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Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet

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Summary Genotypes of pearl millet (*Pennisetum americanum* L. Leeke) were examined for differences in vesicular-arbuscular mycorrhizal (VAM) colonization and response to inoculation. For thirty genotypes tested across three field locations there was a range of mycorrhizal colonization intensity between 25 and 56%. In another experiment with two male-sterile lines, restorer lines and their derived crosses, grown in pots filled with non-sterilized soil there were significant differences between genotypes for colonization by mycorrhiza. This showed host-genotype dependence for mycorrhizal colonization.

Root growth rates, mycorrhizal root length, percentage root colonization and plant growth and P uptake were studied in ten genotypes. A set of 3 genotypes with similar root lengths varied significantly with regard to mycorrhizal root length and the percentage colonization. This supports the suggestion that VAM colonization and spread is dependent on the host genotype. The growth responses differed significantly between the genotypes and they also differed in their responses to P uptake and VAM inoculation. The utility of host-genotype dependent differences in VAM symbiosis in plant breeding is discussed.

Introduction

Plant growth response (efficiency) to mycorrhizal symbiosis depends on three major components, the plant, the mycorrhizal fungus and the soil environment. An improvement in this efficiency can be made by manipulating any of these three components. Many workers have attempted to identify and select mycorrhizal fungi with improved performance in particular environments[14]. For example, Carling and Brown[5] screened various fungal isolates under low and high soil fertility regimes. Soil factors affecting mycorrhizae fungi have also been examined[11], but there is little work on plant dependent variation in mycorrhizal symbiosis.

An essential requirement for good host plant response to mycorrhiza is a rapid colonization of roots during the early growth stages of the

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host plant. Mosse[17] describes variation in colonization between plant species; some were colonized freely, while certain grasses rarely showed 50% colonization. Powell and Sithamparanathan[19] found that when legumes and grasses were inoculated under similar conditions, with three separate VAM fungal species, the legumes averaged 67% root colonization but the grass species had only 30%. There could also be differences in VAM colonization between cultivars of the same crop species[2].

Genotype dependent variation in nutrient uptake, for example phosphorus[8], has been well documented[7]. Certain genotypes of sorghum are adapted better than others to P deficient conditions and are efficient in the uptake of this element. Reports on genotype differences in P uptake have not examined the VAM status of the plants. It is possible that such adaptations to nutrient stress are mediated by mycorrhiza. Since mycorrhiza are considered to play a role in P uptake, it would be reasonable to expect that genotype differences for mycorrhizal symbiosis and responses might also exist. In this paper we examine the root colonization of several pearl millet genotypes for differences in the extent of root colonization, and plant growth and P uptake response in the glasshouse and field.

Materials and methods

Field trials

In order to examine if VAM colonization was host dependent, thirty genotypes of pearl millet were tested across three Alfisol field locations (A, B and C) at the ICRISAT Center in India. All sites carried a natural population of four mycorrhiza genera (*Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis* (Table 1).

The first trial (Alfisol A) was conducted during the rainy season (June to October) of 1982 with no irrigation and the other two during the summer (Jan to May) of 1983 with furrow irrigation as required. The experimental area was divided into 4 blocks of 30 plots each of two 3 m rows, spaced 75 cm apart. Treatments (genotypes) were arranged in a randomized block design. Seeds were hand sown 3–5 seeds per hill with a distance of 10 cm between hills. Thinning to one plant per hill was carried out 21 days after planting. Urea at the rate of 20 kg N/ha was top dressed as a band placement 25 days after emergence for all trials. Roots were sampled at 60 days after planting. Five plants were selected and the shoot portion was cut off at the collar zone close to the soil surface. Roots from each plant were carefully dug up to a depth of 30 cm taking care to avoid roots from the neighbouring plants. Sampled roots were placed in plastic bags and taken to the laboratory, where they were cut to 3 cm lengths and mixed thoroughly. Four random sub-samples (2–3 g fresh sample) for each plant were transferred into 30 ml screwcap Bijou bottles and processed for the determination of percentage colonization[18].

Pot trials

General method. The VAM fungus *Gigaspora calospora* Nicol. and Gerd. was maintained on *Cenchrus ciliaris* L. Link., a perennial host, grown in a sterilized sand:alfisol soil mixture (1:1 v/v) for a minimum period of 90 days. For VAM inoculation, 50 ml sand:soil mixture containing *ca.* 2000 extramatrical chlamydospores were distributed uniformly into the soil

Table 1. Soil characteristics of the three Alfisol field locations

Location	Soil type	Total N (%)	$NaHCO_3$ extractable P (ppm)	pH	Previous crop	VAM flora Genera*	VAM flora Spores/ 25 ml soil
A	Alfisol	0.03	3.5	6.2	Fallow	G, Gig, A, Sc	105
B	Alfisol	0.04	5.0	6.7	*Arachis hypogaea*	G, Gig, A, Sc	263
C	Alfisol	0.07	20.0	7.0	*Arachis hypogaea*	G, Gig, A, Sc	560

*G = Glomus; Gig = Gigaspora; A = Acaulospora; Sc = Sclerocystis

meant for each pot by mixing in a sealed plastic bag. Spore washings were given to the uninoculated control treatments. Plants were grown in alfisol soil, partially sterilized by steaming for 1 h in a Lindig soil sterilizing apparatus or non-sterilized, in 20 cm pots filled with 5 kg soil. Three seeds were centrally sown and two weeks later seedlings were thinned to one per pot. Pots were weighed and watered regularly to 80% of the field capacity. Nitrogen, as urea, at the rate of 60 kg N/ha (275 mg urea/pot) and phosphorus, as triple super phosphate, at the rate of 5 kg P/ha (175 mg triple super phosphate/pot) was incorporated into the soil, prior to sowing. The experiments were conducted in a glasshouse with temperatures ranging from 26 to 35 c. Pots were arranged on tables as randomized blocks. The position of the replicates was rotated every week among the benches in the glasshouse to reduce any positional effects within the glasshouse. Shoot and root dry weights were recorded after drying in a hot-air oven at 60 C for 72 h.

Roots from each plant were washed free of adhering soil on a sieve, and cut into 3 cm segments and mixed thoroughly. Four sub-samples containing between 3–5 g roots were removed, pooled, and transferred into bottles, 10% KOH added and the tissue cleared by steaming in a steam sterilizer at 100 C for 5 min. Followed by staining with 0.08% trypan blue[18]. The percentage VAM colonization, the total mycorrhizal root length and the total root length were estimated using a grid-line intersect method[10]. Phosphorus in the plant tissue was estimated according to Jackson[13], nitrogen by the macro-Kjeladhal method[3] and the mycorrhizal dependencies were calculated according to the method of Menge, Johnson and Platt[15].

Parent-progeny comparison. We compared the VAM colonization of two selected male sterile (female parent) lines (5141A and 111A), 5 R lines (restorer lines, the male parents) and their derived crosses. Plants were grown in an alfisol soil, with pH 6.0 and 4.0 ppm NaHCO extractable P; and they were harvested 60 days after planting. Pots were arranged in a randomized block design with 3 replications.

Growth response variation. The second pot experiment used ten pearl millet genotypes selected from the 30 used in the field trials to examine if genotypes varied with respect to VAM colonization and whether they responded to inoculation. For each genotype, there were 3 treatments: 1) uninoculated control in sterilized soil, 2) inoculated with *Gigaspora calospora* cloned from a single spore isolate, and 3) non-sterilized soil representing the natural VAM flora. The soil had a pH of 6.0 and contained 1.5 ppm NaHCO-extractable phosphorus. Plants were harvested 60 days after planting. Pots were arranged in a randomized block design with 6 replications.

Another pot trial examined the response of three West African pearl millet lines for dry matter, and phosphorus uptake. *Gigaspora calospora* was inoculated in both sterilized and non-sterilized alfisol soil and pots without inoculation served as control. The soil had a pH of 6.8 and contained 4.5 ppm NaHCO extractable P, and VAM fungi of the genera Glomus, Gigaspora, Acaulospora and Sclerocystis, at a total population of 135 spores per 150 ml soil. Pots were arranged in a randomized block design with 4 replications.

Results

The 3 field trials aimed to study the variation between 30 genotypes of pearl millet to mycorrhizal colonization by the indigenous VAM fungi. The mean mycorrhizal colonization of the genotypes, across locations, varied between 26 and 57% (Table 2). The ranges of percentage VAM root colonization for locations A, B and C were 13 to 44, 10 to 59 and 35 to 77 respectively. Overall, the genotypes tended to show similar rankings for VAM colonization. Genotype times location interaction was highly significant ($P \leqslant 0.01$).

In the first pot experiment the male sterile (MS) line, 5141A was more colonized by VAM than the other MS line, 111A (Table 3). Two crosses made using 111A as female parent and 631 P-3 and 733 P-1 as male parents resulted in hybrids with more VAM colonization than either of the two parents. A cross between 5141A and 623 P-2 resulted in F1 progeny which had less VAM colonization than either of the parent lines. Colonization of many other crosses derived using different male parent lines was not affected. A single representation of this group (612P-1) has been shown in Table 3.

The relation between root growth (total root length), the total mycorrhizal root length, the percentage VAM colonization of the ten genotypes, and the growth and P uptake response to inoculation were determined in another pot trial with 10 genotypes. Analysis of variance revealed that the genotypes varied significantly ($P \leqslant 0.05$) in VAM colonization by a single inoculum strain and also when grown in non-sterilized soil containing several indigenous VAM fungal species (Table 4). The total root length of the 10 genotypes grown in sterilized soil with *Gigaspora calospora* was statistically similar, but the total length of mycorrhiza in the roots (mycorrhizal root length) varied significantly ($P \leqslant 0.05$) between genotypes. Similarly, under the unsterilized condition, a group of genotypes (IP 5140, IP 4937 and MBH 110) recorded different amounts of total mycorrhizal root length.

Total root length and the level of mycorrhizal colonization varied between cultivars (Table 4). Generally root growth was doubled by the presence of VAM, presumably because of the better plant nutrition. There was an interaction between cultivars and soil treatment with respect to VAM infection level. Most cultivars had similar lengths of infected root between treatments, but for cv IP 4937 more VAM development occurred in the non-sterilized inoculated soil than in the non-sterile soil. Total root length did not differ between soil treatments.

The plant growth (Plate 1) and dry-matter responses to mycorrhizal inoculation (*Gigaspora calospora*) of the 10 pearl millet genotypes

Table 2. Genotype dependent variation in the mycorrhizal colonization of pearl millet grown at three different field locations with genotypes ranked according to overall means

Genotype	Country of origin	VAM colonization (%)[a]			
		Alfisol A	Alfisol B	Alfisol C	Mean
IP 3277	India	15	11	51	25
IP 3476	India	13	20	49	27
IP 5781	Nigeria	16	32	39	29
IP 3595	India	22	10	56	30
IP 5150	Niger	24	30	35	30
IP 4382	India	15	22	56	31
IP 6891	Tanzania	25	32	40	32
IP 6045	Niger	21	33	41	32
IP 6114	Cameroun	25	28	49	34
IP 4807	India	23	28	57	36
IP 5427	Niger	26	27	56	36
IP 5335	Niger	22	33	55	37
WC-C75	India	17	23	73	38
IP 6590	Malawi	33	40	42	38
IP 6139	Cameroun	28	35	53	39
IP 4861	Lebenon	22	25	70	39
ICH 220	India	30	36	52	39
BJ 104	India	27	34	58	40
MBH 110	India	23	30	67	40
IP 5420	Niger	15	42	62	40
IP 3840	India	27	42	52	41
IP 3120	India	25	26	77	43
IP 5692	Nigeria	29	33	70	44
IP 5306	Niger	22	44	70	45
IP 5009	Nigeria	23	47	74	48
IP 5310	Niger	44	43	61	50
IP 4937	Uganda	30	50	66	48
IP 6538	Mali	33	41	74	50
IP 5140	Niger	37	49	77	54
IP 5921	Senegal	42	59	67	56
	SE (Genotypes)	± 3.1	± 4.4	± 4.2	± 2.1
	Mean	26	34	57	39
	SE (Location)		± 1.5		
	CV %	24	12	14	18

Alfisol A – Low fertility; not cultivated previous two seasons; 3.5 ppm Olsen's P.
Alfisol B – Low fertility; cultivated previous season; 5.0 ppm Olsen's P.
Alfisol C – High fertility; under long term cultivation; 20.0 ppm Olsen's P.
[a] – each value is a mean of 20 observations made from each of 4 replicate plots of 2 rows and 3 m in length.

Table 3. Mycorrhizal colonization of parent genotypes and some selected crosses of pearl millet grown in natural field soil under greenhouse condition

Genotype	Percentage colonization[b]
Female parent	
5141A	51
111A	37
Crosses and male parent	
4141A × 631 P-3	51
111A × 631 P-3	57[a]
631 P-3	37
5141A × 733 P-1	41
111A × 733 P-1	54[a]
733 P-1	38
5141A × 612 P-3	50
111A × 612 P-3	50[a]
612 P-3	65
5141A × 623 P-2	44[a]
623 P-2	56
5141A × 612 P-1	66
111A × 612 P-1	54
612 P-1	63

CV (%) 15

[a] Crosses showing significantly higher/lower colonization compared with either of the parents ($P \leq 0.05$) using Duncan's multiple range test.

[b] Values are means from 3 replicate pots with one plant each.

grown in sterilized soil varied significantly (Table 5). IP 5921 gave maximum response to inoculation and recorded very low dry matter without mycorrhiza. There was little difference between the growth of genotypes in sterilized soil with a single VAM isolate and the growth in non sterilized soil. ICH 220 and IP 4382 produced more plant dry matter after inoculation in sterilized soil, than in non-sterilized soil. Analysis of variance showed that phosphorus uptake of the ten genotypes inoculated with single VAM isolate in sterilized soil or due to indigenous VAM flora in non-sterilized soil also differed significantly ($P \leq 0.05$) (Table 5). There was also a significant ($P \leq 0.05$) difference between genotypes in P uptake for all 3 inoculation regimes.

Total mycorrhizal root length and the percentage colonization were correlated ($P \leq 0.01$ df 28) with total root length, shoot and total plant dry matter, and total phosphorus uptake (Table 6). Root dry weight was not correlated with total mycorrhizal root length. The tissue phosphorus concentration was not related to mycorrhizal colonization of roots. This is presumably because genotypes vary in their strategy for coping with low P availability in the non-mycorrhizal state.

Table 4. Genotype dependent variation in percentage VAM colonization, total mycorrhizal root length and total root length as influenced by mycorrhizal inoculation

Genotype	VAM colonization*				Total mycorrhizal root length* (m/plant)				Total root length (m/plant)			
	Sterile Inoc.	Non-sterilized	Mean[a]	Sterile Non-Inoc.	Sterile Inoc.	Non sterilized	Mean[a]	Sterile Non. Inoc.	Sterile Inoc.	Non-sterilized	Sterile Inoc.	Mean
IP 5921	46	36	41	0	63	70	67	0	132	162	39	108
IP 4937	18	44	31	0	34	95	21	0	187	221	137	182
IP 5140	19	30	24	0	15	23	65	0	78	76	31	62
MBH 110	36	29	42	0	85	53	54	0	239	104	63	136
WC C75	32	47	39	0	74	72	73	0	207	157	76	147
ICH 220	18	25	22	0	44	63	70	0	239	232	94	189
IP 4861	22	26	25	0	30	30	20	0	134	110	94	113
BJ 104	17	24	21	0	20	19	31	0	115	75	78	90
IP 4382	29	23	26	0	59	63	60	0	200	229	68	166
IP 4807	35	30	33	0	39	15	28	0	112	47	103	148
SE (Geno × Inoc.)	± 3.2		± 2.3		± 17.4		± 12.3			± 58.6		± 33.8
Means	27	33			47	50			182	142	78	
SE (Inoc.)	± 1.1				± 5.5					± 18.5		
CV (%)		18				17				15		

* Statistical analysis performed without considering sterile non-inoculated treatment since all the values were zeros.
[a] Genotypes differed significantly for mean VAM colonization and total mycorrhizal root length ($P \leqslant 0.05$), by ANOVA.
Non-Inoc = No inoculation; Inoc. = Inoculated with a single VAM isolate (*Gigaspora calospora*); Non sterile = Natural VAM flora.

Table 5. Genotype dependent variation in growth response and phosphorus uptake due to VAM inoculation

Genotype	Plant dry-matter (g/plant)				Mycorrhizal dependency	Phosphorus uptake (mg/plant)			
	Sterile Non-Inoc.	Sterile Inoc.	Non Sterile	Mean		Sterile Non-Inoc.	Sterile Inoc.	Non Sterile	Mean
IP 5921	6.2	15.2	15.4	12.2	251	11	28	29	23
IP 4937	14.1	17.2	16.8	16.0	122	10	25	15	17
IP 5140	14.2	15.6	14.7	14.8	110	22	31	17	24
MBH 110	13.4	15.4	15.5	14.7	115	6	14	11	10
WC-C 75	15.1	16.4	16.6	16.0	108	11	11	11	11
ICH 220	16.6	18.5	16.8	17.3	112	7	16	15	13
IP 4861	12.6	15.4	13.9	14.0	122	6	15	6	8
BJ 104	10.2	13.1	14.7	12.7	128	6	14	18	13
IP 4382	12.7	16.9	14.3	14.6	133	7	16	12	11
IP 4807	11.2	13.4	14.1	12.9	120	11	15	10	12
SE (Geno × Inoc)		± 0.81		± 0.47			± 2.8		± 1.6
Mean	12.7	15.7	15.3			9	19	13	
SE (Inoc)		± 0.25					± 0.9		
CV (%)		15					25		

Non Inoc. = No inoculation; Inoc. = Inoculated with a single VAM isolate *Gigaspora calospora;* Non Sterile = Natural VAM flora.

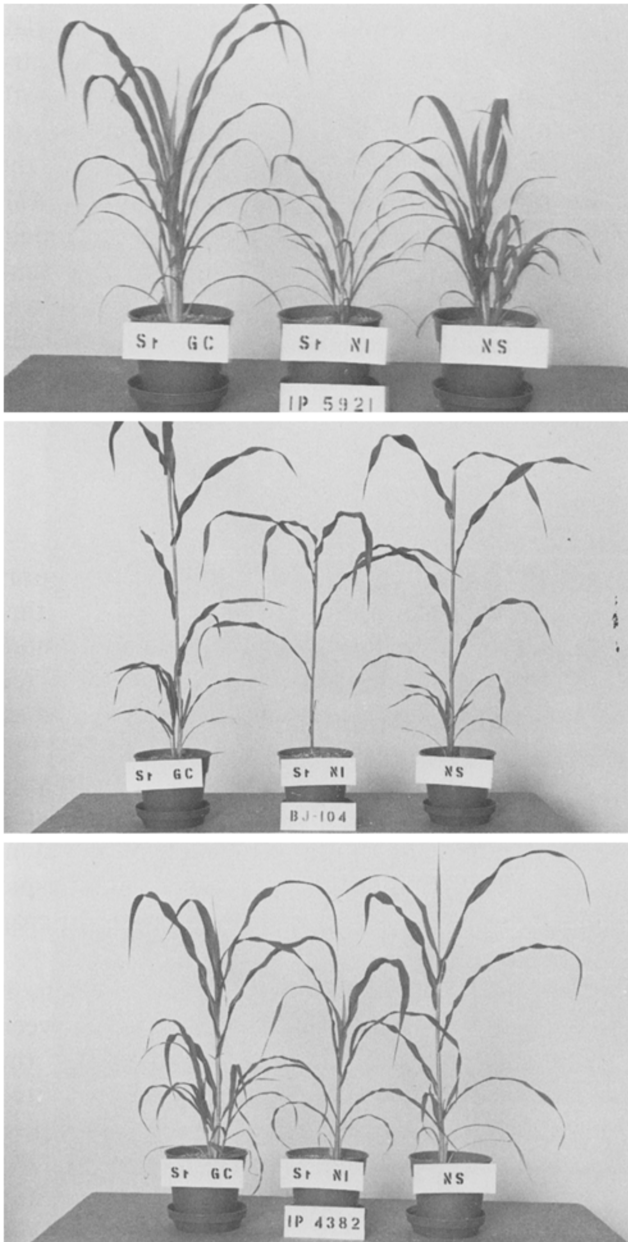

Plate 1. Three pearl millet genotypes showing differential response to inoculation with a single VAM isolate *Gigaspora calospora* (St Gc) indigenous VAM flora *i.e.* non-sterilized soil (NS) compared with uninoculated control in sterilized soil (St NI). Note higher growth of IP 5921 when inoculated compared to naturally available VAM fungi.

The response to inoculation with *Gigaspora calospora* resulted in significant ($P \leqslant 0.05$) increase in dry matter and P uptake by the two West African cultivars, IP 5921 and ZAN but the third cultivar SDL did not respond (Table 7). The cultivar ZAN produced most plant dry matter, and had the largest response to VAM inoculation in both sterilized and non-sterile soil. The cultivar IP 5921 also responded to inoculation in non-sterile soil. Cultivar ZAN was dependent on the presence of VAM for P uptake, and grew poorly without the VAM. In the non-sterile soil plant growth, however varied between inoculated and noninoculated treatments even though they had the same level of mycorrhizal colonization, suggesting that *G. calospora* was more competitive and efficient fungus that the strains of the indigenous population. The other two cultivars were able to extract sufficient P from the soil for reasonable plant growth in the absence of VAM.

Discussion

This study shows that the genotypes of pearl millet can support different amounts of mycorrhizal colonization when exposed to either a single VAM isolate or a mixture as found in the field or in non-sterilized soil in pots. Comparing host crop species, Mosse[17] reported that certain legumes showed greater levels of colonization than grasses. Root colonization levels vary a great deal between plant species. For pearl millet genotypes grown in the same field colonization can vary from 25 to 56%. Menge[14] believes that rapid and high levels of colonization may be the prime determinant of the efficiency of the symbiosis. Thus, screening and selection of genotypes which allow rapid and high levels of colonization may be a factor to incorporate in crop breeding programs.

Huisman[12] pointed out that percentage colonization, is often, if not always confounded by differential root growth rates between genotypes or species. Our study suggests that 34% (Table 6) of the variation in percentage colonization of pearl millet can be attributed to variation in root length. However, for several genotypes with a similar root length there were large differences in the extent of VAM colonization. Genotype variation in root colonization and response to VAM could be due to an interaction between host genotype and VAM strain preferences[17]. The number of infection sites on the root could be a factor[4,20]. Different levels of colonization between genotypes could arise from differences in the rate of growth of the fungus through the root cortex[20] and our present single harvest experiment

Table 6. Correlation matrix showing relationships between VAM, plant growth and phosphorus uptake parameters (second pot experiment)

Parameter	Coefficient	
	% Colonization	Mycorrhizal root length
Percentage colonization	1.000	
Total mycorrhizal root length	0.991**	1.00
Total root length	0.584**	0.640**
Shoot dry weight	0.500**	0.520**
Root dry weight	0.331*	0.250
Plant dry weight	0.560**	0.585**
Percentage P in tissue	0.170	0.207
Total phosphorus uptake	0.444*	0.460**

Significance: * $P < 0.05$, ** $P < 0.05$.

Table 7. Influence of mycorrhizal inoculation on the growth and phosphorus uptake of three West African pearl millet genotypes

		Shoot dry weight (g/plant)		Phosphorus uptake (mg/plant)		VAM colonization (%)	
Soil	Genotype	M	NM	M	NM	M	NM
	IP 5921	6.91	4.74	16.3	13.0	48^{c}	0
Sterilized	SDL	4.96	4.20	16.3	13.8	14^{a}	0
	ZAN	7.83	2.93	19.3	8.3	31^{b}	0
	IP 5921	5.23	4.73	15.2	14.3	78^{d}	44^{bc}
Non-sterilized	SDL	4.91	3.92	11.7	10.2	46^{bc}	35^{bc}
	ZAN	5.47	4.03	12.7	11.0	53^{cd}	52^{cd}
SE (Genotype X inoculation)		± 0.334		± 0.81			
Mean		5.82	4.10	15.25	11.77		
SE (inoculation)		± 0.162		± 0.620			
CV((%)		20		20		10	

Each value is a mean of 8 observations.
IP 5921 = Germplasm line from Senegal; SDL = Sadore local; ZAN = Zanfarwa.
M = Inoculated with mycorrhiza – *Gigaspora calospora;* NM = Non inoculated.
For VAM colonization, values with similar superscripts are not significantly different at $P \leqslant 0.05$; analysed after Log (x + 1) transformation.

cannot differentiate between the two factors. The latter would be partly related to the balance of inhibitory substances produced in response to the infection such as phenolics, phytoalexins, and promoting substances such as carbohydrates. Root tissue R concentration may also affect the rate of colonization[16].

The VAM colonization level varied between location increasing with VAM spore populations in the soil and soil P level. However, the differences in colonization could also be due to strain variation between sites within the VAM soil inoculum. The location with the

greatest level of VAM colonization had been in continuous cultivation for the past 8 years, given high rates of N and P fertilizer, with rotation between crops of sorghum, groundnut and pearl millet. The pearl millet genotypes grew most rapidly at this site and presumably soluble carbohydrate levels in the roots were also greatest.

The plant growth response to VAM inoculation was also genotype dependent. For example the genotype ZAN, had a large response to inoculation in both sterilized and non-sterile soil. Cultivars of wheat have differed in the dry-matter response to VAM inoculation[1]. There were differences between genotypes in their response to VAM colonization some being much more dependent than others on the VAM (Table 5). The cultivar WC C 75 was unique with no effect of the VAM on plant growth or P uptake.

While there was a significant correlation between the extent of mycorrhizal root length and plant dry weight, this accounted for only 34% (Table 6) of the variation in plant dry weight. Mycorrhizal infection can affect the biochemical and physiological activities of plants[6,9], and this response is likely to be genotype dependent. Pearl millet showed differences between genotypes in P uptake in response to VAM infection. Thus the VAM activity with regard to P uptake and translocation may be under the control of host genetic constitution and the physiological need for this element. A VAM fungus identified as highly efficient for P uptake on one host, perhaps even a genotype within a crop species, may not perform efficiently when tested on another host.

The differences in percentage VAM colonization between parents and progenies suggests that it is a heritable trait and therefore the possibility to breed for increased mycorrhizal colonization exists.

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