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African Journal of Agricultural Research

Full Length Research Paper

Effects of tillage practices, cropping systems and organic inputs on soil nutrient content in Machakos County

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Low use efficiencies of inorganic fertilizers coupled with their rising costs has diverted attention of farmers towards organic sources. A study was conducted in Yatta sub-county between October 2012 to February 2013 short rains and April-August 2013 long rainy seasons to evaluate how tillage, cropping and organic inputs influenced soil nutrient status. A randomized complete block design with a split-split plot arrangement replicated three times was used. The main plots were tillage practices (TP): Split-plots comprised the cropping systems (CS) while split-split plots were organic inputs, plus the control. The test crops were sorghum and sweet potatoes (*Impomea batata*) with Dolichos (*Dolichos lablab*) and chickpea (*Cicer arietinum* L.) added either as intercrops or in rotation. Soil was randomly sampled at 0 to 30 cm depth at the onset of the experiment and at maturity of test crop for NPK and % organic carbon (OC) analysis. Significant (P≤0.05) high level of K (1.91 Cmol/+kg), available P (51.45 ppm), total N (0.19%) and OC (2.19%), in combined TR, intercrop sorghum/chickpea with application of Minjingu rock phosphate (MRP)+ farmyard manure (FYM) during SRS of 2012 compared to the other treatment combinations was observed. Comparing different organic inputs, tillage practices and cropping systems combined tied ridges (TR), intercrop of sorghum/chickpea and MRP+FYM and FYM increased the soil nutrients status. Therefore, soil organic inputs such as MPR and FYM are viable alternatives to inorganic fertilizers for improving the soil nutrient status. The study therefore recommends incorporation of the organic inputs in combination with TR, as well as intercropping with legumes in their cropping systems to improve soil health and resilience.

Key words: Cropping systems, tillage practices, organic inputs, semi-arid, soil nutrients.

INTRODUCTION

Low soil fertility and moisture deficits are major constraints to crop production in the semi-arid areas of Kenya. Many interrelated factors, both natural and managerial, lead to soil fertility decline either through leaching, erosion, and crop harvesting (Donovan and Casey, 1998). The low soil fertility is majorly contributed by agriculture intensification particularly in developing countries (Rezig et al., 2012) due to the ever-increasing

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food demand for the rising population. Unless the nutrients are replenished using organic or mineral fertilizers, partially returned through crop residues or rebuilt more comprehensively through traditional fallow systems that allow restoration of nutrients and reconstruction of soil organic matter, soil nutrient levels will decline continuously. Therefore, the use of species different from the main crop such as legumes contributes to the nutrient balance, which may consequently increase soil fertility level over time. Leguminous species are known for their capacity to fix atmospheric di-nitrogen in association with rhizobia bacteria and hence narrow the C: N ratio, resulting in faster residue decomposition (Aita and Giacomini, 2003) with consequent release of accumulated N, P and K to the soil (Borkert et al*.,* 2003). Legume green manures are also efficient at mobilizing P from the soil (Knight and Shirtliffe, 2005) pool through decomposition and release in a labile form that enhances P nutrition of succeeding crops (Cavigelli and Thien, 2003).

Farmers in the Eastern part of Kenya use farmyard manure (FYM) as a cheaper alternative source of plant nutrients as opposed to the more expensive inorganic fertilizers (Gichangi et al*.,* 2007). Farmyard manure acts as an alternative source of fertility enhancement as they release nutrients more slowly and steadily over a period of time and also improve the soil fertility status by activating the soil microbial biomass (Belay et al*.,* 2001; Karuku and Mochoge, 2016). Consequently, inputs from organic sources such as FYM, play a pivotal role in the productivity of many farming systems by providing nutrients through decomposition and substrate for the synthesis of soil organic matter (SOM). SOM has been shown to improve crop growth and yield by supplying nutrients or by modifying soil physical properties (Rees et al*.,* 2000; Karuku et al*.,* 2012, 2014) and environment.

Furthermore, SOM acts as a bonding and dispersing agent by increasing inter-particle hydrophobicity and cohesion within aggregates (Mullins, 2000; Abiven et al., 2009). It is well known that manures are sources of all-necessary macro- and micro-nutrients in available forms, thereby improving the physical, chemical and biological properties of the soil (El-Magd et al., 2005; Zhang, 2005). Due to the slow process of decomposition, manures are usually applied at higher rates, relative to that of inorganic fertilizers, to meet crop nutrient requirements and the excess have positive residual effects on the growth and yield of succeeding crops (Makinde and Ayoola, 2008). Application of manures to soil similarly provide other potential benefits such as improved fertility and structure, increased soil organic matter buildup and improved water holding capacity (Phan et al*.*, 2002; Blay et al*.*, 2002). In addition, tillage practices such as Tied ridges and Furrows and Ridges may allow rainwater retention on open furrows for longer duration as the water infiltrates into the soil or soil management techniques that favor prolonged rainwater infiltration and retention, thus raising the overall soil moisture availability and hence improved crop production in arid and semi-arid lands (ASALs) (Itabari et al., 2003). This study evaluated effects of tillage practices, cropping systems and organic inputs on NPK and organic carbon in Yatta sub County.

METHODOLOGY

Study site

The study was carried out in Yatta Sub County, Kenya, coordinates: 1.4667°S and 37.8333°E at 944 m asl. The sub-county falls under agro-ecological zones IV, classified as semi-arid lands (Jaetzold and Schmidt, 2006). Yatta Sub County comprises a suite of soils that includes Acrisols and Luvisols in association with Ferrallisols (WRB, 2015). In most places, the topsoil is loamy sand to sandy loam, sandy clay to clay with low nutrient availability (Kibunja et al*.,* 2010).

The mean annual temperature vary from 18 to 24°C; also, the area experiences bimodal rainfall with long rains in early April to May (about 400 mm) and short rains commencing from early October to December (500 mm). Most farmers in the Sub County practice small-scale mixed farming with crops grown ranging from maize (*Zea mays)*), beans (*Phaseolus vulgaris*), pigeon pea (*Cajanus cajan*), green grams (*Vigna radiata*), sorghum (*Sorghum bicolor*), and cowpea (*Vigna unguiculata*) (Macharia, 2004).

Initial soil analysis indicated that the soils at the site were acidic sandy clay, low in fertility, with low amounts of total nitrogen (TN), organic carbon (OC) and available P (Table 1). This was attributed to farmers' reliance on one continuous cropping system without application of organic inputs. Continuous cropping of land with little or no nutrient returns lead to their depletion, hence decline in soil fertility (Smalling et al*.*, 1997).

Farm yard manure used in the study was slightly alkaline at a pH of 8.2, OC of 18.6%, TN at 2.1% and P and K contents of 0.4% and 2.7%, respectively. The Ca content was 3.4% (Table 2).

Treatments and experimental design

The treatments were tillage practices (Oxen plough, tied ridges, and furrows and ridges), cropping systems (mono cropping, intercropping, and crop rotation), organic inputs (farmyard manure, rock phosphate, and combined farmyard manure and rock phosphate) and control. The experiment was in a randomized complete block design with split-split plot arrangement. The main plots (150 m × 60 m) were tillage practices (Oxen plough, tied ridges and furrows, and ridges). Split plots (10 m × 4 m) were cropping systems (mono cropping, intercropping, and crop rotation) and split-split plots (2.5 m × 1 m) were organic inputs (farmyard manure, rock phosphate and combined FYM and rock phosphate). A control (no organic inputs applied) was also included as a split-split plot. The test crops were sweet potatoes (*Ipomea batatas* lam) and sorghum (*sorghum bicolor* L.) with Dolichos (*Dolichos lablab)* and chickpea (*Cicer arietinum* L.) either as intercrops or in rotation.

Field practices

Land was prepared manually using oxen plough in late September and planted in October short rain of 2012 and April long rain season of 2013. Manure was broadcasted at a rate of 5 tha^{-1}, Minjingu rock phosphate (MRP) at 498 $kgha^{-1}$ equivalent to 60 $kgP\ ha^{-1}$, and

Table 1. Initial soil physical and chemical properties.

Parameter	%OC	%TN	P (ppm)	K (Mol/Kg)	pH H_20	pH $CACL_2$	CEC
Soil chemical properties	1.86	0.1	26.84	1.91	5	5.63	14.65
	%Clay	**% loam**	**% sand**	**Textural class**			
Soil physical properties	54	22	24	Sand clay			

Table 2. Salient properties of FYM used in the study.

Parameter	%OC	%Total N	%P	%K	%Ca	%Na	pH H_20
chemical properties of FYM	18.6	2.1	0.4	2.7	3.5	0.8	8.2

thoroughly mixed with the soil before the vines and seeds were placed in the holes. Sweet potatoes (wabolinge variety) were propagated through 30 cm long cuttings at a spacing of 90 cm between rows and 30 cm within rows. Weeding was done 5 weeks after planting and harvesting after 6 months when the leaves turned yellow and dry. The harvesting was done using a sharp hoe to remove all tubers. Sorghum (serendo variety) was sown at spacing of 75 cm by 30 cm while Dolichos and chickpea were planted at a spacing of 30 cm within the sorghum and sweet potato rows. Weeding was done after 5 weeks of planting and harvesting after three months when it had reached physiological maturity.

Soil sampling and analysis

Soil samples were collected in a transect (in a zigzag manner from one edge of the field) for initial soil analysis. Soil samples were later taken at maturity of sweet potato and sorghum as main crops, at three samples per treatment which were then composited into a single sample and mixed thoroughly. The sample was air-dried by spreading it out in a clean, warm, dry area for two days before being analyzed for N by micro-Kjeldahl method as described by Bremner (1996); P by double acid method; K by flame photometry and organic carbon determined following Walkley and Black (1934) as described by Nelson and Sommers (1996). Soil pH-H_2O and pH-$CaCl_2$ was determined with a pH meter in a 1:2.5 ratio extract. Electrical conductivity (ECe) was measured on a 1:2.5 ratio extract with an EC meter.

Statistical analysis

Data was subjected to general analysis of variance using GenStat statistical software (Payne et al*.,* 2005) version 18. Means were separated using least significant difference at a probability level of 5%.

RESULTS AND DISCUSSION

Effects of tillage practice, cropping systems and organic inputs on soil nutrients status

Potassium (K) content was significantly (P≤0.05) affected by the organic inputs as increased level were recorded with application of MRP + FYM in all tillage practices and cropping systems, compared to the other organic inputs MRP, FYM and their controls. Increased K content was observed under combined oxen plough (OP), sorghum mono cropping with application of MRP+FYM (3.37 Cmol+/kg) and intercropping of sweet potato/dolichos (3.08 Cmol+/kg) as compared to other tillage practices, combined furrows and ridges, intercropping of sorghum/dolichos with application of MRP+FYM (2.01 Cmol+/kg) and intercropping of sweet potato/dolichos (2.14 Cmol+/kg) and tied ridges along with intercropping of sorghum/chickpea (1.91 Cmol+/kg) and intercropping of sweet potato /chickpea (2.06 Cmol+/kg).

Increased K content under MRP+FYM application was attributed to the fact that when farmyard manure and Minjingu rock phosphate are mixed, it enhances release of other nutrients such as K through increased microbial activity in the soil. Same applies when FYM was applied. Low K content under Tied ridges (1.91 Cmol+/kg); Furrows and ridges (2.11 Cmol+/kg) compared to OP (2.95 Cmol+/kg) could be attributed to increased soil moisture content leading to loss of the nutrients down the profile due to leaching of K in the upper profile as compared to OP. Under different cropping systems, increased K content was observed under intercrop and crop rotation of both chickpea and dolichos in all tillage practices. This was attributable to the effects of exudates such as H^+ and other organic acids released by the legumes in the rhizosphere along with works on the organic materials applied, thus mineralizing more nutrients to the soil. Root-secreted chemicals mediate multi-partite interactions in the rhizosphere, where plant roots continually respond to and alter their immediate environment. Increasing evidence suggests that root exudates initiate and modulate dialogue between roots and soil microbes. For example, root exudates serve as signals that initiate symbiosis with rhizobia and mycorrhizal fungi. In addition, root exudates maintain and

support a highly specific diversity of microbes in the rhizosphere of given particular plant species, thus suggesting a close evolutionary link (Dayakar and Jorge, 2009; Sunita, 2017). Moreover, inclusion of legumes in crop rotations protects the fragile soil surface by restoring the organic matter content and organic fertility of these soils and this would also help to restore the natural fertility of these soils (Ahmad et al., 2010a; Liu et al., 2006).

Increased potassium level under intercropping and crop rotation of chickpea and dolichos was also reported by Ahmad et al. (2010b) who found out that use of green manure especially legumes in a cropping pattern could help restore crop productivity. In addition, Aziz et al. (2010) reported that manure application significantly increases soil K contents due to increased microbial activity in the soil. Another similar observation was made by Suge et al. (2011), who found that addition of organic fertilizers improve soil water holding capacity as well as the CEC and nutrients are released slowly to crop plants, thus impacting on nutrients availability. The inclusion in a rotation of cover crops or green manures can also enhance the efficient use of nutrients by plants, mainly owing to the increase in soil microbial population and activity (Watson et al., 2002).

Changes in potassium content Cmol+/Kg across the seasons (SRS 2012 and LRS 2013)

Changes in potassium content across the two seasons was observed with increase during the LRS (3.65 Cmol+/kg) and (3.39 Cmol+/kg) as compared to the SRS (3.37 Cmol+/kg) and (3.09 Cmol+/kg) under oxen plough in sorghum mono cropping and intercropping of sweet potato/dolichos with the application of MRP+FYMin sorghum and sweet potato plots, respectively (Tables 3 and 4). During the LRS of 2013, the soil moisture content increased as a result of prolonged rainfall as opposed to SRS of 2012. Soil moisture content affects the availability of K in soil, with greater efficiency of K fertilizer with increasing soil moisture (Kuchenbuch et al., 1986) since it influence microbial activities responsible for decomposition for release of potassium.

Decomposition of organic matter is chiefly carried out by heterotrophic microorganisms. This process is influenced by temperature, moisture and ambient soil conditions leading to the release and cycling of plant nutrients, especially nitrogen (N), potassium and phosphorus (Murphy et al., 2007; Sunita, 2017).

Available phosphorous

The soil available P level increased significantly ($P \leq 0.05$) in plots with MRP+FYM compared to other treatments FYM, MRP and control. Accordingly, combined TR, intercropping sweet potato and sorghum/dolichos with application of MRP + FYM had highest P levels (51.45 ppm and 46.31 ppm), respectively in the SRS of 2012 (Tables 5 and 6). Increased available P with application of MRP+FYM was due to the enhanced release from MRP when mixed with FYM since decomposition of FYM releases humic acid which further promote the release of P from the rock. Organic exudates of soil microbes and roots of grain legume crops can mobilize phosphorus from unavailable soil-P pool and increase its availability for P inefficient plant species grown in inter-cropping or crop rotation. Legume crops adopt different strategies such as development of cluster roots, exudation of carboxylates, protons and acid phosphatase to render the P available from inorganic and organic P sources (Hawkins et al., 2005; Lambers et al., 2006; Sunita, 2017).

Thus, inter-cropping or crop rotation of cereal crops with such legumes that have improved mechanisms to gain access to this fixed P will contribute toward more sustainable agriculture (Sunita, 2017). In addition, it implies that MRP underwent considerable dissolution leading to release of P in the MRP applied. Addition of FYM results into an increased microorganism decomposition rates and thus release phosphorous into the soil. Organic manures after decomposition may also provide organic acids and increase P-bioavailability after dissolution of MRP when combined with FYM. Sunita (2017) reported similar findings in India. This also conforms to a study by Mengel and Kirkby (2001), and Marschner (2011) who noted an increase in P contents with addition of FYM and attributed it to mineralization and increased water holding capacity, thus making P readily available to crops. A study by Kari (1996) stated that application of FYM affect available P content considerably. In addition, FYM increase soil moisture content (Boateng et al., 2006), increase microbial activity and resultant biochemical transformations of P in soil. The added organic manures may lead to mineralization of more recalcitrant P fraction (Nziguheba et al., 1998) as also reported by Maerere et al. (2001) and Odedina et al. (2011) in their studies.

There was a significant difference ($p \leq 0.05$) across the tillage practices with increased available content under Tied ridges (51.45 ppm) compared to Furrows and ridges (48.24 ppm) and Oxen plough (38.59 ppm) under intercropping sorghum/chickpea and with the application of MRP+FYM during the SRS of 2012. The increased P under Tied ridges and Furrows and ridges was attributed to the increased soil moisture content harvested under Tied ridges and Furrows and ridges resulting in reduced runoff, hence less soil loss by erosion and then reduced P losses in soil are less mobile and most losses are due to soil erosion. Kaushik and Gautam (1997) found out that increased soil water retention reduces nutrients

Table 3. Effects of tillage practice, cropping systems and organic inputs on soil potassium Cmol+/kg sorghum based plots during SRS of 2012 and LRS of 2013.

TP	CS	CROP	SRS 2012				LRS 2013			
			CTRL	FYM	MRP	MRP+FYM	CTRL	FYM	MRP	MRP+FYM
FR	Crop rotation	CP-SOR	1.29^{bc}	1.4^{de}	1.48^{def}	1.67^{gh}	1.4^{bc}	1.52^{de}	1.6^{def}	1.81^{gh}
	Crop rotation	DOL-SOR	1.08^{a}	1.13^{a}	1.18^{ab}	1.34^{cd}	1.17^{a}	1.22^{a}	1.28^{ab}	1.45^{cd}
	Inter cropping	SOR/DOL	1.62^{gh}	1.7^{ghi}	1.77^{ghi}	2.01^{k}	1.75^{gh}	1.84^{ghi}	1.92^{ghi}	2.18^{k}
	Inter cropping	SOR/CP	1.55^{defg}	1.72^{gh}i	1.87^{ghi}	2.11^{k}	1.68^{defg}	1.84^{ghi}	1.92^{ghi}	l2.18^{k}
	Mono cropping	SOR	1.19^{ab}	1.29^{bc}	1.36^{cd}	1.54^{defg}	1.29^{ab}	1.4^{bc}	1.47^{cd}	1.67^{defg}
OP	Crop rotation	CP-SOR	2.54^{m}	2.66^{mn}	2.78^{o}	3.15^{q}	2.75^{m}	2.88^{mn}	3.01^{o}	3.41^{q}
	Crop rotation	DOL-SOR	2.64^{mn}	2.77^{o}	2.9^{p}	3.28^{qr}	2.86^{mn}	3^{o}	3.14^{p}	3.56^{q}
	Inter cropping	SOR/DOL	2.54^{m}	2.66^{mn}	2.78^{o}	3.15^{q}	2.75^{m}	2.88^{mn}	3.01^{o}	3.41^{q}
	Inter cropping	SOR/CP	2.37^{ln}	2.49^{m}	2.6^{mn}	2.95^{p}	2.57^{l}	2.69^{m}	2.82^{mn}	3.19^{p}
	Mono cropping	SOR	3.08^{q}	3.82^{t}	3.22^{qr}	3.37^{s}	3.33^{q}	4.14^{t}	3.49^{qr}	3.65^{s}
TR	Crop rotation	CP-SOR	1.45^{def}	1.58^{defg}	1.66^{gh}	1.88^{ghij}	1.58^{def}	1.71^{defg}	1.8^{gh}	2.04^{ghij}
	Crop rotation	DOL-SOR	1.16^{a}	1.26^{bc}	1.33^{cd}	1.51^{def}	1.26^{a}	1.37^{bc}	1.44^{cd}	1.63^{def}
	Inter cropping	SOR/DOL	1.33^{cd}	1.44^{de}	1.52^{def}	1.72^{ghi}	1.44^{cd}	1.56^{de}	1.64^{def}	1.86^{ghi}
	Inter cropping	SOR/CP	1.47^{def}	1.61^{gh}	1.68^{gh}	1.91^{ghij}	1.6^{def}	1.75^{gh}	1.82^{gh}	2.07^{ghij}
	Mono cropping	SOR	1.13^{a}	1.23^{ab}	1.29^{bc}	1.46^{def}	1.22^{a}	1.33^{ab}	1.4^{bc}	1.59^{def}

SOR-sorghum, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OP-oxen plough, FYM-farm yard -manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system. Under rotation legumes were harvested during the short rain season 2012 whereas sweet potatoes and sorghum were harvested during the long rain season 2013. Means followed by the same letters in the same season in a column are not significantly different at P ≤ 0.05.

losses through erosion. Oxen ploughed plots may have had lower P due to increased loss through erosion and leaching. Use of the oxen plough tillage practice could increase erosion due to the inappropriate width adjustment on the plough which led to formation of plough furrows acceleration on the rate of rill erosion, especially in sloping lands causing nutrients losses as documented by Kaumbutho and Simalenga (1999).

There was also a significant difference (p≤0.05) across all the cropping systems with increased P content under the intercropping of chickpea (51.45 ppm) and dolichos (46.88 ppm) in Tied ridges with the application of MRP+FYM during SRS of 2012. This was due to enhanced release of the nutrients from the organic inputs as presence of the legumes enhanced release, fixation of nutrients and increased biological activity rotation (Suge et al*.,* 2011; Larkin, 2008). Suge et al. (2011) attributed increased available P to crop while Larkin (2008) indicated that crop rotation help in pests and diseases control, thus increasing soil biological activity. Christen and Sieling (1995) observed increased water use efficiency, which in turn increased P content in the soil under crop rotation. This conforms to a study that crop rotation along with increasing soil organic matter increased biodiversity and soil biological community (Kamkar and Mahdavi, (2009).

Changes in phosphorous content ppm across the season (SRS 2012 and LRS 2013)

Changes in P content across the two season

Table 4. Effects of tillage practice and organic cropping systems on soil potassium Cmol+/kg sweet potato based plots during SRS of 2012 and LRS of 2013.

TP	CS	CROP	SRS 2012				LRS 2013			
			CTRL	FYM	MRP	MRP+FYM	CTRL	FYM	MRP	MRP+FYM
FR	Crop rotation	CP-SP	1.58^{h}	1.66^{hi}	1.73^{hij}	1.96^{m}	1.74^{h}	1.82^{hi}	1.91^{hij}	2.16^{m}
	Crop rotation	DOL-SP	1.04^{a}	1.09^{b}	1.14^{b}	1.29^{bcd}	1.14^{a}	1.2^{b}	1.25^{b}	1.42^{bcd}
	Inter cropping	SP/DOL	1.73^{hij}	1.81^{k}	1.89^{l}	2.14^{o}	1.9^{hij}	1.99^{k}	2.08^{l}	2.36^{o}
	Inter cropping	SP/CP	1.13^{b}	1.19^{bc}	1.24^{bcd}	1.41^{ef}	1.25^{b}	1.31^{bc}	1.37^{bcd}	1.55^{ef}
	Mono cropping	SP	1.09^{b}	1.18^{bc}	1.24^{bcd}	1.41^{ef}	1.19^{b}	1.3^{bc}	1.37^{bcd}	1.55^{ef}
OP	Crop rotation	CP-SP	2.28^{q}	2.38^{r}	2.49^{s}	2.82^{v}	2.5^{q}	2.62^{r}	2.74^{s}	3.11^{v}
	Crop rotation	DOL-SP	1.19^{bc}	1.24^{bcd}	1.3^{bcde}	1.47^{efg}	1.31^{bc}	1.37^{bcd}	1.43^{bcde}	1.62^{efg}
	Inter cropping	SP/DOL	2.48^{s}	2.6^{t}	2.72^{u}	3.08^{w}	2.73^{s}	2.86^{t}	2.99^{u}	3.39^{w}
	Inter cropping	SP/CP	1.29^{bcd}	1.36^{ef}	1.42^{efg}	1.61^{h}	1.42^{bcd}	1.49^{ef}	1.56^{efg}	1.77^{h}
	Mono cropping	SP	2.21^{p}	2.75^{u}	2.32^{q}	2.42^{r}	2.43^{p}	3.02^{u}	2.55^{q}	2.67^{r}
TR	Crop rotation	CP-SP	1.27^{bcd}	1.38^{ef}	1.45^{efg}	1.65^{hi}	1.4^{bcd}	1.52^{ef}	1.6^{efg}	1.81^{hi}
	Crop rotation	DOL-SP	1.02^{a}	1.11^{b}	1.16^{bc}	1.32^{bcde}	1.12^{a}	1.22^{b}	1.28^{bc}	1.45^{bcde}
	Inter cropping	SP/DOL	1.57^{h}	1.71^{hij}	1.8^{k}	2.04^{n}	1.73^{h}	1.88^{hij}	1.98^{k}	2.24^{n}
	Inter cropping	SP/CP	1.58^{h}	1.73^{hij}	1.81^{k}	2.06^{n}	1.75^{h}	1.89^{hij}	1.99^{k}	2.26^{n}
	Mono cropping	SP	1.03^{a}	1.12^{b}	1.18^{bc}	1.34^{bcde}	1.13^{a}	1.23^{b}	1.3^{bc}	1.47^{bcde}

SP-Sweet potato, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OP-oxen plough, FYM-farm yard -manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system.. Means followed by the same letters in the same season in a column are not significantly different at $P \leq 0.05$.

indicated an increase during the LRS (58.8 ppm) and (52.92 ppm) compared to the SRS (51.45 ppm) and (46.31 ppm) under OP in intercropping of sorghum/chickpea and sweet potato/ chickpea with the application of MRP+FYM in sorghum and sweet potato plots, respectively (Tables 7 and 8). The higher amounts of soil available P in the LRS 2013 than SRS 2012 was attributed to the residual effects of the organic inputs applied (MRP, MRP+FYM and FYM). According to Rowell (1994), the rapid adsorption of P onto soil particle is followed by a slower conversion into less available forms including mineral phosphates; thus, P in the MPR and most phosphate fertilizers is available in the first season after application but the rest remains over long periods of time, hence their residual effects.

Total nitrogen

Total N increased significantly ($P \leq 0.05$) through application of FYM in all the tillage practices and cropping systems compared to other treatments. Significant ($P \leq 0.05$) increased % TN (0.19) was recorded under FYM with the intercrop of dolichos/sorghum in furrows and ridges (Tables 7 and 8). The increase in soil TN after FYM application was due to addition of N by decomposing FYM. These results conform to the findings of Thamaraiselvi et al. (2012) who reported increases in soil TN with FYM application. Nyambati (2000) also reported that MRP and organics (FYM) combinations provide cheap N sources. Also, solubilization of MRP through formation of favorable acid environments that results when organics are in contact with MRP decompose in soils releasing N to the soil.

Percent TN content increased significantly

Table 5. Effects of tillage practice and organic inputs on soil available phosphorous sorghum based plots during SRS of 2012 and LRS of 2013.

TP	CS	CROPS	SRS 2012				LRS 2013			
			CTRL	FYM	MRP	MRP+FYM	CTRL	FYM	MRP	MRP+FYM
FR	Crop rotation	CP-SOR	27.1^{g}	29.04^{j}	30.25^{k}	34.28^{p}	30.97^{g}	33.19^{j}	34.57^{k}	39.18^{p}
	Crop rotation	DOL-SOR	30.78^{l}	32.98^{lmn}	34.35^{p}	38.93^{s}	35.18^{l}	37.69^{lmn}	39.26^{p}	44.5^{s}
	Inter cropping	SOR/DOL	34.75^{p}	37.23^{r}	38.78^{s}	43.95^{x}	39.71^{p}	42.55^{r}	44.32^{s}	50.23^{x}
	Inter cropping	SOR/CP	38.14^{s}	40.86^{v}	42.56^{v}	48.24^{y}	43.58^{s}	46.7^{v}	48.64^{v}	55.13^{y}
	Mono cropping	SOR	25.17^{ef}	26.96^{g}	31.83^{lm}	28.09^{ghi}	28.76^{ef}	30.82^{g}	36.38^{lm}	32.1^{ghi}
OP	Crop rotation	CP-SOR	21.68^{b}	23.23^{d}	24.2^{e}	27.42^{gh}	24.78^{b}	26.55^{d}	27.66^{e}	31.34^{gh}
	Crop rotation	DOL-SOR	24.62^{e}	26.38^{g}	27.48^{gh}	31.15^{l}	28.14^{e}	30.15^{g}	31.41^{gh}	35.6^{l}
	Inter cropping	SOR/DOL	27.8^{gh}	29.78^{k}	31.03^{l}	35.16^{q}	31.77^{gh}	34.04^{k}	35.46^{l}	40.19^{q}
	Inter cropping	SOR/CP	30.51^{k}	32.69^{lmn}	34.05p	38.59^{s}	34.87^{k}	37.36^{lmn}	38.91^{p}	44.1^{s}
	Mono cropping	SOR	20.13^{a}	22.47^{c}	21.57^{b}	25.47^{ef}	23.01^{a}	25.68^{c}	24.65^{b}	29.1^{ef}
TR	Crop rotation	CP-SOR	28.91^{j}	30.97^{l}	32.26^{lm}	36.57^{r}	33.04^{j}	35.4^{l}	36.87^{lm}	41.79^{r}
	Crop rotation	DOL-SOR	32.83^{lmn}	35.18^{q}	36.64^{r}	41.53^{v}	37.52^{lmn}	40.2^{q}	41.88^{r}	47.46^{v}
	Inter cropping	SOR/DOL	37.07^{r}	39.71^{t}	41.37^{v}	46.88^{y}	42.36^{r}	45.39^{t}	47.28^{v}	53.58^{y}
	Inter cropping	SOR/CP	40.68^{u}	43.58^{w}	45.4^{x}	51.45^{z}	46.49^{u}	49.81^{w}	51.89^{x}	58.8^{z}
	Mono cropping	SOR	26.84^{g}	28.76^{j}	33.95^{o}	29.96^{k}	30.68^{g}	32.87^{j}	38.81^{o}	34.24^{k}

SOR-Sorghum, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OP-oxen plough, FYM-farm yard -manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system. Means followed by the same letters in the same season in a column are not significantly different at $P \leq 0.05$.

($p \leq 0.05$) across cropping system with crop rotation dolichos/sorghum at 0.21% and intercrop sorghum/chickpea at 0.19% under Tied ridges and application of FYM (Table 7). This same trend was observed under Furrows and ridges (0.19% and 0.17%) and OP (0.15% and 0.13%) under crop rotation of dolichos/sorghum and intercrop though the content was lower compared to sorghum planted plots. Increase in TN under dolichos intercrop and rotation was attributed to the legumes ability to fix Nitrogen and the amount obtained from the legumes residues which led to increased soil organic matter (SOM) as opposed to the mono-cropping. Aita and Giacomini (2003) observed that leguminous species have capacity to fix atmospheric nitrogen and narrow the C/N ratio, resulting in faster residue decomposition and consequent release of accumulated N and other nutrients such as P and K to the soil. Crop rotations usually increase organic matter and prompt changes in N sources, affecting their availability to plants and, as a consequence, the N efficiency is greater when a crop rotation is adopted (Montemurro and Maiorana, 2008). In this study, it was observed that ridges and furrows enhanced infiltration thus reducing runoff and consequently prevented nutrient losses, a fact consistent with FAO (1993). The lower TN content in OP compared to ridges and furrows and Tied ridges was attributed to higher soil erosion and runoff (Kaumbutho and Simalenga, 1999) leading to their loss.

Changes in total nitrogen across the season

Changes in % TN across the two season was observed to increase during the LRS at 0.23 and 0.21% compared to SRS at 0.19 and 0.19% under

Table 6. Effects of tillage practice and organic inputs on soil available phosphorous sweet potato based plots during Short Rain Season 2012 and Long Rain Season 2013.

TP	CS	CROPS	SRS 2012				LRS 2013			
			CTRL	FYM	MRP	MRP+FYM	CTRL	FYM	MRP	MRP+FYM
FR	Crop rotation	CP-SP	24.39^{f}	26.13^{fghi}	27.22^{fghij}	30.85^{m}	27.88^{f}	29.87^{fghi}	31.11^{fghij}	35.26^{m}
	Crop rotation	DOL-SP	27.7^{fghij}	29.68^{jkl}	30.92^{m}	35.04^{p}	31.66^{fghij}	33.92^{jkl}	35.33^{m}	40.05^{p}
	Inter cropping	SP/DOL	31.27^{m}	33.51^{o}	34.9^{p}	39.56^{u}	35.74^{m}	38.29^{o}	39.89^{p}	45.21^{u}
	Inter cropping	SP/CP	34.32^{p}	36.77^{r}	38.31^{t}	43.41^{x}	39.23^{p}	42.03^{r}	43.78^{t}	49.62^{x}
	Mono cropping	SP	22.65^{de}	24.27^{f}	28.65^{jkl}	25.28^{fgh}	25.89^{de}	27.73^{f}	32.74^{jkl}	28.89^{fgh}
OP	Crop rotation	CP-SP	19.51^{b}	20.91^{bc}	21.78^{d}	24.68^{fg}	22.3^{b}	23.89^{bc}	24.89^{d}	28.21^{fg}
	Crop rotation	DOL-SP	22.16^{d}	23.74f	24.73^{fg}	28.03^{jk}	25.33^{d}	27.14^{f}	28.27^{fg}	32.04^{jk}
	Inter cropping	SP/DOL	25.02^{fg}	26.81^{fghi}	27.92^{jk}	31.65^{mn}	28.59^{fg}	30.64^{fghi}	31.91^{jk}	36.17^{mn}
	Inter cropping	SP/CP	27.46^{fghij}	29.42^{jkl}	30.65^{m}	34.73^{p}	31.38^{fghij}	33.62^{jkl}	35.02^{m}	39.69^{p}
	Mono cropping	SP	18.12^{a}	20.22^{bc}	19.41^{b}	22.92^{de}	20.71a	23.11^{bc}	22.19^{b}	26.19^{de}
TR	Crop rotation	CP-SP	26.02^{fgh}	27.88^{jk}	29.04^{jkl}	32.91^{o}	$29.73f^{gh}$	31.86^{jk}	33.19^{jkl}	37.61^{o}
	Crop rotation	DOL-SP	29.55^{jkl}	31.66^{mn}	32.98^{o}	37.38^{rs}	33.77^{jkl}	36.18^{mn}	37.69^{o}	42.72^{rs}
	Inter cropping	SP/DOL	33.36^{o}	35.74^{pq}	37.23^{r}	42.2^{w}	38.12^{o}	40.85^{pq}	42.55^{r}	48.22^{w}
	Inter cropping	SP/CP	36.61^{r}	39.23^{u}	40.86^{v}	46.31^{y}	41.84^{r}	44.83^{u}	46.7^{v}	52.92^{y}
	Mono cropping	SP	24.16^{f}	25.89^{fgh}	30.56^{m}	26.96^{fghij}	27.61^{f}	29.58^{fgh}	34.92^{m}	30.82^{fghij}

SP-Sweet potato, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OP-oxen plough, FYM-farm yard-manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system.. Means followed by the same letters in the same season in a column are not significantly different at $P \leq 0.05$.

Tied ridges of intercropped sorghum/chickpea and crop rotation of dolichos/sweet potato with the application of MRP+FYM in sorghum and sweet potato plots, respectively (Tables 7 and 8). This implies that TN increased with increased soil moisture during the LRS of 2013; hence mineralization was dictated by soil moisture content. The study therefore show a correlation between soil moisture and soil N mineralization, which agree with previous studies (Li et al., 1995; Zhou and Ouyang, 2001).

Organic carbon

The level of organic carbon increased significantly ($P \leq 0.05$) across all cropping system and tillage practices compared to initial soil analysis where FYM and MRP + FYM were added. An increased % OC (2.45) and (3.15) was observed in combined OP, intercrop of sorghum/chickpea and dolichos/sweet potato rotations, respectively where FYM was applied (Tables 9 and 10). This was due to high carbon content present in applied FYM as opposed to MRP application alone plus additional residue from legumes that further raised the carbon content. Across the cropping systems, increased % OC was observed under sorghum/dolichos intercropping (2.45%), then rotation of sorghum/chickpea (2.26%) and rotation of sorghum/dolichos (2.19%) under oxen plough with the application of FYM. Cover crops are generally grown to provide soil cover, thus preventing soil erosion by wind and rainwater, which increases organic matter content in the long run (Karuku et al., 2014; Karuku, 2018). Komatsuzaki (2004) indicated that cover crop utilization is a technique that limits nutrient leaching, scavenging the soil residual N and making it available for subsequent cultivation.

Among the three tillage practices, a significant difference ($p \leq 0.05$) was observed with improved % OC under OP followed by furrows and ridges though under different cropping systems and application of FYM. The observed % OC level conform to the study by Bayu et al. (2006) who

Table 7. Effects of tillage practices and organic cropping systems on soil total N on sorghum based plots during SRS of 2012 and LRS of 2013.

TP	CS	CROPS	SRS-2012				LRS-2013			
			CTRL	FYM	MRP	MRP+FYM	CTRL	FYM	MRP	MRP+FYM
FR	Crop rotation	CP-SOR	0.1^{c}	0.13^{f}	0.1^{c}	0.11^{d}	0.1^{d}	0.14^{h}	0.11^{e}	0.12^{f}
	Crop rotation	DOL-SOR	0.15^{h}	0.19^{l}	0.16^{i}	0.16^{i}	0.16^{j}	0.2^{n}	0.17^{k}	0.18^{l}
	Inter cropping	SOR/DOL	0.09^{b}	0.12^{e}	0.09^{b}	0.1^{c}	0.09^{c}	0.13^{g}	0.1^{d}	0.11^{e}
	Inter cropping	SOR/CP	0.13^{f}	0.17^{j}	0.14^{g}	0.15^{h}	0.14^{h}	0.18^{l}	0.15^{i}	0.16^{j}
	Mono cropping	SOR	0.09^{b}	0.11^{d}	0.12^{e}	0.15^{h}	0.1^{d}	0.12^{f}	0.13^{g}	0.16^{j}
OP	Crop rotation	CP-SOR	0.08^{a}	0.11^{d}	0.08^{a}	0.09^{b}	0.08^{b}	0.12^{f}	0.09^{c}	0.1^{d}
	Crop rotation	DOL-SOR	0.12^{e}	0.15^{h}	0.12^{e}	0.13^{f}	0.13^{g}	0.16^{j}	0.14^{h}	0.14^{h}
	Inter cropping	SOR/DOL	0.07^{a}	0.1^{c}	0.07^{a}	0.08^{a}	0.07^{a}	0.11^{e}	0.08^{b}	0.09^{c}
	Inter cropping	SOR/CP	0.1^{c}	0.13^{f}	0.1^{c}	0.11^{d}	0.11e	0.14^{h}	0.12^{f}	0.12^{f}
	Mono cropping	SOR	0.08^{a}	0.12^{e}	0.09^{b}	0.09^{b}	0.08^{b}	0.13^{g}	0.09^{c}	0.1^{d}
TR	Crop rotation	CP-SOR	0.11^{d}	0.15^{h}	0.11^{d}	0.13^{f}	0.12^{f}	0.16^{j}	0.12^{f}	0.14^{h}
	Crop rotation	DOL-SOR	0.16^{i}	0.21^{m}	0.17^{j}	0.18^{k}	0.18^{l}	0.23^{o}	0.19^{m}	0.2^{n}
	Inter cropping	SOR/DOL	0.1^{c}	0.14^{g}	0.1^{c}	0.12^{e}	0.12^{f}	0.16^{j}	0.12^{f}	0.14^{h}
	Inter cropping	SOR/CP	0.14^{g}	0.19^{l}	0.15^{h}	0.16^{i}	0.18^{l}	0.23^{o}	0.19^{m}	0.2^{n}
	Mono cropping	SOR	0.11^{d}	0.12^{e}	0.13^{f}	0.16^{i}	0.11^{e}	0.13^{g}	0.14^{h}	0.18^{l}

SOR-Sorghum, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OI-Organic Inputs OP-oxen plough, FYM-farm yard -manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system. Means followed by the same letters in the same season in a column are not significantly different at $P \leq 0.05$.

Table 8. Effects of tillage practices and organic cropping systems on soil total N sweet potato based plots during SRS of 2012 and LRS of 2013.

TP	CS	CROPS	SRS 2012				LRS 2013			
			CTRL	FYM	MRP	MRP+FYM	CTRL	FYM	MRP	MRP+FYM
FR	Crop rotation	CP-SP	0.08^{c}	0.12^{g}	0.09^{d}	0.1^{e}	0.09^{c}	0.13^{g}	0.09^{c}	0.11^{e}
	Crop rotation	DOL-SP	0.13^{h}	0.17^{l}	0.14^{i}	0.15^{j}	0.14^{h}	0.19^{m}	0.15^{i}	0.16^{j}
	Inter cropping	SP/DOL	0.07^{b}	0.11^{f}	0.08^{c}	0.09^{d}	0.08^{b}	0.12^{f}	0.08^{b}	0.1^{d}
	Inter cropping	SP/CP	0.11^{f}	0.15^{j}	0.12^{g}	0.13^{h}	0.12^{f}	0.17^{k}	0.13^{g}	0.14^{h}
	Mono cropping	SP	0.08^{c}	0.09^{d}	0.1^{e}	0.13^{h}	0.09^{c}	0.1^{d}	0.11^{e}	0.14^{h}
OP	Crop rotation	CP-SP	0.07^{b}	0.11^{k}	0.08^{c}	0.09^{d}	0.08^{b}	0.12^{f}	0.09^{c}	0.1d
	Crop rotation	DOL-SP	0.12^{g}	0.16^{k}	0.13^{h}	0.14^{i}	0.13^{g}	0.18^{l}	0.14^{h}	0.15^{i}

Table 8. Contd.

	Inter cropping	SP/DOL	0.06^{a}	0.1^{e}	0.07^{b}	0.08^{c}	0.07^{g}	0.11^{e}	0.08^{b}	0.09^{c}
	Inter cropping	SP/CP	0.1^{e}	0.14^{i}	0.11^{f}	0.12^{g}	0.11^{e}	0.16^{j}	0.12^{f}	0.13^{g}
	Mono cropping	SP	0.07^{b}	0.12^{g}	0.08^{c}	0.09^{d}	0.08^{b}	0.13^{g}	0.09^{c}	0.1^{d}
	Crop rotation	CP-SP	0.09^{d}	0.13^{h}	0.09^{d}	0.11^{f}	0.1^{d}	0.14^{h}	0.1^{d}	0.12^{f}
	Crop rotation	DOL-SP	0.14^{i}	0.19^{m}	0.15^{j}	0.16^{k}	0.16^{j}	0.21^{n}	0.17^{k}	0.18^{l}
TR	Inter cropping	SP/DOL	0.08^{c}	0.12^{g}	0.08^{c}	0.1^{e}	0.09^{c}	0.13^{g}	0.09^{c}	0.11^{e}
	Inter cropping	SP/CP	0.12^{g}	0.17^{l}	0.13^{h}	0.14^{i}	0.14^{h}	0.19^{m}	0.15^{i}	0.16^{j}
	Mono cropping	SP	0.09^{d}	0.1^{e}	0.11^{f}	0.14^{i}	0.09^{c}	0.16^{j}	0.12^{r}	0.11^{e}

SP-Sweet potato, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OI-Organic Inputs, OP-oxen plough, FYM-farm yard -manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system. Means followed by the same letters in the same season in column are not significantly different at P ≤ 0.05.

Table 9. Effects of tillage practices and organic cropping systems on % soil Carbon sorghum based plots during SRS of 2012 and LRS of 2013.

TP	CS	CROPS	Organic Inputs-SRS 2012				Organic Inputs -LRS 2013			
			CTRL	MRP+FYM	MRP	FYM	CTRL	MRP+FYM	MRP	FYM
	Crop rotation	CP-SOR	1.2^{a}	1.29^{b}	1.34^{bc}	1.52^{de}	1.74^{m}	1.87^{o}	1.94^{opq}	2.2^{tu}
	Crop rotation	DOL-SOR	1.51d^{e}	1.62^{f}	1.69^{g}	1.91^{ghijk}	1.35^{fg}	1.45^{hi}	1.51^{hij}	1.71^{m}
FR	Inter cropping	SOR/DOL	1.74^{g}	1.87^{ghij}	1.95^{ghijkl}	2.21^{p}	1.9^{op}	2.03^{r}	2.12^{t}	2.4^{w}
	Inter cropping	SOR/CP	1.82^{ghi}	1.95^{ghijkl}	2.03^{lmn}	2.3^{pqr}	1.47^{hi}	1.58^{k}	1.64^{l}	1.86^{o}
	Mono cropping	SOR	1.93 ^{ghijkl}	2.06^{lmn}	2.14^{p}	2.43^{s}	1.16^{cde}	1.25^{f}	1.3^{fg}	1.47^{hi}
	Crop rotation	CP-SOR	1.78^{gh}	1.91^{ghijk}	1.99^{lm}	2.26^{pq}	2.06^{s}	2.21^{tu}	2.3^{tuv}	2.61^{y}
	Crop rotation	DOL-SOR	2.52^{t}	2.7^{u}	2.81^{v}	2.19^{p}	0.89^{a}	0.95^{b}	0.99^{b}	1.12^{cd}
OP	Inter cropping	SOR/DOL	1.94^{ghijkl}	2.08^{lmn}	2.16^{p}	2.45^{s}	2.25^{tuv}	2.41^{w}	2.51^{x}	2.84^{z}
	Inter cropping	SOR/CP	0.97^{b}	1.04^{c}	1.08^{cd}	1.23^{f}	1.63^{f}	1.74^{g}	1.82^{ghi}	2.06^{lmn}
	Mono cropping	SOR	1.7^{m}	2.16^{t}	1.83^{o}	1.9^{op}	2.67^{y}	2.38^{w}	2.87^{w}	2.98^{x}
	Crop rotation	CP-SOR	1.47^{d}	1.52^{de}	1.6^{f}	1.82^{ghi}	1.29^{fg}	1.33^{fg}	1.4^{h}	1.59^{k}
	Crop rotation	DOL-SOR	1.17^{a}	1.21^{a}	1.27^{b}	1.44^{d}	1.02^{c}	1.06^{c}	1.11^{cd}	1.26^{f}
TR	Inter cropping	SOR/DOL	1.7^{g}	1.76^{gh}	1.85^{ghij}	2.1^{lmno}	1.85^{o}	1.91^{op}	2.01^{r}	2.28^{tuv}
	Inter cropping	SOR/CP	1.77^{gh}	1.83^{ghi}	1.93^{ghijk}	2.19^{p}	1.45^{hi}	1.48^{hij}	1.56^{k}	1.77^{mn}
	Mono cropping	SOR	1.88^{ghij}	1.95^{ghijkl}	2.05^{lmn}	2.33^{pqr}	1.13^{cd}	1.17^{cde}	1.23^{f}	1.4^{h}

SOR-Sorghum, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OP-oxen plough, FYM-farm yard -manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system.. Means followed by the same letters in the same season in a column are not significantly different at P ≤ 0.05.

Table 10. Effects of tillage practices and organic cropping systems on % soil Carbon sweet potato based plots during SRS of 2012 and LRS of 2013.

TP	CS	CROPS	Organic Inputs-SRS 2012				Organic Inputs-LRS 2013			
			CTRL	MRP+FYM	MRP	FYM	CTRL	MRP+FYM	MRP	FYM
FR	crop rotation	CP-SP	1.3^{a}	1.39^{b}	1.45^{bc}	1.65^{de}	1.91^{m}	2.05^{o}	2.14^{opq}	2.43^{tu}
	crop rotation	DOL-SP	1.64^{de}	1.75^{f}	1.83^{g}	2.07^{ghijk}	1.48^{fg}	1.59^{hi}	1.66^{hij}	1.88^{m}
	inter cropping	SP/DOL	1.89^{g}	2.02^{ghij}	2.11^{ghijkl}	2.39^{p}	2.09^{op}	2.24^{r}	2.33^{t}	2.64^{w}
	inter cropping	SP/CP	1.97^{ghi}	2.11^{ghijkl}	2.2^{lmn}	2.5^{pqr}	1.62^{hi}	1.74^{k}	1.81^{l}	2.05^{o}
	mono cropping	SP	2.1^{ghijkl}	2.25^{lmn}	2.34^{p}	2.66^{s}	1.28^{cde}	1.37^{f}	1.43^{fg}	1.62^{hi}
OP	crop rotation	CP-SP	1.93^{gh}	2.07^{ghijk}	2.16^{lm}	2.44^{pq}	2.26^{s}	2.42^{tu}	2.53^{tuv}	2.87^{y}
	crop rotation	DOL-SP	2.73^{t}	2.92^{u}	3.05^{v}	3.15^{y}	0.98^{a}	1.05^{b}	1.09^{b}	1.24^{cd}
	inter cropping	SP/DOL	2.11^{ghijkl}	2.26^{lmn}	2.35^{p}	2.67^{s}	2.47^{tuv}	2.65^{w}	2.76^{x}	3.13^{z}
	inter cropping	SP/CP	1.76^{f}	1.89^{g}	1.97^{ghi}	2.23^{lmn}	1.06^{b}	1.14^{c}	1.19^{cd}	1.35^{f}
	mono cropping	SP	1.87^{m}	2.37^{t}	2.01^{o}	2.09^{op}	2.89^{u}	3.66^{z}	3.1^{w}	3.23^{x}
TR	crop rotation	CP-SP	1.59^{d}	1.65^{de}	1.74^{f}	1.97^{ghi}	1.41^{fg}	1.47^{fg}	1.54^{h}	1.75^{k}
	crop rotation	DOL-SP	1.27^{a}	1.31^{a}	1.38^{b}	1.56^{d}	1.12^{c}	1.16^{c}	1.23^{cd}	1.39^{f}
	inter cropping	SP/DOL	1.84^{g}	1.9^{gh}	2^{ghij}	2.27^{lmno}	2.03^{o}	2.1^{op}	2.22^{r}	2.51^{tuv}
	inter cropping	SP/CP	1.92^{gh}	1.99^{ghi}	2.09^{ghijk}	2.37^{p}	1.57^{h}	1.63^{hij}	1.72^{k}	1.95^{mn}
	mono cropping	SP	2.04^{ghij}	2.11^{ghijkl}	2.23^{lmn}	2.52^{pqr}	1.24^{cd}	1.29^{cde}	1.36^{f}	1.54^{h}

SP-Sweet potato, DOL-dolichos, CP-chickpea, TP-tillage practice, TR-tied ridges, FR-furrows and ridges, OP-oxen plough, FYM-farm yard -manure, MRP-Minjingu rock phosphate, CTRL-control, LRS-long rain season, SRS-short rain season, CS-cropping system.. Means followed by the same letters in the same season in a column are not significantly different at $P \leq 0.05$.

noted that FYM application increased soil SOC content by up to 67% over the control. The crop residues from the legumes further act as manures thus increasing %OC and other nutrients. This agrees with Knight and Shirtliffe (2005) where legume green manure increased benefits such as atmospheric N_2 fixation and P mobilization from the soil, facts also observed in this study.

Changes in % organic carbon across the season (SRS 2012 and LRS 2013)

Data on % OC changes across seasons indicate an increase during the LRS (2.28%) and (2.27%) compared to the SRS (2.1%) and (2.51%) under Tied ridges with intercropping sorghum/dolichos and intercropping sweet potato/dolichos applied with MRP+FYM in sorghum and sweet potato plots, respectively (Tables 8 and 9). The higher % OC observed during LRS is attributed to higher biomass production, which upon decomposition, releases CO_2, thus raising carbon levels. Devi and Yadava (2006, 2009) in earlier studies, had reported that high rate of CO_2 release during the LRS could be due to a congenial environment for microorganisms dwelling in the soil decomposing organic matter. The low % OC in the SRS seen in the study is attributed to low soil moisture content, temperature and relative humidity, thereby inhibiting the microbial activity (Devi and Yadava, 2006; Kosugi et al., 2007). Ginting et al. (2003), for example, found that 4 years after the last application of FYM, the residual effects resulted in 20 to 40% higher soil microbial biomass C.

Conclusions

Soil organic inputs, MPR and FYM are viable alternatives to the expensive inorganic fertilizers for improving the soil nutrient status in Matuu,

Yatta Sub County. Combined TR, intercropping of sorghum and sweet potato with dolichos and with application of MRP + FYM significantly increased soil K and P whereas combined TR, intercropping of dolichos with sorghum and sweet potatoes and with application of FYM led to an increase in soil % OC and TN. Moreover, the MRP and FYM are locally available, thus making it an ideal source of nutrients for smallholders economically.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Chemical and biological managment of white mold (*Sclerotinia sclerotiorum*) disease in irrigated common beans (*Phaseolus vulgaris*) cultivation

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The aim of this work was to study the effect of fungicides and biological agents on the control of white mold (*Sclerotinia sclerotiorum*) in common beans (cv. Pérola). Nine treatments were applied in six blocks (54 experimental units) using a randomized block design (RBD). The treatments were: T1 (control); T2, *Bacillus subtilis* strain QST 713 (4 L / ha); T3, *B. subtilis* strain QST 713 (4L / ha); T4, *B. subtilis* strain QST 713 (2 L / h); T5, *B. subtilis* strain QST 713 trifloxystrobin + prothioconazole (4 L / ha, 0.5 L / ha); T6, *B. subtilis* strain QST 713 trifloxystrobin + prothioconazole (2 L / ha, 0.5 L / ha); T7, *B. subtilis* strain QST 713 trifloxystrobin + prothioconazole, fluazinam (2 L / ha, 0.5 L / ha, 1 L / ha); T8- trifloxystrobin + prothioconazole, fluazinam (0.5 L / ha, 1L / ha); T9- *Trichoderma harzianum,* difenoconazole and azoxystrobin fluazinam + (1.5 L / ha, 0.5 L / ha, 1 L / ha). White mold (WM) incidence was evaluated at 39 days after planting (DAP), with subsequent evaluations at 39, 46, 53, 60, 67 and 74 DAP. Average yield from T5, T6, T7 and T8 was statistically higher than in the other treatments and consequently, treatments T7, T8 and T9 had the lowest mean area under disease progress curve values. The combined chemical and biological treatment was an effective white mold management strategy that increased yield and decreased disease incidence in common beans.

Key words: Active ingredient, white mold, *Bacillus subtilis*, *Trichoderma harzianum*, triflloxystrobin, prothioconazole.

INTRODUCTION

The common bean [*Phaseolus vulgaris* L. (Fabaceae)] is one of 55 species in the genus *Phaseolus* sp. It is one of the most important, oldest and most cultivated crops worldwide. It is extremely important in Brazil where, along

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with rice, it is a dietary staple (Santos and Gavilanes, 1998).

White mold (WM) in common beans is caused by the soil fungus, *Sclerotinia sclerotiorum* (Lib.) De Bary (1884) and can trigger epidemics with annual losses exceeding 50%. WM mainly occurs in crops irrigated by central pivot (Oliveira, 2005; Soule et al., 2011). The disease thrives in cool temperatures and / or micro-climatic conditions and during a period of intense bean planting in Brazil called the third growing season (especially in the Southeast and Center-West regions of Brazil). Other conditions that favor the disease include the presence of various fungi hosts, scleroids in the soil and transmission by seeds (Faria et al., 2011).

Innitial symptoms of WM include sparse plants with wilted upper leaves and cottony structures in the stems, leaves and pods formed by fungus mycelium. This last characteristic has led to the name "white-mold" (Paula et al., 2015).

Plant pathogens such as *S. sclerotiorum* can also colonize seed endosperm. The pathogens can then be transported over long distances by these propagules, proliferate and provide a source of inoculum in new fields. Resistant structures from previous crops or infested soil can also be transported with seeds, machines and agricultural implements, such as tractors, seeders and harvesters when not properly cleaned. Irrigation water, floods and wind can also disseminate the plant pathogen. Infections that begin in the myceliogenic cycle, followed by the ascogenous cycle, can multiply during the crop cycle (secondary cycle) and cause reinfestations, which lead to new resistant structures and increased inocula in the soil (Paula et al., 2015).

The fungus, *S. sclerotiorum*, identified in 1884, has been studied ever since. It is present throughout the world. It can infect more than 408 species of plants, monocots and dicots (Görgen, 2009). Changes in pigment, leaf wrinkling, wilt, chlorosis, atrophy, necrosis or abscission of parts of the plant are signs of this pathogen-host interaction (Prabhakar et al., 2013).

Solarization is an alternative method for reducing inoculum and controlling fungal plant pathogens in the soil. While this practice has shown promise in small crop areas it may not be practical in larger ones (Ferraz et al., 2003).

Fungicide application is the most common control method because it can be easily adapted to crop management plans and because it effectively prevents, controlls and reduces disease severity (Mueller et al., 2002).

WM can be controlled by physiological resistance and escape mechanisms, a consequence of the growth habit of the plant, which provides favorable soil aeration and climatic conditions. Neither of these mechanisms provide adequate control of the disease (Kim et al., 2000; Kolkman and Kelly, 2002; Huang et al., 2003; Soule et al., 2011). These mechanisms are mainly found in sources of genetic resistance and could be incorporated in commercial cultivars mainly by retro-crossings (Görgen et al., 2003).

Fungicide applications are recommended when flowering begins and again after 10 to 14 days if the disease progresses. Applications via boom sprayers may be hindered by canopy closure between rows and the consequent need for higher volume applications (Tu, 1989). Therefore, to achieve economically viable and effective disease control, growers must pay careful attention to application timing and positioning. The spray should create a uniform layer over the plant surface and act as a barrier to the host-pathogen. Systemic fungicide applications can also provide protection from contact (Oliveira, 2005).

Spraying should be uniformly diffused over the entire plant and soil surface where apotecia develops. Initial spraying should be carried out preventively at the opening of the first flowers. Subsequent applications should occur when apotecia appears and when the crop presents other favorable conditions for disease (Oliveira, 2005). Integrated disease management can lower costs and reduce diverse production risks (Ferreira et al., 2013). Azoxystrobin (estrobirulin chemical group) has been registered for disease control in 32 crops, including beans, and is the active ingredient (ai) in 23 registered commercial products. Another fungicide, trifloxystrobin (strobilulins) has been registered to control diseases in 24 crops, including beans, and is the main in six commercial products; while protioconazol (triazolitione) has been registered to control diseases in 3 crops, in addition to beans, and is the main in two commercial products (Paula et al., 2009).

B. subtilis QST strain 713 is an organic fungicide but is the active ingredient in only one commercial product. While it is not intended for any specific crop it can be used on various (Silva et al., 2015).

Diphenoconazole (triazole group) has been registered in Brazil for WM control in 37 crops, including beans, and 10 commercial products. Diphenoconazole (phenylpyridinylamine group) has been registered for disease control in eight crops, whereas the biological agent *Trichoderma harzianum* has been registered for disease control in beans and is the main in three commercial products (Agrofit, 2016).

The two main WM control practices in beans involve conventional fungicide use, which is expensive and has strong environmental impacts related to toxic waste (Rocha and Oliveira, 1998). Another practice involves the use of various species of *Trichoderma* spp. to control not only *S. sclerotiorum* but various soil pathogens (Lobo and Abreu, 2000).

Our objective is to evaluate the use of chemical and biological managment on *Sclerotinia sclerotiorum* in

Table 1. Chemical and biological agents for WM control applied on different days after planting and water volume control aiming bean cv. Perola cultivated in condition by Central Pivot in the crop 2015.

Treatments	Active ingredients and commercial fungicides	Dosages (L ha¹)	Day after planting	Volume of spray (L ha¹)
T1	Negative control	empty	empty	empty
T2	*Bacillus subtilis* lineage QST 713-Serenade© (CB)	4	1st. spray 18 (CB)	200
T3	*B. subtilis* lineage QST 713- Serenade© (BC)	4	1st. spray 26 (CB)	200
T4	*B. subtilis* lineage QST 713- Serenade© (BC)	2	1st. spray 18 (CB); 2nd spray 26(CB)	200
T5	*B. subtilis* lineage QST 713- Serenade© (BC) and Trifloxistrobina + protioconazol- Fox© (CC)	4 and 0,5	1st. spray 18(CB); 2nd spray 26 (CQ); 3rd spray 34 (CQ); 4th spray 46 (CQ)	200
T6	*B. subtilis* lineage QST 713- Serenade© (BC) and Trifloxistrobina + protioconazol- Fox© (CC)	2 and 0,5	1st. Aplic. 18(CB); 2nd spray 26 (CB-CQ); 3rd spray 34 (CQ) and 4th spray 46 (CQ)	200
T7	*B. subtilis* lineage QST 713- Serenade© Trifloxistrobina + protioconazol - Fox© (CC1) and fluazinam- Frowcide (CC2)	2, 0.5 and 1	1st. Aplic. 18(CB); 2nd spray 26 (CB-CQ1-CQ2); 3rd spray 34 (CQ1-CQ2) and 4th spray 46 (CQ1)	200
T8	Trifloxistrobina + protioconazol- Fox© (CC1) and fluazinam- Frowcide© (CC2)	0,5 and 1	1st. spray 26(CQ1-CQ2); 2nd spray 34 (CQ1-CQ2); 3rd spray 46 (CQ1-CQ2)	200
T9	*Tricoderma harzianum*- Trchodermil SC 1306© (BC), azaxistrobina + difenoconazol- Amistar Top© (CC1) and fluazinam- Frowncide© (CC2)	1,5; 0,5 and 1	1st. spray 18(CB); 2nd spray 26 (CB-CQ1-CQ2); 3rd spray 34 (CQ1-CQ2) and 4th spray 46 (CQ1)	200

*BC, biological control, CC chemical control.

irrigated common bean crops (*Phaseolus vulgaris*).

MATERIALS AND METHODS

We set up our experiment during the dry season of 2015 on an irrigated (central pivot) crop of cv. Pearl at a farm called Fazenda São José in Cristalina, GO, Brazil. The field was situated at 17 ° 5'56 "S and 47 ° 38'44" W (GPS) and at an altitude of 861 m.

The soil was prepared using the no-tillage system and was preceded by soybean and corn crops. The crop was fertilized after planting by broadcast fertilization using 270 kg ha^{-1} of formulated 05-37-00 potassium chloride (KCl, Triton©). The crop was managed according to Carneiro et al. (2015).

The beans were sown in the first week of October. Nine types of treatments were applied from 1 to 4, times during the crop cycle. Some of these treatments were biological and chemical combinations (Table 1). A randomized block design was used with six replicates, totaling 54 experimental units or plots (Table 2).

Each plot measured 6x6 m (36 m^2), spaced at 0.5 m between rows and 0.2 m between plants. The last 0.5 m from the ends of the two central rows was discarded (9 m^2 total). The evaluations were carried out on the ten centermost rows (useful area). There were 30 plants plants per row and a total of 300 plants per plot.

White mold incidence (% WM) was evaluated at 39 days after planting (DAP) and again at 46, 53, 60, 67 and 74 DAP (Table 3). The numbers of symptomatic plants (white mold symptoms) were counted five times divided by the total number of evaluated plants (10 plants).

The area under white mold progress curve (AUDPC) was calculated by integrating the disease progress curve for each plot (% white mold incidence at x days), using the formula:

$$\mathrm{AUDPC} = \Sigma_i^{n-1} \frac{(X_i + X_{i+1})(t_{i+1} - t_i)}{2}$$

Where, n is the number of the severity ratings, Xi is the severity of the disease and $(t_{i+1} - t_i)$ is the number of days between consecutive evaluations (Campbell and Madden, 1990). The value *of* AUDPC synthesizes all the WM impact assessments into a single value representing the crop-cycle epidemic.

The yield (kg / ha) of the plots was evaluated at 87 DAP (desiccation was carried out 2 days before harvest, affecting 70% of the leaves). The number of plants in 4 rows (2.5 m each) was counted; it was divided by the line spacing used and multiplied by 10, giving the number of plants per ha. Next, the number of pods in 10 consecutive plants in a row was counted and divided by 10, yielding the mean number of pods per plant. Fifty pods were collected, and the number of beans counted. This value was then divided by 50 to find the average number of beans per pod. Next, 1000 beans were weighed. Then, yield was estimated as the mathematical product of the number of plants per ha, the number of pods per plant, the average number of seeds per pod and weight of 1000 beans, divided by 60,000 (Koss and Lewis, 1993).

Control efficiency (CE) is the percent reduction in AUDPC due to a treatment application, relative to the AUDPC values of the control treatment (without applications). Yield efficiency (YE) represents the relationship between yield increases relative to the yield of the control treatments (without application of chemical and biological

Table 2. Biological and chemical treatments (T) for the control of white mold a bean cv Pérola crop uder central pivot during the winter crop.

Line	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
L1	T2 - Serenade© (4 L/ha)	T 3 - Serenade© (4 L/ha)	T 3 - Serenade© (4 L/ha)	T2 - Serenade© (4 L/ha)	T 5 - Serenade© (4 L/ha) + Fox© (500 mL/ha)	T 9 - Tricodermil (1,5 kg/ha) + Amistar Top© (500 mL/ha) + Frownсide© (1 L/ha)
L2	T 5 - Serenade© (4 L/ha) + Fox© (500 mL/ha)	T 6 - Serenade© (2 L/ha) + Fox© (500 mL/ha)	T 6 - Serenade© (2 L/ha) + Fox© (500 mL/ha)	T 8 - Fox© (500 mL/ha) + Frownсide© (1 L/ha)	T 7 - Serenade (2 L/ha) + Fox© (500 mL/ha) + Frownсide© (1 L/ha)	T 3 - Serenade© (4 L/ha)
L3	T 4 - Serenade© (2 L/ha)	T 4 - Serenade© (2 L/ha)	T 1 - Control	T 3 - Serenade© (4 L/ha)	T 8 - Fox© (500 mL/ha) + Frownсide© (1 L/ha)	T2 - Serenade© (4 L/ha)
L4	T 9 - Tricodermil© (1,5 kg/ha) + Amistar Top© (500 mL/ha) + Frownсide© (1 L/ha)	T 5 - Serenade© (4 L/ha) + Fox© (500 mL/ha)	T 5 - Serenade© (4 L/ha) + Fox© (500 mL/ha)	T 1 - Control	T 1 - Control	T 4 - Serenade© (2 L/ha)
L5	T 8 - Fox© (500 mL/ha) + Frownсide© (1 L/ha)	T2 - Serenade© (4 L/ha)	T 8 - Fox© (500 mL/ha) + Frownсide© (1 L/ha)	T 6 - Serenade© (2 L/ha) + Fox© (500 mL/ha)	T 4 - Serenade© (2 L/ha)	T 5 - Serenade© (4 L/ha) + Fox© (500 mL/ha)
L6	T 7 - Serenade© (2 l/ha) + Foxv (500 ml/ha) + Frownсide© (1 l/ha)	T 8 - Fox© (500 mL/ha) + Frownсide© (1 L/ha)	T2 - Serenade© (4 L/ha)	T 5 - Serenade© (4 L/ha) + Fox© (500 mL/ha)	T 6 - Serenade© (2 L/ha) + Fox© (500 mL/ha)	T 1 - Testemunha
L7	T 3 - Serenade© (4 L/ha)	T 1 - Control	T 4 - Serenade© (2 L/ha)	T 4 - Serenade© (2 L/ha)	T 3 - Serenade© (4 L/ha)	T 7 - Serenade© (2 L/ha) + Fox© (500 mL/ha) + Frownсide© (1 L/ha)
L8	T 1 - Control	T 7 - Serenade© (2 L/ha) + Fox© (500 mL/ha) + Frownсide© (1 L/ha)	T 9 - Tricodermil© (1,5 kg/ha) + Amistar Top© (500 mL/ha) + Frownсide© (1 L/ha)	T 9 - Tricodermil© (1,5 kg/ha) + Amistar Top© (500 mL/ha) + Frownсide© (1 L/ha)	T 9 - Tricodermil© (1,5 kg/ha) + Amistar Top© (500 mL/ha) + Frownсide© (1 L/ha)	T 8 - Fox© (500 mL/ha) + Frownсide© (1 L/ha)
L9	T 6 - Serenade© (2 L/ha) + Fox (500 mL/ha)	T 9 Tricodermil© (1,5 kg/ha) + Amistar Top© (500 mL/ha) + Frownсide© (1 L/ha)	T 7 - Serenade© (2 l/ha) + Fox (500 ml/ha) + Frownсide© (1 l/ha)	T 7 - Serenade© (2 L/ha) + Fox (500 mL/ha) + Frownсide© (1 L/ha)	T2 - Serenade© (4 L/ha)	T 6 - Serenade© (2 L/ha) + Fox (500 mL/ha)

combinations) (Silva, 2018).

The crop health and yield variables were subjected to analysis of variance and the means compared by the Tukey test at 5% probability (Assistat ® version 7.7 Beta).

RESULTS AND DISCUSSION

No symptoms of white mold were observed during the first evaluation (39 DAP); however, symptoms of fusarium wilt *(Fusarium oxysporum* f.sp. *phaseoli)* were observed. Furthermore, mean incidence values did not differ significantly among the various treatments. Similarly, Boechat et al. (2014), using spectral analysis, also did not detect white mold within the same DAP range and suggested that crop phase or the residual effects of previous crop management practices could explain the lack of white mold.

At 46 DAP, when the beans were in the R5 stage, the T5 treatment *(B. subtilis strain* QST 713 - Serenade© + prothioconazole and trifloxystrobin - Fox© - 4 L ha^{-1} and 0.5 L ha^{-1} - V3, V4, R5, R5 +10 days*)* showed statistically lower incidence of white mold than did the other treatments. Wutzki

Table 3. Area under below progress curve disease (AUDPC), control efficiency (CE), productivity (kg ha^{-1} and sc ha^{-1}) and yield efficiency (YE) in different combinations of biological and chemical treatments (T) applied to the bean cv. Pérola in the winter crop under central pivot irrigation (2015).

Code	Treatments	AUDPC	CE (%)	Produc. kg / ha (sc / ha)	YE (%)
T1	*Control*	397.4[a]	Empty	2093 (34.8)[b]	Empty
T2	*Bacillus subtilis* strain QST 713 - Serenade © (CB)	313.7[ab]	21.3	2371 (39.5)[b]	13.2
T3	*B. subtilis* QST 713 strain - Serenade© (CB)	303.2[ab]	23.9	2400 (40.0)[b]	14.6
T4	*B. subtilis* QST 713 strain - Serenade © (CB)	325.6[ab]	18.3	2244 (37.3)[b]	7.2
T5	*B. subtilis* QST 713 strain - Serenade © (CB) and trifloxystrobin + prothioconazole - FOX © (CQ)	208.0[b]	48	2780 (46.3)[ab]	32.8
T6	*B. subtilis QST 713* strain - Serenade © (CB) and trifloxystrobin + prothioconazole - FOX © (CQ)	211.5[b]	47	2725 (45.4)[ab]	30.2
T7	*B. subtilis QST 713 strain* - Serenade ©, trifloxystrobin + prothioconazole - FOX © (CQ1) and fluazinam - Forwcide © (CQ2)	87.6[d]	77.9	2840 (47.3)[ab]	35.7
T8	Trifloxystrobin + prothioconazole - FOX © (CQ1) and fluazinam - Frownicide © (CQ2)	109.0[c]	72.4	3178 (53.0)[in]	51.8
T9	*Trichoderma harzianum* - Trichodermil SC 1306 © (CB), azoxystrobin + difenoconazole - Amistar Top © (CQ1) and fluazinam - Frownicide © (CQ2)	127.6[c]	67.8	2683 (44.7)[ab]	28.2

*Means followed by same letter vertically to the test Tukey P ~ 0.05.

et al. (2016) found that chemical control applications at these phenological stages did not differ statistically from the control and were therefore not effective. Chromatography–mass spectrometry showed that the bioagent *Trichoderma longibrachiatum* T6 achieved the same antifungal potential as *Trichoderma* in the control of *Verticillium* sp. (Zhang et al., 2018).

At 53 DAP, when the beans were in the R6 stage, the T5, T7, T8 and T9 treatments showed statistically lower incidence of white mold than the other treatments. As expected, the highest incidence occurred in the control (T1) and in the T2, T3 and T4 treatments (Figure 1A, B). Lower performance from the biological treatments is expected, given that this is the first time they had been used during this crop cycle. Continuous use of biological control achieves better results, whereas the first application is only the starting point for results that should continue to improve (Pomella and Ribeiro, 2009).

At 60 DAP, when the beans were in the R6 stage, the T7, T8 and T9 showed the lowest incidence of white mold relative to the other treatments. As expected, the highest incidence of white mold occurred in the control (T1) and in the T2, T3 and T4 treatments (Figure 1C). Meyer et al. (2014) showed that chemical control of WM in soybean crops was efficient and that the active ingredient fluazinam was the most efficient. The chemical treatments in the present study also yielded the best results.

At 67 DAP, when the beans were in the R7 stage, the lowest, statistically different incidence of white mold was found in the T7, T8 and T9 treatments. Again, as expected, the highest incidence occurred in the control (T1) and in T2, T3, T4 and T5 (Figure 1D). Although the *T. harzianum* treatment showed statistically significant results at this stage we can not say that it was effective, given that it was used in concert with fungicides that were producing much better results. Contrary to Silva et al. (2015), who examined these two biological agents in the control of *S. sclerotiorum* in lecttuce, we found that *T. harzianum* provided better control of WM (Silva et al., 2015). Not only was biological control (*Trichoderma* spp.) of WM studied, but also, edornaviruses, which are specific to fungi, were studied in *Vicia faba* (Khalifa and Pearson, 2014).

At 74 DAP, when the beans were in the R8 stage, the lowest, statistically different incidence of white mold was found in T8. The highest incidence of white mold occurred in the control (T1) while statistically similar

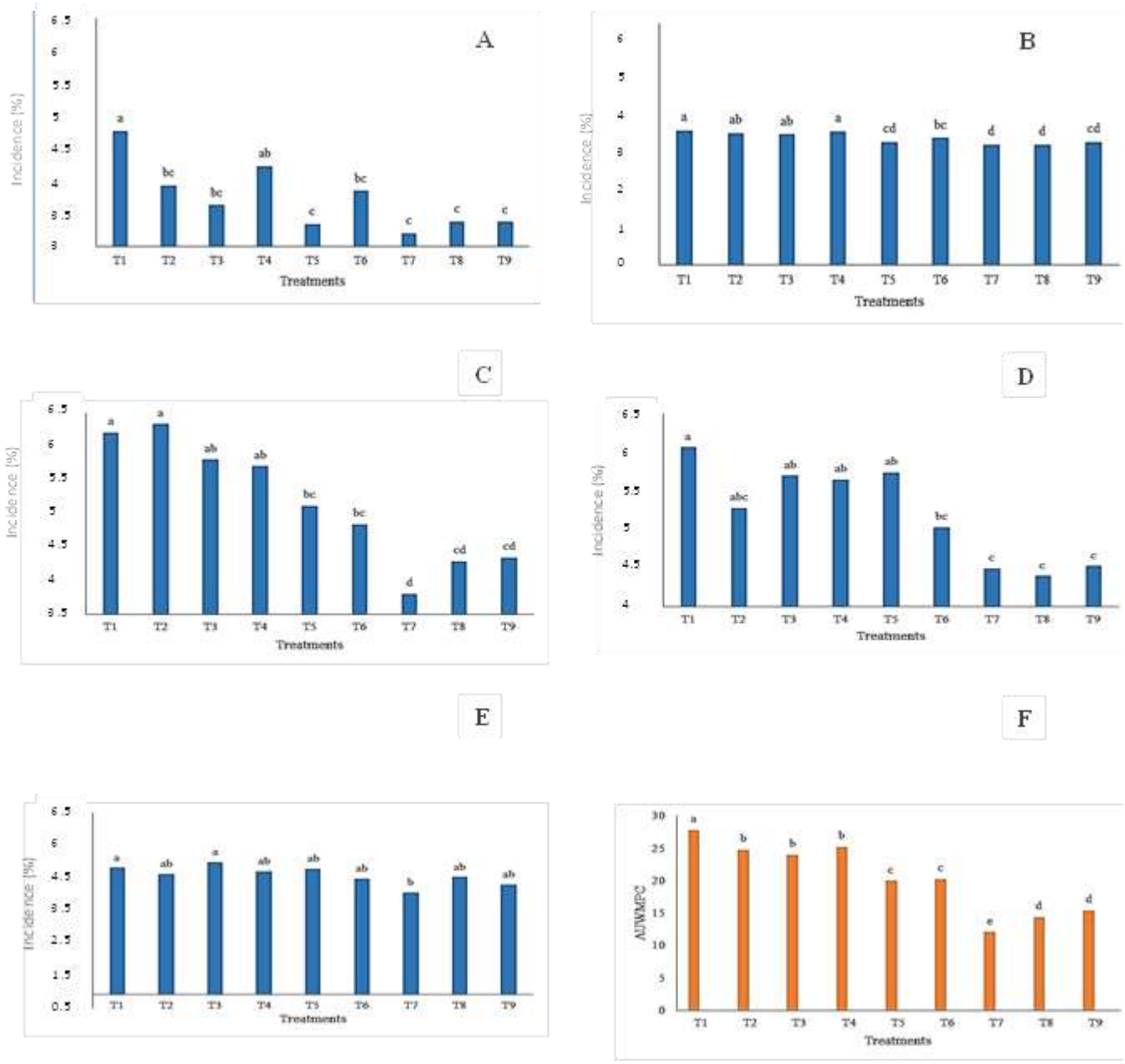

Figure 1. Mean of the transformed incidence √ (x + 10) of white mold in the common bean cv. Pearl during the winter harvest under central pivot irrigation (2015), submitted to varioud biological and chemical control combinations. **A.** incidence at 46 days after planting DAP. **B.** incidence at 53 DAP, C. incidence of white mold at 60 DAP. **D.** incidence of white mold after 67 DAP. **E.** incidence of white mold after 74 DAP. **F.** Area under the white mold progress curve (AUDPC).

results were found in T2, T3, T4, T5, T6, T8 and T9 (Figure 1E). This shows that biological control of WM was not as effective as chemical control in both seed treatment and in post-emergence applications (Moraes and Teixeira, 2008).

AUDPC, which summarizes the extent of the white mold epidemic, had the lowest value in the T7 treatment, followed in ascending order by T8 and T9, and shortly after by T5 and T6, T2, T3 and T4, which were statistically similar. Finally, the highest incidence of white mold was in the control (T1) (Figure 1F). A commercial product based on *Coniothyrium minitans* combined with low doses of fungicides was effective at managing white bean mold (Elsheshtawi et al., 2016).

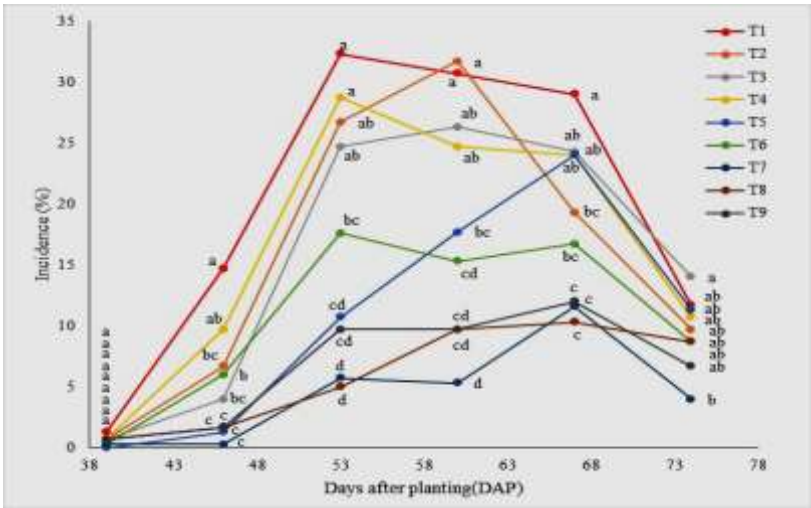

Figure 2. Temporal progress curves of white mold incidence in beans cv. Pérola using different combinatons of treatments during the 3rd. harvest under center pivot irrigation (2015) [Means followed by the same letter do not differ by Tukey test relative to the progress curve (P ≃ 0.05)].

The progress curve expressed the critical limits of disease development in the different treatments, with the control treatment producing the upper limit of incidence (Figure 2). Thus, the best treatment (T7) reduced disease incidence by 0-13 % (control 0-33%) (Figure 2). When pyrisoxazole (rarely used in Brazil) was used to control 166 strains of *Sclerotinia sclerotiorum*, it provided excellent protection and reduced disease in oleaginous plants (Duan et al., 2018). The fungicides procymidone and fluazinam (commonly used in Brazil) combined with benzalkonium chloride were more efficient in controlling WM in soybeans (73.1 - 71.6%, 2010 crop; 75.7 - 77.6 %, 2011 crop) than isolated applications of *T. harzianum* (Sumida et al., 2015).

From 48 to 53 DAP, disease development was considered critical due to progressive growth in all the chemical treatments. In the T8 treatment, reductions in incidence began to decrease at 60 DAP (Figure 2). Single chemical applications are not effective over long crop periods; however, efficacy can be extended by combining treatments chemical and biological (Moraes et al., 2008; Mueller et al., 2002; Paula Junior et al., 2006).

After harvesting, we compared bean yields from the various treatments. The statistically highest yields were in T8 (3590 kg / ha) and T5, T6, T7 and T9, which were statistically similar. The control (T1) and T2, T3 and T4 produced the lowest yields (<3490 kg / ha) (Figure 3). Chemical fungicides provided better WM control and consequently higher yields. Similar conclusions were drawn by Paula Junior et al. (2009).

The highest control efficiency, as measured by AUDPC, was observed in T7 (77.9%), followed by T8 (72.4%) and then T9 (67.8 %), which demonstrates that control efficiency greater than 50% was achieved in these treatments (Carneiro et al., 2015).

The highest yield efficiency was T8 (51.8%), followed by T7 (35.7 %), T5 (32.8 %), T6 (30.2 %), T9 (28.2 %), T3 (14.6 %), T2 (13.2 %) and T4 with the lowest percentage (7.2 %) (Table 1), showing that control efficiency is linked to yield (that is the lower the WM intensity, the higher the yield). The fact that this was the first time these biological controls were used to control this pathogen may partly explain why these treatments produced the lowest yield and control efficiencies (Vinale et al., 2008).

The highest average yield was 3178 kg ha^{-1} or 53 sc ha^{-1} for treatment T8 (trifloxystrobin + prothioconazole - Fox© and fluazinam - Frowncide©) (Table 1). The treatment with the combined chemical-biological application yielded 1085 kg ha^{-1} (18 sc ha^{-1}) more than the untreated control. Thus, 100 ha, under the same conditions, could yield an additional 108,500 kg (1800 sc 100 ha^{-1}) / 100 ha of beans, which, at current prices (R$ 205.00 sc) would provide an additional R$ 369,000.00. Given spraying costs per hectare of R$ 11.10 (Richetti and Roese, 2008), the spraying costs on 100 hectares would be R $ 1110.00 per application.

In the T8 treatment (Table 1), the fungicide trifloxystrobin + prothioconazole - Fox© for 1 h costs R$ 65.00 per hectare (R$ 130.00 per liter; dosage 0.5 L / ha^{-1}) or R$ 6,500.00 per application on 100 ha. Similarly, the fungicide fluazinam - Frowncide© also costs R$ 65.00 per ha (R$ 130.00 per liter; dosage of 0.5 L / ha), which would cost an additional R$ 6500.00 per application on 100 ha. Thus, a single application on 100 ha of the fungicides in the T8 treatment would cost R$ 13,000.00

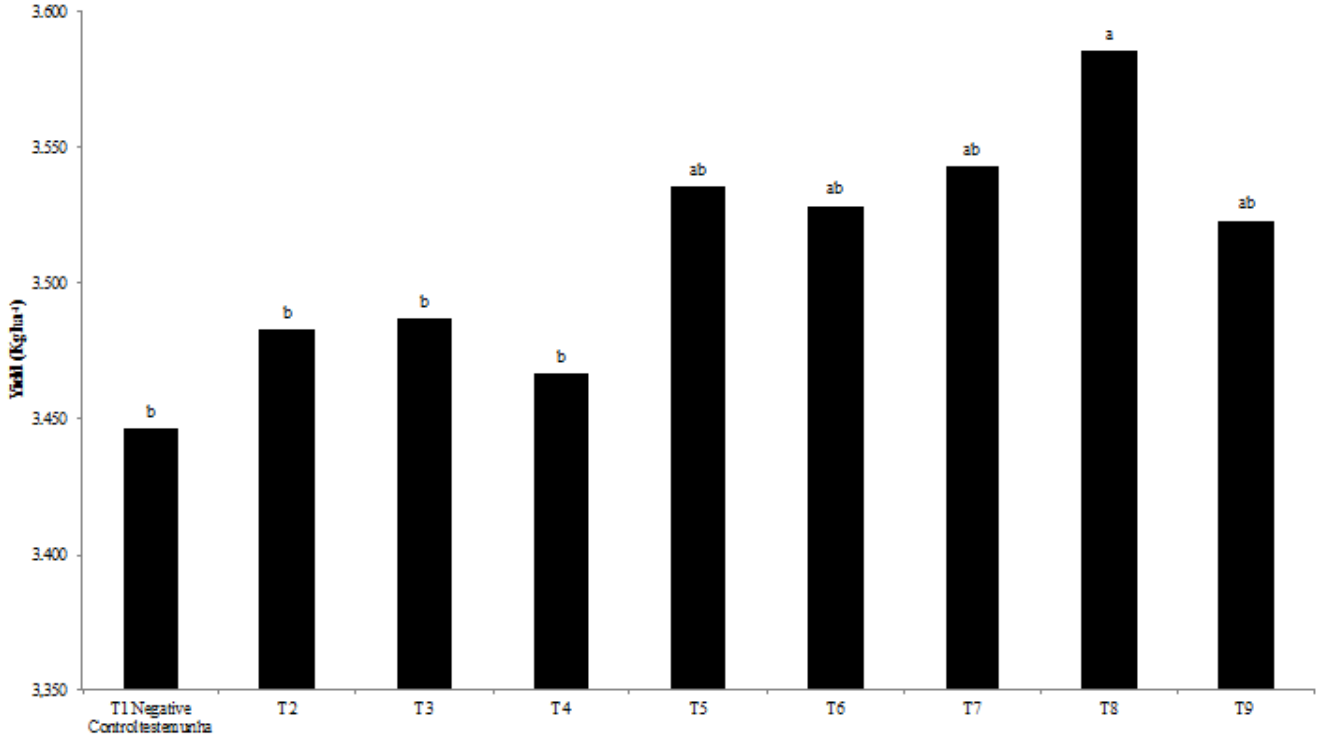

Figure 3. Average yields (kg / ha) transformed by √ (x + 10) using different types of biological and chemical combinations applied on beans cv. Pérola during the winter crop under central pivot irrigation (2015) [Means followed by the same letter do not differ by Tukey test (P ~ 0.05)].

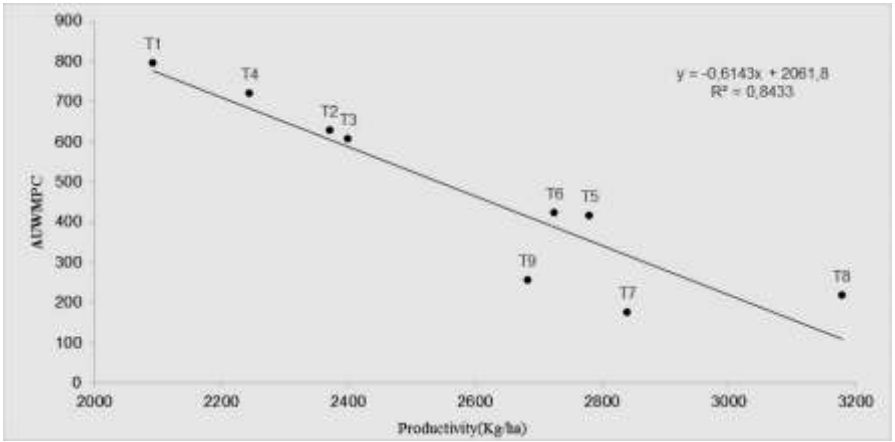

Figure 4. Mean area under the white mold progress curve (AUWMPC) versus yield (kg / ha) of different combinations of biological and chemical treatments to bean cv. Pérola in the winter crop under central pivot system.

(Carneiro et al., 2015).

Finally, the total cost of three applications of T8 on 100 ha would be R$ 58,500.00 (3 x (spraying cost of R$ 1110 + fungicide cost of R$ 13,000). Therefore, the net revenue on 100 ha gained by using the T8 treatment, rather than the untreated control no treatment, would be R$ 307,170.00 per 100 ha (Carneiro et al., 2015).

Yield increases in the experiment were explained by AUDPC (84.3%) (Figure 4), including highly correlated variables (growth rate of -0.6143% day^{-1}) fit to a linear model. The control treatment (without any applications) showed that higher AUDPC was related to lower yields.

Contrary to our study, isolated fungicide active ingredients (not mixed with other active ingredients) and isolated treatments of *T. harzianum* in two consecutive harvests were shown to be more efficient at controlling the severity and incidence of WM and improving yield than treatments containing pure fungicides (fluazinam) in the 2009-2010 crop (Sumida et al., 2015).

T7 and T9 were strongly correlated with higher yield and lower AUDPC (Figure 4). Decreased WM, which was influenced by physiological resistance and plant architecture, had little influence on yield but reduced AUDPC (Görgen et al., 2003).

Conclusion

Single applications of biological control agents (T2, T3 and T4), applied at different rates and times, had statistically similar effects on WM incidence in common beans throughout the evaluation period.

A combination of a biological control agent (*B. subtilis*) and two active chemical control ingredients (trifloxystrobin + prothioconazole and fluazinam) produced the greatest reduction in AUDPC. The treatments using only biological control agents produced greater reductions in AUDPC than did the treatment without a combination of controls strategies.

T8 (three applications), with two chemical treatments and 3 applications over the bean growth cycle, produced numerically higher yields that were statistically equal to the yields of the treatments with combinations of biological and chemical agents (T5, T6, T7 and T9, four applications).

The highest control efficiency related AUDPC (77.9%) and yield efficiency (51.8%) were in T7 and T8 respectively.

The yield increases from the combined chemical and biological treatments reduced AUDPC by 84.3%. T8 increased yields, lowered final production costs and reduced the incidence of white mold, which the crop converted into yield gains.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Impact of irrigation adoption on rural farmer's welfare in Eastern Cape Province of South Africa: A propensity score matching approach

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The aim of the present study was to assess the impact of irrigation adoption on farmer's welfare measured by consumption expenditure and food consumption pattern. The paper was based on cross sectional farm household data collected in 2015 from a sample of 200 households in rural Eastern Cape of South Africa. The study used a stratified random sampling method to select farming households from three district municipalities of the province. A Propensity Score Matching method was adopted for data analysis. Estimates of the Average treatment of the treated (ATT) suggests that irrigation participation decrease food expenditures, increases consumption and income significantly at 5% level. The study concludes that irrigation participation is one of the viable solutions to increase farmer's welfare in the study area. Therefore this study recommends a continued public and private investment in irrigation schemes in Eastern Cape.

Key words: Irrigation technology, smallholder farmers, propensity score matching, consumption, South Africa.

INTRODUCTION

In recent years, a significant progress towards generating new ideas and technologies to deal with technical and institutional constraints facing African agriculture has been made and notably the impressive growth rates of 7% and more per annum in some instances. But extreme poverty persists and Sub-Saharan Africa has been regarded as the worst undernourished (International Fund for Agricultural Development, 2014). For this reason, many African governments have put agricultural transformation top of their developmental agenda. The Comprehensive African Agricultural Development Program (CAADP) launched in 2003 has driven the commitment by 42 African governments to increase public spending on agriculture by 10% (Forum for Agricultural Research in Africa, 2013). Each country was to adopt an inclusive strategy to boost agricultural growth and speed transformation. This was to be achieved through public-private partnerships in smallholder value chain development and youth employment creation in value chains (Burney and Naylor, 2012).

Similarly, South Africa since the end of Apartheid era have made large investments of resources to build up their smallholder sectors as a response to the strong belief that agriculture will drive much of inclusive

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economic growth (Rukuni, 2011). These programmes include the Land reform programme comprising tenure reform, land redistribution and restitution, a Massive Food Production Programme in the Eastern Cape, and the Micro Agricultural Financial Institution of South Africa (MAFISA). Sadly, these efforts have not produced the desired results, and poverty has deepened (Water Research Commission, 2014). There was the expectation that enhanced access to productive resources such as land and technical support would translate into increased agricultural productivity for the black farmers who make up the bulk of the smallholders in the country (Water Research Commission, 2014). However, the transformation of the smallholder farming practiced in the former independent homelands of Eastern Cape remains virtually stagnant at best (Vongai and Thamaga-Chitja, 2018).

In the face of climate changes and drought, the majority of smallholder farmers depend on rainfed agriculture for food production which leads to low productivity (Patrick et al., 2015). Commentators have noted that fresh water is becoming a scarce and over-exploited natural resource (UNESCOWWAP, 2006; Ridoutt and Pfister, 2010). As a result, agriculture is under intense pressure to reduce depletion of water sources, the pollution of water systems and its contribution to soil infertility and erosion (FAO, 2010). Fanadzo et al. (2010) and Mnkeni et al. (2010) noted that the decline in available freshwater resources in South Africa is caused by ignorance, and improper measurement and monitoring of water use in smallholder irrigation schemes. Some impact assessment studies that were conducted following implementation of such irrigation schemes reported positive direct and indirect effects of irrigation adoption on the farm households. The question that can be asked is: What is it that is odd to the South African smallholder agricultural sector that all these initiatives by government targeting smallholder farmers have not achieved desired results? Thus, the aim of the present study was to assess the impact of irrigation adoption to household welfare in Eastern Cape, South Africa.

Characteristics that differentiates smallholder farmers from commercial farmers

The primary characteristics of smallholder agriculture in semi-arid developing countries are its diversity in space, its variability through time, and its multidimensionality in terms of the ways it operates and survives (Cousins, 2013). This is largely because dry land smallholders must be highly responsive to a varied, changeable and hazardous environment. Thus, their operations are very different from those of large-scale farms driven by commercial goals, equipped with credits and efficiency oriented technologies and covered by insurance systems against hazards and losses. This diversity, variability and multidimensionality mean that each particular system must be approached with careful attention to its unique mix of characteristics. Smallholder farmers in most parts of the world, especially developing nations are 'rational allocators of available resource' but have limited technical and economic opportunities. As a consequence, these farmers remain 'poor but efficient'. In addition Pauw (2005) noted that due to poverty, smallholder farmers often struggle to support themselves with inadequate income from agricultural activities. Hence, they rely on other sources of income such as wage remittances and pension as well as government transfer or non-agricultural labour income.

According to the National Department of Agriculture (2008), the major characteristics of production systems of smallholder farmers are of simple, outdated technologies, labour intensity, high seasonal fluctuations and women playing a vital role in production. Smallholder farmers differ in individual characteristics, farm size, resource distribution between food and cash crops, livestock and off- farm activities, their use of external inputs and hired labour, the proportion of food crops sold and household expenditure patterns. These differences highlighted above are even considered to be constraints that smallholder farmer's face and are the typical characteristics of smallholder farmers in the Eastern Cape province of South Africa. Smallholder agriculture is the main source food for the rural populations as well as an income generating occupation because it is the main activity for many rural parts of developing countries. This implies that smallholder agricultural productivity is very crucial in alleviating poverty and hunger (Pote, 2008). In recognizing this potential of the role of smallholder farmers, it is necessary to have a deeper insight into their key characteristics that differentiates smallholder farmers from commercial farmers which include the following:

Outdated technologies

The smallholder farm sector of South Africa is characterized by rudimentary production technology (Limpopo Department of Agriculture, 2008). Kalibwani (2005) argues that smallholder farmers in Southern Africa mainly use traditional production techniques and production levels are often low. Given this condition, a narrow production base often characterize smallholder farming. The rudimentary technology status can be explained by the fact that the sector is also labour intensive with minimal usage of machinery (Cousins, 2005).

High seasonal fluctuation

Smallholders are further differentiated from high-input commercial farmers by their need to manage multiple risks. Almost all of their inputs and outputs are subject to large variation and uncertainty, such as labour, which is often the most critical variable. Another critical risk arises

from the high variability in rainfall, which itself has two major consequences as far as sequestration is concerned.

One is variation in the timing of bio productivity, which means that planting and harvesting (and most other agricultural and non-agricultural activities) may have to be readjusted rapidly, sometimes within a season, and often between seasons. For example, fallows that appeared secure for years may have to be cleared after a particularly poor season. The other consequence is variability between fields, some of which may receive sufficient rainfall, and some of which may not. There are other risks that have similar consequences. These include: attacks by pests (against which pesticides are too expensive); illness, resulting in the unavailability of labour at some critical point in the season; and variability regarding prices of inputs such as seed, labour, food, and of outputs, mainly crops.

Labour intensity

The smallholder agriculture in South Africa is characterised by intensive use of labour which is mainly derived from family members. Smallholder farmers do the farming work themselves with the help of their family members. It is sometimes the cases that some family members such as siblings or grown up children are paid in order to help out on the farm. In this case, there is limited usage of external inputs such as machinery and fertilizers. Use of labour in smallholder farming is in some case a form of self- exploitation arising from the fact that the majority are poor hence cannot afford external farm inputs and cost of labour, hence they have to do with family labour.

Subsistence

Production in smallholder farming is mainly for subsistence purposes and to a lesser extent marketable surplus (Limpopo Department of Agriculture, 2008). Cousin (2005) also confirms this characteristic by asserting that output from smallholder farming for some rural households constitutes a greater proportion of their total livelihoods. Given this picture, production in smallholder farming is mainly to meet household subsistence/survival needs. In fact, it is because of such low production levels that there are calls by researchers and policy makers alike, for smallholder farmers to produce beyond subsistence in order to meet national food security goals.

Democratization of Agriculture in South Africa

Obi (2006), observed that government has changed the direction of agrarian policy to explicitly support black population with a view to fully integrating them into the mainstream economy since the advent of self-rule in 1994. The concern of government since then is how to empower the black farmers by providing them with the enabling environment which will allow them to participate in the agricultural economy. Among these empowerment programmes include, rural development programs, Settlement Land Acquisition Grant (SLAG), revitalization of irrigation schemes, the land Re-distribution for Agricultural Development (LRAD), Proactive Land Acquisition Strategy(PLAS), Farm Equity Schemes, the land restitution programme (LRP), Comprehensive Agricultural Support Programme (CASP), Municipal Commonage Programmes (Aliber and Hall, 2009).

In the modern day agriculture, accessing market with ease is the best form of support that can be given to smallholder farmers. The strategy of unlocking the market potentials of these farmers into the country agricultural economy is the priority of the National Department of Agriculture (DoA, 2001). Roe (2003), Magingxa (2006), Pote (2008), Obi and Pote (2011) were among many experts that have a similar view. In view of this, the government of South Africa has started on the veritable idea which will enhance market development for smallholder farmers. Along with various reforms on markets, government has come up with a range of policies that are tailored towards strengthening the deprived black farmers, not minding if they are producing for subsistence purpose or making attempt at operating on commercialize level (FANRPAN, 2012). However, the recent assessment of government efforts at reforming markets for smallholder farmers has not yielded any tangible outcome. These measures have not produced any improvement in the issue of rural smallholder farmers whose conditions have remain stagnated (Aliber and Hart, 2009). According to Obi (2011) smallholders, especially in less developed countries, have encountered several challenges in gaining access to market. Market access includes the ability to obtain necessary farm inputs and farm services and the ability to deliver farm products to buyers. From the outcome, the measures introduced to liberalize the local food markets and the strategy to integrate the economy of the country into an international system have hurt the poor smallholder farmers living in the former homelands of South Africa rather than being a source of help to them (Makhura and Mokoena, 2003; Van Schalkwyk et al., 2003). In addition, Pauw (2005) and Pote (2008) said that the remarkable success recorded both on macro economy and the commercial agricultural sector did not get to smallholder farmers because they were mainly the victims of discriminatory and harsh policies used under apartheid.

The homestead food gardeners

Going down memory lane, people formed, established and then depended on subsistence homestead food gardens in the former homelands of South Africa due to

betterment planning and homeland settlement policies and lastly apartheid (McAllister, 2010). Perry (2012), described a homestead as a very old concept, where the Bantu settlers in the Eastern Cape Province designed their homesteads as a function of their location to natural resources especially water resources. These people were mostly agro pastoral farmers with little enthusiasm. The uniqueness of this arrangement was that it accommodated both livestock rearing and crop production. Communal efforts were used in performing some farming tasks such as planting, ploughing, weeding and even harvesting thereby reducing the cost of production considerable, further to all these their farming work depended greatly on nature. Till date some of the local cultural practices still stand amongst these rural smallholder farmers in the Eastern Cape and the indigenous knowledge used then on how to know seasons and time, still evolve among most villagers.

MATERIALS AND METHODS

Description of study areas

Amathole is a rich region lying between longitude 27.3616° E and latitude 32.5842° S. The land area covered is 21,595 km^2. Rainfall on the high ground is around 1000 mm per annum whereas it is much lower in the valley bottom (600 mm) where it can only support limited rain fed cultivation (Figure 1). The district has an estimated population of 892,637, representing 14.7% of the Eastern Cape's population.

Chris Hani

Chris Hani is a rich region lying between longitude 26.7968° E and latitude 31.8743° S. The district population is predominantly Xhosa speaking and has a population of 795 461 people. The district covers area of 36,144 km², which is mostly Grassland and Subarid Thorn Bushveld vegetation types. The maximum temperature often exceeds 40°C during summer and it's a frost region during winter. The rainfall varies between 200mm and 300mm.

OR Tambo

OR Tambo lies between 31.4632° S and 29.2321° E coordinates. The district population is predominantly Xhosa speaking and has a population of 1,364,943 people. The district covers area of 12,096 km², which is grasslands and thicket to forests and bushveld. OR Tambo is characterized by moderate, humid and subtropical coastal climate from an average maximum of 25°. The annual rainfall varies between 1100 and 1400 mm per annum.

Research design

This study used a cross sectional survey design where data were collected at a single point in time. The method is less costly, less time consuming, and reliable.

Sampling technique and sample size

For the purpose of the study, stratified sampling was used to select farmers in the study areas. The farmers were stratified into two strata: irrigation users and non-users. From each stratum, random sampling was done to obtain 100 irrigators and 100 non-irrigators. Data collection was done through structured surveys using a full administered questionnaire. Since the number of household heads in the two groups is proportional, equal number of sample were drawn from each group that is 100 household heads will be selected from each group. In total, 200 household heads were interviewed. Relevant secondary data was obtained from reports from central statistics office, ministry of agriculture, extension officers, and the meteorology station.

Impact evaluation and econometric framework

The common impact evaluation problem is the inference of causal connection between the treatment and the outcome. The two specific problems with regards to impact evaluation of an intervention to targeted individuals are (i) the selection bias problem and (ii) the problem of missing data for the counterfactual. The selection bias problem emanates from the fact that most program interventions are targeted at specific groups with specific characteristics and that the intervals targeted are not randomly selected whilst the missing data problem is caused by the fact that it is not possible to measure the impact on the same individuals as at each moment in time each individual is either under the intervention being evaluated or not and thus he or she cannot be in both. Therefore, one cannot observe the outcome variable of interest for the targeted individuals had they not participated at the same time. However, there is extensive literature describing developments in addressing such problems. For example, empirical literature categorises evaluation methods in five categories (i) The pure randomised experiments (ii) the natural experiment (iii) the matching method (iv) the selection or instrumental variable model which relies on the exclusion restriction and (v) the structural simulation model.

This study assessed the impact of irrigation adoption on consumption expenditure and pattern using non-experimental data. As consequence, we follow from the work of Ravallion (2001), Godtland et al. (2004) and Bernard et al. (2007) by applying the Average Treatment Effect on the Treated (ATT). Accordingly, the study assessed whether or not the adoption of irrigation brings change to household income, total expenditures and household consumption patterns. The present study developed stochastic model following:

$$Yi = a_0 + \sum_{i=1}^{n} \quad \beta i Xi + \mu \qquad (1)$$

Where, *Yi* is the dependent variable meaning household welfare (household income, total expenditure and household consumption pattern, *Xi's are* the independent variables of the study (for example social grants, involvement in crop production and sales, Livestock ownership, Non-farm activities and remittances).

In this case, there is an endogeneity problem since irrigation is one of the observed characteristics. The question is to estimate the treatment effect of this observational (non-experimental) study by comparing the average treatment effect between adopters and non-adopters. And it can be expressed as follows:

$$ATTi \; = \; E\,\{Y1i - Y0i/\; D \; = \; 1, P\;(Xi)\} \qquad (2)$$

Where, ATT = Estimation of Average Treatment Effect between the treated and control using predetermined variable (X_i); Y_{1i} and Y_{0i} = Potential outcomes of the treated and untreated.

Since there might be bias problem, the study used propensity score matching wherein it selects a control group that do not adopt to resemble them with the treated group on the basis of similarity in the observed data. Because, the ATT might not be observed for

Figure 1. Map of the study area.
Source: Google maps, 2016.

some respondents, propensity matching method sets a conditional independency assumption that all relevant differences between the two groups be captured by their observable variables (X_i). Both adopters and non-adopters are matched on the basis of propensity scores.

$$Yi0 \perp / X \rightarrow E\,(Yi0 / Pi = 1, Xi) = E\,(Yi0 / Pi = 0, Xi) \quad (3)$$

The propensity match can use different models to estimate the propensity score. The study uses the probit model because adopting irrigation technology is not random rather affected by observed, unobserved or both factors. The model estimates the adoption level and can be expressed as follows:

$$P\,(Di = 1 / Xi) = \phi\,(f\,(Xi) = a_0 + \sum_{i=1}^{n} \beta i Xi + \mu \quad \mu \approx N(0, \delta 2) \quad (4)$$

Where, ϕ denotes the normal cumulative distribution function and $f(X_i)$ represents a specification of the respondent adopted *(D_i = 1)* for those who adopted irrigation) determinants (X_i) of which includes all the observed covariates as linear terms without interaction of higher orders terms that have effect on the tendency to adopt and household welfare. Every sampled adopters and non-adopters have an estimated propensity score, which is a continuous variable and can be expressed as follows:

$$\hat{z}\,(Xi / T = 1) = \hat{z}\,(Xi) \quad (5)$$

The difference between the average outcomes of the two groups is the estimated effect of the adoption if the resemblance is satisfactory (Caliendo and Kopeinig, 2008). For the matching of adopters to non-adopters on the basis of the propensity score, the study assumed two alternatives: nearest-neighbor and kernel matching which were used to calculate a weight for each matched adopters to non-adopters set.

Accordingly, the impact of the irrigation is the mean difference in the outcomes between the treated and untreated group for each stratum. In each stratum or block, the average difference between outcomes of treated observations and control observations is estimated as follows:

$$ADsq = \frac{\sum_{izt(q)} Y_i^T}{N_q^T} - \frac{\sum_{jzt(q)} Y_j^C}{N_q^C} \quad (6)$$

Where, AD^S_q is the average difference block q, I(q) is the set of units in a bloc q; N_q^T and N_q^C are the number of treated control units in the block (q).

Consequently, the estimator of ATT is computed as an average of each AD (UNDP, 2009) and is given by the following equation: where, Q is the total number of blocks.

$$ATT = \sum_{q=1}^{Q} AD_q^S \frac{\sum_{izt(q)} D_i}{\sum_{\forall i} D_i} \quad (7)$$

The kernel matching method used weighted averages of all

individual in the comparison group to make the counterfactual effect. The weights are calculated based on the distance between each individual from the comparison group and the treated observation of which the counterfactual is estimated (Caliendo and Kopeining, 2008). The Kernel matching ATT estimator is given by:

$$ATT = \frac{1}{N^T}\sum_{i=iT}\{Y_i^T - \frac{\sum_{jzC} Y_j^C\ G\left(\frac{P_{j-P_i}}{h_n}\right)}{\sum_{kzC}\ G\left(\frac{P_{k-P_i}}{h_n}\right)} \quad (8)$$

Where, G, is the Kernel function and h_n is a bandwidth parameter. The choice of bandwidth parameter is more important because it defines the fitness and the variance between the estimated and true underlying the density function. The researcher needs considered the variance and the bias of the estimation at the same time while choosing the bandwidth parameter. After the matching process and producing significant propensity scores, the study compared the average outcomes of the matched respondent groups (treated vs control) based on some comparable variables (such as total expenditures, consumption patterns, and household incomes) to estimate whether there is a statistically significant effect of the treated on the outcome.

Ethical consideration

An ethical clearance was sought for from the university. Furthermore, permission was asked from governmental agencies and local leaders before gathering all the farmers in the community. The respondents for this research were not subjected to any risk that could harm them physically or mentally. Lastly, respondents were notified that there are no monetary benefits and rewards from participating in the study

Trustworthiness

The results of this study were triangulated with other sources such as relevant published reports and articles, and the feedback meetings also helped to validate the findings.

RESULTS AND DISCUSSION

In this section, descriptive statistics of the variables and the estimation results of the probit regression are presented. The results will facilitate to identify the factors that influence a decision to participate on irrigation schemes.

Descriptive analysis of the household endowment by adoption status

Household endowments are normally used to measure the wealth of farming households and can reveal the living conditions. An assumption is that, a well-endowed household would better adopt technology than otherwise. A comparison of assets for adopters and non-adopters was made to check if irrigation has any effect on household assets. The results analysis is presented in Table 1.

The results indicated that, in overall, the few (12%) farmers in the study area owned land. Only 9% of adopters and 15% of adopters owned the land. This suggests that, access to land in the study areas is still a problem which could limit production and adoption of irrigation for farming.

Livestock ownership is used as a supplemental household's income especially during off season. The analysis revealed that about 70 and 58% of adopters and non-adopters owned livestock respectively. Household's assets such as ownership of cellphone and access to electricity were regarded as important means of accessing information about new technologies and were assumed to have influenced irrigation adoption. Only 30% of non-adopters had access to cellphones compared to 53% of adopters. In terms of access to electricity, only 41% of non-adopters and 66% of adopters had access to electricity. Therefore, access to electricity could be one amongst the factors affecting adoption.

Household endowments such as type of a house, type of roofing, number of rooms, good sanitation and access to water, all combined could improve the well-being of a household and consequently encourage adoption of irrigation technology. Not many household were endowed in terms of these assets. This was observed by only 20 and 32% of non-adopter and adopters had access to water for household consumption. The adopters seemed to be better off in terms of roofing type and the number of rooms as a larger number (67%) used zink and had 10 rooms.

Descriptive analysis of the impact of irrigation adoption by farmers

Table 2 presents the descriptive statistics of the impact of irrigation adoption on income from crop production, total agricultural expenditure, and consumption expenditure and farm size. The average area under cultivation by all the farmers was 5.95 ha, while the difference test showed that the area cultivated by non-adopters (6.1 ha) was significantly higher than that of adopters (5.8 ha). However, despite the higher area cultivated by non-adopters, they seem not better off in terms of household income from crop production. For instance, the adopters had a significantly higher income from both the production and wage income than non-adopters. Consequently, adopters were able to spend more on agriculture (95151.92) than non-adopters (R77215.08).

In terms of the impact of irrigation on welfare status of farmers, a comparison was done between adopters and non-adopters. Per capital expenditure reflects the effective consumption of households and therefore provides welfare information. The results revealed that consumption expenditure of adopters (9877.71) was higher than that of non-adopters (9588.92). This implies that adopters had a better welfare than non-adopters.

Table 1. Household endowment by adoption status.

Household endowments	Adopters percentage (%)	Non-adopters percentage (%)	Overall percentage (%)
% Household owning land	9	15	12
% Zink Roofed type	67	44	55.5
Number of rooms	10	6	8
Access to household water	32	20	26
Access to good sanitation	40	57	48.5
Owns mobile cellphone	53	30	41.5
Access to electricity	66	41	53.5
Number of livestock owned	70	58	64

Source: Results from SPSS (Version 20) generated from field survey, 2016.

Table 2. Descriptive analysis by adoption status.

Variable	Adopters	Non adopters	Mean difference
Income from crops	2044.01	622.12	1421.89
Wage income	4819.35	2653.36	2165.99
Agricultural expenditure	95151.92	77215.08	17936.83
Consumption expenditure	933.3	990.1	-56.8
Farm household size (ha)	5.8	6.1	5.95

Source: Results from SPSS (Version 20) generated from field survey, 2016.

Table 3. Crops grown by Smallholder Farmers in Eastern Cape.

Crops	Adopters		Non-adopters		Main season
	Frequency	%	Frequency	%	
Vegetables	61	65	75	70	Winter
Maize	77	72	71	76	Summer
Beans	48	45	50	54	Summer
Potato	75	70	67	72	Winter
Butternut	53	57	73	68	Summer

Source: Results from SPSS (Version 20) generated from field survey, 2016.

Types of crops grown in Eastern Cape

Farmers in both groups appeared to have different goals for engaging in crop and vegetable production. Table 3 shows the different crops cultivated by households in the sample in 2014/2015. Large plots of smallholder farmers in the area planted maize, beans, potatoes, butternut and vegetables with an average of 65%. Comparing the two groups, irrigators grew mostly maize (77%), potato (75%), vegetables (61), butternut (53%) and beans (48%) while non-irrigators grew mostly maize (76%), potato (72%), vegetables (70%), butternut (68%) and beans (54%). The results are presented in Table 3.

Although most of the selected crops are grown throughout the year, most of vegetables are grown during winter season especially potatoes and maize with beans in summer. Farmers in the study area do crop rotation, where they grow maize and beans from October end to December and usually harvest during winter season around May. Vegetable crops such as potatoes, spinach and cabbages are grown during May to the end of August (Cousins, 2013).

In general, irrigating households tend to produce cash crops and vegetables for the market. A portion of the produce was consumed at home while the bulk of production was sent to the market. These results confirm findings by De Cock et al. (2013) that households produce for both home consumption and the market. These findings endorse statements that agriculture plays a major role in the livelihoods of rural people. It was, however, difficult to assess the true contribution of own food production as households did not keep records.

Table 4. Propensity Score Matching to measure the impact of access to irrigation on household welfare.

Matching method	Outcome variable	Household welfare			Standard Error	t-test
		Adopter	Non-Adopter	ATT		
Nearest neighbour	Farm income	2044.01	622.12	1421.8	124.14	0.055**
	Wage income	4819.35	2653.36	2165.9	266.56	0.126*
	Food expenditure	933.30	990.10	-56.8	31.6	0.165
	Food consumption pattern	4.04	3.42	0.64	0.44	0.03**
Kernel Matching	Farm income	1977.6	643.41	1334	206.13	0.053**
	Wage income	4687.22	2568.40	2118	167.3	0.110*
	Food expenditure	926.7	983.2	-56.5	26.8	0.142
	Food consumption pattern	4.0	3.38	0.62	0.36	0.02**

***, ** and * means significant at 1%, 5% and 10% levels, respectively.
Source: Results from Stata (Version 13) generated from field survey, 2016.

They could not recall the exact quantities that were taken for home consumption and marketing.

Econometric analysis of the Impact of adoption on household welfare

As indicated above, the PSM model was used because of its strength to minimise selection bias which may arise as a result of unobservable. In the process of running the model, the balancing property was selected in estimating propensity scores to ensure that the two group's characteristics are distributed equally. Heckman et al. (1996a) encouraged dropping treatment observations with weak common support as inferences can be made about causality only in the area of common support. In addition, all standard errors were bootstrapped with 1000 repetitions following Smith and Todd (2005), Dillon (2011) and Sinyolo (2013).

The nearest neighbour and Kernel matching methods were used to estimate the impact of irrigation participation on farm income from crop production. Table 4 presents results from the PSM model that was estimated for comparison purposes with the treatment effect model results. The matching results indicate that irrigation has a positive significant impact on the livelihoods status of irrigators. Two matching estimators, the Nearest neighbouring and the Kernel based matching algorithms were employed as robustness checks.

Table 4 indicates that both the nearest neighbour and Kernel matching methods point to the fact that irrigation access has a positive impact on total farm income. The nearest neighbour matching method indicated that irrigator received high farm income (R2044.01) and non-irrigating farmers (R622.12). There is no much difference in terms of the incomes generated using Kernel Matching method. These positive results indicate that participating on irrigation helps to improve farm incomes of households and is significant at 5% level. This is consistent with the findings of previous studies (Tesfaye et al., 2008; Gebregziabher et al., 2009; Bacha et al., 2011; Kuwornu and Owusu, 2012; Senyolo et al., 2009; Sithole et al., 2014).

The result for wage income are positive both in the case of NNM and KM, indicating that adopting irrigation farming leads to high wages in the household. The wage incomes are high for irrigating farmers compared to counter parts in both methods. This could be as a result of the fact that irrigation also creates jobs for their members. The ATT on total food expenditure was negative both in the case of NNM and KM algorithms, indicating that participation on irrigation can decrease the expenditure levels on food from (R933.30) to (R926.70). This could be due to the fact that irrigating farmers grew up enough for home consumption than non-irrigators. Food consumption pattern was positive and significant in both case of NNM and KM, indicating that participation on irrigation farming increases food consumption patterns per week. Food was categorised into vegetables, fruit, meat, eggs and dairy. Food consumption patterns for irrigator was high at an average of (4 times per week) compared to (3 times a week) for non-irrigators.

Conclusions

The overarching goal of African agricultural development programmes and policies is increasing productivity for accelerated economic growth. An example of these programmes is CAADP which was launched in 2003 to address agricultural and food security issues in Africa praising irrigation as one step towards achieving this goal. Recognizing this, South Africa government under CASP has undergone rehabilitation and revitalization of irrigation schemes, however progress of the impact of such programme is not known. This study has tried to assess the impact of irrigation technology on welfare among the crop farmers in Eastern Cape. Among the

many findings, the results indicated that adoption of irrigation significantly impacted total household expenditure. The impact on all the outcomes of interest was also higher among the female headed households than the male headed households. In conclusion, improved agricultural technology adoption can lead to the much desired increase in productivity, ensure households food security and can also be away out of the menace of rural poverty in Eastern Cape.

RECOMMENDATIONS

Based on the above findings, the study recommends that efforts should be geared toward making adequate irrigation schemes available to the rural farmers in order to meet welfare status.

1) There is a need for increased access to credit and information that will ultimately increase diffusion and level of technology adoption.
2) The provincial department of agriculture should consider giving a special attention in strengthening the capabilities of the existing extension system by assigning additional extension agents as well as equipping them with the necessary technologically appropriate equipment such as smart pens, cars and cell phones to name the few.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Correlation and path coefficient analysis in mid-altitude sesame (*Sesamum indicum* L.) germplasm collection of Ethiopia

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The current investigation was aimed to study the genetic association of seed yield and its components in 81 mid-altitude sesame accessions based on morphological traits. The genotypes were evaluated in 9 × 9 simple lattice design at Melkassa Agricultural Research Center, Ethiopia, during the 2014 cropping season. The study mainly focused on determining the nature and extent of phenotypic and genotypic correlation and path coefficient analysis among 13 quantitative traits. Analysis of variance revealed significant difference among genotypes for all traits studied. Mean performance of genotypes revealed that the highest mean seed yield/plant (8.6 g) recorded for Oromia-22 and the lowest mean seed yield/plant (2.6g) for Oromia-9; with overall mean of 5.33 g/plant. Whereas, the highest mean oil content (52.15%) noted for Oromia-13 and the lowest (43.35%) mean oil content was recorded for Am-SW-7 genotypes, with overall mean of 47.1%. Characters viz., number of capsules, biomass yield, harvest index and 1000 seed weight showed highly significant and high positive correlation with seed yield. Plant height and number of seeds/capsule also showed highly significant but moderately positive significant association with seed yield; indicating that these traits are reliable yield components and seed yield can be improved through direct selection of these traits. Maximum positive direct effect on seed yield was exerted by number of capsules, biomass yield, days to maturity and harvest index; showing that these traits can be used for selection to improve the primary trait. Hence, the use of these traits in sesame improvement program would increase seed yield.

Key words: Correlation coefficient, mid-altitude, quantitative traits, path coefficient analysis.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an annual plant of Pedaliaceae family considered to be the oldest oilseed crop cultivated by man, having been grown in the Near East and Africa for over 5,000 years for cooking and medicinal purposes (Sharma et al., 2014). Generally, 65% of world sesame production is used for edible oil extraction and 35% for confectionary purpose (Pham et al., 2011). The fatty acid composition is rather attractive,

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due to the high level of unsaturated fatty acids. Sesame seed is the single readily available source of protein high in sulfur containing amino acids (Bradley, 2002). It is the major cash crop for smallholder farmers and a valuable foreign exchange revenue commodity for different countries. The remaining cakes of sesame are used as a source of crude protein for animal feed.

In Ethiopia, sesame is used as cash crop, export commodity, raw material for oil industries and as source of employment opportunity. Now a day, it becomes the primary export oil crop playing a role in the agricultural Gross Domestic Product (GDP) of the country. A sizable proportion of the population, therefore, generates income from oilseed farming, trade and processing (Abate et al., 2015). However, production and extension in Ethiopia is quite limited, particularly because of its low yield. One of the major problems facing sesame production in Ethiopia has been growing of inferior sesame varieties with low yield and poor quality. To overcome the problems of low productivity of sesame, there must be a sound procedure for selection of high yielding varieties adapted to the local environment (Tadele, 2005).

Seed yield in sesame like other field crops is a multifacet character and direct selection for this trait may often be misleading. The components that determine the yield are best indices for selection. Therefore, knowledge of relationship between important yield traits and seed yield may help the researchers to identify suitable donors for a potential and successful breeding program (Kumaresan and Nadrajan, 2002). Estimation of character associations could identify the relative importance of independent traits contributing to dependent ones and suggest upon the traits that may be useful as indicator for other traits. In other words, character associations between yield components can be used as the best guide for successful yield improvement by indirect selection. Achievement of such success depends upon sort and accuracy of estimated correlation coefficient, plant materials, environmental conditions and their interaction. Among several factors, yield related traits highly influence the amount of grain yield that can be obtained (Salah et al., 2013). Some of the yield related traits in sesame include: days to flower, days to maturity, plant height, number of branches, capsules per plant, capsule length, number of seeds per capsule, thousand-seed weight and seed yield per plant. These traits affect yield positively and/or negatively and their effect on yield depends on the influence of environment on them.

The knowledge of nature and magnitude of genetic variability is of immense value for planning efficient breeding programme to improve the yield potential of crop species. Likewise, information on genetic association of plant traits with seed yield has great importance to breeder in selecting desirable genotypes (Parameshwarappa et al., 2009). Phenotypic selection of parents based on their performance alone may not always be reliable procedure since phenotypic expression is highly influenced by environmental factors, which are non-heritable. It is therefore, essential to select genotypes on the basis of their genetic worth (Salah et al., 2013). Thus, correlation helps in selection of superior genotype from diverse genetic populations (Jogdhande et al., 2017). However, in correlation studies indirect associations become more complex and confusing but path analysis can avoid this complication by measuring the direct influence of one trait on other as well as permits the partitioning of a given correlation coefficients into its components of direct and indirect effects (Manisha et al., 2018; Jogdhande et al., 2017). The path coefficient analysis is an effective means of analyzing direct and indirect causes of association and permits critical examination of the specific traits that produce a given correlation. It provides information about magnitude and direction of direct and indirect effect of the yield components (Chaudhary et al., 2005; Bizeti et al., 2004).

However, lack of information (particularly agro-ecological based) on character association of yield and its contributing traits, believed to limit the genetic improvement of sesame in Ethiopia. Hence, the present investigation was focused to gather adequate information on genetic association of yield and yield related traits in sesame accessions collected from mid-altitude areas of Ethiopia.

MATERIALS AND METHODS

Description of the study site

The experiment was conducted at Melkassa Agricultural Research Center (MARC), Ethiopia, during the 2014 cropping season. Melkassa is located along the Upper Awash valley between 8° 33' N and 39° 17' E. The altitude of the Center is 1550 m.a.s.l. and the minimum and maximum annual temperature ranges from 14.35 to 28.22°C, respectively. The mean annual rain fall in the area is 704.8 mm with verti-cambisol soil type of pH = 7.6.

Experimental materials and design

The materials for the study comprised 81 sesame accessions (including three released varieties as standard checks) representing the mid-altitude areas of Ethiopia (Table 1) that were obtained from Ethiopian Biodiversity Institute (EBI) and Werer Agricultural Research Center (WARC). The experiment was laid out in 9 × 9 simple lattice designs with two replications and each genotype was planted in a plot consisting of four rows of 2.5 m long at a distance of 40 cm between rows and 10 cm between plants. All cultural practices were applied as required throughout the season.

Data collection

Morphological data *viz*., days to 50% flowering (DF), days to 75% maturity (DM), number of primary branch/plant (PBPL), number of capsules/plant (CPPL), number of seeds/capsule (SDPC), capsule length (CL) (cm), plant height (PH) (cm), biomass/plant (BMPL) (g),

Table 1. List of sesame accessions collected from mid-altitude areas of Ethiopia used in the experiment.

S/N	Genotype	Altitude	/ District	S/N	Genotype	Altitude	Region/ District
1.	Am-NSh-1	1395	Amhara North Shoa	42.	SNNP-2	1300	North Omo
2.	Oromia-1	1500	East Wollega	43.	SNNP-3	1290	Bench-Maji
3.	Oromia-2	1395	East Wollega	44.	SNNP-4	1310	North Omo
4.	Oromia-3	1640	West Wellega	45.	Am-NG-2	1460	Amhara North Gonder
5.	Oromia-4	1520	West Wellega	46.	Oromia-13	1610	Bale Zone
6.	Am-NG-1	1635	Amhara North Gonder	47.	Oromia-14	1560	Jimma Zone
7.	Am-SW-1	1590	Amhara South Wollo	48.	Oromia-15	1500	Jimma Zone
8.	Oromia-5	1580	Bale	49.	Am-NG-3	1440	Amhara North Gondr GGGonder
9.	Oromia-6	1680	Bale	50.	Am-NG-4	1440	"
10.	Oromia-7	1565	Arsi	51.	Am-NW-16	1630	Amhara North Wollo
11.	Oromia-8	1560	Arsi	52.	Tigray-1	1400	Debubawit
12.	Am-SW-2	1673	Amhara South Wollo	53.	Am-SW-9	1570	Amhara South Wollo
13.	Am-SW-3	1565	"	54.	Am-NG-5	1530	Amhara North Gonder
14.	Am-SW-4	1440	"	55.	Oromia-16	1600	East Wollega
15.	Am-SW-5	1462	"	56.	Oromia-17	1690	Arsi Zone
16.	Am-SW-6	1624	"	57.	Oromia-18	1700	East Harrarge
17.	Am-NW-1	1522	Amhara North Wollo	58.	Oromia-19	1630	East Harrarge
18.	Am-NW-2	1270	"	59.	Tigray-2	1650	Debubawit
19.	Am-NW-3	1400	"	60.	Tigray-3	1640	Debubawit
20.	Am-NW-4	1450	"	61.	Oromia-20	1560	East Wollega
21.	Am-NW-5	1430	"	62.	Am-SW-10	1540	Amhara South Wollo
22.	Am-NW-6	1700	"	63.	Am-SW-11	1500	"
23.	Am-NW-7	1550	"	64.	Am-SW-12	1660	"
24.	Am-NW-8	1580	"	65.	Oromia-21	1570	West Wellega
25.	Am-NW-9	1645	"	66.	Oromia-22	1435	West Harrarge
26.	Am-NW-10	1555	"	67.	Oromia-23	1450	West Harrarge
27.	Am-NW-11	1490	"	68.	Oromia-24	1500	West Harrarge
28.	Am-NW-12	1460	"	69.	Oromia-25	1380	Illubabor
29.	Am-NW-13	1500	"	70.	Oromia-26	1380	Illubabor
30.	Am-NSh-2	1395	Amhara North Shoa	71.	Oromia-27	1462	Illubabor
31.	Am-NW-14	1640	Amhara North Wollo	72.	Am-NG-6	1335	Amhara North Gonder
32.	SNNP-1	1520	Gurage Zone	73.	Am-NG-7	1360	"
33.	Oromia-9	1635	Jimma	74.	Am-NG-8	1360	"
34.	Oromia-10	1590	East Wollega	75.	Am-NG-9	1445	"
35.	Oromia-11	1680	East Harrarge	76.	Am-NG-10	1470	"
36.	Oromia-12	1580	East Wollega	77.	Tigray-4	1534	Mirabawit
37.	Am-NSh-3	1565	Amhara North Shoa	78.	Tigray-5	1545	Mirabawit
38.	Am-NSh-4	1560	"	79.	T-85	-	Check variety
39.	Am-SW-7	1673	Amhara South Wollo	80.	E	-	"
40.	Am-SW-8	1565	"	81.	Tate	-	"
41.	Am-NW-15	1440	Amhara North Wollo				

Note: Region = Administrative Zone, SNNP = Southern Nation Nationality people.

harvest index/plant (HIPL) (%), 1000 seed weight (TSW) (g), seed yield/plant (SYPL) (g) and seed yield/ha (SYH) (kg) were collected for each plot by selecting 5 plants at random from the central rows leaving aside rows from the top and bottom to take care of boarder effects. Finally, oil content OC (%) was determined for each genotype from 5 g of seeds using Nuclear Magnetic Resonance Spectroscope; as the proportion of oil in the seed to the total oven dried seed weight × (100).

Data analysis

Analysis of variance was carried out for the data with SAS statistical software (9.2); to test for significant differences among the genotypes according to the standard statistical procedure described by Gomez and Gomez (1984). Phenotypic and genotypic correlations between agro-morphological traits were estimated using the method described by Miller et al. (1958) as follows:

Table 2. Mean squares from analysis of variance for 13 agro-morphological traits in 81 sesame genotypes.

Trait	Rep (1)	Block (8)	Gen (72)	Error (72)	CV (%)	SE
Days to Flowering	4.17	19.88	17.57*	7.87	2.76	2.03
Days to Maturity	1.04	14.70	12.72*	5.83	1.57	1.91
No. of Pr. Branches	3.56	4.17	8.53*	5.27	25.25	2.29
No. of Capsules/plant	0.01	79.53	428.83*	184.03	13.74	10.55
No. of Seeds/capsule	111.50	18.44	17.46*	6.98	6.50	2.08
Capsule Length (cm)	0.04	0.10	0.08*	0.07	10.60	0.27
Plant Height (cm)	1063.12	262.38	242.88**	115.17	8.78	10.73
Biomass/plant (g)	9.18	1.05	3.12**	1.51	19.67	1.17
Harvest Index (%)	235.45	41.34	83.26**	29.96	6.29	4.77
1000 Seed weight (g)	0.57	0.07	0.13*	0.06	11.29	0.21
Seed Yield/plant (g)	9.98	1.05	3.13**	1.46	24.63	1.14
Seed Yield/ha (kg)	24545.89	2575.81	7846.69**	3651.85	24.65	57.06
Oil Content (%)	0.83	11.71	8.11*	3.35	2.54	1.20

**, * significance at P < 0.01 and P < 0.05 level, respectively; figures in parenthesis refer to degrees of freedom; CV= coefficient of variation, Gen= genotypes, SE= standard error.

Phenotypic correlation coefficient $(rp_{xy}) = \frac{Covpxy}{\sqrt{(\sigma^2 px)(\sigma^2 py)}}$

Genotypic correlation coefficient $(rg_{xy}) = \frac{Covgxy}{\sqrt{(\sigma^2 gx)(\sigma^2 gy)}}$

Where, r_{pxy} is phenotypic correlation coefficient and genotypic correlation coefficient (rg_{xy}) between character x and y; Cov_{pxy} and Cov_{gxy} are phenotypic covariance and genotypic covariance between character x and y; σ^2_{gx} and σ^2_{gy} are genotypic variances traits x and y; σ^2_{px} and σ^2_{py} are phenotypic variances of traits x and y, respectively.

The correlation coefficient was analyzed based on the tabulated data, using META-R Version- 6. 01 (Alvarado et al., 2017). The path coefficient analysis was computed for 12 yield related traits using the phenotypic and genotypic correlation results following the procedure suggested by Dewey and Lu (1959). Seed yield per plant was used as dependent character in path coefficient analysis and the remaining traits were used as independent variables.

$$R_{ij} = P_{ij} + \sum r_{ik} P_{kj}$$

Where, r_{ij} is mutual association between the independent character (i) and dependent traits (j) as measured by correlation coefficients, p_{ij} is components of direct effects of the independent traits (i) on the dependent traits (j), $\sum r_{ik} P_{kj}$ = summation of components of indirect effect of a given independent character (i) on the dependent traits (j) via all other independent traits (k).

The residual effect (R) was computed using the formula suggested by Dewey and Lu (1959) as:

$R = \sqrt{1-R^2}$ Where, $R^2 = \sum r_{ij} p_{ij}$

RESULTS AND DISCUSSION

Analysis of variance and mean performance of accessions

The analysis of variance revealed significant difference among the genotypes for all traits, indicating the presence of sufficient variability among the tested accessions for the traits under consideration (Table 2). This result was in agreement with the previous results reported by, Parameshwarappa et al. (2009), Salah et al. (2013) and Ahmed and Ahmed (2013). Mean performance of 81 sesame genotypes for 13 traits (data not shown) showed that seed yield per plant was ranged from 2.6 g (Oromia-9) to 8.6 g (Oromia-22) with overall mean of 5.33 g/plant. Generally, 44% of the genotypes had greater mean seed yield than the overall mean of genotypes. The highest mean seed yield/plant was recorded for Oromia-22 (8.66 g) followed by Am-NW-13 (8.41 g), Am-SW-5 (8.23), Oromia-25 (7.62 g), Am-NW-14 (7.60 g), Am-SW-10 (7.53) and Oromia-1 (7.51 g). These genotypes were found to be superior even to the released varieties. The highest (52.15%) and lowest (43.35%) mean oil content was recorded for Oromia-13 and Am-SW-7 genotypes, respectively, with overall genotypes mean of 47.1%. The genotypes Oromia-13 (52.15%) and Am-NSh-2 (51.20%) had the highest mean oil content and were found to be superior as compared to the standard checks. A high variation in number of capsules/plant, primary branches/plant and plant height was found among the studied genotypes. Similarly, high variation in number of primary branches, plant height and 1000 seed weight was reported by Gidey et al. (2013).

Correlation coefficient

Phenotypic and genotypic correlation coefficients of the various traits are presented in Table 3. The phenotypic and genotypic correlations in general were higher than the environmental correlation for the studied traits.

Table 3. Genotypic correlation above diagonal and phenotypic correlation below diagonal among 13 traits in 81 sesame accessions.

Trait	DF	DM	PBPL	CPPL	SDCP	CL	PH	BMPL	HIPL	TSW	SYPL	SYP	OC
DF		0.69***	0.05	-0.02	-0.04	-0.13*	-0.03	-0.01	0.01	0.09	-0.01	-0.01	-0.05
DM	0.70***		0.05	0.03	0.00	-0.09	0.02	0.02	0.03	0.04	0.02	0.02	-0.02
PBPL	0.06	0.10		0.35***	-0.04	0.01	0.25***	0.27***	0.21***	0.06	0.27***	0.27***	-0.04
CPPL	-0.02	0.04	0.42***		0.13*	0.15**	0.33***	0.79***	0.73***	0.11	0.79***	0.79***	0.01
SDCP	0.00	0.08	-0.01	0.24*		0.00	0.08	0.48***	0.53***	0.22***	0.48***	0.48***	-0.07
CL	-0.08	-0.09	0.04	0.22	0.03		0.00	0.13*	0.16**	-0.01	0.13*	0.13*	-0.04
PH	0.01	0.03	0.38**	0.46***	0.30*	0.00		0.28***	0.22***	0.03	0.28***	0.28***	0.00
BMPL	0.02	0.05	0.31*	0.83***	0.53***	0.21	0.43***		0.94***	0.60***	1.00***	1.00***	-0.11*
HIPL	0.05	0.05	0.25*	0.78***	0.57***	0.24	0.37**	0.96***		0.62***	0.94***	0.94***	-0.13*
TSW	0.17	0.06	0.07	0.21	0.24*	-0.01	0.06	0.65***	0.62***		0.60***	0.60***	-0.21*
SYPL	0.02	0.05	0.31*	0.83***	0.53***	0.21	0.43***	0.99***	0.96***	0.65***		1.00***	-0.11*
SYP	0.02	0.05	0.31*	0.83***	0.52***	0.22	0.42***	0.98***	0.96***	0.65***	1.00***		-0.11*
OC	-0.02	-0.01	0.06	0.16	-0.06	-0.02	0.03	-0.08	-0.12	-0.06	-0.08	-0.08	

***, **, *= significant at P < 0.001, < 0.01 and < 0.05 respectively. DF = Days to flowering, DM= Days to maturity, PBPL= Number of primary branches per plant, CPPL= Number of capsules per plant, SDPC= Number of seeds per capsule, CL= capsules length, PH= Plant height, BMP= Biomass per plant, HIPL= Harvest index per plant, TSW= 1000 seed weight, SYPL= Seed yield per plant, SYP= Seed yield per hectare, and OC= Oil content.

Number of capsules/plant, biomass/plant, harvest index and 1000 seed weight exhibited highly significant (<0.001) and high positive association with seed yield/plant at both phenotypic and genotypic level, indicating that these traits are reliable yield components and seed yield can be improved through direct selection of these traits. Similar results were reported by Muhamman et al. (2010) and Haruna et al. (2012), who found highly significant correlation of sesame seed yield with number of capsules/plant, biomass/plant and 1000 seed weight. Plant height and number of seeds/capsule showed highly significant and moderate positive correlation with seed yield/plant at both phenotypic and genotypic level. Number of primary branch/plant showed significant (<0.05) and low positive genotypic association with plant height, biomass/plant, harvest index and seed yield/plant. Hence, indirect selection in favor of these traits can improve seed yield in sesame. Similar results were reported by Sumathi et al. (2007) and Sumathi and Muralidharan (2010) for plant height, number of branches, number of capsules, days to 50% flowering, days to maturity and 1000 seed weight. However, oil content showed significant (<0.05) negative genotypic correlation with most of the yield traits (Table 3). This implies that indirect selection for high oil content would reduce seed yield/plant. Hence, simultaneous improvement for seed yield and oil content is difficult in the studied germplasm. Similar result was found by Daniya et al. (2013). In the contrary, Onginjo and Ayiecho (2009) found insignificant positive correlation of oil content with seed yield and suggested that selection for oil content had no any effect on seed yield.

Generally, correlation analysis revealed the presence of highly significant positive association between seed yield and the yield related traits such as number of capsules, biomass/plant, harvest index, 1000 seed weight and number of seeds/capsule. This finding is in line with Muhamman et al. (2010), Tamina and Tapash (2011), Haruna et al. (2012) and Daniya et al. (2013) who reported significant positive correlations between yield traits and final seed yield in sesame.

Path coefficient analyses

According to Bhatt (1973), correlation analysis may not be sufficient to explain the extent of associations in a manner that will enable one to decide on either a direct or an indirect selection strategy. Because of this, path coefficients

Table 4. Direct (diagonal) and indirect effect (off diagonal) of quantitative traits on seed yield/plant at phenotypic level in 81 sesame genotypes.

Trait	DF	DM	PBPL	CPPL	SDCP	CL	PH	BMPL	HIPL	TSW	OC	pr
DF	**0.02**	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.01	-0.04	0.00	0.02
DM	0.01	**0.00**	0.00	-0.02	-0.01	0.00	0.00	0.07	0.01	-0.02	0.00	0.05
PBPL	0.00	0.00	**0.01**	-0.17	0.00	0.00	-0.00	0.43	0.05	-0.02	0.01	0.31
CPPL	0.00	0.00	0.00	**0.53**	-0.1	-0.02	-0.37	0.85	0.17	-0.25	0.02	0.83
SDCP	0.00	0.00	0.00	-0.10	**-0.16**	0.00	-0.00	0.73	0.12	-0.06	-0.01	0.53
CL	0.00	0.00	0.00	-0.09	0.00	**-0.04**	-0.00	0.29	0.05	0.00	0.00	0.21
PH	0.00	0.00	0.00	-0.19	-0.05	0.00	**-0.00**	0.59	0.08	-0.02	0.00	0.43
BMPL	0.00	0.00	0.00	-0.04	-0.09	-0.01	-0.00	**1.08**	0.21	-0.17	-0.01	0.99
HIPL	0.00	0.00	0.00	-0.31	-0.09	-0.01	-0.00	0.80	**0.75**	-0.16	-0.01	0.96
TSW	0.00	0.00	0.00	-0.08	-0.04	0.00	0.00	0.90	0.13	**-0.26**	-0.01	0.65
OC	0.00	0.00	0.00	-0.06	0.01	0.00	0.00	-0.11	-0.03	0.02	**0.10**	-0.1

DF= Days to flowering, DM= Days to maturity, PBPL= Number of primary branches/plant, CPPL= Number of capsules/plant, SDPC= Number of seeds/capsule, CL= Capsules length, PH= Plant height, BMPL= Biomass/plant, HIPL= Harvest index/plant, TSW= 1000 seed weight, SYPL= Seed yield/plant and OC= Oil content. Residual = 0.15.

Table 5. Direct (diagonal) and indirect effect (off diagonal) of quantitative traits on seed yield/plant at genotypic level in 81 sesame genotypes.

Trait	DF	DM	PBPL	CPPL	SDCP	CL	PH	BMPL	HIPL	TSW	OC	gr
DF	**-0.01**	0.00	0.00	-0.01	-0.01	0.00	0.00	-0.01	0.00	0.02	0.00	-0.01
DM	0.00	**0.00**	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.02
PBPL	0.00	0.00	**0.00**	0.10	-0.01	0.00	0.00	0.17	-0.02	0.01	0.00	0.27
CPPL	0.00	0.00	0.00	**0.60**	0.02	0.00	0.00	0.20	-0.05	0.02	0.00	0.79
SDCP	0.00	0.00	0.00	0.04	**0.13**	0.00	0.00	0.31	-0.04	0.04	0.00	0.48
CL	0.00	0.00	0.00	0.04	0.00	**0.02**	0.00	0.08	-0.01	0.00	0.00	0.13
PH	0.00	0.00	0.00	0.10	0.01	0.00	**0.00**	0.18	-0.02	0.01	0.00	0.28
BMPL	0.00	0.00	0.00	0.23	0.06	0.00	0.00	**0.64**	-0.07	0.12	0.00	0.99
HIPL	0.00	0.00	0.00	0.22	0.07	0.00	0.00	0.20	**0.34**	0.12	0.00	0.94
TSW	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.27	-0.05	**0.31**	0.00	0.60
OC	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	-0.07	0.01	-0.04	**0.00**	-0.11

DF= Days to flowering, DM= Days to maturity, PBPL= Number of primary branches/plant, CPPL= Number of capsules/plant, SDPC= Number of seeds/capsule, CL= Capsules length, PH= Plant height, BMPL= Biomass/plant, HIPL= Harvest index/plant, TSW= 1000 seed weight, SYPL= Seed yield/plant and OC= Oil content. Residual = 0.13.

analysis was carried out between the different traits at both phenotypic and genotypic levels to partition correlation coefficients into direct and indirect effects; permitting a critical examination of the specific forces acting to produce a given correlation and measuring the relative importance of the causal factors. The result of phenotypic path analysis (Table 4) revealed that biomass/plant (1.08) had maximum positive direct effect on seed yield/plant followed by harvest index (0.75) and capsules/plant (0.53). Similarly, at genotypic level (Table 5), biomass/plant (0.64), capsules/plant (0.60) and harvest index (0.34) imposed highest direct effect on seed yield/plant. These traits also had strong positive correlation with seed yield at both phenotypic and genotypic level. Therefore, these traits can be considered as the principal traits while selecting for seed yield. In other words, selection indices may be formed by considering all these traits for improvement of seed yield. This result was in agreement with previous studies on association of traits in sesame accessions of different countries (Mothilal, 2005; Ahadu, 2008; Goudappagoudra et al., 2011; Ibrahim and Khidir, 2012).

The traits viz., 1000 seed weight (0.90), capsules/ plant (0.85), harvest index (0.80) and number of seeds/capsule (0.73) showed maximum indirect effects on seed yield at phenotypic level. Thousand seed weight and seeds/capsule also showed high indirect effect at genotypic level (Table 5). Therefore, this finding strongly emphasized that these two traits (1000 seed weight and seeds/capsule) made the greatest indirect contribution

to seed yield. However, both these traits had negative direct effect to seed yield/plant at phenotypic level, suggesting that direct selection in favor of these traits can affect seed yield. This result agreed with those of Azeez and Morakinyo (2011), Ibrahim and Khidir (2012).

Conclusion

The analysis of variance revealed significant difference among the sesame accessions for all traits considered, indicating the presence of sufficient variability among the tested genotypes. Mean performance of 81 sesame genotypes for 13 traits generally showed that 44% of the genotypes had greater mean seed yield than the overall mean of genotypes. Genotypes Oromia-22, Am-NW-13, Am-SW-5, Oromia-25 and Am-NW-14, exhibited the highest mean seed yield and were superior even to the check varieties. Whereas, the genotypes Oromia-13 and Am-NSh2 had the highest mean oil content and were found to be superior as compared to the checks. Hence, these genotypes should be given emphasis while intending to improve sesame yield (seed and oil) in mid-altitude areas of Ethiopia. The phenotypic and genotypic correlations in general were higher than the environmental correlation for the studied traits. Number of capsules/plant, biomass/plant, harvest index and 1000 seed weight exhibited highly significant and high positive association with seed yield at both phenotypic and genotypic level, suggesting that these traits are reliable yield components and seed yield can be improved through direct selection of these traits. On the other hand, Plant height and number of seeds/capsule showed highly significant and moderate positive correlation with seed yield/plant at both phenotypic and genotypic level; hence, indirect selection in favor of these traits can improve seed yield in sesame. Number of capsules/plant, biomass/plant, days to maturity and harvest index imposed maximum positive direct effect on seed yield. Hence, they can be considered as the principal traits while selecting for seed yield. In other words, selection indices may be formed by considering all these traits for improvement of sesame seed yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

OsHKT1;3 gene sequence polymorphisms and expression profile in rice (*Oryza sativa* L.)

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Rice is sensitive to salt stress, but its sensitivity varies among genotypes, indicating natural variation in regulatory mechanisms and genetic makeup. High-affinity potassium transporters (HKTs) that transport cations across membranes play important roles in stress responses of plants. In this study, the gene sequence polymorphisms and expression level of *OsHKT1;3* which is a member of the rice *HKT* gene family was assessed. Sequence analysis indicated 5 single nucleotide polymorphisms (SNPs) in the coding sequence; 4 nucleotide substitutions, one nucleotide deletion and one nucleotide insertion in the promoter region of *OsHKT1;3* gene. Among 5 SNPs in the coding sequence, one was non-synonymous (C598G) which caused the change in amino acid L200V and 4 were synonymous substitutions (A798C, G2083A, T2101C, C2122T). The substituted amino acid L200V was predicted to locate in the third transmembrane segment of OsHKT1;3 protein. In the promoter region, 3 nucleotide substitutions at position -879, -453, and -202 caused the change in cis-elements with 8 deletions and 3 additions. Expression levels of *OsHKT1;3* were analyzed in the leaves and the roots under 2 different salt concentrations and showed a tendency of reduction in most of the conditions.

Keywords: *OsHKT1;3*, salt stress, rice, polymorphism.

INTRODUCTION

Salt stress is a severe abiotic stress reducing the productivity of crop plants. Salinity affects plant growth by both osmotic and ionic stresses (Zhu, 2002; Tester and Davenport, 2003; Bressan et al., 2009). To overcome with the toxicity of elevated Na^+ level, plants developed different mechanisms to regulate the Na^+ content by minimizing Na^+ influx into cells, maximizing Na^+ efflux out of the cells, and promoting Na^+ sequestration into the vacuole. These activities are mediated by specific transporters (Chinnusamy et al., 2005).

High-affinity potassium transporters (HKTs) are plant-specific proteins that transport cations across membranes (Almeida et al., 2013). The *HKT* gene family is segregated into 2 sub-classes. Class 1 consists of Na^+-selective transporters having a serine at

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the first pore domain, whilst members of class 2 have a glycine at this position (with an exception of OsHKT2;1) and comprise transporters permeable to Na^+ and K^+ (Platten et al., 2006; Hauser and Horie, 2010). The gene number of class I differs amongst plants (Garciadeblas et al., 2003; Huang et al., 2008). Though arabidopsis has only one *AtHKT1;1* gene, rice contains 4 to 5 depending on cultivar, including *OsHKT1;1, OsHKT1;2, OsHKT1;3, OsHKT1;4* and *OsHKT1;5* (Garciadeblas et al., 2003; Platten et al., 2006). Some members of the class I play roles in salt tolerance of the plants by retrieving Na^+ from the xylem sap and preventing Na^+ to accumulate in the shoots (Berthomieu et al., 2003; Byrt et al., 2007; Sunarpi et al., 2005; Ren et al., 2005; Sun et al., 2018), and by excluding Na^+ from leaf blades (Cotsaftis et al., 2012; Wang et al., 2015; Suzuki et al., 2016; Kobayashi et al., 2017).

Natural genetic polymorphisms are proven to contribute to stress tolerance in plants. Hence, investigation of these natural variations helps to illustrate the underlying mechanisms of stress responses (Baxter et al., 2010; Brady et al., 2005; Rus et al., 2006). In the report of Ren et al. (2005) by using a population derived from the salt-tolerant cultivar Nona Bokra and the salt-sensitive cultivar Koshihikari, they could identify *OsHKT1;5* as the candidate gene for salt QTL and the allelic variation of the Nona Bokra potentially contributed to an increase in Na^+ transport activity. Later, Cotsaftis et al. (2012) proved that the V395L substitution present in OsHKT1;5 transporter protein of Nona Bokra could be responsible for the change in Na^+ transport activity. Additionally, in the study of Negrão et al. (2013) other two substitutions in OsHKT1;5 were shown to be significantly associated with salt-tolerance related traits. By analyzing genetic variation of *OsHKT2;1* gene in a collection of 49 rice cultivars, Oomen et al. (2012) identified in total nine SNPs, but no considerable effect on transport properties was found. However, a new rice HKT *OsHKT2;2/1*gene was identified in the highly salt-tolerant cultivar Nona Bokra (Oomen et al., 2012). Recently, using a rice diversity panel for genome-wide association mapping, Campbell et al. (2017) detected three non-synonymous variants within *OsHKT1;1* gene associated with altered Na^+ accumulation in the root. Using the same approach of association mapping, Jiang et al. (2018) could detect 2 SNPs present in the coding region of *ZmHKT1;5* which were significantly associated with salt tolerance in maize.

OsHKT1;3 is a member of class I of HKT gene family in rice. This gene is expressed in both the roots and the leaves and encodes protein which transports selectively Na^+ (Jabnoune et al., 2009). Till now, to our knowledge, there is only one study on the nucleotide polymorphisms of *OsHKT1;3* gene sequence which focused on wild rice (Mishra et al., 2016). Hence, in this study, variations in the *OsHKT1;3* gene sequence including the promoter region using different rice genotypes was analyzed. The polymorphisms present in the coding and the promoter sequences were further analyzed *in silico* to elucidate the potential effect on either protein properties or transcriptional regulatory via cis-regulatory elements, respectively. The expression profile of *OsHKT1;3* gene in the roots and the leaves under different salt conditions were analyzed using real-time RT-PCR.

MATERIALS AND METHODS

Plant cultivation and salt treatment

Seeds of 7 rice (*Oryza sativa* L.) cultivars, consisting of Nipponbare, Chiem Rong, Nuoc Man 1, Nuoc Man 2, Cuom 2, Chanh Trui, and Pokkali, were kindly supplied by Vietnam National University of Agriculture (Hanoi, Vietnam). The seeds were germinated for 4 days. Then, the rice seedlings were grown in Yoshida solution (Yoshida, 1976) and placed either in a greenhouse for phenotyping or in a growth chamber (12 h days with 500 µE m^{-2} s^{-1} at 26°C and 12 h night at 22°C) for gene expression analysis. The growth media were renewed every week. After 14 days of normal growth, the media were replaced for media with the appropriate salt concentrations (0, 50, and 100 mM NaCl). Stress treatment was performed for 7 days at 50 and 100 mM NaCl in a gene expression experiment, and for 14 days at 100 mM NaCl in phenotyping experiment.

Evaluation of salt tolerance

The leaf scoring was performed for salt-treated plants based on modified standard evaluation score (SES) of visual injury symptom at seedling stage of rice as described in Gregorio et al. (1997) and Bado et al. (2016).

Total DNA extraction from the leaves

The DNA extraction was carried out using the CTAB method. About 200 mg leaf powder was thoroughly mixed with 500 µL CTAB buffer. After incubating at 65°C for 20 min, 500 µL CI (chloroform: isoamylalcohol) was added. The collected supernatant was mixed with cold isopropanol for 15 min. After centrifugation, the DNA pellet was collected and washed with 70% ethanol. Then, the DNA pellet was left to dry at room temperature. The DNA was dissolved in Tris-EDTA buffer and stored at -20°C for further usage.

Amplification of entire *OsHKT1;3* gene by PCR

The 1942-bp promoter fragment and 2465-bp fragment covering entire gene sequence of *OsHKT1;3* were amplified separately from genomic DNA material by PCR technique. Primers used for amplifying the *OsHKT1;3* coding sequence are cds-FW (5′-CACCACTAACTCTTTGATGCTGA-3′) and cds-RW (5′-GCTAAGCTCGAATCTGTCGC-3′); and for amplifying the *OsHKT1;3* promoter region are Pro-FW (5′-TCGTCTAAAGGATGGCAATGA -3′) and Pro-RW (5′-CAGCAAAGGAGATCAGGGCAA-3'). The PCR reaction contained DNA (20 to 50 ng), dNTPs mixture (0.2 mmol/L), $MgCl_2$ (1.5 mmol/L), primers (0.4 µmol/L), Dream Taq polymerase (1 U), and Dream Taq polymerase buffer (1×). The thermal cycle of PCR reaction was 95°C for 5 min, 35 cycles of 95°C for 30 s, 58°C (cds

primers)/59°C (promoter primers) for 30 s and 72°C for 2 min, and 72°C for 5 min. Then, 5 µL of PCR products were run on 1% agarose gel. After purified using GeneJET PCR Purification Kit (Thermo Fisher Scientific), the PCR products were sent to the First BASE DNA sequencing service (Singapore) for sequencing. The sequences were submitted to GenBank database under accession numbers MH727499 for Nipponbare cds, MH727500 for Nuoc Man 2 cds, MH727501 for Chiem Rong cds, MH727502 for Nuoc Man 1 cds, MH727503 for Chanh Trui cds, MH727504 for Cuom 2 cds, MH727505 for Pokkali cds, MH727492 for Nipponbare upstream region, MH727493 for Chiem Rong upstream region, MH727494 for Nuoc Man 1 upstream region, MH727495 for Nuoc Man 2 upstream region, MH727496 for Cuom 2 upstream region, MH727498 for Chanh Trui upstream region, and MH727497 for Pokkali upstream region.

Sequence analysis

Bioedit (Hall, 1999), Multalin webserver (Corpet, 1988), and Expasy web server (http://web.expasy.org/translate/) were used to analyzed sequences. The coding and promoter sequences of *OsHKT1;3* of all cultivars were compared to those of Nipponbare.

Construction of 3D model of OsHKT1;3 protein

The PHYRE2 program (Kelley et al., 2015) and SWISS-MODEL (http://swissmodel.expasy.org; Biasini et al., 2014) were used to predict the 3D model of OsHKT1;3 protein. The discovery studio 4.5 visualizer was used to visualize the protein structure.

Prediction of putative cis-regulatory elements in the promoter regions of *OsHKT1;3*

Nipponbare 2-kb upstream sequence from the start codon of *OsHKT1;3* gene was taken from the MSU rice genome annotation database (http://rice.plantbiology.msu.edu/; Kawahara et al., 2013) (this region was re-sequenced in this study also). The putative cis-elements present in the promoter regions of Nipponbare and other cultivars were predicted by using PLACE database (Higo et al., 1999; http://www.dna.affrc.go.jp/PLACE/).

RNA isolation and first-strand cDNA synthesis

The leaves and the roots of both control and salt-stressed seedlings were harvested at days 1, 3, and 7 of salt treatments. The leaf/root materials of different plants per cultivar and treatment were homogenized using a ball mill (Mixer Mill MM 400, Retsch, Germany) and equally pooled. Total RNA was extracted using GeneJET Plant RNA purification Kit (Thermo Fisher Scientific, USA). RNA concentration was examined photometrically using NanoDrop ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, Wilmington, DE). The genomic DNA was removed by using DNase I (Thermo Fisher Scientific, USA) and the absence of DNA was checked by PCR for amplification of OsHKT2;1 intron sequence (FW: 5'-ATCATCAGGTGTGTTCCTCTCTC-3', RW: 5'-CATTGGCTTGATGCCCAGTGT-3'). 1 µg of purified RNA was used to transcribe into cDNA using Revert-Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA).

Quantitative real-time PCR for gene expression analysis

The transcript levels of *OsHKT1;3* gene in different samples were quantified by real-time PCR analysis using specific primers for *OsHKT1;3* (FW: 5'- TTTGCATCACAGAACGGGAC-3', RW: 5'-TCCATATGCACTGACGACTTC-3'). The reference gene, *Actin1*, was used to normalize variance in the amount of input cDNA. Each real-time PCR reaction contained 1 µL of diluted cDNA, 10 µL of SYBR Green Master Mix 2X (Luminaris Higreen low ROX qPCR master mix, Thermo Fisher Scientific, USA), 0.6 µL of primer mix (10 µM) in a total reaction volume of 20 µL. The thermal cycle of PCR was performed as: 95°C for 10 min, 40 cycles of (95°C for 15s, 60°C for 1 min) in 96-well optical reaction plates employing ABI Fast 7500 System (Applied Biosystems, Foster City, CA). The relative mRNA levels of *OsHKT1;3* gene (described as fold change) in different samples were computed using the $2^{-\Delta\Delta Ct}$ method as described previously by Livak and Schmittgen (2001).

RESULTS

Classification of salt tolerance

The rice plants were subjected to salt stress at the vegetative stage using hydroponic culture. The modified standard evaluation score (SES) of visual injury symptom at seedling stage has been proven to be the reliable parameter for discriminating amongst the susceptible, the tolerant, and the moderate groups (Gregorio et al., 1997). Thus, in this study, modified SES was used for classification of salt tolerance of rice. As shown in Table 1, the cultivars Pokkali, Chanh Trui, Cuom 2, and Nuoc Man 1 were classified as salt-tolerant; while Chiem Rong and Nuoc Man 2 were moderate. Nipponbare was sensitive.

Polymorphisms in the coding sequence of *OsHKT1;3* gene

The *OsHKT1;3* gene sequence is 2325 bp in length with 3 exons and 2 introns. To investigate the genetic variation in the *OsHKT1;3* coding sequence, the entire gene sequence of *OsHKT1;3* was amplified by PCR using specific primers. The amplified DNA fragments were further sequenced allowing the detection of five SNPs in the coding sequence (Figure 1). Among 5 SNPs in the coding sequence, one was non-synonymous (C598G) and 4 were synonymous substitutions (A798C, G2083A, T2101C, C2122T). These variants were found in Chanh Trui, and Nuoc Man 1.

The non-synonymous C598G led to the amino acid change of L200V. To elucidate the putative effect of the non-synonymous (C598G) on protein structure, the 3D molecular model of OsHKT1;3 transporter was predicted and the position of substituted amino acid L200V on protein domains was analyzed. The 3D models of OsHKT1;3 shows the presence of 3 glycine residues (Gly247, Gly371, and Gly471) and one serine residue (Ser93) forming a selectivity filter (Figure 2B). The substituted amino acid (L200V) locates in the third transmembrane segment of OsHKT1;3 (Figure 2A and C). It was concluded that this substitution unlikely interferes with the capacity of Na^+ transport of the variant

Table 1. Salinity score of rice plants under salt treatment.

Cultivar	Salinity score	Reaction to salinity
Pokkali	2.3	Tolerant
Chanh Trui	2.3	Tolerant
Cuom 2	3.7	Tolerant
Nuoc Man 1	3.7	Tolerant
Chiem Rong	4.3	Moderate
Nuoc Man 2	5.7	Moderate
Nipponbare	7.7	Susceptible

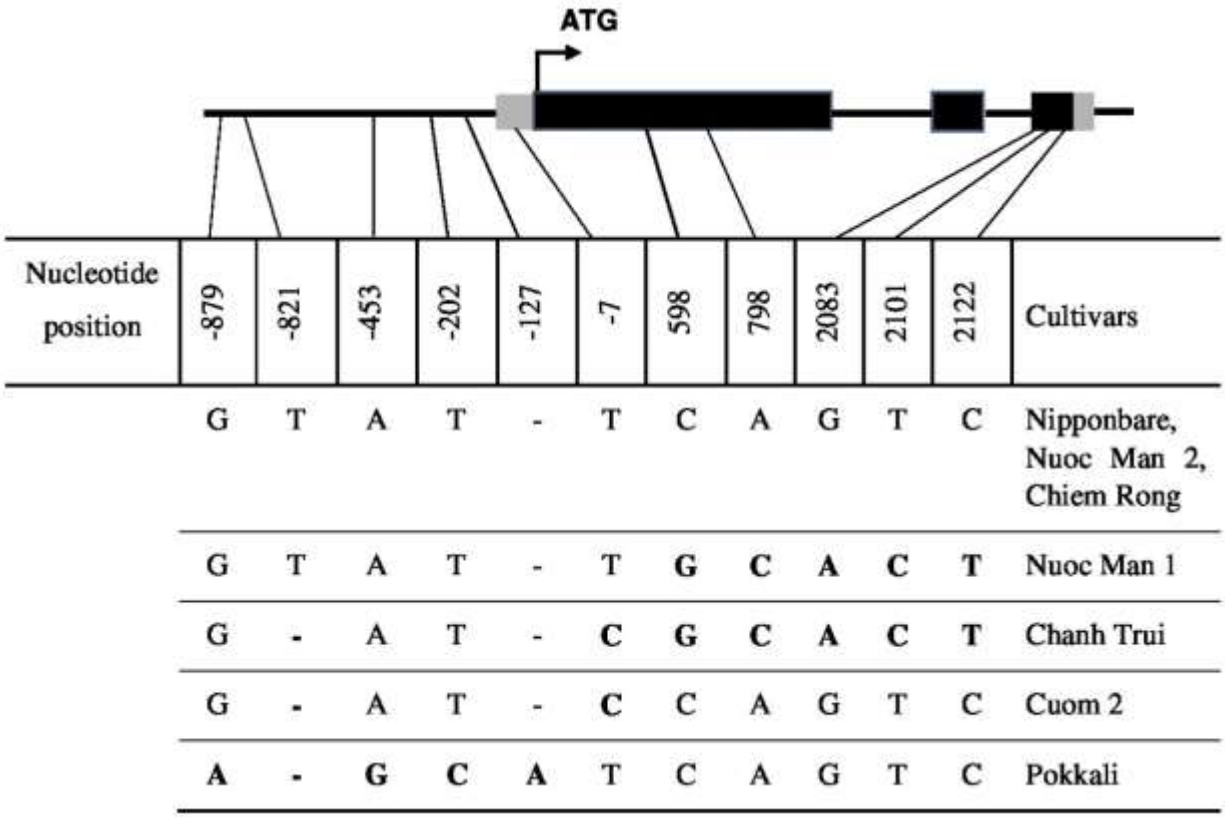

Figure 1. Nucleotide polymorphisms in the *OsHKT1;3* coding sequence and upstream region. The *OsHKT1;3* coding and upstream sequences of different cultivars were compared to those of Nipponbare. Letters in bold indicate the polymorphisms.

OsHKT1;3 transporter.

OsHKT1;3 gene upstream region polymorphism

The 1942-kb upstream region from the start codon of *OsHKT1;3* was amplified by PCR following direct sequencing. By comparing the obtained sequences, 4 nucleotide substitutions (T-7C, T-202C, A-453G, and G-879A), one deletion of T at position -821, and one addition of A at position -127 in the upstream sequence of *OsHKT1;3* gene were identified (Figure 1). To dissect the consequent effect of polymorphisms on transcriptional regulatory functions of the promoter, the cis-elements were predicted using PLACE database. It has been shown that the nucleotide substitution at position -202 and -453 caused deletions of 3 cis-elements, consisting of 2 CAATBOX1 and one RAV1AAT (Table 2); while nucleotide substitution at position -879 caused replacement of 5 cis-elements by other 3 cis-elements involving to stress responses (Table 2). The changes in cis-elements occurred in only cultivar Pokkali.

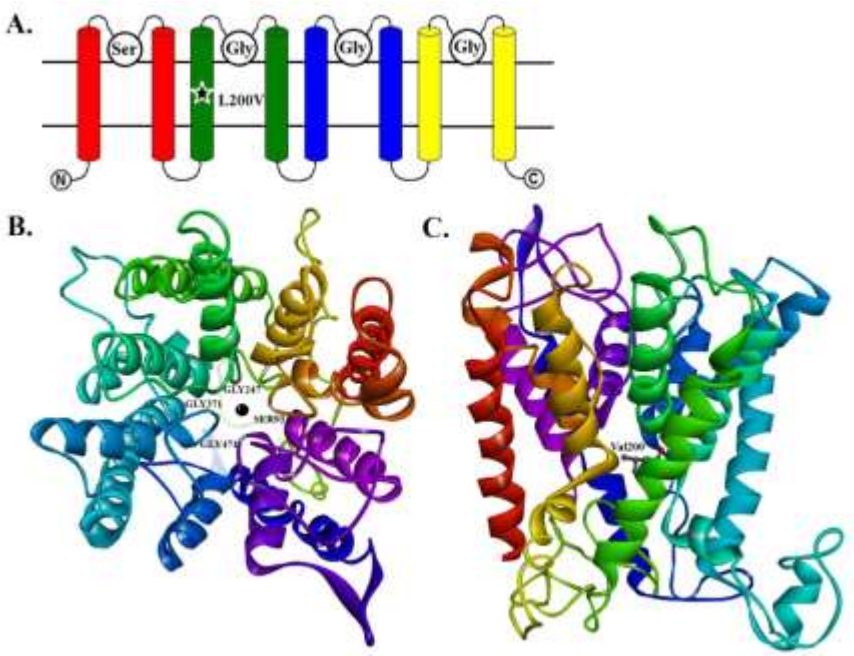

Figure 2. Molecular model of the OsHKT1;3 transporter. A: Schematic representation of 4 transmembrane–pore loop–transmembrane domain model. The location of changed amino acid is marked with a star. B: Visualization of OsHKT1;3 protein model from the top. The ion filter pore is formed by 4 amino acid residues, including Ser, Gly, Gly, and Gly. The black dot located at the pore center is indicated for Na^+ ion. C: Visualization of OsHKT1;3 protein model from the side with an indication of the L200V polymorphism site.

Expression profile of *OsHKT1;3* gene

Expression analysis of *OsHKT1;3* gene was carried out using Real time RT-PCR in 2 rice cultivars, namely Pokkali and Nippponbare, under control and salinity conditions (50 and 100 mM NaCl) to determine the different responses in expression level of *OsHKT1;3* gene. As shown in Figure 3, the expression of *OsHKT1;3* gene was decreased in both leaf and root samples of both rice cultivars. The reduction in expression of *OsHKT1;3* was more pronounced in the roots than in the leaves.

DISCUSSION

Rice (*O. sativa* L.) is an important crop, but its productivity has been limited by salinity. Investigation of novel genes/alleles for salt tolerance in rice is of necessity. In this study, the natural allelic variation in sequence and expression of rice *OsHKT1;3* encoding the Na^+- selective transporter belonging to HKT family using rice cultivars varying in salt-tolerant levels was explored (Table 1). In total, eleven nucleotide variations were identified in the gene sequence and the upstream region of the *OsHKT1;3* (Figure 1).

In the coding sequence of the gene, one non-synonymous substitution (C598G) was detected, leading to an amino acid substitution (L200V). It has been previously reported that SNPs in the coding sequence of *HKT* genes affect the functions of transporters and associated with salt-tolerant traits (Rubio et al., 1995; Ren et al., 2005; Baxter et al., 2010; Ali et al., 2016; Mishra et al., 2016; Campbell et al., 2017; Jiang et al., 2018). Therefore, the 3D models of OsHKT1;3 was predicted to elucidate the putative effect of the C598G polymorphism on functions of the variant transporter. In the predicted protein model, 4 amino acid residues, including 3 glycine and one serine, which form a selectivity filter were identified (Figure 2B). That structure determines the Na^+-selective transport property of OsHKT1;3, which agrees with the finding of the highly selective Na^+ transporter of OsHKT1;3 reported by Jabnoune et al. (2009). The substituted amino acid L200V locates in the third transmembrane domain (Figure 2A and C), and is unlikely to interfere with Na^+ transport in the variant transport.

The upstream region of the gene, where the cis-elements present, plays important roles in controlling the expression of the gene. Identification of cis-elements may elucidate expression patterns of the gene (Mariño-Ramírez et al., 2009). Thus, variation in the cis-element

Table 2. Polymorphisms in the upstream region of *OsHKT1;3* and putative cis-elements.

Position	Polymorphic type	Cis-element	Change in number	Sequence (5'-3')	Function of cis-element	References*
-879	G/A	CAATBOX1	-1	CAAT	Responsible for tissue specific promoter activity	Shirsat et al. (1989)
		WBOXATNPR1	-1	TTGAC	Salicylic acid (SA)-induced element	Yu et al. (2001)
		WBOXHVISO1	-1	TGACT	Sugar-responsive elements	Sun et al. (2003)
		WBOXNTERF3	-1	TGACY	Wounding-responsive elements	Nishiuchi et al. (2004)
		WRKY71OS	-1	TGAC	GA-responsive elements	Zhang et al. (2004)
		MYB2AT	+1	TAACTG	Responsive to water stress	Urano et al. (1993)
		MYB2CONSENSUSAT	+1	YAACKG	MYB recognition site involved in dehydration responsiveness	Abe et al. (2003)
		MYBCORE	+1	CNGTTR	Binding site of MYB proteins involved in the regulation of dehydration-responsive genes and flavonoid synthesis genes	Urano et al. (1993)
-453	A/G	CAATBOX1	-1	CAAT	Responsible for tissue specific promoter activity	Shirsat et al. (1989)
-202	T/C	CAATBOX1	-1	CAAT	Responsible for tissue specific promoter activity	Shirsat et al. (1989)
		RAV1AAT	-1	CAACA	The binding site of transcription factor RAV1	Kagaya et al. (1999)

*The reference for function of cis-element.

number and pattern in the upstream region of the rice cultivars can have a decisive impact on *OsHKT1;3* gene expression, and thereby on a response of plants to salt stress. In the current study, in total 4 nucleotide substitutions (T-7C, T-202C, A-453G, and G-879A), one deletion of T occurred at site -821, and one addition of A at position -127 were detected in the upstream sequence of *OsHKT1;3* gene (Figure 1). Further *in silico* analysis revealed that the nucleotide substitution at position -202 and -453 caused deletions of 3 cis-elements, consisting of 2 CAATBOX1 and one RAV1AAT (Table 2). The cis-element CAATBOX1 determines tissue-specific activity of the promoter (Shirsat et al., 1989), while RAV1AAT is the binding site of the transcription factor RAV1 (Kagaya et al., 1999). The nucleotide substitution at position -879 caused replacement of 5 cis-elements, including CAATBOX1, WBOXATNPR1, WBOXHVISO1, WBOXNTERF3, and WRKY71OS, by other 3 cis-elements, consisting of MYB2AT, MYB2CONSENSUSAT, and MYBCORE (Table 2). The 3 replacing cis-elements (MYB2AT, MYB2CONSENSUSAT, and MYBCORE) are involved in water stress responses (Abe et al., 2003; Urao et al., 1993); while the 5 replaced cis-elements CAATBOX1, WBOXATNPR1, WBOXHVISO1, WBOXNTERF3, and WRKY71OS have different roles. CAATBOX1 is responsible for tissue-specific promoter activity (Shirsat et al., 1989); while WBOXATNPR1, WBOXHVISO1, WBOXNTERF3, and WRKY71OS are responsible for salicylic acid, sugar, wounding and gibberellin, respectively (Yu et al*.,* 2001; Sun et al., 2003; Nishiuchi et al*.,* 2004; Zhang et al., 2004). These cis-element changes might affect the expression pattern of *OsHKT1;3* gene in response to stress conditions.

Since the polymorphisms in the upstream region of *OsHKT1;3* gene that lead to the changes in cis-elements occurred only in salt-tolerant cultivar Pokkali, then the difference in gene expression response to salt stress of 2 contrasting rice cultivars, Pokkali and Nipponbare were next examined. The results showed that *OsHKT1;3* gene expression was decreased in both leaves and roots samples of both rice cultivars (Figure 3). In the previous study, the *OsHKT1;3* gene was found to express in both the roots and the mature leaves (Jabnoune et al., 2009; Abdulhussein et al., 2018). The expression of *OsHKT1;3* gene in the roots and the leaves was not changed upon the different growth conditions (Jabnoune et al., 2009).

OsHKT1;3 is found to be located in the Golgi membrane, not in the plasma membrane (Rosas-Santiago et al., 2015). OsHKT1;3 can mediate both inward and outward currents but displays weak inward rectification in *Xenopus* oocytes (Jabnoune et al., 2009). Thus, it might be that the reduced expression of *OsHKT1;3* under salinity conditions help decrease the transport of Na^+ from

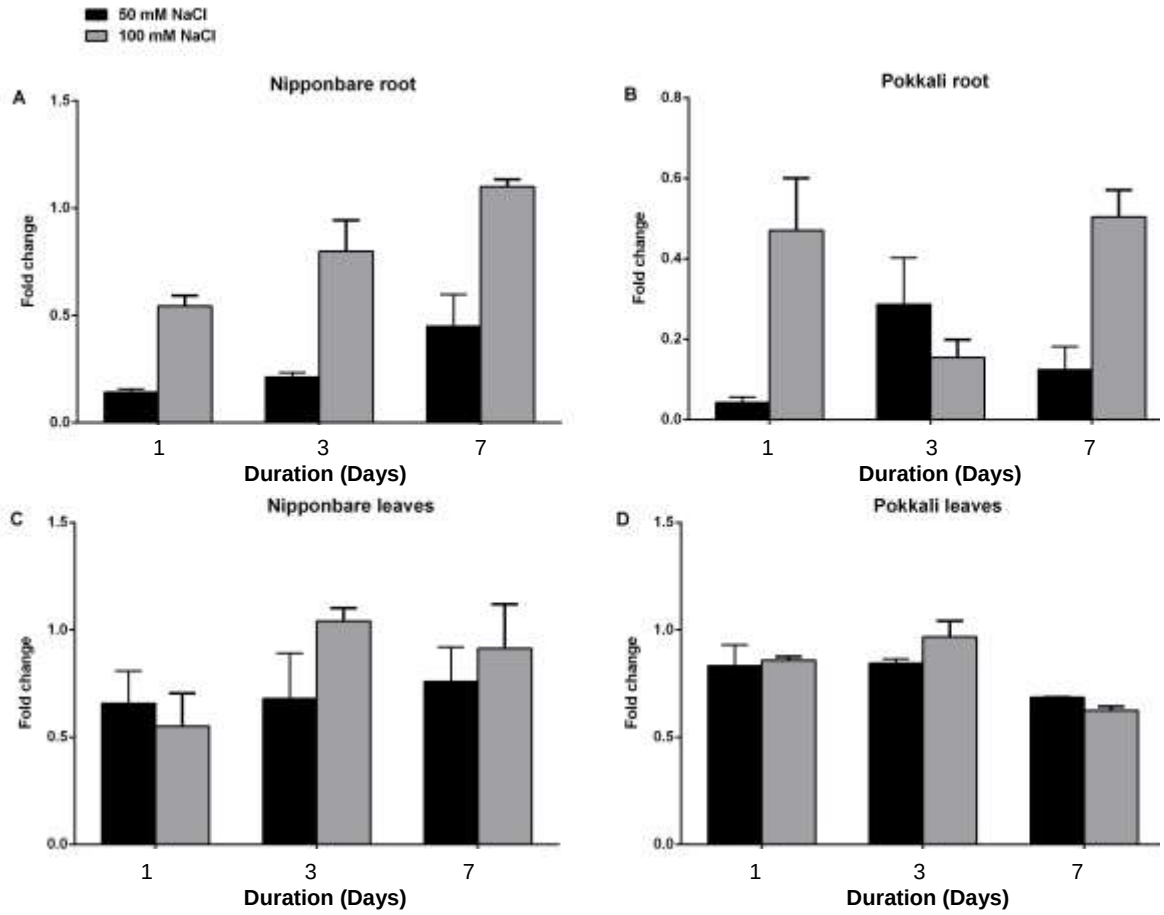

Figure 3. Expression of *OsHKT1;3* gene under control and salinity conditions in two contrasting rice cultivars. The expression of *OsHKT1;3* gene in different samples was normalized with the expression of rice actin 1, the internal control gene. Fold changes in *OsHKT1;3* expression in different rice genotypes at different time points were calculated to corresponding control using ΔΔCt method (Livak and Schmittgen, 2001). Values represent the mean and standard deviation of 3 technical replica.

Golgi to the cytoplasm, which in turn protects the cell from the toxicity of accumulated Na^+ in the cytoplasm. This hypothesis is supported by the findings of Rosas-Santiago et al. (2015) that the yeast is more susceptible to Na^+ when the *OsHKT1;3* is expressed.

In conclusion, polymorphisms were detected in the coding and promoter sequences of *OsHKT1;3* gene of the salt-tolerant cultivars (Pokkali, Chanh Trui, Cuom 2, and Nuoc Man 1), but not of the moderate cultivars (Chiem Rong, Nuoc Man 2) and sensitive cultivar Nipponbare. Amongst those polymorphisms, SNP C598G caused the change in amino acid L200V in tolerant cultivars Chanh Trui and Nuoc Man 1; and SNP G-879A led to the addition of several water stress related cis-elements in the tolerant cultivar Pokkali which showed more reduction in *OsHKT1;3* expression level in the roots than that of the sensitive cultivar Nipponbare. Thus, it shall be useful to further characterize these 2 SNPs to demonstrate their decisive roles in salt tolerance. Site-directed mutagenesis study using modern techniques such as CRISPR-Cas9 would help resolve whether SNP C598G affects transport activity of protein or SNP G-879A alters gene expression level, which subsequently clarifies the roles of these 2 SNPs to plant stress tolerance. Rice plants responded to salinity by reducing the expression of *OsHKT1;3* gene.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Genetic diversity of rhizobia and plant growth promoting rhizobacteria of soil under the influence of Piliostigma reticulatum (DC.) Hochst and their impact on shrub growth

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Piliostigma reticulatum **shrub is a native legume found in fallow areas in dry and semi-dry savanna soil and is used in intercropping systems. The aim was to understand the functioning of the rhizosphere, particularly the involvement of symbiotic and free living-N fixing bacteria. Soil extracts collected from** ***P. reticulatum*** **roots were sampled in two contrasting areas and endophytic bacterial communities were isolated using three trap host species (*F. albida*, *A. bivenosa* and *V. seyal*). Potential endophytic bacteria (PEB) were characterized by RFLP, *nifH* PCR and by 16S rRNA gene sequencing. The subsequent behavior of *P. reticulatum* was monitored *in vitro* by measuring leaf weight, biomass and chlorophyll content, after inoculation with PEB. This approach enabled isolation of 59 bacteria belonging to different genotypes. The most abundant genera were *Cohnella* (27.65%) among which 11 isolates clustered together and could represent a new species closely related to *C. plantaginis*. The other dominant genera were *Paenibacillus* (21.27%), *Bradyrhizobium* (14.89%) and *Ensifer* (8.5%). The nitrogen fixing gene (*nifH*) was detected in 21 strains and in particular, detected in a single isolate (PZS_S04) close to *Cohnella xylanilytica*. The strains PZS_S05 (*Ensifer*) and PZG_A18 (*Cohnella*) significantly increased certain parameters including shoot dry weight, shrub height at 90 days and photosynthetic activity (SPAD), compared to non-inoculated controls.The result obtained showed that soil under the influence of *P. reticulatum* roots harbored a specific diversity of endophytic bacteria including two free living-N fixing bacteria with the potential to improve the growth of *P. reticulatum* in natural conditions.**

Key words: *Piliostigma reticulatum*, microbiology, phylogeny, Potential endophytic bacteria (PEB), nitrogen-fixing bacteria.

INTRODUCTION

The rhizosphere of legumes is a fertile zone due to the accumulation of different organic compounds released

from their roots (Bowen and Rovira, 1999; Barea et al., 2005). For this reason, the soil under the influence of plant roots is very favorable for the growth and activity of microbial communities, which plays a significant role in carbon and nitrogen biogeochemical cycles (Vitousek and Howarth, 1991; Toal et al., 2000). Plant-associated bacteria can also have a beneficial impact on legume nutrition as nitrogen is biologically fixed by nitrogen-fixing symbiotic bacteria or by free living-N fixing bacteria in the rhizosphere. Nitrogen-fixing symbiotic bacteria (or rhizobia) can establish symbiotic relationships and can reduce the atmospheric nitrogen (N_2) into ammonium (NH_4^+), which can be directly assimilated by plants. In addition, it is known that rhizobia can behave like non-symbiotic endophytes of legumes or non-legume plants such as maize, rice, cotton and wheat (Ueda et al., 1995; Engelhard et al., 2000) and can establish non-specific relationships with plants. However, the effects of the micro-environment formed by the plant rhizosphere on nitrogen-fixing symbiotic bacteria diversity remain unclear, in particular, on the subfamilies of *Leguminosae* such as *Cercidoideae* (LPWG, 2017). This new subfamily proposed by the Legume Phylogeny Working Group (LPWG) currently contains 12 genera (including *Piliostigma* genus) which mainly grow in tropical regions and have no root nodules (LPWG, 2017).

P. reticulatum (DC.) *Hochst* is a pioneer species, able to grow on degraded land, and to increase plant succession by other species. The shrub induces spatial heterogeneity of soil chemical properties in arid and semi-arid environments (Diedhiou et al., 2009). Twigs and wood fragments of *P. reticulatum* can also be used as dead ground cover (Diedhiou et al., 2009; Yélémou et al., 2013), to restore degraded land and improve crop yields (Bright et al., 2017). The use of *P. reticulatum* residues may improve the ability of soils to respond to saline conditions (Sall et al., 2015). Soil under the influence of the shrub is described in terms of islands of fertility (Wezel et al., 2000; Housman et al., 2007) due to a shift in soil microbial community diversity and enzymatic functions beneath the rhizosphere of the shrub compared to bulk soil (Diedhiou et al., 2009; Diedhiou-Sall et al., 2013). However, how microbial communities including symbiotic and non-symbiotic bacteria present in the soil under the influence of *P. reticulatum* contribute to the growth of this shrub remains unclear. Some non-symbiotic endophytic bacteria have several beneficial effects on host plants. During colonization of the roots or soil rhizosphere, they stimulate and promote plant growth (Bai et al., 2003; Aserse et al., 2013). Microorganisms isolated from the rhizosphere of various crops have been shown to produce phytohormones such as indole acetic acid as secondary metabolites. The different biosynthetic pathways of this hormone are already well described for some bacterial genera such as *Azospirillum*, *Azotobacter* and *Paenibacillus* (Shokri and Emtiazi 2010). Endophytes play an important role in the degradation of plant litter and organic pollutants, which in turn, actively increases soil fertility (Wang and Dai, 2011). Some works (James et al., 2000; Turner et al., 2013) suggest that endophytic bacteria may increase nitrogen fixation.

The aims of the study were to understand the functioning soil under the influence of *P. reticulatum* to: 1) evaluate the diversity of bacteria (symbiotic and non-symbiotic nitrogen fixing); and 2) study their impact on the growth of *P. reticulatum* plants.

MATERIALS AND METHODS

Sites and sampling of shrub

This study was performed at two climatically and environmentally contrasted sites. The first site Zone Sudano-Sahelian (ZSS) is located near Ndiassane (14°55'N-16°49' W), Senegal. The area is characterized by a Sudano-Sahelian climate with mean annual rainfall ranging from 400 to 600 mm. The second site Zone Sudano-Guinean (ZSG) is located in the village of Sare Yorobana (12°50'N-14°50'W), Senegal. This southern area is characterized by a Sudano-Guinean climate with more rain, and the mean annual rainfall reaches between 800 and 1,200 mm. At each site, three *P. reticulatum* shrubs ~ 1.5 m high were chosen and the soil under the shrub was sampled to a depth of 25 cm. The soil samples were composed of rhizosphere soil and extended to soil located around the roots, as soil adhering to the roots and around the roots are both under the influence of the shrub.

Trapping potential endophytic bacteria (PEB) associated with P. reticulatum.

The PEB present in the soil under the influence of *P. reticulatum* roots were studied using three trap host species (*Faidherbia* (syn Acacia) *albida*, *A. bivenosa* and *Vachellia* (syn Acacia) seyal) belonging to the mimosoid clade and the subfamily of Caesalpinioideae (LPWG, 2017). Firstly, the seeds of these species were scarified and the surface sterilized by soaking them in 95% (v/v) sulfuric acid for 30 min. The seeds of *F. albida*, *A. bivenosa* and *V. seyal* were thoroughly rinsed and soaked in sterile distilled water for 5 h, 7 h and 2 h for F. albida, A. bivenosa and V. seyal, respectively. The seeds were left to sprout in Petri dishes containing 2% agar and then incubated at 28°C for 48 h. The sprouting seeds were transferred under sterile conditions to Gibson tubes (Gibson 1963) containing a sterile Jensen nitrogen-free nutrient medium adjusted to pH 7 (Vincent 1970). The seedlings in the tubes were maintained in a growth chamber in the following conditions (at 28 ± 1°C with a 16 h day/8 h night photoperiod, relative humidity of 45 ± 5% and a 74 µmol.m^{-2}. s^{-1} light intensity). After one week, the young plants were inoculated with 5 ml soil suspensions (10 g of soil in 90 ml of physiological water) influenced by *P. reticulatum*. Four repetitions were performed for each trap host species and four non-inoculated plants were used as controls.

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Isolation of endophytic bacteria

After 45 days of growth, the nodules growing on the roots of the trap host species (*F. albida*, *A. bivenosa*, *V. seyal*) were harvested and enumerated. The nodules were then sterilized using a solution of $HgCl_2$ (2% w/v) for 3 min, followed by several soakings in sterile water. The endophytic bacteria were isolated by streaking a loop-full of the crushed nodule on yeast mannitol agar (YMA) medium (Vincent, 1970) and incubated at 28°C for 24 h to 72 h. Similar morphological colonies to rhizobia were pricked out on YMA. Pure colonies were obtained after successive transplanting of single colonies on new plates.

After the strains were identified by sequencing, sub-groups of strains showing strong similarity with known bacterial species belonging to the family of *Rhizobiaceae* and *Bradyrhizobiaceae* were retested for their ability to nodulate their respective hosts, using Gibson tubes (Gibson, 1963) as described above.

DNA extraction and PCR of 16S rRNA and nifH genes

The genomic DNA of isolated bacteria was extracted using the NucleoSpin Tissue 96 kit (Macherey Nagel) according to the manufacturer's instructions. All PCR were performed using the kit illustra Hot Start Mix RTG (GE Health care, Bucking Hampshire, UK). The 16S rRNA gene was amplified using pA (AGAGTTTGATCCTGGCTGAG) and pH (AAGGAGGTGATCCAGCCGCA) primers (Edwards et al., 1989). The reaction was carried out with 35 cycles as follows: denaturation for 15 s at 94 °C, primer annealing for 30 s at 55°C, and polymerization for 90 s at 72°C, plus a preheating phase of 5 min at 94°C and a terminal extension for 3 min at 72 °C. The *nifH* gene was amplified using the primers PolR (ATSGCCATCATYTCRCCGGA) and PolF (TGCGAYCCSAARGCBGACTC) (Poly et al., 2001) under the following conditions: 30 cycles each, denaturation for 60 s at 94 °C, annealing for 60 s at 60 °C, and elongation for 60 s at 72 °C. The cycles began with 95 °C denaturation for 15 min and ended with an extension phase for 10 min at 72 °C. PCR products were submitted to electrophoresis on 1% agarose gel for 30 min at 100 V and stained with ethidium bromide.

RFLP of 16S rRNA gene

The PCR products of the 16S rRNA gene were digested with two restriction endonucleases (Fast Digest enzyme) *HaeIII* and *MspI* (Thermo Fisher Scientific). For each PCR product, digestion was carried out in a volume of 20 µl containing 0.5 µl of enzyme, 2 µl of buffer, 5 µl of PCR products and 12.5 µl of water, and the mixtures were incubated at 37 °C for 30 min. Electrophoresis was performed on 2% agarose gel for 60 min at 100 V and stained with ethidium bromide. The results were analyzed by comparing bands, which enabled the clustering of profiles according to the position and size of bands.

Taxonomic identification of endophytic bacteria

The PCR products of 16S rRNA gene of isolates were sequenced (GATC Biotech, Constance, Germany). The nucleotide sequences were verified and corrected using SeqMan Pro (DNASTAR - Software for Molecular Biology - Sequence Analysis) and aligned with Clustal W Multiple alignment (Thompson et al., 1994). The resulting sequences were blasted against reference sequences in the NCBI database (http://www.ncbi.nlm.nih.gov/Blast.cgi). The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). The percentage of replicate trees in which the associated taxa were clustered together in the bootstrap test (1000 replicates) was shown next to the branches. All sequences were submitted to NCBI with accession numbers ranging from KY992880 to KY992923.

Growth of *P. reticulatum* in *in vitro* conditions

The seeds of *P. reticulatum* were scarified in sulfuric acid for 1 h, then rinsed several times and soaked for 24 h in sterile water. The seeds were grown on 2% agar at 28°C for 48 h and then transferred into Gibson tubes. The seedlings in tubes were maintained in a growth chamber under the conditions described above. After 5 days, the seedlings were inoculated with 1 ml of bacterial culture (approximately 109 cfu ml^{-1}) of each of the seven PEB. Five replicates were made for each strain and five non-inoculated plants were used as controls. The level of free-nitrogen nutritional plant was readjusted regularly in the tubes.

After three months of growth, the height of the plants was measured and chlorophyll content of the leaves was measured with a SPAD-502 chlorophyll meter (SPAD-502, Minolta Corp.; Ramsey, NJ, USA). Shoots and roots were harvested separately and dried at 70 °C to constant dry weight, after which dry weight was measured.

Statistical analyses

Statistical analyses were performed on the different growth parameters to investigate the effect of each treatment. ANOVA was followed by comparison with controls using Dunnett's test. The multcomp package (Hothorn et al., 2008) was used with one-sided. Dunnett tests (strains tested had positive effects on these growth parameters).

RESULTS

Capture of PEB from soil under the influence of *P. reticulatum*

Soil under the influence of *P. reticulatum* sampled from the two contrasting sites contained different numbers of nodules depending on the three trap plants (*F. albida*, *A. bivenosa* and *V. seyal*). A total of 140 nodules were recovered at Sudano-Sahelian site (ZSS), of which 83 nodules came from the roots of *F. albida*, 43 nodules from the roots of *V. seyal* and only 14 from the roots of *A. bivenosa*. At the Sudano-Guinean site, a greater number of nodules (412) formed on the roots of the three trap plants. A total of 337 nodules formed on the roots of *F. albida*, 74 nodules on the roots of *V. seyal* and only one nodule formed on roots of *A. bivenosa*. Next, 10 nodules were sub-sampled on each trap plant to isolate the PEB, except for *A. bivenova* from the Sudano-Guinean site. Morphological screening under a magnifying glass enabled the selection of 59 PEB including 26 from the site. Sudano-Sahelian site and 33 from the Sudano-Guinean.

The 16S rRNA gene PCR was performed on the 59 PEB. RFLP profiles revealed 14 different clusters (I to

Table 1. Distribution of 16S RFLP profiles of Potential Endophytic Bacteria (PEB) isolated from nodules of three trap host species (*Faidherbia albida, Acacia bivenosa* and *Vachellia seyal*).

	Distribution of profiles (number)						Distribution of Grps (%)
	ZSS			ZSG			
	F. alb	***A. biv***	***V. Sey***	***F. alb***	***A. biv***	***V. Sey***	
I	2	1	5	5	1	6	34
II	7	-	-	3	-	1	18
III	-	-	1	1	-	2	7
IV	1	-	2	-	-	7	17
V	-	1	-	-	-	1	3
VI	1	-	1	-	-	1	5
VII	-	-	1	-	-	1	3
VIII	-	1	-	-	-	-	2
IX	-	-	-	1	-	-	2
X	-	-	-	1	-	-	2
XI	-	-	-	1	-	-	2
XII	1	-	-	-	-	-	2
XIII	-	1	-	-	-	-	2
XIV	-	-	-	-	-	1	2

XIV) based on the number and size of the bands (Table 1). Cluster I was the most abundant (34%) and was found with three plant hosts. Cluster II was the second biggest group with 18% of profiles most isolated from nodules of *F. albida*. The profile of cluster IV was characteristic of the Sudano-Guinean site and isolated from nodules of *V. seyal.* The other groups were poorly represented, regardless of the host plant or the origin of the soil.

The PCR of *nifH* gene was also performed on 59 PEB, of which 35.6% (21 isolates) showed a positive amplification signal (Table 2), with nine positive signals of PEB at the Sudano-Sahelian site and 12 at the Sudano-Guinean site. According to the three trap host species used, the 21 *nifH* genes were distributed as follows: 15 positive signals (71.4%) were obtained with *V. seyal*; four positive signals (19%) with *F. albida* and only two positive signals (9.5%) with *A. bivenosa*.

Taxonomic diversity of potential endophytic bacteria (PEB)

The results (Table 2) revealed 25 different species of PEB distributed in four clusters (Figure 1). These PEB belong to nine (9) families: *Paenibacillaceae*, *Bacillaceae*, *Rhizobiaceae*, *Phyllobacteriaceae*, *Bradyrhizobiaceae*, *Moraxellaceae*, *Pseudomonadaceae*, *Burkholderiaceae* and *Micrococcaceae*.

Cluster I included 25 strains and was separated in two sub-clusters (SC1 and SC2). The SC1 group contained 22 strains with strong similarity to the *Paenibacillaceae* family. Thirteen (13) strains were closely related to the *Cohnella* genus, most of which (11) were grouped in a subgroup closely related to *Cohnella plantaginis* isolated from plantain in China (Wang and Dai 2011). One strain (PZG_A13) was close to *C. rhizosphaerae* isolated from the rhizosphere environment of *Zea mays* (Kämpfer et al., 2015) and one (PZS_S04) formed a set with *C. xylanilytica* (Khianngam et al., 2010). Nine strains were closely related to *Paenibacillus glycanilyticus* (Kajiyama et al., 2002), to *P. humicus* (Vaz-Moreira et al., 2007), and to *P. rigui*, isolated from a freshwater wetland (Baik et al., 2011). The SC2 grouped three strains, including two strains (*Bacillus aryabhattai* and *Bacillus sp strains* (HM212416.1)), closely related to the Bacillus genus and one strain close to *Rummeliibacillus suwonensis*. These strains were isolated from different environments; *Bacillus aryabhattai* was isolated from the rhizosphere region of Lemna sp of East Kolkata wetlands of the Indian sub-continent (Ray et al., 2012), *Bacillus sp* strains (HM212416.1) and *Rummeliibacillus suwonensis* isolated from soil collected in a mountain area of South Korea (Her and Kim, 2013).

Cluster II contained a single isolated strain (PZS_A07), which was closely related to *Kocuria marina* sp, which is a novel Actinobacterium isolated from marine sediment (Kim et al., 2004).

Cluster III contained 12 strains separated in two sub-clusters (SC3 and SC4). Five strains were grouped in the SC3 cluster with almost 100% similarity with *Bradyrhizobium elkanii* (Kuykendall et al., 1992) and *Bradyrhizobium liaoningense* isolated from the root

Table 2. PCR signal of the nifH gene and sequence similarities of 16S rRNA gene of Potential Endophytic Bacteria (PEB) isolated from nodules of three trap host species (Faidherbia albida, Acacia bivenosa and Vachellia seyal).

Name of PEB	nifH PCR	Accession number	Closest related organism	Accession n°	% Similarity
PZS_B04	-	KY992880	*Acinetobacter.pittii*	CP017938	100
PZG_S16	+	KY992881	*Bacillus aryabhattai*	EF114313	100
PZS_B03	+	KY992882	*Bacillus sp. GIMN1.006*	HM212416	100
PZS_S08	+	KY992883	*Bradyrhizobium elkanii*	JQ911631	100
PZG_S14	+	KY992884	*Bradyrhizobium elkanii*	JQ911632	100
PZG_S20	+	KY992885	*Bradyrhizobium elkanii*	JQ911633	100
PZS_A08	+	KY992886	*Bradyrhizobium elkanii*	JQ911633	100
PZG_S09	+	KY992887	*Bradyrhizobium liaoningense*	AB698736	99
PZG_A19	-	KY992888	*Paraburkholderia insulsa*	KF733462	100
PZS_S06	-	KY992889	*Caballeronia zhejiangensis*	HE983367	100
PZS_A03	-	KY992890	*Caballeronia zhejiangensis*	HE983367	100
PZS_B01	-	KY992891	*Cohnella plantaginis*	JN982125	98
PZS_S02	-	KY992892	*Cohnella plantaginis*	JN982125	98
PZS_S07	-	KY992893	*Cohnella plantaginis*	JN982125	97
PZS_A06	-	KY992894	*Cohnella plantaginis*	JN982125	98
PZG_B05	-	KY992895	*Cohnella plantaginis*	JN982125	98
PZG_S10	-	KY992896	*Cohnella plantaginis*	JN982125	97
PZG_S15	-	KY992897	*Cohnella plantaginis*	JN982125	97
PZG_S18	-	KY992898	*Cohnella plantaginis*	JN982125	98
PZG_A12	-	KY992899	*Cohnella plantaginis*	JN982125	98
PZG_A13	-	KY992900	*Cohnella plantaginis*	JN982125	99
PZG_A18	-	KY992901	*Cohnella plantaginis*	JN982125	98
PZG_A20	-	KY992902	*Cohnella plantaginis*	JN982125	98
PZS_S04	+	KY992903	*Cohnella xylanilytica*	HE866503	97
PZS_S05	+	KY992904	*Ensifer adhaerens*	AJ420774	100
PZG_S11	+	KY992905	*Ensifer adhaerens*	AJ420774	100
PZG_S19	+	KY992906	*Ensifer adhaerens*	AJ420774	100
PZG_A15	+	KY992907	*Ensifer sp. JNVU CB6*	JN832576	100
PZS_A07	-	KY992908	*Kocuria marina*	KP345974	100
PZS_B02	+	KY992909	*Mesorhizobium plurifarium*	JQ039741	100
PZS_A02	-	KY992910	*Paenibacillus glycanilyticus*	NR_024759	99
PZS_A09	-	KY992911	*Paenibacillus humicus*	AM411529	100
PZS_A10	-	KY992912	*Paenibacillus humicus*	AM411529	100
PZG_A14	-	KY992913	*Paenibacillus glycanilyticus*	NR_024759	99
PZG_A17	-	KY992914	*Paenibacillus glycanilyticus*	NR_024759	99
PZS_A01	-	KY992915	*Paenibacillus humicus*	AM411529	99
PZG_A11	-	KY992916	*Paenibacillus rigui strain*	NR116517	97
PZS_A05	+	KY992917	*Paenibacillus sp. JG-TB13*	FR849925	99
PZG_S12	+	KY992918	*Paenibacillus sp. JG-TB13*	FR849925	99
PZG_A16	-	KY992919	*Pseudomonas azotoformans*	KT375344	100
PZG_S17	-	KY992920	*Ralstonia pickettii*	CP001069	99
PZG_S21	-	KY992921	*Rhizobium sp. Lv6.1Se*	DQ422964	98
PZS_S01	-	KY992922	*Rhizobium sp. ORS 3441*	EU584258	100
PZG_A21	-	KY992923	*Rummeliibacillus pycnus*	NR041521	99

PEB: Potential endophytic bacteria; (+): positive signal of nifH; (-): negative signal of nifH; Twelve (12) other strains were not sequenced and the results by nifH amplification were as follows: PZG_A22 (-); PZG_S22 (-); PZG_S23 (-); PZG_S24 (-); PZG_S25 (-); PZG_S26 (+); PZG_S27 (+); PZG_S28 (+);PZS_A23 (-); PZS_A24 (-);PZS_S29 (-); PZS_S30 (-). Three slow sequences are not included in the table: PZG_S13 (+), PZS_S03 (+), PZS_A04 (+).

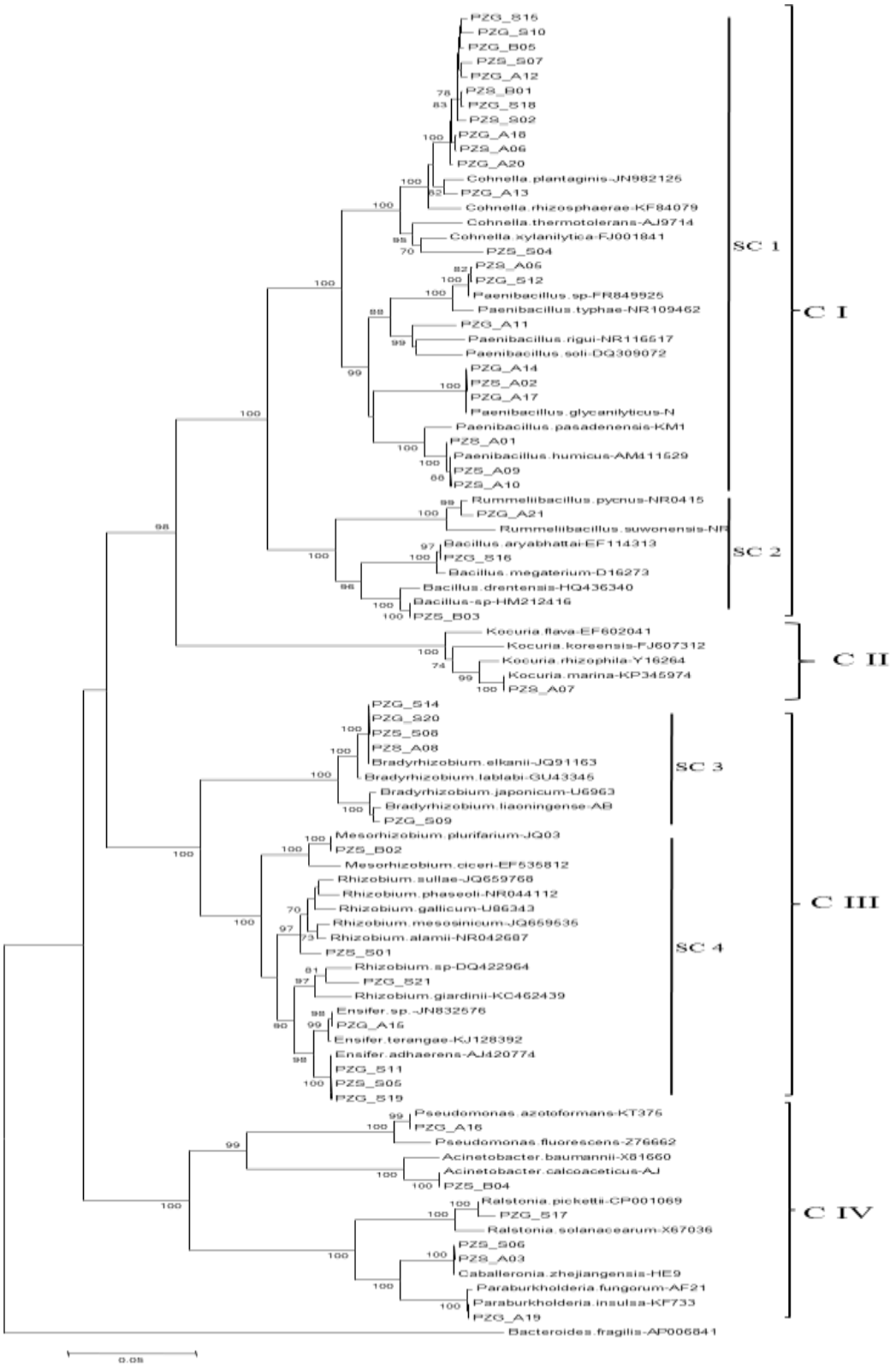

Figure 1. Phylogenetic tree of Potential Endophytic Bacteria (PEB) based on aligned sequences of 16S rRNA gene. Phylogeny history was inferred using the neighbor-joining method. Only bootstrap probability values >70% (1000 replicates) are shown at the branching points. Phylogenetic analyses were conducted in MEGA version 6; C: cluster; SC: sub-cluster

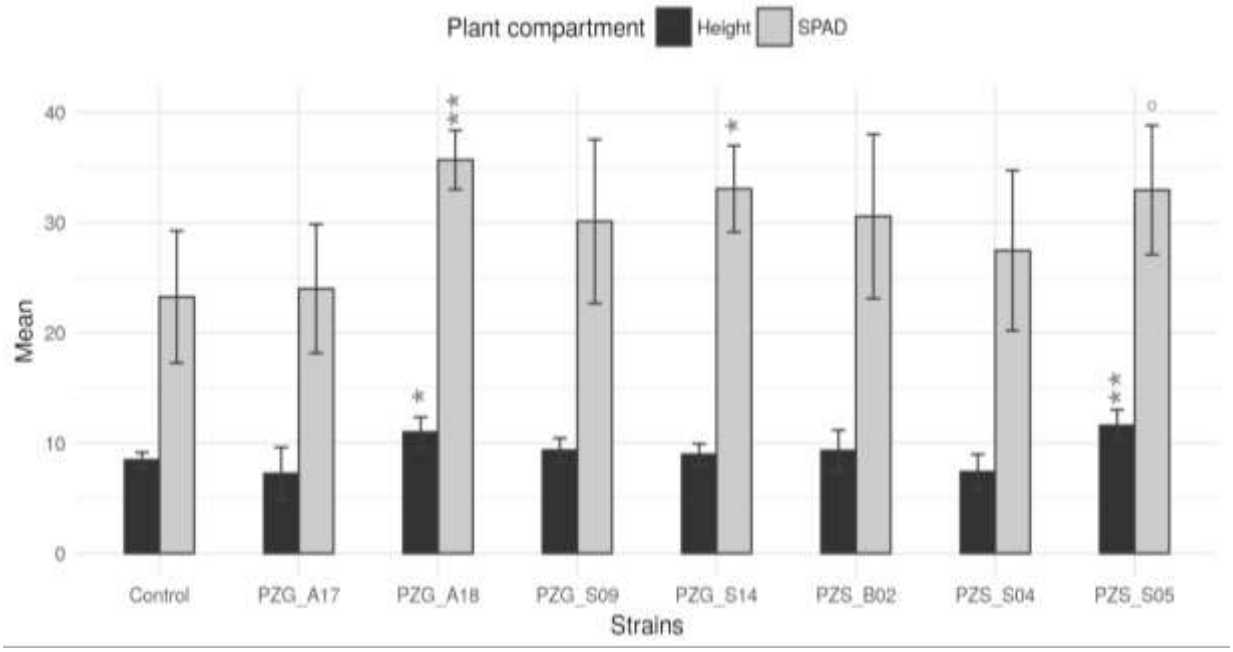

Figure 2. Response of *P. reticulatum* inoculated with 7 potential endophytic bacteria (PEB) in terms of chlorophyll content (SPAD) and height at 90 days (cm); The histograms represent the means and the errors represent the standard deviations (n=5); Signification of codes: 0.001 '**', 0.01 '*' and 0.05 '°' according to ANOVA and Dunnett's.

nodules of *soybean* (AB698736). The SC4 grouped seven fast growing strains, which were close to *Mesorhizobium plurifarium* (de Lajudie et al., 1998), to *Rhizobium giardinii sp* isolated from *Phaseolus vulgaris* nodules (Amarger et al., 1997) and to *Ensifer adhaerens* (Casida Jr, 1982).

Cluster IV contained six strains, which were close to different bacterial species: *Pseudomonas azotoformans* (Iizuka and Komagata 1963), *Acinetobacter calcoaceticus* (AJ888983), *Ralstiona pickettii* (Ralston et al., 1973), *Caballeronia zhejiangensis* (HE983367), and *Paraburkholderia insulsa* (KF733462).

Impact of PEB on the growth of *P. reticulatum*

In order to evaluate the impact of PEB isolated from soil under the influence of *P. reticulatum*, seedlings of the shrub were inoculated with seven strains (PZG_A17, PZG_A18, PZG_S09, PZG_S14, PZS_B02, PZG_S04 and PZS_S05), mainly chosen on the basis of the presence of the *nifH* gene (Table 2). After three months, *P. reticulatum* shrubs inoculated with the three strains significantly increased the chlorophyll contents of leaves measured by SPAD (Figure 2), compared to the inoculated shrub. These were strains PZG_A18 close to *Cohnella*, PZG_S14 close to *Bradyrhizobium elkanii* and to a lesser extent, PZS_S05 close to *Ensifer* ($p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively). Analyses of plant height showed that (Figure 2) the endophytic bacteria PZS_S05 and PZG_A18 significantly improved ($p < 0.001$ and $p < 0.01$, respectively) the growth of the shrub, compared to controls.

In terms of shoot dry weight (Figure 3), only the endophytic bacteria PZG_A18 differed significantly ($p < 0.001$) from inoculated plants. The dry weight of roots (Figure 3) revealed no significant difference between the inoculated plants and the controls, except for PZS_S05, which increased root weight ($p < 0.05$). In terms of total dry weight, no significant difference was observed between inoculated plants and controls (Figure 3). In total, the strains PZS_S05 close to *Ensifer* and PZG_A18 close to *Cohnella* induced a significant increase in certain parameters including dry weight of roots, growth at 90 days and photosynthetic activity, compared to the inoculated shrub.

DISCUSSION

The use of three host trap species (*F. albida*, *V. seyal* and *A. bivenosa*) provided access to the diversity of potential endophytic bacteria (PEB) present in the soil under the influence of *P. reticulatum* roots in two contrasting areas (one Sudano-Sahelian and one Sudano-Guinean). Indeed, the number of nodules formed on the roots of host plants showed that the soil from Sudano-Guinean site induced more nodules than the soil

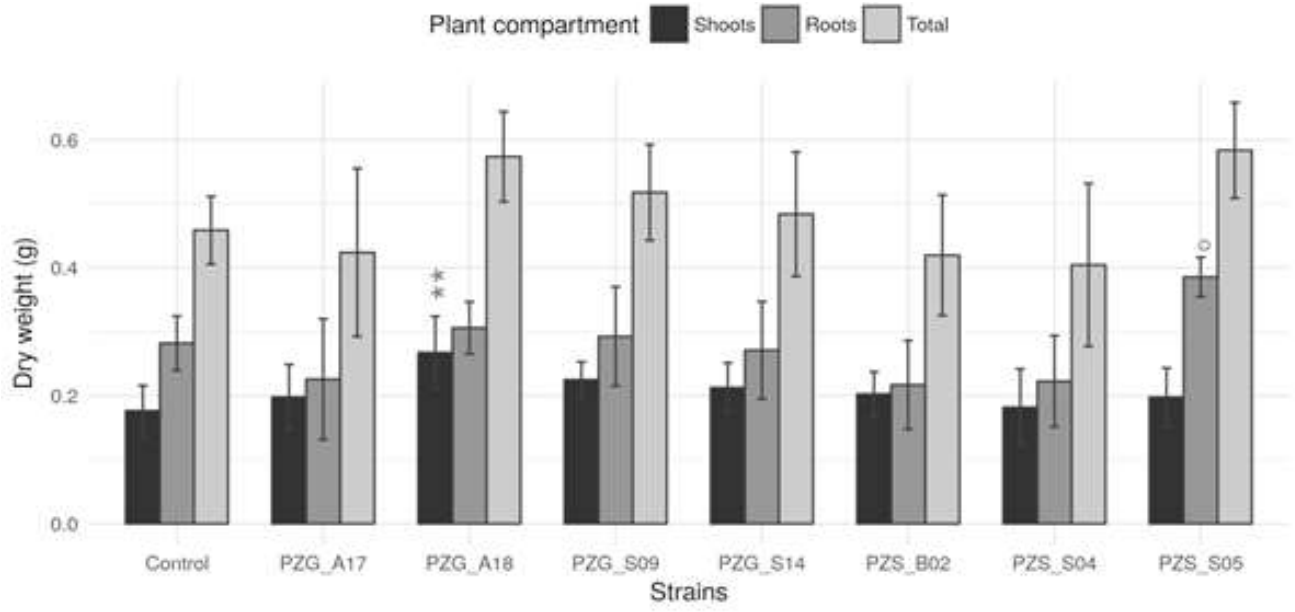

Figure 3. Dry weight of shoots (g) roots (g) and total of P. reticulatum inoculated with 7 endophytic bacteria. The histograms represent the means and the errors represent the standard deviations (n=5). Signification of codes: 0.001 '**', 0.01 '*' and 0.05 '°' according to ANOVA and Dunnett's.

Table 3. Soil properties at the ZSS site (Sudano-Sahelian climate) and at the and at the ZSG site (Sudano-Guinean climate)

Soil components	ZSS soil	ZSG soil
% Clay	11.93[a]	9.02[a]
% Silt	11.8[a]	18.28[a]
% Sandy	73.14[a]	71.92[a]
% Total N	0.084[a]	0.040[a]
% Total C	0.940[a]	0.622[a]
Total P (mg·kg−1)	1486.25[a]	57.75[b]
Ratio C:N	11.52[b]	15.69[a]

from Sudano-Sahelian site. These differences seem to be due mainly to the contrasting soil properties (Table 3) at the two sites. In fact, soils from the two regions of Senegal used in this study are both sandy, but the soil from the Sudano-Guinean site had a higher percentage of silt and clay than the soil from the Sudano-Sahelian site. Similar results were found using *Senegalia senegal* inoculated with soils from arid and semi-arid regions of Senegal (Bakhoum et al., 2014) and *A. saligna* inoculated with soils from northern and southern areas of Algeria (Amrani et al., 2010). As expected, the diversity of RFLP profiles was also higher in the soil from Sudano-Guinean site than the soil from Sudano-Sahelian site.

On the other hand, the results showed that the number of nodules formed with *F. albida* was higher than the number obtained with *A. bivenosa* and *V. seyal* regardless of the origin of the soil. The largest number of taxonomic groups was obtained on *V. seyal* host despite the fact that the same number of nodules was sub-sampled. This is in agreement with the fact that *V. seyal* belongs to the group of plant species that can be nodulated by the *Ensifer* genus (*Sinorhizobium*) (Odee et al., 2002; Romdhane et al., 2005; Cordero et al., 2016; Sankhla et al., 2017) and *Mesorhizobium* (Diouf et al., 2007). In contract, *A. bivenosa* and *F. albida* are frequently nodulated by the *Bradyrhizobium* genus (Odee et al., 2002; Perrineau et al., 2012; Sprent et al., 2017). Even though the trap plants are selective with respect to soil bacteria, they have the advantage of selecting endophytic bacteria, including Rhizobia, more easily.

The results showed that the isolated bacterial belonged to nine families: *Paenibacillaceae, Bacillaceae,*

Rhizobiaceae, Phyllobacteriaceae, Bradyrhizobiaceae, Moraxellaceae, Pseudomonadaceae, Burkholderiaceae and *Micrococcaceae*. More than 60% of these bacterial species were denoted non-symbiotic bacteria. A similar study carried out by Burbano et al. (2015) found fewer families of bacteria associated with *Colophospermum mopane*. As *P. recticulatum*, *Colophospermum mopane also* belongs to the family of non-nodulate plants (LPWG, 2017). Thus, the trap species used in this study clearly revealed the presence of symbiotic and nitrogen-fixing bacteria (*Mesorhizobium*, *Bradyrhizobium* and *Rhizobium*) in soil under influence of *P. reticulatum*. In addition, all the strains identified as belonging to the large group of rhizobia were able to form nodules on roots of their respective host plants (data not shown). This shows that *P. reticulatum* is unable to form visible nodules, but could influence the bacterial diversity of soil. This is in agreement the work of Diedhiou et al. (2013) who used phospholipid fatty acid analysis (PLFA) of microbial communities and also reported that Gram-positive bacteria (non-symbiotic) were in the majority in the soil under the influence of *P. reticulatum*.

To study the growth of *P. reticulatum* in in-vitro conditions, nitrogen fixing genes among the isolated strains were investigated. Among nitrogenase proteins, the *nifH* sub-unit is the most conserved. In this study, the screening of 59 PEB showed that a little less than half the isolates produced positive signals to *nifH* gene amplification, including almost endophytic bacteria belonging to the rhizobia group (except PZS_S01, *Rhizobium sp*). The absence of amplification of the latter strain may be due to a sequence divergence between the primers used and the gene present in the genome.

Strains closely related to *Cohnella* species were the most abundant endophytes sampled. The majority of these isolates clustered together with a high bootstrap value and was closely related to *C. plantaginis*. This cluster could represent a new *Cohnella species* (Figure 4). All the strains in this sub-cluster showed negative signals for the *nifH* gene. However, Wang et al. (2012) recently reported *Cohnella plantaginis* to be a novel nitrogen-fixing species, but by using the acetylene reduction assay. In contrast to this sub-cluster, PZS_S04, similar to *Cohnella xylanitytica* (97%), showed positive amplification of the *nifH* gene. *C. xylanitytica* is a xylan-degrading bacterium and was recently proposed as a new *Cohnella* species (Khianngam et al., 2010). It is known that many members of the *Bacillus* and *Peanibacillus* genera are diazotrophic bacteria. This is certainly the case of strains PZS_B03, PZG_S16 (belonging to the *Bacillus genus*), PZS_A04 and PZG_S12 (belonging to the *Peanibacillus genus*). In fact, the *Bacillus* genus also comprised a high percentage of endophytic bacteria associated with the roots of *Colophospermum mopane* (Burbano et al., 2015). Comparison of the results of this study and those of Burbano et al., showed that PEB from *P. reticulatum* were more diversified than those associated with the roots of *C. mopane* and *Cohnella sp*. was not found associated to with roots of *C. mopane*. The different approaches used in the two studies (direct isolation or functional approach) could explain these differences.

In addition, the presence of nitrogen-fixing species suggests that these species could be involved in the supply of nitrogen to the plant, as well as enriching the soil with nitrogen. These groups are frequently encountered in the nodules of legumes (Zakhia et al., 2006). Their role in the nodules remains obscure, but they probably play a role in biological fixation in the nodules and/or beneficial effects via production of plant hormones that promote plant growth. In all, seven PEB were selected and inoculated on young plants of *P. reticulatum*. Compared to controls, PZS_S05 (*Ensifer* sp.) and PZG_A18 (*Cohnella* sp.) were able to significantly increase the height of the shrub. These two same strains also significantly increased chlorophyll contents, as is the case for PZG_S14 strain. Regarding dry weight, only strains PZG_A18 and PZS_S05 significantly increased dry shoot and root dry weight, respectively, but to a lesser extent. The mechanisms involved in these significant increases in plant growth should now be investigated, although many studies have shown that non-symbiotic endophytic bacteria can been used as inoculants to promote plant growth, nodulation, and to increase yields (Bai et al., 2003; Liu et al., 2010; Stajkovic et al., 2011). Stajković and collaborators (2011) also showed that shoot dry weight and nitrogen content in common bean plants were improved after co-inoculation of *Rhizobium phaseoli* and *Bacillus sp*. On the other hand, it appears that the increase of chlorophyll (SPAD) increased dry weight of leaves for PZG_A18, whereas in contrary the dry weight of the roots is increasend with PZS_S05. The current state of our knowledge on this shrub does not know the mechanism involved, which seems not related to bacterial strains. It will be interesting to test these strains in field conditions and to measure their impact on the growth of associated millet. As recently shown by Diakhaté and collaborators (2016) *P. reticulatum* appears to promote greater diversity of microorganisms in the root zone of cereal millet.

Conclusion

P. reticulatum is an important shrub for soil fertilization. Thus knowledge of bacterial diversity and their impacts on growth and nitrogen status of this shrub could help make better use of *P. reticulatum* in the arid and semi-arid zones of the Sahel. The results of this study revealed that the soil under the influence of *P. reticulatum* is associated with greater bacterial diversity, the extent of which varies depending on its area of origin (northern or southern Senegal).

The methods used including trapping bacteria, PCR-

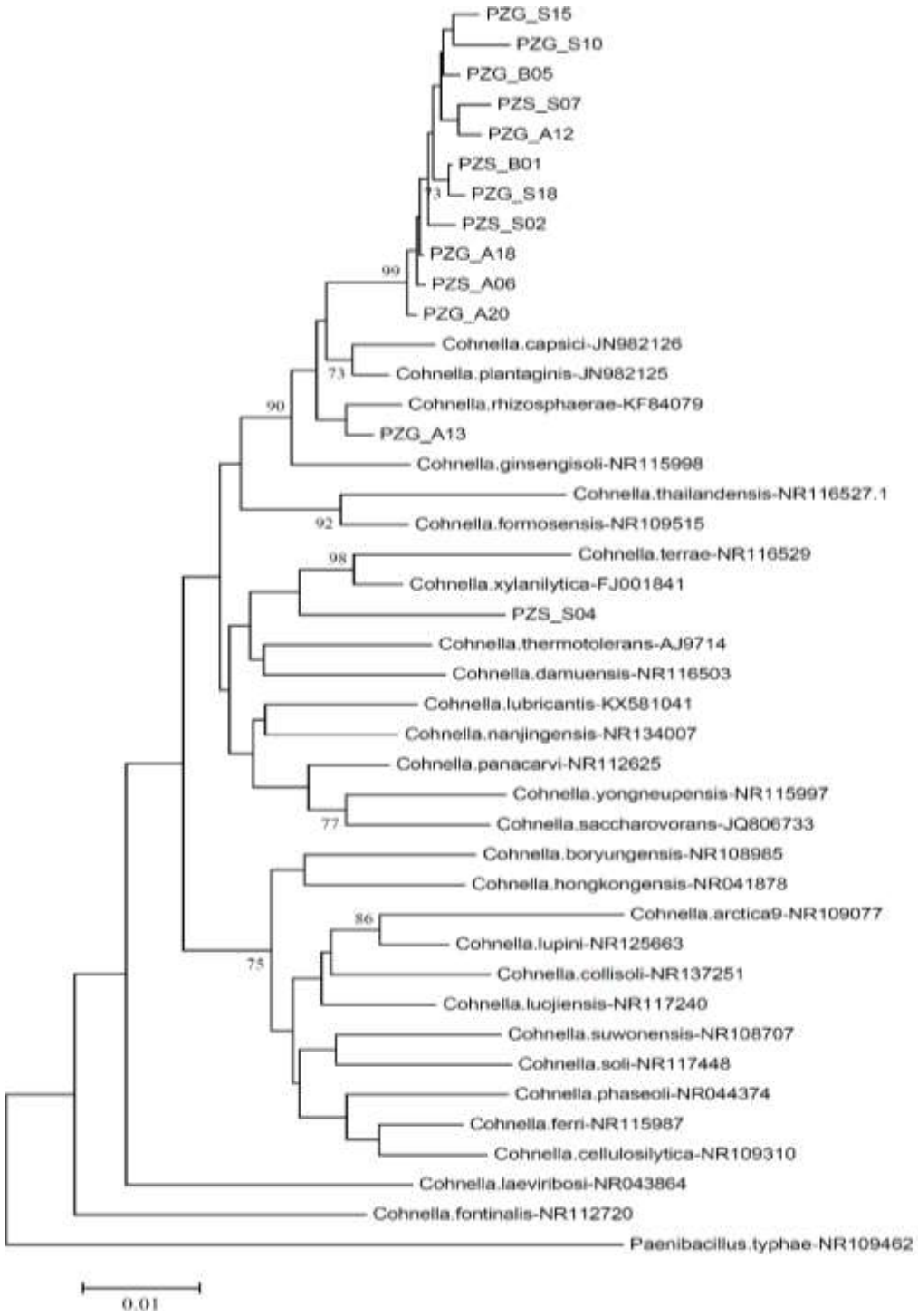

Figure 4. Phylogenetic tree based on aligned sequences of 16S rRNA gene of Cohnella species. Phylogeny history was inferred using the neighbor-joining method. Only bootstrap probability values >70% (1000 replicates) are indicated at the branching points. Phylogenetic analyses were conducted in MEGA.

RFLP and 16S rRNA gene sequencing efficiently demonstrated that *P. reticulatum* exerts an impact on the bacterial communities of the soil. The strains PZS_S05 (*Ensifer*) and PZG_A18 (*Cohnella*) were able to improve *P. reticulatum* growth and increase chlorophyll content. These results pave the way for the use of the endophytic

bacteria / *P. reticulatum* association to improve the growth of the shrub.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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