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Arbuscular mycorrhizal fungi, rhizobia, available soil P and nodulation of groundnut (*Arachis hypogaea*) in Zimbabwe

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Abstract

Arbuscular mycorrhizal fungal (AMF) colonization and nodulation of groundnut were examined in nine soils collected from subsistence farmers' fields in Zimbabwe. Nodule number, shoot dry weight, shoot N and P contents, and AMF colonization were assessed after 6 weeks growth. Both nodule number and AMF colonization differed by an order of magnitude among the nine soils. Soil available P explained almost all the variability in nodule number ($r^2 = 0.98$), but had no significant effect on percent AMF colonization. By adding P to one soil, nodule numbers increased four-fold resulting in a significantly higher N content in the shoots. Similar, but smaller, effects were obtained by increasing the abundance of AMF through an inoculation with *Glomus intraradices*, suggesting that nodulation in this soil was limited by AMF abundance and that the fungi could, to a limited extent, substitute for P fertilizer.

Keywords: Arbuscular mycorrhizal fungi; Rhizobia; Nodule number; Available soil P; Subsistence farming; Zimbabwe

1. Introduction

In Zimbabwe, maize (*Zea mays* L.) is grown as a staple, but production on subsistence farmers' fields is often limited by nitrogen (Snapp, 1998). To improve soil N fertility, there has been a renewed interest in using N_2-fixing legumes in intercropping and rotational cropping systems. Groundnut (*Arachis hypogaea* L.), cowpea (*Vigna unguiculata* (L.) Walp.) and bambara groundnut (*V. subterranea* (L.) Thou.) are currently grown for human consumption and for animal feed in Zimbabwe. However, their capacity to improve soil N fertility may be limited by the low concentrations of available soil phosphorus in the old, highly weathered soils of sub-Saharan Africa (Buresh et al., 1997) because good nodulation and high rates of N_2-fixation require substantial amounts of P (Giller, 2001).

Arbuscular mycorrhizal fungi (AMF), which colonize roots of most plant families (Smith and Read, 1997), can increase plant P uptake from the soil (Koide, 1991). Additive and sometimes synergistic effects on legume performance are frequently seen when both rhizobia and AMF are present (Goss and de Varennes, 2002; Sanginga et al., 1999; Fitter and

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Garbaye, 1994). While AMF are components of most natural ecosystems, their abundance and efficacy can be severely reduced by common agricultural practices, such as fallowing, soil disturbance caused by tilling and weed management and prolonged cultivation of non-host plants (Boswell et al., 1998; Kabir et al., 1997; Douds et al., 1995; Harinikumar and Bagyraj, 1989).

There were three objectives of this work: (1) to determine the relationship between nodule numbers on groundnut and available soil P in a wide range of soils from subsistence farmers' fields in southern Zimbabwe (Experiment 1), (2) to survey the AMF abundance in these soils (Experiment 1) and (3) to determine the effect of an increased AMF and rhizobia abundance, and a P and fungicide application on groundnut performance (Experiment 2).

2. Materials and methods

2.1. Experiment 1

Soils were collected from eight subsistence farmers' fields in southern Zimbabwe, and from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) research station in Matopos, Zimbabwe. The collections were made during 18–24 December 1999, which was at the beginning of the growing season. Soil was collected from approximately five randomly chosen stations per field at least 1 m away from the immediate root system of growing plants and where no fertilizers had been added that year. In no case was soil collected from around groundnut plants. The soil was bulked from each field and transported to the research station where it was passed through a 2 mm sieve and stored in the cool shade prior to the experiment. The pH (water 1:1, w:v), texture and available P of eight of the nine soils are listed in Table 1. Texture was determined using the hydrometer method according to Gee and Bauder (1986). Available soil P was determined using the iron oxide-impregnated filter paper technique according to Bramley and Roe (1993) with some modifications. The filter papers (12 cm^2, Whatman no. 541) were pulled quickly through a 10% aqueous solution of $FeCl_3 \cdot 6H_2O$. After air-drying for at least 1 h, the strips were drawn through a 5% ammonia solution, and then rinsed three times in distilled water. Approximately 4 g of air-dry soil was placed in a 50 ml polyethylene screw-top tube and shaken with 40 ml 0.01 M $CaCl_2$ solution and one filter paper strip for 24 h. The filter strip was then washed carefully in distilled water to remove any adhering soil. Adsorbed P was removed from the filter strip by shaking the filter strip in 20 ml 0.1 M H_2SO_4 for 30 min. An aliquot was taken, neutralized with NaH_2CO_3 solution and available P was determined using the method of Watanabe and Olsen (1965). A sub-sample of each soil was dried at 105 °C overnight to correct for the moisture in the air-dried soil samples.

Groundnut seeds (*A. hypogaea* var. Falcon) were planted in 1.6 l pots on 28 December 1999, and thinned to two plants per pot after emergence. Plants were grown in a greenhouse in Matopos under ambient conditions for 6 weeks and watered as needed. There were five replicate pots per soil. At harvest, shoots were separated from the roots, and

Table 1
Characteristics of soils used in Experiment 1

Area	Location	Texture (%) (clay:silt:sand)	P availability (mg P kg^{-1}soil)	pH (water)
Tsholotsho	1	41:11:48	1.3	7.9
	2	3:7:90	1.8	6.2
	3	4:6:90	3.0	5.0
Matopos	1	33:29:38	4.4	6.5
Gwanda	1	5:22:73	18.8	7.2
	2	6:17:77	5.7	6.0
	3	13:19:68	8.9	6.1
Zimuto	1	2:10:88	3.7	4.7
	2[a]			

[a] Not enough soil was collected for analyses of chemical and physical properties.

dried at 65 °C to constant weight. Visible nodules were counted and percent AMF colonization was determined using the gridline intersect method on cleared roots that had been stained in trypan blue (Brundrett et al., 1996).

2.2. Experiment 2

Soil was collected from the second location in Tsholotsho on 20 December 1999, and transported to the research station in Matopos. Groundnut (var. Falcon) was planted on 28 December 1999, in 1.6 l pots and grown for 6 weeks in a greenhouse under ambient conditions in non-sterile soil amended with either P (2 g single superphosphate pot^{-1} (19% P_2O_5)), AMF (2000 spores pot^{-1} of *Glomus intraradices*, Schenk and Smith (originally isolated from Utah, USA)), a fungicide Benomyl (200 ml of 0.1% solution added 1 day prior to planting), a commercially available *Bradyrhizobium* isolate (MAR 1510, originally isolated from *Macrotyloma* spp., a wild legume in Zimbabwe) for groundnut sold in Zimbabwe (seeds soaked in a solution consisting of 5 g of commercial inoculum in 200 ml of H_2O plus 1 ml of solution added in each hole prior to planting), or left unamended. Each of the five treatments was replicated five times. For the rhizobia treatment, one replicate was lost during handling and shipping from Zimbabwe to the USA. No additional fertilizers were added and the plants were watered as needed. At harvest, nodule numbers were determined along with shoot N (Nessler method; Jensen, 1962) and P concentrations (Watanabe and Olsen, 1965) following a digestion of tissue in concentrated H_2SO_4 for 1 h at 400 °C. AMF colonization was determined as before.

2.3. Statistical analyses

Data were transformed when necessary and analyzed using a one-way analysis of variance (ANOVA) or regression in Minitab Release 11 (Minitab Inc., State College, PA, USA). Transformation failed to generate data that fulfilled the underlying assumptions of the ANOVA for nodule numbers in Experiment 2. These data were, therefore, analyzed using the Kruskal–Wallis test for non-parametric data. Mean separations following the ANOVA were accomplished using the least significant difference method or by the Mann–Whitney pair-wise comparison test for the non-parametric data.

3. Results

3.1. Experiment 1

Nodule number and AMF colonization levels varied 10-fold among the nine soils (Table 2). AMF colonization was lowest where either pH was low or soil available P was high, but the relationships between AMF colonization and either pH or available P was not significant (i.e. the slopes did not differ from zero). There was a significant positive relationship ($r^2 = 0.98$, $p < 0.001$, $\text{d.f.}_{\text{error}} = 6$) between nodule number and available soil P (Fig. 1). Shoot weight was also positively related to available soil P ($r^2 = 0.62$,

Table 2
Mean (S.E.) of nodule number, arbuscular mycorrhizal fungal colonization (AMF) and shoot dry weight of groundnut in soils collected from four areas in southern Zimbabwe, including the ICRISAT research station in Matopos (Experiment 1)

Area	Location	Nodules (per gram root, FW)	AMF colonization (% of root length)	Shoot DW (g $plant^{-1}$)
Tsholotsho	1	23 (3.5)^{d}	30 (7.3)^{abc}	0.84 (0.14)^{d}
	2	28 (8.6)^{cd}	25 (6.3)^{bcd}	0.95 (0.09)^{bcd}
	3	34 (6.6)^{cd}	7.8 (1.1)^{ef}	0.99 (0.07)^{abcd}
Matopos	1	57 (8.5)^{bc}	38 (5.2)^{ab}	1.03 (0.02)^{abcd}
Gwanda	1	240 (21)^{a}	13 (2.3)^{def}	1.23 (0.41)^{a}
	2	82 (13)^{b}	40 (7.3)^{a}	1.12 (0.08)^{ab}
	3	90 (9.7)^{b}	10 (4.6)^{def}	1.21 (0.07)^{a}
Zimuto	1	33 (5.0)^{cd}	3.7 (1.5)^{f}	0.90 (0.07)^{bcd}
	2	40 (8.8)^{cd}	22 (4.8)^{cde}	0.93 (0.11)^{bcd}

Different letters (a–f) in superscripts indicate a significant ($p \leq 0.05$) difference between means across all locations, $n = 5$.

Table 3
Effect of P and fungicide applications, and AMF and rhizobia inoculations on groundnut grown in soil from the Tsholotsho 2 location (Experiment 2)

Treatment	Shoot DW (g)	Nodules (per plant)	AMF colonization (% of root length)	P content (mg shoot^{-1})	N content (mg shoot^{-1})
Control	1.0 (0.1)^{bcd}	75 (25)^{c}	25 (6.3)^{cd}	1.1 (0.12)^{c}	27 (2.3)^{cd}
Rhizobia	1.1 (0.1)^{abc}	56 (9.2)^{c}	35 (12)^{bc}	1.3 (0.13)^{bc}	29 (1.6)^{bc}
Fungicide	0.7 (0.1)^{d}	43 (14)^{c}	13 (5.6)^{de}	0.9 (0.08)^{c}	20 (1.6)^{d}
AMF	1.2 (0.1)^{ab}	144 (7.2)^{b}	57 (7.4)^{a}	1.9 (0.31)^{b}	36 (3.7)^{b}
Phosphorus	1.3 (0.1)^{a}	290 (75)^{a}	1.4 (0.6)^{e}	6.9 (1.2)^{a}	46 (3.8)^{a}

Different letters (a–e) in superscripts indicate a significant ($p \leq 0.05$) difference between treatment means (L.S.D.). Nodule numbers were analyzed using a non-parametric test. Mean (S.E.), $n = 5$ (except in the rhizobia treatment where $n = 4$).

$p = 0.012$, d.f.$_{error} = 6$), but a stepwise regression with available soil P and shoot DW as predictors showed that the variability in nodule number was driven by available soil P and not plant size, because this parameter was the sole predictor regardless of the loading sequence.

3.2. Experiment 2

P application significantly increased nodule number and shoot N content (Table 3). Inoculation with *G. intraradices* significantly increased AMF colonization, nodule numbers and shoot N content. A regression of AMF colonization against nodule number with the +P treatment excluded showed that the percent AMF colonization explained 44% of the variation in nodule numbers (Fig. 2). Percent AMF colonization also explained 61% of the variability in shoot N content in the same samples. Applications of fungicide and rhizobia had no significant effect on any of the variables tested (Table 3).

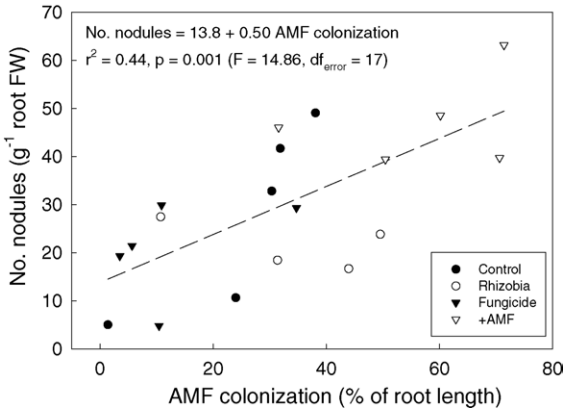

Fig. 2. Percent AMF colonization vs. nodule number on 6-week-old groundnut in Experiment 2. Values from the +P treatment were excluded from the analysis.

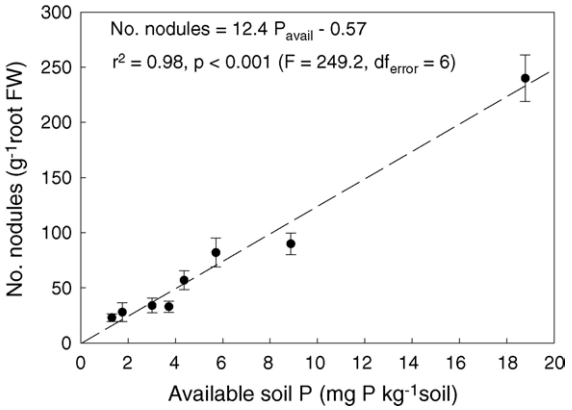

Fig. 1. Available soil P vs. nodule number on 6-week-old groundnut grown in eight Zimbabwean soils (Experiment 1).

4. Discussion

This study showed that nodule formation in groundnut was strongly associated with available soil P in a wide range of soils. By adding P to one of the soils, both nodule number and shoot N content increased significantly, indicating the importance of P for N_2 fixation. In the current study, additional AMF increased AMF colonization, which resulted in a 73% higher shoot P content and almost twice as many nodules per gram root fresh weight (47.4 ± 4.3, mean $\pm$ S.E.) compared to the control (27.8 ± 8.6). By using the graph in Fig. 1, this frequency of nodules corresponded to an available soil P level of around

3.8 mg P kg soil^{-1}, which is about twice that found in the soil from Tsholotsho 2 (Table 1). This indicates that the utilization of P was improved by the increased AMF colonization. Even though the response to an increased AMF colonization was considerably smaller than the response to P fertilizers, it could be of importance for subsistence farmers where access to inorganic fertilizers is difficult.

Large-scale AMF inoculation projects should not be proposed as an outcome of this study. Even though this practice was shown to benefit nodulation and growth of legumes (Ganry et al., 1985), it is not the best solution due to the quantities of inoculum needed, and thus, the high cost to the subsistence farmer. Rather, the abundance of indigenous fungi could be managed in soils where it is currently low. In temperate agro-ecosystems, fungal abundance is affected by tillage and fallow practices (Boswell et al., 1998; Kabir et al., 1997; Douds et al., 1995; Harinikumar and Bagyraj, 1989), but little is known about the effects of common management practices by subsistence farmers in the semi-arid tropics, which needs to be addressed. In this study, the AMF colonization differed by an order of magnitude between the soils and seemed to be negatively affected by low pH and higher soil P. The negative effect of P on mycorrhizal colonization has been known for quite some time (Baylis, 1972; Nicolson, 1967) and was clearly shown in Experiment 2 (Table 3). Also, there are some indications that colonization levels are lower in low pH soils (van Aarle et al., 2002; Hamel et al., 1996). In such cases, liming could be effective in increasing the inoculum potential of the soil. Because N_2 fixation acidifies the soil, liming could also help maintain soil pH if the soil has a low buffering capacity (Jarvis and Hatch, 1985).

Inoculations with rhizobia in Experiment 2 had no significant effect on either nodule numbers or shoot N content, presumably because the nodulation and N_2 fixation was P limited. The viability of the commercial inoculum was not explicitly tested, but because nodule numbers increased four-fold with the P application, nodulation may not have been limited by the number of rhizobia in the soil.

This study indicated that a single element, P, controlled the number of nodules when groundnuts were grown in pots in a wide range of soils, and that an increased abundance of AMF could to a limited extent substitute for P fertilizers under these conditions. Increased P availability led to greater nodulation and N accumulation, which could improve soil N fertility if crop residues are incorporated in the field.

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