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Influence of mycorrhization and phosphate fertilizer on growth of Voandzou (*Vigna subterranea* (L.) Verdc.)

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ABSTRACT

Many strains of arbuscular mycorrhizal fungi (AMF) have the ability to improve the water and mineral nutrition of plants and in particular the supply of phosphorus (P). The objective of this study was to determine the influence of mycorrhization and simple superphosphate (SSP) on growth of Voandzou, also called Bambara groundnut (*Vigna subterranea* (L.) Verdc.). Five SSP doses (0, 30, 60, 90 and 120 mg $P_2O_5.kg^{-1}$ of substrate) were administered on seedlings of two Voandzou landraces inoculated with AMF composite (*Gigaspora margarita* and *Acaulospora tuberculata*). Plants were harvested eight weeks after sowing. The results showed that mycorrhization and SSP favored vegetative growth and increased biomass. Thus, the number of leaves of mycorrhized plants was significantly higher than that of control by 5.0%. The highest leaf P-content was observed with 60 mg P_2O_5 + AMF treatment; but was reduced with 120 mg P_2O_5 + AMF treatment. Mycorrhizal intensity was highest without SSP. High P levels were observed to reduced mycorrhizal efficiency. However, low SSP doses could be associated with AMF to optimize Voandzou growth. In conclusion, mycorrhization and SSP enhance the growth of Voandzou.

Keywords: Bambara groundnut, growth, arbuscular mycorrhizal fungi, phosphorus.

INTRODUCTION

Voandzou (*Vigna subterranea* (L.) Verdc.) is a neglected plant that originated from northeastern Nigeria and northern Cameroon (Begemann, 1988). Its highly nutritious content and high content of essential amino acids make it an important crop to consider for food security (Mbaiogaou et al., 2013; Yao et al., 2015). Despite the consumption needs and the range of their properties, a decline in legume production has been observed in Cameroon since the 1980s. This low production is reflected in rising market prices and seed scarcity (IRAD, 2013). Nutritional deficiencies like phosphate deficiency are also among the main causes of this drastic decline in yields and are important limiting factors for crop growth, production and survival (Sánchez-Coronado et al., 2007; Ouoba et al., 2016). Indeed, P concentrations

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in tropical soils are generally less than half of those in temperate soils (Syers et al., 2008; Temegne et al., 2015a; 2017a). To increase production, farmers amend the soil with chemical phosphate fertilizers (Mutetwa et al., 2010). However, these are costly and harmful to the environment if leached (Cordell et al., 2009). In order to reduce the use of phosphate fertilizers, the search for productive crop varieties under limited P supply, as well as alternatives of sustainable phosphate fertilization are considered. Numerous studies have shown that soil microorganisms, especially mycorrhizal fungi, improve the water and mineral nutrition of plants and in particular the P supply of plants (Ngonkeu, 2003; Taffouo et al., 2014). Thus, the objective of this study was to evaluate the influence of mycorrhization and phosphorus on Voandzou growth.

MATERIALS AND METHODS

Plant material

Two Voandzou landraces, chosen because they were largely appreciated by the consumers, were used: V1 (ivory cream seed coat) and V2 (ivory cream seed coat with grey eyes).

Experimental set up

A randomized factorial device with three factors: landraces (V1 and V2), simple superphosphate (SSP) doses (0, 30, 60, 90 and 120 mg $P_2O_5.kg^{-1}$ of substrate) (Rotaru, 2010) and AMF (M-: control and M+: mycorrhizae) was used. AMF used was a composite of *Gigaspora margarita* and *Acaulospora tuberculata* (99 spores/100 g) supplied by the Regional Laboratory for Biological Control and Applied Microbiology of IRAD Nkolbisson. Each treatment was repeated six times. The experiment was set up in the open during the dry season at the Faculty of Sciences of the University of Yaounde I. The germinated seeds (length of the radicle of 2 mm) were sown together with the AMF inoculum (15 g/sachet) and SSP in polyethylene bags (23*17 cm) containing the autoclaved (120 °C/4 h) substrate (2/3 soil + 1/3 river sand (2 mm)) at the rate of one seed per sachet. The substrate characteristics are shown in Table 1. It was sandy-clay. The sachets were watered every two days until harvest. The number of leaves produced and plant height were evaluated every 2 weeks from the date of sowing until eight weeks after sowing. At harvest (2 months after sowing), plant weight and plant water content were evaluated. Leaf P-content (Benton and Vernon, 1990), frequency and intensity of mycorrhization (Trouvelot et al., 1986) were measured.

Data analysis

The data were processed by an analysis of variance (ANOVA) using IBM SPSS version 20 software. Means were compared using the Student-Newman Keuls test at 5% threshold.

Table 1: Physical and chemical characteristics of substrate.

Sand	Silt	Clay	pH	C	N	C/N	P	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	CEC
	%		-	%		-	$\mu g.g^{-1}$			$cmol.kg^{-1}$		
74	2	24	5.5	0.75	0.04	18.7	3.42	1.14	0.25	0.11	0.07	2.37

RESULTS

Growth

AMF (Arbuscular Mycorrhizal Fungi) significantly increased the number of leaves ($p<0.001$) and plant height ($p<0.01$) of Voandzou over time (Table 2). But P_2O_5 did not significantly affect them ($p>0.05$) (Table 3).

Biomass and P-content

Phosphate fertilizer (SSP) and AMF significantly increased ($p<0.001$) the biomass (shoot fresh weight, root fresh weight and plant fresh weight) and leaf P-content; however, they did not affect ($p>0.05$) Voandzou water content (Table 4). Thus at 60 mg P_2O_5, AMF significantly increased shoot fresh weight by 68.77%. The highest P-content was observed at 60 mg P_2O_5 + AMF. But it was reduced with 120 mg P_2O_5 + AMF treatment (Table 4). Root/shoot ratio was significantly highest in P_2O_5 deficient plants.

Frequency and intensity of mycorrhization

SSP doses did not significantly influence ($p>0.05$) the frequency of mycorrhization of Voandzou. However, it significantly ($p<0.05$) influenced the intensity of mycorrhization, with the greatest intensity (35.4%) being observed at 30 mg of P_2O_5 (Table 5).

Landrace effect

The landrace significantly influenced the number of leaves ($p<0.001$) and plant height ($p<0.001$) of Voandzou over time. V4 had a number of leaves and height significantly higher than that of V1 over time (Table 6). Leaf P-content significantly ($p<0.001$) varied as a function of landrace (Table 7). However, it did not significantly ($p>0.05$) influence the biomass, water content, frequency and intensity of mycorrhization of Voandzou. Thus, the leaf P-content of V1 significantly exceeds that of V4 by 21.43% (Table 7).

Table 2: Relationship between number of leaves or plant height (y) and time (x in weeks) up to eight weeks after sowing, depending on AMF.

Parameters	Treatment	Time (weeks)	Regression equation	R^2
Number of leaves	M-	$2 \leq x \leq 8$	$y = 6.06x^{0.75}$ (***)	0.98
	M+	$2 \leq x \leq 8$	$y = 7.04x^{0.86}$ (***)	0.98
Plant height (cm)	M-	$6 \leq x \leq 8$	$y = 20.95x^{0.10}$ (**)	0.91
	M+	$6 \leq x \leq 8$	$y = 19.84x^{0.18}$ (**)	0.98

AMF: arbuscular mycorrhizal fungi, M-: without AMF, M+: with AMF. **, ***: significant at 0.01 and 0.001 probability levels, respectively; ns: not significant at the 0.05 probability level.

Table 3: Relationship between number of leaves or plant height (y) and time (x in weeks) up to eight weeks after sowing, depending on P_2O_5.

Parameters	P_2O_5 (mg/kg)	Regression equation	R^2
Number of leaves	0	$y = 6.79x^{0.73}$ (ns)	0.99
	30	$y = 6.03x^{0.92}$ (ns)	0.99
	60	$y = 7.04x^{0.73}$ (ns)	0.98
	90	$y = 5.97x^{0.88}$ (ns)	0.99
	120	$y = 6.91x^{0.81}$ (ns)	0.98
Plant height (cm)	0	$y = 20.11x^{0.13}$ (ns)	0.97
	30	$y = 20.23x^{0.17}$ (ns)	0.91
	60	$y = 20.54x^{0.13}$ (ns)	0.97
	90	$y = 20.61x^{0.13}$ (ns)	0.99
	120	$y = 20.46x^{0.13}$ (ns)	0.93

Table 4: Effect of P_2O_5 and AMF on biomass and leaf P-content.

P_2O_5 ($mg.kg^{-1}$)	AMF	Shoot fw (g)	Root fw (g)	Plant fw (g)	R/S ratio	Leaf P (%)	Plant WC (g/dw)
0	M-	9.2[a]	14.7[ab]	23.9[a]	1.7[b]	0.063[a]	3.97[b]
	M+	10.9[ab]	16.7[b]	27.6[ab]	1.5[b]	0.083[abc]	2.7[a]
30	M-	9.8[a]	11[ab]	20.8[ab]	1.1[a]	0.065[a]	3.4[ab]
	M+	11.6[ab]	13.2[ab]	24.8[ab]	1.2[a]	0.095[bc]	3.98[b]
60	M-	9[a]	10.5[a]	19.5[a]	1.2[a]	0.062[a]	3.7[b]
	M+	15.2[c]	15.4[ab]	30.6[b]	1[a]	0.103[c]	3.8[b]
90	M-	10.9[ab]	11.4[ab]	22.3[ab]	1.1[a]	0.074[ab]	3.7[b]
	M+	11.4[ab]	14.3[ab]	25.6[ab]	1.3[a]	0.093[bc]	3.3[ab]
120	M-	12.9[abc]	15.4[ab]	28.3[ab]	1.2[a]	0.061[a]	3.2[ab]
	M+	14.5[bc]	16.1[ab]	30.6[b]	1.1[a]	0.08[abc]	3.2[ab]
P_2O_5		**	**	**	***	**	ns
AMF		***	**	***	ns	***	ns
P_2O_5 * AMF		*	ns	ns	ns	*	*

P_2O_5 :phosphate, AMF: arbuscular mycorrhizal fungi, M-: without AMF, M+: with AMF, fw: fresh weight, R/S: root/shoot, WC: water content. Means followed by the same letter in a column are not significantly different at the 5% threshold. *, **, ***: significant at 0.05, 0.01 and 0.001 probability levels, respectively; ns: not significant at 0.05 probability level.

Table 5: Effect of P_2O_5 on mycorrhization.

P_2O_5 $mg.kg^{-1}$	Frequency (%)	Intensity (%)
0	90[a]	17.5[a]
30	98.3[a]	35.4[b]
60	90[a]	19.4[ab]
90	83.3[a]	10.5[a]
120	91.7[a]	15.7[a]
p value	ns	*

P_2O_5: phosphate. Means followed by the same letter in a column are not significantly different at the 5% threshold; *: significant at 0.05 probability level, ns: not significant at 0.05 probability level.

Table 6: Relationship between number of leaves or plant height (y) and time (x in weeks) up to eight weeks after sowing depending on landraces.

Parameters	Landrace	Time (weeks)	Regression equation	R^2
Number of leaves	V1	$x = 2$	$y = 7.14x^{0.75}$ (***)	0.99
	V2	$x = 2$	$y = 5.95x^{0.89}$ (***)	0.99
Plant height (cm)	V1	$2 \leq x \leq 8$	$y = 17.78x^{0.18}$ (***)	0.98
	V2	$2 \leq x \leq 8$	$y = 23.01x^{0.11}$ (***)	0.90

V1: ivory cream seed coat, V2: ivory cream seed coat with grey eyes. ***: significant at 0.001 probability level.

Table 7: Effect of landrace on measured parameters.

Land-races	Shoot fw (g)	Root fw (g)	Plant fw (g)	R/S ratio	Leaf P (%)	Plant WC (g/dw)	F (%)	I (%)
V1	11.3[a]	13.2[a]	24.5[a]	1.2[a]	0.09[b]	3.5[a]	92[a]	20.9[a]
V2	11.8[a]	14.5[a]	26.3[a]	1.3[a]	0.07[a]	3.5[a]	89.3[a]	18.4[a]
p value	ns	ns	ns	ns	***	ns	ns	ns

fw: fresh weight, R/S: root/shoot, WC: water content, F: mycorrhizal frequency, I: mycorrhizal intensity. Means followed by the same letter in a column are not significantly different at 5% threshold. ***: significant at 0.001 probability level; ns: not significant at 0.05 probability level.

DISCUSSION

The plant height and number of leaves per plant increased over time under different treatments applied. AMF significantly improved Voandzou height and number of leaves over time. It significantly increased the biomass and leaf P-content. This result is similar to that of Bourou et al. (2011) who found that *Glomus aggregatum* increased the height of tamarind. The increase in the P-content of cowpea leaves (*Vigna unguiculata* (L.) Walp) inoculated with *Glomus clarum* was observed by Megueni et al. (2011). This result could be explained by the fact that AMF certainly secrete enzymes that could hydrolyze the minerals indirectly accessible (strongly-bonded) to the roots. The improvement of water and mineral nutrition as well as the best development of the Voandzou inoculated with AMF are due to the development by these fungi of a network of hyphae. These hyphae take water and nutrients to allocate them to plants.

P_2O_5 significantly increased the biomass and leaf P-content of Voandzou. This result can be explained by the N and P interaction in the rhizosphere; because a sufficient P supply promotes nodulation and consequently the plant's growth. Indeed, N and P are among the essential elements needed for plant metabolism. P is needed in significant quantities, and is involved in the key functions of several plants, including energy transfer, photosynthesis, sugar and starch transformation, nutrient movement in the plant and transfer of genetic characteristics (Jemo et al., 2010). Silva et al. (2012) reported that inorganic fertilizer rapidly provides important elements at the early growth stage and at the plant development stage.

The result showed that the root/shoot ratio was significantly highest in P_2O_5 deficient plants. The reduction of root/shoot ratio by the increase in P_2O_5 doses is explained by the fact that the plants do not make an effort for their nutrition, and therefore do not develop their roots under sufficient P supply; so they have a luxury consumption (Shen et al., 2013; Temegne, 2014). However, under limited P supply, root/shoot ratio was high, which is explained by an important development of roots for the search of nutrients. This important development of the roots under limited P_2O_5 supply could also be explained by a preferential translocation of the resources towards the growth of the roots at the expense of the leaves. This result is similar to those of Temegne et al. (2015a).

P_2O_5 did not significantly affect mycorrhizal frequency. This result is in line with those of Mbogne et al. (2015) on pumpkins and Temegne et al. (2017b) on Bambara groundnuts. De Oliveira et al. (2015) also showed that AMF increases plant development at low P levels.

Apart from the number of leaves, plant height and leaf P-content, the landrace did not significantly influence the other parameters. The varied responses of landraces to P_2O_5 and AMF supply could be explained by the genotypic difference between them (Temegne et al., 2015 a, b).

Conclusion

The objective of the study was to evaluate the effect of arbuscular mycorrhizal fungi (AMF) and phosphorus (P_2O_5) supply on Voandzou growth. P_2O_5 enhanced biomass accumulation and leaf P-content. With or without P_2O_5 addition, AMF inoculation improved growth, biomass and leaf P-content of Voandzou. However, low P_2O_5 levels could be associated with AMF for optimal yield and sustainable production of Voandzou. Further studies on the effect of different doses of P_2O_5 fertilizers on the mycorrhization of Voandzou in farmers' fields are warranted to complement these current results.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. NCT designed the study, elaborated the research protocol, facilitated the work, performed statistical analysis and wrote the draft of manuscript. TDNF performed the data collection and contributed to literature review. VDT and EY corrected the research protocol and supervised the work. GN-N facilitated the work and corrected the draft of manuscript.

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