumulation in foliar tissue and P in corn grains. For the N contents in the foliar tissue and N and K in the grains, a significant effect of the treatments was observed. The foliar N content was higher when 160 kg ha<sup>-1</sup> N was used, however, it did not differ statistically from the treatments that received inoculation with *A. brasilense*. When the N and K contents were observed, the treatments 160 kg ha<sup>-1</sup> N and turfous inoculant were superior to the control (Table 6).

Regarding the production components for Site 2, no significant differences were observed in the DE and NFGE. For the CE, NGF and MMG the lowest averages were provided by the control, treatments with inoculation of the seeds with *A. brasilense* showed the highest averages, except for the standard inoculant, which promoted intermediate results (Table 7).

Productivity was significantly influenced. Seed inoculation treatment with *A. brasilense* promoted higher means, and fertilization with 160 kg ha<sup>-1</sup> N was used. Seed inoculations with standard, liquid and turfous inoculant exceeded the treatment with 80 kg ha<sup>-1</sup> N promoting increments of 15.65; 23.16 and 26.22%, respectively (Table 7).

In the experiment conducted at Site 3 no effect of treatments on N accumulation in foliar tissue as well as N, P and K on the grains was observed. For the accumulation of foliar P the control obtained higher average values, while for the accumulation of K the highest average was observed with the fertilization of 160 kg ha<sup>-1</sup> N, however differentiating only from the control (Table 8).

At Site 3 all the productive variables were significantly

**Table 5.** Length of cob (CE), cob diameter (DE), number of row of grain per cob (NFGE), number of grains per row (NGF), mass of grains (MMG) and productivity (PROD) of Plants of hybrid corn Pioner® 30F53 YH at Site 1 during the months of October 2013 to February 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha <sup>-1</sup> )
Control	19.84 <sup>ns1</sup>	51.24 <sup>b</sup>	16.55 <sup>ns</sup>	36.57 <sup>ns</sup>	298.31 <sup>b</sup>	11.595.31 <sup>b</sup>
80 kg ha <sup>-1</sup> N	20.05	52.09 <sup>ab</sup>	16.65	37.48	327.31 <sup>ab</sup>	12.560.80 <sup>ab</sup>
160 kg ha <sup>-1</sup> N	19.93	52.71 <sup>a</sup>	16.45	37.46	346.59 <sup>a</sup>	13.564.43 <sup>a</sup>
Inoculum Pattern <sup>2</sup>	20.20	51.79 <sup>ab</sup>	16.05	37.34	338.57 <sup>ab</sup>	12.585.91 <sup>ab</sup>
Inoculum Liquid <sup>3</sup>	20.62	51.75 <sup>ab</sup>	16.40	38.91	329.77 <sup>ab</sup>	13.245.25 <sup>a</sup>
Inoculum Peaty <sup>4</sup>	20.84	51.88 <sup>ab</sup>	16.00	39.93	333.14 <sup>ab</sup>	13.446.86 <sup>a</sup>
Média	20.24	51.86	16.35	37.95	328.94	12.834.75
C.V. (%)	3.08	0.83	2.86	5.26	5.40	6.16

<sup>1</sup> Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. <sup>ns</sup> not significant at 5% error; <sup>2</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

**Table 6.** Nitrogen, phosphorus and potassium contents in foliar tissue and grain of Pioner® 30F53 YH hybrid corn in Site 2 during the months of November 2013 to March 2014.

Treatments	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	----- (g kg <sup>-1</sup> ) -----					
Control	22.05 <sup>b</sup>	3.11 <sup>ns</sup>	24.72 <sup>ns</sup>	8.59 <sup>b</sup>	1.38 <sup>ns</sup>	3.08 <sup>b</sup>
80 kg ha <sup>-1</sup> N	23.13 <sup>b</sup>	3.55	22.72	10.83 <sup>ab</sup>	1.26	3.65 <sup>ab</sup>
160 kg ha <sup>-1</sup> N	28.52 <sup>a</sup>	3.58	23.15	12.89 <sup>a</sup>	1.44	3.78 <sup>a</sup>
Inoculum Pattern <sup>2</sup>	26.31 <sup>ab</sup>	3.51	24.31	11.22 <sup>ab</sup>	1.28	3.62 <sup>ab</sup>
Inoculum Liquid <sup>3</sup>	25.32 <sup>ab</sup>	3.56	26.32	10.25 <sup>ab</sup>	1.30	3.58 <sup>ab</sup>
Inoculum Peaty <sup>4</sup>	26.25 <sup>ab</sup>	3.59	25.52	12.25 <sup>a</sup>	1.29	3.70 <sup>a</sup>
Média	25.09	3.66	24.46	11.14	1.33	3.57
C.V. (%)	10.25	8.56	9.56	12.56	18.54	13.25

<sup>1</sup> Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. <sup>ns</sup> not significant at 5% error; <sup>2</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

**Table 7.** Spigot length (CE), ear diameter (DE), number of grain row per cob (NFGE), number of grains per row (NGF), mass of grains (MMG) and productivity (PROD) of Pioner® 30F53 YH hybrid corn plants at Site 2 during the months of November 2013 to March 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha <sup>-1</sup> )
Control	15.61 <sup>c1</sup>	48.92 <sup>ns</sup>	17.02 <sup>ns</sup>	33.20 <sup>b</sup>	340.68 <sup>c</sup>	6.255.56 <sup>c</sup>
80 kg ha <sup>-1</sup> N	17.28 <sup>ab</sup>	51.00	17.21	35.07 <sup>ab</sup>	373.63 <sup>b</sup>	7.025.63 <sup>bc</sup>
160 kg ha <sup>-1</sup> N	17.30 <sup>ab</sup>	53.19	17.42	35.15 <sup>ab</sup>	386.96 <sup>a</sup>	9.164.23 <sup>a</sup>
Inoculum Pattern <sup>2</sup>	16.45 <sup>bc</sup>	52.79	17.11	33.60 <sup>b</sup>	374.50 <sup>b</sup>	8.125.36 <sup>a</sup>
Inoculum Liquid <sup>3</sup>	17.66 <sup>ab</sup>	53.72	17.65	36.35 <sup>a</sup>	390.07 <sup>a</sup>	8.653.21 <sup>a</sup>
Inoculum Peaty <sup>4</sup>	17.78 <sup>a</sup>	53.53	17.44	37.52 <sup>a</sup>	392.10 <sup>a</sup>	8.868.23 <sup>a</sup>
Média	17.01	52.19	17.28	35.15	376.32	8.015.37
C.V. (%)	5.55	5.21	3.31	5.47	1.97	10.52

<sup>1</sup> Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. <sup>ns</sup> not significant at 5% error; <sup>2</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

**Table 8.** Nitrogen, phosphorus and potassium contents in foliar tissue and grain of Pioneer® 30F53 YH hybrid corn in Site 3 during the months of October 2013 to March 2014.

Treatments	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	----- (g kg <sup>-1</sup> ) -----					
Control	12.91 <sup>ns1</sup>	3.44 <sup>a</sup>	14.10 <sup>b</sup>	8.10 <sup>ns</sup>	1.47 <sup>ns</sup>	5.46 <sup>ns</sup>
80 kg ha <sup>-1</sup> N	13.78	1.93 <sup>d</sup>	17.00 <sup>ab</sup>	7.88	1.28	4.58
160 kg ha <sup>-1</sup> N	13.35	2.81 <sup>b</sup>	22.46 <sup>a</sup>	8.32	1.47	4.82
Inoculum Pattern <sup>2</sup>	14.00	2.17 <sup>cd</sup>	17.90 <sup>ab</sup>	8.10	1.34	4.20
Inoculum Liquid <sup>3</sup>	13.91	2.78 <sup>bc</sup>	17.56 <sup>ab</sup>	8.31	1.44	5.48
Inoculum Peaty <sup>4</sup>	13.52	2.25 <sup>bcd</sup>	21.09 <sup>a</sup>	8.53	1.42	5.01
Média	13.31	2.56	18.35	7.89	1.40	4.91
C.V. (%)	9.08	15.40	20.74	17.99	15.39	17.64

<sup>1</sup> Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. <sup>ns</sup> not significant at 5% error; <sup>2</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

**Table 9.** Cob length (CE), cob diameter (DE), number of row per cob (NFGE), number of grains per row (NGF), mass of grains (MMG) and productivity (PROD) of Pioneer® 30F53 YH hybrid corn plants at Site 3 during the months of November 2013 to March 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha <sup>-1</sup> )
Control	43.98 <sup>c</sup>	10.70 <sup>c</sup>	13.94 <sup>b</sup>	21.13 <sup>c</sup>	273.40 <sup>c</sup>	1954.38 <sup>c</sup>
80 kg ha <sup>-1</sup> N	48.50 <sup>b</sup>	48.50 <sup>b</sup>	15.15 <sup>a</sup>	26.75 <sup>b</sup>	293.50 <sup>bc</sup>	6501.10 <sup>b</sup>
160 kg ha <sup>-1</sup> N	52.18 <sup>a</sup>	52.18 <sup>a</sup>	15.85 <sup>a</sup>	35.43 <sup>a</sup>	316.65 <sup>a</sup>	9175.55 <sup>a</sup>
Inoculum Pattern <sup>2</sup>	48.58 <sup>b</sup>	48.58 <sup>b</sup>	15.38 <sup>a</sup>	26.65 <sup>b</sup>	297.85 <sup>ab</sup>	7935.18 <sup>ab</sup>
Inoculum Liquid <sup>3</sup>	48.58 <sup>b</sup>	48.90 <sup>b</sup>	15.20 <sup>a</sup>	26.35 <sup>b</sup>	299.30 <sup>ab</sup>	7813.90 <sup>ab</sup>
Inoculum Peaty <sup>4</sup>	49.28 <sup>b</sup>	49.28 <sup>b</sup>	15.58 <sup>a</sup>	28.25 <sup>b</sup>	301.83 <sup>ab</sup>	8308.83 <sup>a</sup>
Média	14.75	48.40	15.17	27.43	291.42	6114.82
CV (%)	7.96	2.82	4.50	10.50	4.72	15.93

<sup>1</sup> Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. <sup>ns</sup> not significant at 5% error; <sup>2</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

influenced by the treatments with nitrogen fertilization and inoculation of seeds with *A. brasilense*. The variables CE, DE and NGF were superior to the other treatments when the fertilization with 160 kg ha<sup>-1</sup> N.

When evaluating MMG and productivity seed inoculations promoted averages similar to fertilization with 160 kg ha<sup>-1</sup> N, which was the highest average. Seed inoculations with standard, liquid and turfous inoculant exceeded the treatment with 80 kg ha<sup>-1</sup> N promoting increases of 22.05, 20.19 and 27.80%, respectively (Table 9).

For the experiment conducted at Site 4, N, P and K foliar contents as well as N content in maize grains were not influenced by the treatments used (Table 10).

Unlike the previous site, at Site 4, the variables CE, DE, NFGE, NGF and MMG were not influenced by the treatments used. Productivity was statistically influenced

by the treatments employed, and the highest average was provided by fertilization with 80 kg ha<sup>-1</sup> N, which differed from the control and fertilization treatments with 160 kg ha<sup>-1</sup> N (Table 11).

## DISCUSSION

Results of the interaction between diazotrophic bacteria and maize in terms of agronomic potential, nitrogen fixation or growth promotion, depends on many biotic and environmental factors such as plant genotype, soil microbiological community and nitrogen availability (Roesch et al., 2006).

It is important to note that the effect of inoculation with *Azospirillum* in the experiments conducted can not be correlated only with the increase of N, but also with other

**Table 10.** Nitrogen, phosphorus and potassium contents in foliar tissue and grain of hybrid corn plants Pioneer® 30F53 YH at Site 4 during the months of December 2013 to April 2014.

Treatment	Level on foliar tissue			Level on the seed		
	N	P	K	N	P	K
	----- (g kg <sup>-1</sup> ) -----					
Control	25.40 <sup>ns</sup>	3.00 <sup>ns</sup>	22.50 <sup>ns</sup>	14.00 <sup>ns</sup>	1.38 <sup>ns</sup>	4.44 <sup>ns</sup>
80 kg ha <sup>-1</sup> N	26.00	3.00	22.50	13.30	1.40	4.58
160 kg ha <sup>-1</sup> N	25.90	3.00	20.60	13.70	1.51	5.56
Inoculum Pattern <sup>2</sup>	24.70	3.10	20.60	13.50	1.41	4.57
Inoculum Liquid <sup>3</sup>	26.00	3.10	25.60	13.60	1.44	5.10
Inoculum Peaty <sup>4</sup>	26.60	3.40	25.60	13.90	1.46	5.25
Média	25.76	3.10	22.90	13.66	1.43	4.92
CV (%)	5.61	7.22	11.50	7.98	13.45	15.52

<sup>1</sup> Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. <sup>ns</sup> not significant at 5% error; <sup>2</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

**Table 11.** Cob length (CE), cob diameter (DE), number of row of grain per cob (NFGE), number of grains per row (NGF), mass thousand grains (MMG) and productivity (PROD), of plants of hybrid corn Pioneer® 30F53 YH at Site 4 during the months of December 2013 to April 2014.

Treatments	CE (cm)	DE (mm)	NFGE	NGF	MMG (g)	PROD (kg ha <sup>-1</sup> )
Control	13.8 <sup>ns</sup>	4.0 <sup>ns</sup>	15.4 <sup>ns</sup>	30.12 <sup>ns</sup>	218.4 <sup>ns</sup>	4967 <sup>b</sup>
80 kg ha <sup>-1</sup> N	14.8	4.1	16.1	31.85	214.2	5993 <sup>a</sup>
160 kg ha <sup>-1</sup> N	14.5	4.0	15.4	31.36	211.5	4953 <sup>b</sup>
Inoculum Pattern <sup>2</sup>	15.0	4.2	16.0	31.71	231.3	5590 <sup>ab</sup>
Inoculum Liquid <sup>3</sup>	14.4	4.1	15.5	30.92	230.2	5746 <sup>ab</sup>
Inoculum Peaty <sup>4</sup>	14.6	4.1	15.5	30.25	234.0	5586 <sup>ab</sup>
Média	6.26	2.76	4.61	31.04	10.06	19.47
C.V. (%)	2.09	0.26	1.65	1.25	26.3	963

<sup>1</sup> Measures followed by the same lowercase letter in the column do not differ, at 5% probability of error by the Duncan test. <sup>ns</sup> not significant at 5% error; <sup>2</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum PATTERN AzoTotal® 'Liquid'; <sup>3</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses 'Liquid'; <sup>4</sup> 80 kg ha<sup>-1</sup> de N + inoculation of seed with inoculum Nitro 1000 Grasses "Peat".

nutrients (Holguin and Bashan, 1996; Ferreira et al., 2013). The results obtained in the present research for potassium in Site 3 and phosphorus at Sites 1 and 3.

Thus, based on research data with field inoculation experiments (Okon and Vanderleyden, 1997), the genus *Azospirillum* spp. promotes gains in productivity of important crops in the most varied conditions of climate and soil. However, they point out that the gain with inoculation goes beyond simply aiding in the biological fixation of N<sub>2</sub>, also helping in the increase of the absorption surface of the roots of the plant and, consequently, in the increase of the volume of the explored soil, being able to increase the absorption of other nutrients.

According to the authors Okon and Vanderleyden (1997), this finding is justified by the fact that the inoculation modifies the morphology of the root system,

increasing not only the number of radicles but also the diameter of the lateral and adventitious roots.

The modifications of the root system is related that *Azospirillum* spp. In plants, produce and stimulate the production of growth promoting substances, among them auxins, gibberilins and cytokinins, and not only the biological fixation of nitrogen.

According to Cantarella (2007), the N foliar sufficient level is 27.5 to 32.5 g kg<sup>-1</sup> N, our result showed below the critical level for the maize crop. For the contents of the other nutrients, the values are within the appropriate, regardless of the treatments. When considering the critical levels established P and K sufficiency range in foliar tissue is, 2.5 to 4.0, and 17.0 to 22.5 g kg<sup>-1</sup>, respectively (Cruz et al., 2008). In this way, the results of P and K in all studied sites are presented in the range of sufficiency, except for the K content obtained in the

control treatment in Site 3, which was below the critical level.

The results demonstrated for the foliar N content evidenced that the effect of plant growth promotion by the action of *A. brasilense* is not restricted to biological fixation of nitrogen, although it contributes in part to the supply of N to the corn plant. About 50% as demonstrated in the present study. However, part of this 50% may have been supplied via the promotion of root growth, due to the induction of plant hormone synthesis, such as auxins (Radwan et al., 2004; Kuss et al., 2007), increasing the nutrient absorption capacity from the decomposition of the pre-existing straw.

Results similar to the present study are demonstrated (Salomone and Dobereiner, 1996) which verified increases in productivity with the inoculation of *Azospirillum* spp. In different crop conditions. In this same sense Cavallet et al. (2000), verified higher yield indexes in the corn crop, as a consequence of inoculation *A. brasilense*.

Okon and Labandera-Gonzalez (1994), when evaluating twenty years of studies with the inoculation of *Azospirillum* sp. it was found that 60 to 70% of the experiments had positive results. In this sense, the beneficial effects of inoculation with *A. brasilense* have already been reported in several studies in the literature (Dartora et al., 2013; Repke et al., 2013; Quadros et al., 2014; Costa et al., 2015; Marini et al., 2015; Morais et al., 2015), in addition to the increase in productivity, the characteristics that these bacteria have in synthesizing growth-related phyton- mones are shown in Figure, such as auxins, gibber- linins and cytokinins (Kuss et al., 2007; Perrig et al., 2007) and the availability of N<sub>2</sub> present in the soil in absorbable forms for plants.

For the experiment conducted at Site 4, it is worth highlighting that after flowering, the high incidence of foliar diseases in maize was observed, which may have impaired the photosynthetic process and the transsite of assimilates to the grains, explaining in part the average productivity of grains.

In general, the results observed in the present work, involving four sites, developed in three distinct regions, show the agronomic efficiency of *A. brasilense* (strains AbV5 and AbV6) in promoting plant growth, contributing to good maize of the hybrid Pioner® 30F53 YH, which received inoculation via seed, with half of the nitrogen dose recommended.

## Conclusions

The inoculation of the seeds of corn with 100 mL to 25 kg of seed with the liquid and turfous inoculants, based on the bacterium *Azospirillum brasilense*, presents agronomic efficiency.

Inoculation with *Azospirillum brasilense*, regardless of the physical nature of the inoculant (liquid or turfous), allowed to reduce nitrogen fertilization in the corn crop by

50% without compromising final crop yield.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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