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Influence of species of VA mycorrhizal fungi on cassava yield response to phosphorus fertilization

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Summary At three different sites with acid soils in Colombia field trials with cassava were monitored for frequency of VA mycorrhiza. Increasing levels of phosphorus (P) from 0 to 200 kg P/ha had been applied. The fields differed in the composition of species of VA mycorrhizal fungi. At all sites infections of the roots by the total mycorrhizal population decreased with increasing P fertilization, but at two sites the relative frequency and activity of one species, *Glomus manihotis*, increased with increasing P applications. This species was only present at two sites, and only in these sites a cassava yield response to up to 200 kg P was found. The differential activity of fungal species was confirmed in greenhouse trials, where *Entrophospora colombiana* was found to be most effective at 50 kg P and *G. manihotis* at 200 kg P.

Introduction

Cassava, *Manihot esculenta* Crantz, is a crop extremely well-adapted to acid-infertile soils with low phosphorus (P) content, so common in tropical America[17]. On these marginal soils cassava is grown as sole crop or as the last crop in the rotation before a fallow period[3]; however, without fertilization yields are generally very low. P deficiency is the major limitation for production. Yield responses to applications as high as 400 kg P/ha have been found, and levels of 100 to 150 kg P/ha are frequently recommended[3]. It is well known that cassava obligately depends on vesicular arbuscular (VA) mycorrhizal fungi for its P nutrition[10,13,22]. An effective mycorrhizal association is required for P uptake and plant growth in nearly all soils[11]. The VA mycorrhizal fungi take up P from the same sources which are also available to the plant roots[14]. Thus, theoretically, an increase of P into the labile pool by fertilization would be expected to increase the P uptake ability of the mycorrhizal root system of cassava. However, in many trials it was found that the cassava yield response to increasing P fertilization is highly dependent on the trial site[2,7,9,15]. The present study was conducted to determine whether the site specificity for P response is related to the composition of the native mycorrhizal population.

Increasing P supply frequently inhibit the percentage of root length

infected by mycorrhizal fungi and therefore the beneficial activity of the endophytes may be decreased[14]. On the other hand, VA mycorrhizal fungi are known to consist in soils with very high P contents[4], and thus, among the species of mycorrhizal fungi there may be differences in tolerance to low or high soil P conditions or to P fertilization.

Materials and methods

Field trials

Three established NPK trials with cassava were monitored for frequency of VA mycorrhiza. Two trials were located in the mountain region of the Cauca Department in South Colombia (at Agua Blanca with cassava cv M Col 113 and Barranquena, and at CIAT Quilichao with cv M Col 113 and M Col 1684). One trial was conducted in the Eastern Plains of Colombia in the Meta department (at Carimagua with cv M Ven 77 and M Col 638). The soils at the trial sites were acid clay loams with low nutrient availability and high Al saturation (Table 1). From the NPK trials only the following completely randomized treatments were considered: 100–0–100, 100–50–100, 100–100–100 and 100–200–100 kg/ha of N–P–K. Fertilizers were applied at time of planting in short bands at about 10 cm from the planting stake, N as urea, P as triple superphosphate and K as KCl. N and K were fractionated, half at planting and half at two months after planting. Fine roots were collected twice during the rainy seasons, except at Quilichao where the second sampling was conducted at the end of a dry season. At the first sampling roots were collected from 4 or 5 plants towards the center of each plot of each of the four replications at a distance of 5–20 cm from the plant's base and from 0–20 cm depth at 8 (Agua Blanca and Quilichao) or 5.5 (Carimagua) months after planting. The second sample was taken at harvest (14 months after planting at Agua Blanca and 12 months after planting at the other sites) by collecting fine roots from the soil after pulling up the whole plant for determination of yields. Representative subsamples of roots were processed and assessed for infection by VA mycorrhizal fungi, as reported elsewhere[16]. In addition to the infection of the roots by either hyphae, arbuscules and vesicles, the infection by vesicles alone was determined.

At the first sampling, representative soil samples from the zero fertilizer plots of the NPK trials were used to multiply in the greenhouse the native fungal species with *Pueraria phaseoloides* as host plant[5]. After one year, fungal spores were separated from these pots and the different species of VA mycorrhizal fungi were identified using keys[18,21]. The ability of the species to form vesicles in the roots was determined in separate greenhouse trials with cassava cv CM 91-3 grown in sterilized soil from Quilichao and inoculated with pure isolates of the fungi.

Greenhouse trial

Soil from Quilichao was pasteurized with steam for 4 h and incubated with the equivalent of 0, 50, 100 and 200 kg P/ha as triple superphosphate. These applications resulted in extractable P (with 0.1 *N* HCl + 0.03 *N* NH_4F) of 2.9, 6.3, 11.2 and 21.3 µg P/g soil. In order to investigate solely the effect of mycorrhizal fungi on P uptake, each pot of 2 kg soil received a solution application of 285 mg NH_4NO_3, 190 mg KCl, 221 $MgCl_2 . 6\ H_2O$ and 10 mg $ZnCl_2$. Before planting of rooted tip cuttings of cassava cv M Ven 77 (one plant per pot), the whole pot content was mixed with infected soil from pure pot cultures with mycorrhizal fungi. The spore number in this infected soil was determined. Each of three fungal species, *Glomus manihotis*, *Entrophospora colombiana* and *Glomus occultum* was established with 1000 spores per pot; also there was a mixture of the three species with 350 spores of each species per pot. Each of the three mycorrhizal treatments was replicated four times at each P level. After two months of growth, the plant tops were harvested and the P content was analyzed; all roots were separated from the soil for measuring the total root length as well as the mycorrhizal root length per plant using a method described earlier[5].

Table 1. Chemical and physical characteristics of soils at the three trial sites

Site	Organic matter (%)	pH	P (μg/g soil)	meq/100 g soil Al	Ca	Mg	K	Soil classification*
Agua Blanca**	6.2	4.4	0.8	8.5	0.95	0.46	0.16	Dystrandept
Quilichao	7.1	4.3	1.8	2.8	1.76	0.71	0.18	Dystropept
Carimagua	2.2	4.1	1.5	3.1	0.37	0.17	0.08	Haplustox

* After 'Instituto Geográfico Agustin Codazzi'[12].

** Soil samples at Agua Blanca were taken before application of 1 t/ha dolomitic lime; at other sites 15 days after application.

Table 2. Effect of increasing P application on fresh yields (t/ha) of different cassava cultivars grown at different sites in Colombia

Site	Cassava cultivar	Plant part	P application (kg P/ha) 0	50	100	200	LSD 5%
Agua Blanca	M Col 113	Roots	16.1	25.9	21.6	23.0	5.7
		Top growth	5.9	12.8	12.5	11.9	2.3
	Barranqueña	Roots	13.2	25.3	24.8	24.6	9.0
		Top growth	7.3	16.0	19.7	19.8	6.7
Quilichao	M Col 113	Roots	15.1	30.4	34.6	39.4	7.1
		Top growth	4.7	10.2	10.7	15.7	4.5
	M Col 1684	Roots	14.7	29.2	28.2	33.7	7.6
		Top growth	4.2	7.1	6.7	11.1	3.6
Carimagua	M Ven 77	Roots	9.2	17.0	19.7	21.8	4.8
		Top growth	4.9	9.8	12.0	13.3	2.2
	M Col 638	Roots	7.0	12.4	14.1	18.1	4.3
		Top growth	5.3	11.9	14.6	16.6	3.3

Results

Field trials

Whereas no yield response to more than 50 kg P/ha was found at Agua Blanca, both cassava cultivars responded to up to 200 kg P/ha at Quilichao and Carimagua (Table 2).

At each trial site five or more species of mycorrhizal fungi were naturally present (Table 3). Of all species, a clear vesicle formation in the roots was found only with *G. manihotis* and *G. fasciculatum*. Thus each percentage vesicle infection of roots represents the infection with *G. fasciculatum* at Agua Blanca, with *G. manihotis* at Quilichao, and with both *G. manihotis* and *G. fasciculatum*, at Carimagua. The percentage infection by either hyphae, arbuscles or vesicles represent the root infection by the whole mycorrhizal population with its different species. Table 4 shows that at first root sampling with increasing P application the frequency of mycorrhiza decreased, *i.e.* infection by

Table 3. Species of VA mycorrhizal fungi at the three trial sites and information about vesicle formation inside roots of cassava cv CM-91-3

Agua Blanca		Quilichao		Carimagua	
Species	Vesicles formed	Species	Vesicles formed	Species	Vesicles formed
Glomus microcarpum	No	*Entrophospora colombiana*	No	*Entrophospora colombiana*	No
Glomus fasciculatum	Yes	*Glomus occultum*	No	*Acaulospora longula*	No
Glomus occultum	No	*Glomus manihotis*	Yes	*Gigaspora heterogama*	No
Entrophospora colombiana	No	*Acaulospora appendicula*	No	*Glomus occultum*	No
Acaulospora appendicula	No	*Gigaspora heterogama*	No	*Glomus fasciculatum*	Yes
				Glomus manihotis	Yes

all mycorrhizal fungi inclusive of *G. fasciculatum*. At Quilichao the frequency of *G. manihotis* increased more markedly than of all other fungi with application of 50 kg P/ha, and the decrease of this species was less marked at higher P rates. At Carimagua the root infection by *G. manihotis* and/or *G. fasciculatum* increased with increasing P fertilization while the infection by the whole mycorrhizal population decreased. From first to second root sampling the root infection by all non-vesicles forming fungi had increased at Agua Blanca and Carimagua, and decreased at Quilichao. Increasing P fertilization tended to affect the root infection in similar ways as at the first sampling. At second sampling, the root infection by the vesicles forming fungi was inconsistently influenced by increasing P applications. *G. manihotis* was present at low population at Quilichao. At both samplings, no differences in the frequency of all fungi in the roots were found between the two cassava cultivars at Agua Blanca. At Quilichao and Carimagua the frequency of mycorrhiza was lower in cv M Col 1684 and M Col 638, respectively, in comparison to the other cultivar.

Greenhouse experiment

Species of mycorrhizal fungi differed in their effectiveness to enhance growth and P uptake of cassava depending on the P level applied (Table 5). When the three fungal species were mixed in the inoculum, at the 50 kg P level the P uptake was similar to that of plants inoculated with *E. colombiana* alone; at the highest P level the P uptake was similar to those plants inoculated with *G. manihotis*. Total root length and infected root length of the plants had an optimum at 50 or 100 kg

Table 4. Effect of increasing P application on per cent mycorrhizal infection of different cassava cultivars grown at different sites in Colombia

Site	Sampling*	Cassava cultivar	Mycorrhizal frequency	P application (kg P/ha) 0	50	100	200	LSD 5%
Agua Blanca	1st	M Col 113	HAV**	38.6	22.7	16.6	23.0	4.1
			Ves**	15.2	4.3	4.7	7.0	2.0
		Barranqueña	HAV	31.8	12.9	17.3	17.6	3.7
			Ves	10.3	1.7	4.5	1.8	1.9
	2nd	M Col 113	HAV	50.2	50.4	37.5	40.2	4.9
			Ves	3.8	2.2	3.8	4.0	ns
		Barranqueña	HAV	36.6	52.7	36.3	45.1	3.4
			Ves	5.3	6.6	8.9	10.0	1.9
Quilichao	1st	M Col 113	HAV	66.4	79.3	50.9	47.1	5.9
			Ves	31.4	60.8	46.6	34.8	4.6
		M Col 1684	HAV	66.4	67.8	39.7	40.0	6.9
			Ves	27.0	51.6	31.0	24.6	5.9
	2nd	M Col 113	HAV	47.7	45.9	38.6	38.1	3.4
			Ves	2.3	5.3	2.9	3.3	1.0
		M Col 1684	HAV	39.2	47.6	23.5	20.7	3.8
			Ves	5.2	10.6	6.3	3.2	2.4
Carimagua	1st	M Ven 77	HAV	63.8	54.6	35.1	32.9	4.9
			Ves	0.0	6.8	7.1	12.9	2.1
		M Col 638	HAV	58.3	43.3	20.9	36.9	–***
			Ves	0.0	9.6	8.2	11.9	–
	2nd	M Ven 77	HAV	70.7	60.3	56.3	54.3	–
			Ves	8.7	6.0	6.8	11.0	–
		M Col 638	HAV	46.3	30.9	76.0	43.8	–
			Ves	2.1	2.7	36.5	4.5	–

* 1st sampling at 5.5 MAP (Carimagua) or 8 MAP (others); 2nd at harvest.
** Frequency of either hyphae, arbuscules or vesicles (HAV) or of vesicles alone (Ves).
*** No statistical analysis was possible as observations were from bulked samples of all 4 replications.

Table 5. Effect of increasing P application on shoot dry matter and P uptake of cassava cv M Ven 77 inoculated with different species of mycorrhizal fungi

Species of mycorrhizal fungi	Dry matter (g/plant) P application (kg/ha) 0	50	100	200	P uptake (mg/plant) P application (kg/ha) 0	50	100	200
G. manihotis	1.55	9.02	10.93	13.73	2.01	13.31	22.61	31.91
G. occultum	2.05	7.49	9.32	11.21	2.77	10.64	13.41	18.23
E. colombiana	2.77	14.44	15.13	14.21	2.87	17.64	21.14	27.91
Mixture*	2.53	15.52	14.20	14.89	2.68	18.09	26.78	30.91
LSD 5%			2.40				3.96	

* Mixture of the three species

Table 6. Effect of increasing P application on root length, mycorrhizal root length and mycorrhizal frequency in roots of cassava cv M Ven 77 inoculated with different species of mycorrhizal fungi

Species of mycorrhizal fungi	P application (kg/ha)	Total root length (m/plant)	Root length with mycorrhiza (m/plant)	Root length with vesicles (m/plant)	Mycorrhizal frequency (%)	
					HAV*	Vesicles
G. manihotis	0	5.8	3.3	1.2	56.9	20.7
	50	15.5	13.9	11.6	89.7	74.8
	100	20.2	18.3	12.8	90.6	63.4
	200	14.5	12.6	7.2	86.9	49.7
	LSD 5%	4.6	3.9	4.6	17.6	16.2
G. occultum	0	7.7	1.5	0	19.5	0
	50	25.6	3.2	0	12.4	0
	100	22.9	1.9	0	8.3	0
	200	19.7	1.3	0	6.6	0
	LSD 5%	9.9	n.s.*	–	n.s.	–
E. colombiana	0	7.9	3.1	0	39.2	0
	50	12.4	7.8	0	62.9	0
	100	11.0	5.7	0	51.8	0
	200	7.9	5.6	0	70.9	0
	LSD 5%	3.5	2.7	–	n.s.	–
Mixture of three species	0	6.0	5.4	0.9	90.0	15.0
	50	11.9	10.5	6.2	88.2	52.1
	100	8.2	7.3	4.0	89.0	48.8
	200	11.1	8.0	4.1	72.1	36.9
	LSD 5%	4.0	4.3	2.1	n.s.	16.2

* Percentage infection with either hyphae, arbuscules or vesicles.
** n.s. = not significant ($P = 0.05$).

P/ha depending on the fungal species (Table 6). The percentage mycorrhizal frequency only increased with P applications in plants inoculated with *G. manihotis*. With this species, inoculated alone or in mixture the frequency of vesicles had an optimum at 50 kg P.

Competition of species at different P levels

The ability of the vesicles forming fungi, *i.e. G. manihotis* and *G. fasciculatum* to compete with the other fungi for infecting the roots at increasing P application levels was calculated after Sieverding[19] and is shown in Table 7. The competitive ability of *G. manihotis* increased with increasing P level in the mixture of fungi used in the greenhouse trial. In the field, at Agua Blanca the competitive ability of

Table 7. Relation* of the frequency of vesicles to the frequency of either hyphae arbuscles or vesicles; greenhouse trial and field trials (first observation of mycorrhizal frequency)

Trial	Cassava cultivar	Mycorrhizal fungi	P application (kg P/ha) 0	50	100	200
Greenhouse	M Ven 77	*G. manihotis*	0.36	0.83	0.70	0.57
		G. occultum	0	0	0	0
		E. colombiana	0	0	0	0
		Mixture**	0.17	0.59	0.55	0.51
Agua Blanca	M Col 113	Native species	0.39	0.19	0.28	0.30
	Barranqueña	Native species	0.32	0.13	0.26	0.10
Quilichao	M Col 113	Native spceies	0.47	0.77	0.92	0.74
	M Col 1684	Native species	0.41	0.76	0.78	0.62
Carimagua	M Ven 77	Native species	0	0.12	0.20	0.39
	M Col 638	Native species	0	0.22	0.39	0.32

* Calculated as: $\frac{\text{Mycorrhizal frequency of vesicles (\%)}}{\text{Mycorrhizal frequency of either hyphae, arbuscules or vesicles (\%)}}$.

** Mixture of the three species used in the greenhouse trial.

G. fasciculatum decreased by P application. At Quilichao, *G. manihotis* competed strongly with all other fungi in the range of 50–200 kg P. At Carimagua, both or one of the vesicles forming fungi intensified competitive ability with increasing P application: it may be assumed that it was *G. manihotis*, because *G. fasciculatum* was negatively affected by P fertilization at Agua Blanca.

Discussion

In this report we used the relative incidence of vesicles in the roots as an indication of the presence of *G. manihotis* and *G. fasciculatum*. This is feasible because anatomical structures of VA mycorrhizal fungi in the roots serve as such indicators[1]. We concede that the calculations might be an underestimation of the presence of these fungi. From the vesicles forming fungi, *G. manihotis* is known to be one of the most effective species for increasing cassava growth on acid soils; *G. fasciculatum* is of low to moderate effectiveness[11]. As shown by the greenhouse trials, species of VA mycorrhizal fungi may differ in their effectiveness with increasing P fertilization. From the literature there are also some indications that fungal species might differ in their adaptation to certain soil chemical conditions[8,20].

The field trials showed that all naturally occurring endophytes were able to make efficient use of P dressings of 50 kg P/ha added to those very low P containing soils. However, a growth response to higher P applications was not found in Agua Blanca since none of the

endophytes was adapted to those P levels. At the other two sites the P tolerant *G. manihotis* was natively present and there a growth response up to 200 kg P was found. The extent of the response to increasing P application depended on the relative enhancement of this endophyte in the roots, as was clearly visible when the sites are compared (Tables 2, 4 and 7).

The change in mycorrhizal incidence in the roots during the growth period of cassava may be partly due to the different development stages of the plant[14]; however, with cassava the main effect was due to the rainy and dry season[19].

From the results we may conclude that the composition of the native VA mycorrhizal population determines the level of response to P application on acid low P soils. However, site specific recommendations of higher P applications will only be possible if a methodology is developed to determine rapidly the population of P tolerant species of VA mycorrhizal fungi in a specific field sample.

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