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Original Paper

Inoculation of arbuscular mycorrhizal fungi improve soil chemical properties, growth and symbiotic N_2-fixation in soybean (*Glycine max* L.) cultivars under field condition with low phosphorus availability

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Arbuscular mycorrhizal fungi (AMF) play an important role in nutrition of most plants as well improving soil fertility. The present study investigated the effects of different AMF isolates (*Funneliformis mosseae*, *Rhizophagus intraradices* and *Claroideoglomus etunicatum*) and control on soil chemical properties, growth and nitrogen (N_2) fixation in two soybean cultivars (TGx 1448-2E and TGx 1440-1E) in phosphorus (P)-deficient soil. The study was laid in split plot in a randomized complete block design with three replications. The results showed increased root colonization (up to 76%) with AMF inoculation compared to uninoculated control. The inoculation of the AMF isolates enhanced the growth parameters, nodulation and dry weights, which resulted in increased number of pods, 100-seed weight and seed yield. More pronounced effects were observed with *F. mosseae* and *R. intraradices* inoculation compared to *C. etunicatum*. In addition, similar trend was observed for P and N content in the plants as well the N_2 fixation activities, which resulted in increased total N fixed in both cultivars (up to 27.9 and 27.4 kg ha^{-1} respectively). After harvest, the results showed improved soil fertility in terms of soil N, available P, soil pH, organic carbon as well as exchangeable cations (calcium, magnesium, potassium and sodium) with AMF inoculation. TGx 1448-2E inoculated with *F. mosseae* gave the highest seed yield (1,773 kg ha^{-1}). The findings from this study suggest that *R. intraradices* or *F. mosseae* could be used to enhance N_2-fixation, soil fertility and productivity of soybean in phosphorus-deficient soils.

Keywords: arbuscular mycorrhizal fungi, soil phosphorus, relative ureide abundance, soil fertility, soybean productivity

1 Introduction

Soybean (*Glycine max* L. Merrill) is an economically important crop grown in most parts of the world including Nigeria. It contains high quality oil and protein and also play a major role in meeting the global food demand (FAOSTAT, 2019). Most crops including legumes depend on availability of phosphorus (P) and nitrogen (N) in the soil for better growth and yield performance. The low soil fertility of most tropical and sub-tropical soils is a major problem threatening crop productivity in those regions (Wahid et al., 2016). The ability of legumes including soybean to form a symbiotic relationship with the dinitrogen (N_2)-fixing bacteria e.g. rhizobia help in nitrogen (N) fixation in the soil. However, N-fixation in most legumes is regulated by the availability of P in the soil; making P a major constraint of legume N_2 fixation (Vance et al., 2003). P deficiency has been reported to affect nodule development and N-fixation in some legumes (Sulieman et al., 2014; Nasr Esfahani et al. 2016). Previous studies have shown reduction in nodulation and N-fixation under low soil P availability (Sulieman et al., 2013). Thereby, the potential of N-fixation and yield potential of most legumes could be greatly inhibited in areas with low soil P such as tropics and sub-tropics.

Improving the soil fertility in tropical soils mostly depends largely on intensive chemical fertilizer inputs (Makinde et al., 2011). However, the continuous and excessive use of inorganic fertilizer has resulted in environmental pollution as well as increase in soil degradation, soil acidification, and soil nutrient imbalance. There is also report of decrease in organic carbon, N and potassium (K) as well as other basic cations such as calcium

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(Ca) and magnesium (Mg) with continuous chemical fertilizer application (Ayoola et al., 2006). Moreover, the benefits of using chemical fertilizers are short-term and often expensive for poor-resource farmers in sub-Saharan Africa. Thus, the use of beneficial soil microbes, particularly arbuscular mycorrhizal fungi (AMF) could be an alternative to enhance soil fertility and improve productivity of crops in the region.

AMF are the ubiquitous and widespread belonging to the phylum *Glomeromycota* (Smith and Read, 2008). AMF from symbiotic associations with most crops and play a key role in many important ecosystem functions and soil processes, particularly P uptake in exchange for carbohydrates from the host (Kiers et al., 2011; Verbruggen et al., 2013). AMF hyphae proliferate extensively in the soil to acquire and transfer substantial amounts of P and N to the host plant (Brown et al., 2013; Kleinert et al., 2014). In addition, AMF has been used to enhance soybean growth and yield on P-deficient soil in a derived savannah of Nigeria (Sakariyawo et al., 2016; Adeyemi et al., 2017; 2020). Thus, AMF could be a promising tool in enhancing P uptake in crops under low soil P availability.

Despite the importance of AMF in improving soil fertility and availability of essential nutrients for plant uptake, there is scarcity of information on the effect of AMF on N fixation in legumes as well as soil properties such as soil pH, organic C, soil N and P, and exchangeable cations (Ca, K, Mg and Na) in Nigeria. Therefore, there is a need to evaluate the potential benefits of AMF to improve soil fertility, sustainable crop production as well as environmental protection from excessive application of chemical fertilizers. Thus, the present study was aimed to investigate the effects of different AMF isolates on symbiotic nitrogen contribution and soil chemical properties for enhanced soybean productivity in southwest Nigeria

2 Materials and Methods

2.1 Study area

The study was carried out in the experimental field located at Federal University of Agriculture, Abeokuta, Ogun, Nigeria (Lat. 7° 15′N, Long. 3° 28′E), in the cropping season of year 2018. The modified wet sieving and sucrose techniques of Giovanetti and Mosse (1980) was used to determine the initial mycorrhizal spore density in the soil (48 spores/100 g of soil). Initial properties of the soil used are presented in Table 1.

Experimental treatments and design

Two soybean cultivars (TGx 1448-2E and TGx 1440-1E) were inoculated with four arbuscular mycorrhizal fungi (AMF) inoculation treatments [control (no inoculation), *F. mosseae*, *R. intraradices* and *C. etunicatum*], laid out in split plot in a randomized complete block design, with three replications.

Table 1 Selected soil physico-chemical properties

Soil Property	
Texture	Sandy loam
Sand (%)	70.75
Silt (%)	12.75
Clay (%)	16.50
pH (H2O)	5.70
Organic Matter (%)	1.78
Nitrogen (%)	0.09
Available Phosphorus (mg kg^{-1})	6.13
Potassium (cmol kg^{-1})	0.61
Calcium (cmol kg^{-1})	6.68
Magnesium (cmol kg^{-1})	1.47
Sodium (cmol kg^{-1})	0.29
Total exchangeable acidity (cmol kg^{-1})	0.11
Cation exchange capacity (cmol kg^{-1})	9.17
AMF spore density (spores 100 g of $soil^{-1}$)	48

2.2 Cultural practices

Soybean seeds (TGx 1440-1E and TGx 1448-2E) were sourced from Institute of Agricultural Research and Training (IAR & T), Ibadan, Nigeria. The cultivars tested are widely grown in Nigeria because of their promiscuity in nodulation as well as high responsiveness to AMF inoculation (Adeyemi et al., 2017; 2019).The AMF inocula (*F. mosseae*, *R. intraradices* and *C. etunicatum*) were obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria. They are dominant in most agricultural soils. The inoculum were multiplied in roots of maize plants grown on sterilized river sand. Soil inoculum consisting of spores (approximately ~480 per 50 g of soil), hyphae and colonized root pieces was applied at sowing. The experimental plots were marked out using pegs, measuring tape and ropes, and then labelled after double ploughing. Each plot size was 4 × 4 m (16 m^2) and net plot size of 3 × 3 m (9 m^2). The soybean seeds were sown manually in a row at a depth of 2–5 cm and spacing of 50 cm (inter-row) × 10 cm (intra-row) on July 20 in 2018 cropping season to give a total of 369 plants per replicate plot. Mycorrhizal inoculum (200 g) was applied to the base of each planting row before sowing and covered lightly with top soil. The experimental plots were separated by 2 m between plots to restrict AMF influence

to inoculated plot only. Thinning out was carried out 2 weeks after sowing.

2.3 Growth attributes

Five plants from the net plot were tagged to measure the plant height, stem girth, number of leaves and leaf area at 60 days after sowing. Plant height was taken with graduated meter. Stem girth was measured with Vernier calliper. Leaves were counted as the number of trifoliate leaves per plant. Leaf area was calculated according to Weirsma and Bailey (1975) derived equation: A = 0.411 + 2.008 LW; where *A* = trifoliate leaf area, *L* and *W* are the maximum length and width of the terminal leaflet of a trifoliate leaf, respectively, while 0.411 and 2.008 are constants.

2.4 Biomass and nodulation

Five soybean plants were harvested from each plot, separated into leaf, stem + petiole, roots and nodules. The roots were dug to a depth of 20 cm. The plant tissues and nodules were oven-dried at 70 °C for 72 hr. The soybean nodulation was assessed by counting the average number of nodules from the harvested soybean plants.

2.5 AMF root colonization

Soybean roots were washed thoroughly in tap water, cut into approximately 1 cm segments, then cleared in 10% (w/v) KOH at 90 °C for 40 min. The cleared roots samples were stained with 0.05% (w/v) trypan blue in lactoglycerol (Phillips and Hayman, 1970). The percentage root length colonized by AMF (% RLC) was measured on 25 root segments under a stereo microscope at 20 × magnification scoring the presence or absence of arbuscules, vesicles and hyphae (Giovannetti and Mosse, 1980). The % RLC was calculated using the following formula (Adeyemi et al., 2019):

$$\%RCL = \frac{number\ of\ colonized\ root\ segments}{total\ number\ of\ root\ segments} \times 100 \quad (1)$$

2.6 Estimation of N_2 fixation

The full stem + petiole of the soybean plants were oven-dried at 70 °C for 72 hr and ground to pass a 1.0 mm mesh. Subsample of 0.5 g was transferred to 100 mL beakers and 25 mL distilled water was added to each subsample and boiled for 1–2 minutes. The boiled samples were boiled and filtered through a funnel and 15 cm filter paper (Whatman No. 40) into 50 mL volumetric flask. The extracts were stored in a freezer in small flasks for N solutes analysis (Peoples et al., 1989). The molar concentrations of ureide and nitrate (NO_3) in the extract was measured using the ureide assay of Young and Conway (1942) in 0.5 mL 0.5N sodium hydroxide (NaOH) and 1.0 mL 0.65 N HCl. The optical density was read at 525 nm on a spectrophotometer. The blended plant samples (shoot) were digested in concentrated hot acid (H_2SO_4) using Kjeldahl digestion method to determine the shoot N. The relative abundance of ureide-N in extracts of whole stem segments was calculated using the equation:

$$relative\ ureide - N(\%) = \frac{4 \times ureide}{\left[(4 \times ureide) + nitrate\right]} \times 100 \quad (2)$$

The standard curve relating proportion of N derived from N_2 fixation (Ndfa%) to relative ureide nitrogen (RU-N) for soybean during pod-fill was obtained using the equation (Herridge and Peoples, 2002).

$$x = 10.7 + 0.50\,P + 0.0034\,P^2 \quad (3)$$

where:

P – the proportion of plant N from N_2 fixation (% Ndfa)

x – the relative abundance of ureide-N in stem extracts

The shoot N (kg.ha^{-1}) was calculated using the below equation:

$$shoot\ \text{N (kg ha}^{-1}) = [shoot\ N\ concentration\ (\%) \times \times\ shoot\ dry\ matter] \quad (4)$$

To convert shoot N to whole-plant N in this soybean experiment, a multiplication factor of 1.5 was used (assumes one-third of total plant N is below ground, based on the 15 N-labelling experiment of Rochester et al., 1998).

The amount of N_2 fixed was calculated using the equation below:

$$amount\ of\ N_2\ fixed\ \text{(kg ha}^{-1}) = (\%\ \text{N dfa} \times \text{crop N}) \quad (5)$$

Shoot P content

Sub-samples of oven-dried samples were ground to fine particles and digested in a HNO_3-$HClO_4$-H_2SO_4 (5 : 2 : 1) solution, then analyzed colorimetrically by the vanadomolybdate-yellow assay (Murphy and Riley, 1962) to determine P concentration. P accumulation was calculated by multiplying P concentration with the biomass.

2.7 Seed yield and yield attributes

At harvest maturity, the plants were harvested from the plots and manually threshed separately and allowed to dry to about 10% moisture content for the determination of grain yield and estimated in kilogram per hectare.

2.8 Determination of soil chemical properties

Soil samples were randomly collected from each plot from 0–20 cm soil depth using a soil auger. The soil sample was air-dried and ground to pass a 2 mm sieve for laboratory analysis. Soil pH was determined using a pH meter in a 1 : 2.5 soil: water ratio using pH-meter (Rhoades and Oster, 1986). Total N was determined using the Kjeldahl method (Bremner and Mulvaney, 1982), and organic C was determined using the Walkley and Black method (Nelson and Sommers, 1982). Available P was extracted using $NaHCO_3$ at pH 8.3 and determined spectrophotometrically (Murphy and Riley, 1962). The exchangeable cations (Na^{2+}, Mg^{2+}, K^{+} and Ca^{2+}) were determined using the ammonium acetate extraction (Cottenie et al., 1982).

2.9 Statistical analysis

The data were subjected to analysis of variance (ANOVA) with Genstat Release 12.1 (Copyright 2009, VSN International Ltd). Duncan's multiple range test was used to separate significant differences among treatment means at the 5% probability level.

3 Results and discussion

3.1 Growth variables

The three AMF isolates significantly increased the plant height, stem girth, number of leaves and leaf area of the two soybean cultivars compared to un-inoculated plants (Table 2). There was no significant difference among the AMF isolates in all the growth variables measured.

Dry matter accumulation

Inoculation with *F. mosseae* gave the highest shoot, root and total dry weight in both soybean cultivars, followed by inoculation with *R. intraradices*, then *C. etunicatum* compared to the control (Table 3).

3.2 Root colonization

Higher root length colonization were recorded in both cultivars inoculated with the AMF isolates (Figure 1). The highest root colonization in both cultivars were observed in inoculation with *R. intraradices*. Inoculation with *C. etunicatum* had lower root colonization in both cultivars compared to *R. intraradices* and *F. mosseae*

Table 2 Effect of different AMF isolates on plant height, stem girth, number of leaves and leaf area of two soybean cultivars

Cultivar	AMF isolates	Plant height (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)
TGx 1440-1E	un-inoculated	52.9c	0.67c	20.7b	146.0b
	F. mosseae	65.5a	0.81a	26.5a	184.0a
	R. intraradices	64.2a	0.80a	25.3a	181.1a
	C. etunicatum	61.8a	0.76a	24.0a	170.3a
TGx 1448-2E	un-inoculated	58.9b	0.70b	21.5b	159.9b
	F. mosseae	62.7a	0.82a	25.0a	183.7a
	R. intraradices	61.4ab	0.81a	24.3a	182.8a
	C. etunicatum	60.9ab	0.80a	24.0a	178.7a

means in columns followed by different letters show significant differences at $P < 0.05$ among mycorrhizal treatments using Duncan's multiple range test ($n = 5$)

Table 3 Effect of different AMF isolates on shoot, root and total dry weight of two soybean cultivars

Cultivar	AMF inoculation	Shoot dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	Total dry weight (g $plant^{-1}$)
TGx 1440-1E	in-inoculated	6.89c	1.21cd	8.10d
	F. mosseae	9.94a	1.58a	11.5a
	R. intraradices	9.30ab	1.42a	10.7b
	C. etunicatum	8.87b	1.38b	10.3c
TGx 1448-2E	un-inoculated	7.29c	1.17d	8.46d
	F. mosseae	9.81a	1.36bc	11.2ab
	R. intraradices	9.48ab	1.32bc	10.8b
	C. etunicatum	8.78b	1.29bc	10.1c

means in columns followed by different letters show significant differences at $P < 0.05$ among mycorrhizal treatments using Duncan's multiple range test ($n = 5$)

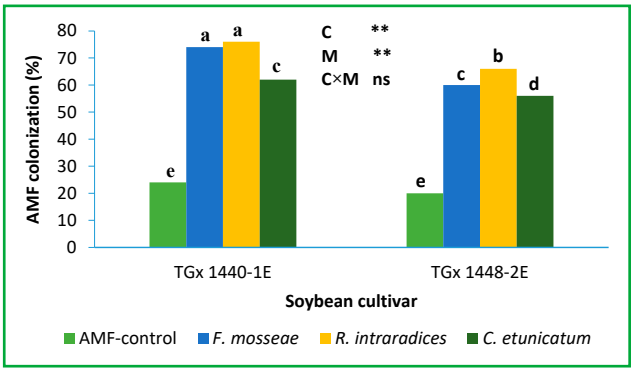

Figure 1 Root colonization of soybean cultivars inoculated with three different AMF isolates on P-deficient soil

C – cultivars; M, AMF inoculation. ** indicates significant differences at P <0.01; ns indicates non-significant differences. Means followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

3.3 Nodulation and nodule dry weight

The number of nodules and nodule dry weight was significantly increased by the AMF isolates compared to the control (Figure 2). The nodule dry weight was significantly influenced by AMF inoculation. Inoculation with *F. mosseae* significantly had the highest nodule dry weight compared to other AMF isolates in both cultivars.

3.4 Shoot N and P content

The soybean shoot N content was significantly influenced by AMF inoculation (Figure 3a). The three AMF isolates significantly increased the total N content of the two soybean cultivars. Inoculation with *F. mosseae* and *R. intraradices* resulted in higher N content in TGx 1440-1E than *C. etunicatum* while inoculation with *R. intraradices* and *C. etunicatum* resulted in higher N content in TGx 1448-2E.

The P content of the soybean cultivars were significantly influenced by AMF inoculation (Figure 3b). In both cultivars, P content was increased with AMF inoculation

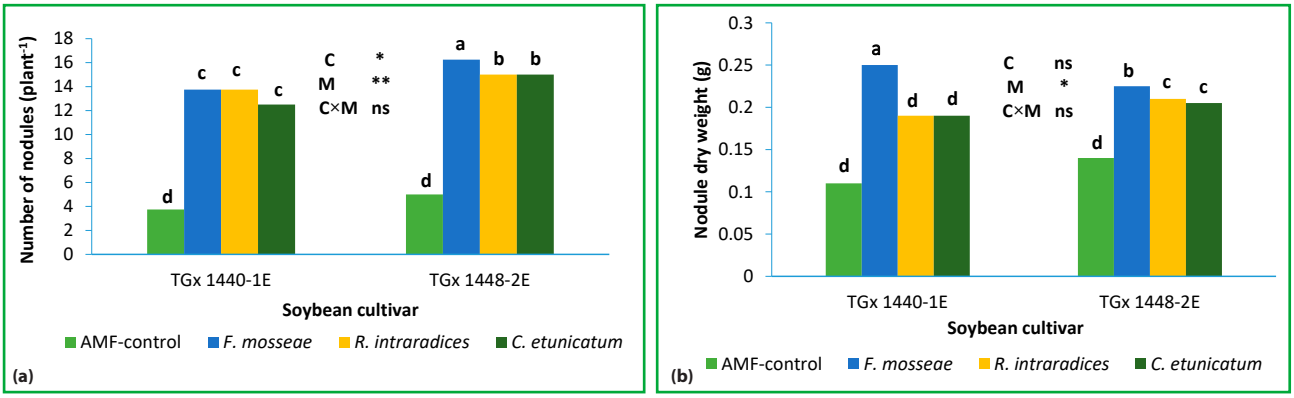

Figure 2 Number of nodules (a) and nodules dry weight (b) of soybean cultivars inoculated with three different AMF isolates on P deficient soil

C – cultivars; M, AMF inoculation. *, ** indicates significant differences at P <0.05 and P <0.01 respectively; ns indicates non-significant differences. Means followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

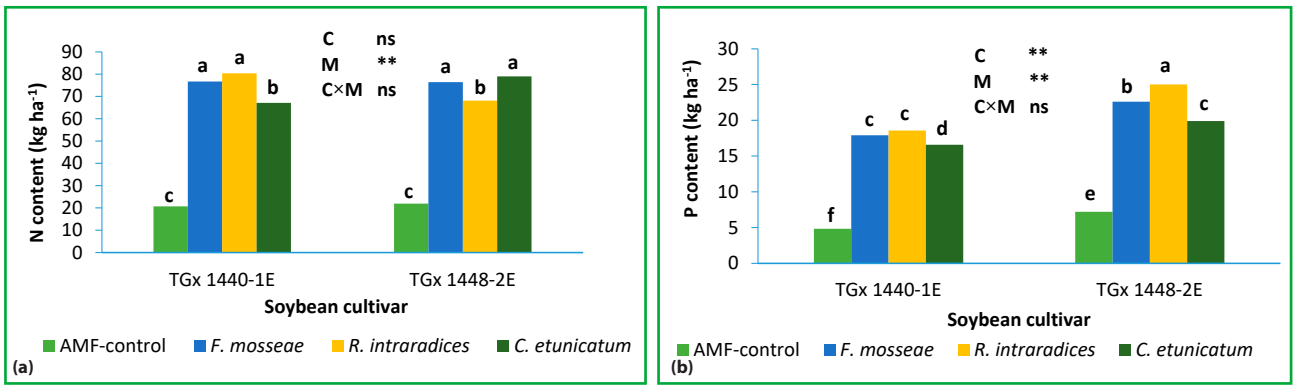

Figure 3 Plant N (a) and P content (b) of soybean cultivars inoculated with three different AMF isolates on P deficient soil

C – cultivars; M, AMF inoculation. ** indicates significant differences at P <0.01; ns indicates non-significant differences. Means followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

Table 4 Effect of different AMF isolates on concentration of nitrate, ureide and relative abundance of ureide-N of two soybean cultivars

Cultivar	AMF isolates	Nitrate (µmol)	Ureide (µmol)	Relative Ureide-N (%)
TGx 1440-1E	un-inoculated	0.49a	0.025b	17.3b
	F. mosseae	0.26b	0.032a	33.8a
	R. intraradices	0.29b	0.030a	29.1a
	C. etunicatum	0.25b	0.025b	29.9a
TGx 1448-2E	un-inoculated	0.47a	0.027b	18.8b
	F. mosseae	0.26b	0.030a	31.9a
	R. intraradices	0.24b	0.034a	36.3a
	C. etunicatum	0.29b	0.033a	30.7a

means in columns followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

with the three AMF isolates compared to un-inoculated treatments. The higher P content was observed in with *R. intraradices* and *F. mosseae* in TGx 1440-1E, while in TGx 1448-2E, inoculation with *R. intraradices* resulted in highest P content.

3.5 Nitrogen fixation

The concentrations of nitrate-N (NO_3-N), ureide-N, and relative abundance of ureide-N were significantly influenced by AMF inoculation (Table 4). The nitrate-N significantly reduced with AMF inoculation in both cultivars. The ureide-N and relative ureide-N abundance significantly increased with AMF inoculation compared to the control, except inoculation with *C. etunicatum* on ureide-N in TGx 1440-1E.

The N derived from the atmosphere (Ndfa) was significantly influenced by AMF inoculation (Figure 4a). Soybean inoculated with *F. mosseae* had the highest Ndfa in TGx 1440-1E while inoculation with *R. intraradices* resulted in the highest Ndfa in TGx 1448-2E. The amount of N fixed in both cultivars was significantly influenced by AMF inoculation (Figure 4b). Soybean inoculated with *F. mosseae* significantly had the highest N fixed in TGx 1440-1E.

3.6 Yield components

The yield components (number of pods, dry pod weight and 100-seed weight) of the soybean cultivars were significantly influenced by the AMF isolates (Table 5). The number of pods significantly increased with AMF inoculation in both cultivars compared to the control, except *C. etunicatum* in TGx 1448-2E. Soybean inoculated with *F. mosseae* significantly had the highest dry pod weight in both cultivars. The 100-seed weight of the soybean cultivars was significantly lowest in un-inoculated treatments in both cultivars. The biomass yield of the soybean cultivars increased with AMF inoculation (Figure 5a). Soybean inoculated with *F. mosseae* significantly had the highest biomass yield in both cultivars. Similar pattern was observed with the seed yield of the soybean cultivars (Figure 5b).

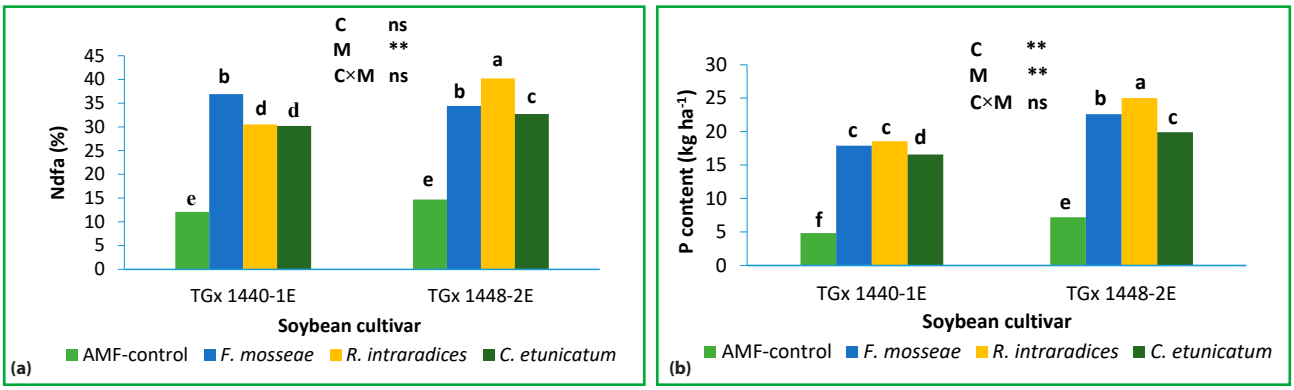

Figure 4 N derived from the atmosphere (Ndfa) (a) and total N-fixed (b) in soybean cultivars inoculated with three different AMF isolates on P deficient soil
C – cultivars; M, AMF inoculation. *, ** indicates significant differences at P <0.05 and P <0.01 respectively; ns indicates non-significant differences. Means followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

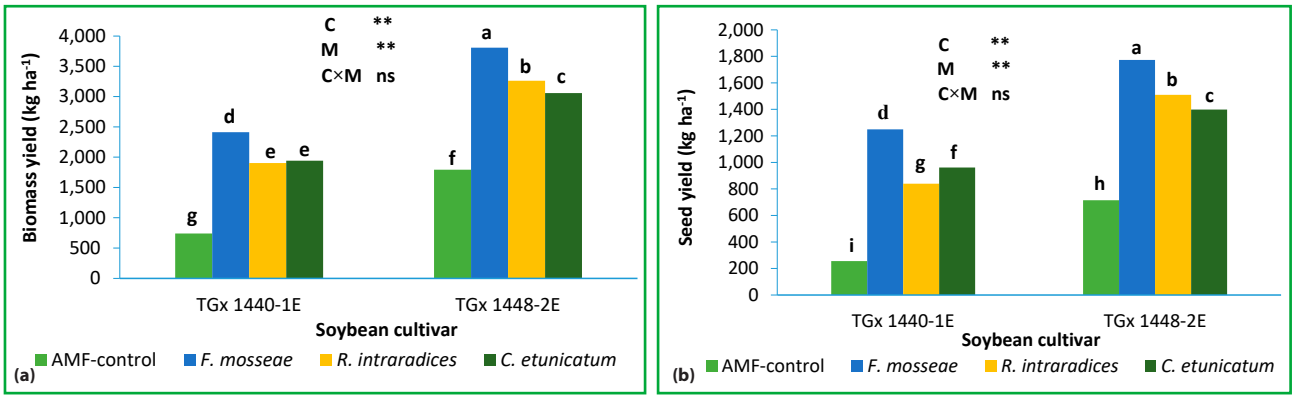

Figure 5 Biomass (a) and seed yield (b) of soybean cultivars inoculated with three different AMF isolates on P deficient soil
C – cultivars; M, AMF inoculation. ** indicates significant differences at P <0.01; ns indicates non-significant differences. Means followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

Table 5 Effect of different AMF isolates on plant height, stem girth, number of leaves and leaf area of two soybean cultivars grown o P-deficient soil

Cultivar	AMF isolates	Number of pods (plant^{-1})	Dry pod weight (g plant^{-1})	100-seed weight (g)
TGx 1440-1E	un-inoculated	14.6d	5.89e	4.15c
	F. mosseae	37.9bc	12.2bc	6.35a
	R. intraradices	28.2c	11.1c	6.18a
	C. etunicatum	33.7bc	10.6c	6.07a
TGx 1448-2E	un-inoculated	32.2bc	8.28d	5.18b
	F. mosseae	54.9a	16.5a	6.35a
	R. intraradices	52.1a	13.7b	6.03a
	C. etunicatum	49.6ab	13.4b	5.87a

means in columns followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

3.7 Soil chemical properties

The AMF isolates increased the soil pH, organic carbon, total N and available soil P compared to non-mycorrhizal treatment in the rhizosphere of the two soybean cultivars (Table 6). Among the AMF isolates, *F. mosseae* and *R. intraradices* significantly increased the organic C and N in the rhizosphere of TGx 1440-1E and available P in the rhizospheres of both cultivars compared to *C. etunicatum*. Similar pattern was observed for the basic cations in the soil (Table 7). *F. mosseae* and *R. intraradices* significantly increased the K^+ in the rhizosphere of both cultivars and Ca^{2+} in the rhizosphere of TGx 1440-1E compared to *C. etunicatum*.

Table 6 Effect of different AMF isolates on soil pH, organic C, total N and available P in rhizosphere of two soybean cultivars

Cultivar	AMF isolates	Soil pH	Organic C (%)	Total N (%)	Available P (mg kg^{-1})
TGx 1440-1E	un-inoculated	6.40b	0.49c	0.17b	32.2c
	F. mosseae	6.93a	0.59a	0.20a	37.2a
	R. intraradices	6.96a	0.58a	0.19a	38.2a
	C. etunicatum	6.67ab	0.54b	0.18ab	33.8bc
TGx 1448-2E	un-inoculated	6.53b	0.52b	0.17b	32.7c
	F. mosseae	7.20a	0.59a	0.19a	37.4a
	R. intraradices	7.00a	0.58a	0.19a	36.6a
	C. etunicatum	6.93a	0.57a	0.19a	35.7b

means in columns followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

Table 7 Effect of different AMF isolates on soil exchangeable cations in rhizosphere of soybean cultivars

Cultivar	AMF isolates	Na (cmol kg^{-1})	K (cmol kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})
TGx 1440-1E	un-inoculated	0.31b	0.50d	0.25c	0.34b
	F. mosseae	0.36a	0.56ab	0.28a	0.39a
	R. intraradices	0.35a	0.55ab	0.28a	0.38a
	C. etunicatum	0.33b	0.52cd	0.26b	0.36ab
TGx 1448-2E	un-inoculated	0.32b	0.51d	0.26b	0.35b
	F. mosseae	0.37a	0.57a	0.28a	0.39a
	R. intraradices	0.36a	0.56ab	0.28a	0.38a
	C. etunicatum	0.35a	0.54bc	0.28a	0.38a

means in columns followed by different letters show significant differences at P <0.05 among mycorrhizal treatments using Duncan's multiple range test (n = 5)

3.8 Discussion

Improving N-fixation in legumes and soil nutrients availability is expected to contribute greatly to crop productivity in poor soils in the tropics. The present study was aimed to investigate the effects of arbuscular mycorrhizal fungi (AMF) on symbiotic nitrogen contribution and soil chemical properties for enhanced soybean productivity in southwest Nigeria. The positive response of the soybean cultivars to AMF inoculation in this study suggests their adaptability and successful inoculation of the AMF isolates. This further corroborates previous studies on the ability of the AMF isolates to compete with native AMF species in the soil (Ortas, 2012; Cely et al., 2016). In addition, the tested AMF isolates in this study have also been reported to be widely distributed in most P- deficient tropical soils (Öpik et al., 2010).

High root colonization ability is an important pre-requisite for AMF isolates to compete with highly competitive indigenous AMF and their selection for mycorrhizal inoculation in crop production. The enhanced root colonization compared to the controls in both cultivars could be attributed to the propagules quality, which is a major factor affecting functional AMF establishment. The variation observed in RLC ability of the AMF isolates in the present study could be attributed to the fungal genotype, which may influence the different steps of mycorrhizal establishment, from spore germination to appressorium formation and intraradical growth. This represents a fundamental survival, which can plug into compatible extraradical networks, gaining immediate access to plant-derived carbon from the host plants (Sbrana et al., 2011). The doubling effect on root colonization observed with the isolates is is supported by the reports of Köhl et al. (2016) on eight different unsterilized field soils who revealed that the isolates, notably *R. intraradices* has a broad niche with ability to compete successfully with indigenous AMF and hence can successfully establish in a wide range of soils with highly variable chemical characteristics.

The improved growth and seed yield performance of both soybean cultivars with AMF inoculation in the present study were consistent with earlier reports (Sakariyawo et al., 2016; Adeyemi et al., 2017, 2020). This could be attributed to the high root colonization, which resulted in increased availability of soil nutrients and content in plants. This could have improved the soybean physiological traits supporting the higher biomass accumulation and allocation of assimilates to the yield components of the soybean cultivars in the study. The increased soil availability and content of N and P might have also favoured leaf area expansion needed for light interception during photosynthesis (Pablo-Barbieri et al., 2008; Otie et al., 2019). The N and P is also essential for yield formation because of their essential role in plant metabolic processes and assimilate partitioning (Akmal et al., 2010).

In the present study, mycorrhizal colonization enhanced the nodulation and N-fixation activities of both soybean cultivars. This corroborates to the findings of Vardien et al. (2014) who reported increased nodulation with AMF inoculation. The reduction in the N-fixing activities of the soybean could reflect the P limitation in maintenance of rhizobia symbiosis and the high energy requirements for N_2 reduction by nitrogenase (Sulieman and Tran, 2015). It may also be attributed to the reduced C delivered to nodules, which consequently affect nodule performance. However, the enhanced N-fixation with the AMF isolates can be explained by AMF ability to enhance P content, which enhances the nodule development and supply high energy needed for N_2 reduction by nitrogenase (Sulieman and Tran, 2015). The increased P accumulation with AMF could be linked with absorption of amino acids and ureides in plant cell of nodules (Hernandez et al., 2009). This could have reflected in the increased relative abundance of ureide-N (RU-N) and N_2 derived from the atmosphere (Ndfa) with AMF inoculation.

Soil organic carbon (SOC) is an important index of rhizosphere quality assessment (Srivastava et al., 2002).

The three AMF isolates significantly increased the SOC in the soybean rhizosphere compared to non-AMF treatment. This could be linked to the increased root colonization and AMF hyphae in the soil. This result supported the fact that AMF play a major role via secretions of organic C from their extraradical hyphae loaded into the rhizosphere (Cheng et al., 2012). Rhizodeposition of organic C by the roots of host plant helps AMF activities in soil aggregate formation (Rillig and Mummey, 2006). The increased soil P after harvest could be attributed to the activities of the AMF species and indigenous AMF to release organic acids such as phosphatase and citrate via their extra-radical hyphae in solubilisation of fixed P in the soil. The soybean plant have similar ability to release the organic acids from their roots. In addition, AMF could also have contributed to N transfer indirectly by stimulating soil bacteria involved in the mineralisation processes of soil organic matter, and therefore also of plant tissues and nodules of the legume (Saia et al., 2014).

The better growth performance and N-fixation in soybean inoculated with *R. intraradices* and *F. mosseae* can be explained in their higher P acquisition efficiency and transfer to the host plant. This confirms previous reports on the ability of these two AMF species to increase P content in diverse crops including soybean (Antunes et al., 2009; Cozzolino et al., 2013; Williams et al., 2013). Several other studies have reported significant variations among AMF species in their high performance in improving P content in plants (Pellegrino et al., 2011; Smith and Smith, 2011). Undoubtedly the relative performance of AMF depends on the isolates or species being compared. However, DNA based identification of how exactly competition between native and introduced AMF species pans out in the soils needs further research.

4 Conclusions

Based on the results from this study, the inoculation of the AMF isolates improved the growth attributes, availability and content of nutrients (N and P), N-fixation activities, and soil chemical properties, which resulted in increased the seed yield of both soybean cultivars. The N-fixation and seed yield of the soybean cultivars were best optimized in plots inoculated with *F. mosseae* and *R. intraradices*. Thus, the use of effective AMF isolates is extremely relevant to increasing N-fixation and soybean yield on P-deficient soils in southwest Nigeria. However, the present study was limited to a year field study in one location, it is therefore recommended that further field studies should be undertaken to in multi-location and different season towards the stable use of those AMF isolates in in enhancing N fixation and soybean productivity in Nigeria.

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