SCREENING FOR THE EFFICIENCY OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI ON PEARL MILLET AND SORGHUM

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ABSTRACT

Six cultures were tested as Vesicular arbuscular mycorrhizal (VAM) inoculants for pearl millet (Pennisetum americanum Leeke) grown in pots containing alfisol soil under glass house conditions. The VAM fungi varied in their ability to stimulate plant growth and P uptake. Inoculation with Gigaspora calospora, G. margarita and Glomus fasciculatum increased shoot dry-matter 1.5 folds over the control. One of the cultures, E3 resulted in growth depression. Rankings of the same six VAM cultures when tested on sorghum was different suggesting specificity between the isolates and the host plant concerned. In the field, four VAM cultures were tested on two genotypes of pearl millet -BJ 104 and MBH 110. The extent of response in plant growth due to VAM cultures differed from that found in the glass house trials, suggesting that rankings from a field screening would be different. Some problems in the screening of VAM fungi for efficiency are discussed and suggestions made.

INTRODUCTION

Cereal crops such as wheat[1], maize[4], barley[9] and finger millet[6] form vesicular-arbuscular mycorrhizal (VAM) symbiosis and acrue benefits from this association. Most of the work on cereal-VAM symbiosis has been done in temperate soil conditions. VAM occur on several semi-arid tropic (SAT) crops[18] and because the SAT soils usually have low fertility status, such associations may have a large effect on plant growth.

There have been situations where response to VAM inoculations has been marked, but at the same time lack of it has also been reported for the same crop . In case of soybean, a single genotype showed wide range in the degree of response to different VAM fungi , including growth depression due to some isolates. Variability in the response of diferent genotypes of a particular crop when inoculated with a single VAM fungal isolate has been studied only for pearl millet (Unpublished). In certain other cases the same fungal isolate-plant genotype combinations have not shown stability in the degree of reponse to inoculation, when put to test in different locations (personal communications). All these facts sum up to underline the immediate need for a well thoughtout screening and selection program if VAM symbiosis is to be succesfully used in the field, and obtain consistant responses for the given crop. Pearl millet and sorghum are major cereal crops of SAT regions and this paper deals with some efforts towards screening and selection of VAM fungi for these two crops.

MATERIALS AND METHODS

Pot trials:

A pot trial was conducted using 6 different VAM fungi- *Glomus fasciculatum*, *G. fasciculatum*(E3), *G. mosseae*, *Gigaspora calospora*, *Gigaspora margarita* and *Acaulospora sp.* Plants of pearl millet hybrid BJ 104 were grown in steam

sterilized alfisol soil mixed with sterilized sand (1:1 v/v), pH 8.2, 10.4 PPM $NaHCO_3$ extractable P and harvested 54 days after planting. Each treatment was replicated five times.

An experiment similar to one described above was run using the same VAM fungal species on sorghum(CSH 5). Soil, growth conditions, experimental design and replications and other procedures were as followed for the first pot trial on pearl millet.

The two VAM fungi giving the greatest response in the first experiment on pearl millet were used as inoculants for BJ 104 grown in sterilized alfisol soil:sand (1:1 v/v, pH 7.2, 7.0 PPM $NaHCO_3$ extractable P). Growth was compared between inoculated plants and plants grown in medium supplemented with P (given 230 mg P per pot as triple super phosphate, equivalent to 8 kg P per ha when extrapolated on a weight basis). Plants were harvested 63 days after planting. Each treatment was replicated eight times.

Mycorrhizal inoculum:

The VAM fungi were maintained on Cenchrus ciliaris, a perennial host, grown in sterilized sand:alfisol soil mixture (1:1 v/v) for a minimum period of 90 days. For VAM inoculation, extramatrical chlamydospores of the particular fungus were collected by wet sieving and ca. 600 of them

were layered 2-3 cm below the planting hole in each pot.

Pot culture:

Plants were grown in alfisol soil alone or mixed with sand (1:1 v/v) in 20 cm pots filled with 5 kg root medium. For P additions finely ground weighed amounts were added to the soil for each pot and mixed thoroughly in a plastic bag. The VAM inoculum was added to each of the 3 centrally placed planting holes followed by seeds of the pearl millet hybrid BJ 104. Two weeks later the seedlings were thinned to one per pot. Plants were watered to 60% moisture holding capacity by weight. The experiments were conducted in a glasshouse maintained at a temperature ranging from 26 to 35 C. Pots were arranged on tables as randomized blocks. Each replicate set containing all the treatments was rotated every week among the benches in the glasshouse (on round robin basin) to reduce positional effects within the glass house.

Field trial:

A factorial experiment was conducted in the field in alfisol soil with 6.7 PPM $NaHCO_3$ extractable P, pH 7.1, and supplemented with urea at 40 kgN/ha. This trial examined the responses of 2 pearl millet hybrids, BJ 104 and MBH 110 to inoculation with four different VAM fungi, Plots consisted of six rows spaced 75 cm apart and 5 m in length and the net plot size was 12 m^2. Treatments were allocated

to form a completely randomised block design with four replications. Four different VAM fungi were inoculated in the form of sand:soil mixture containing extramatrical chlamydospores and VAM colonized root pieces of Cenhrus ciliaris. Five kg of Sand :soil VAM inoculum with a spore density of approximately 600 per 50 g inoculum was applied for each plot of 30 m . The inoculum was placed 3 cm deep in the freshly opened furrow at the top of planting ridge. Seeds of pearl millet hybrids BJ 104 and MBH 110 were hand sown and 3 weeks the seedlings were thinned to give a 10 cm between two plans in a row.

Observations:

In the case of field trial, total dry weight and seed yield were recorded at crop maturity. For pot trials, plant height and dry weight were recorded at harvest. To determine the percentage root colonization by the VAM fungi, roots from each plant were carefully washed free of adhering soil and initially cut into 3 cm segments and mixed thoroughly. Four sub samples containing approximately 3-5 g roots were removed, pooled and cut into smaller segments of 1 cm length. They were then 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 0.05% trypan blue.[16] The percentage root colonization was calculated as follows:

$$\frac{\text{Number of VAM positive segments}}{\text{Total number of segments scored}} \times 100 = \% \text{ VAM colonization}$$

Phosphorus in the plant tissue was estimated by the vanadomolybdate method after digestion with tri-acid mixture (HNO:HCLO:H SO:10:3:1). Statistical analyses of the data were done using ANOVA.

RESULTS

Three of the six cultures tested as VAM inoculants, Glomus fasciculatum, Gigaspora calospora and G margarita produced more than a 1.5 fold increase in plant dry-matter over the uninoculated control (Table 1). Glomus mosseae and Acaulospora sp also increased yield but plant height and dry-matter production was less with G. fasciculatum E3 than those of control and other treatments.

Inoculation also resulted in a 2 to 3 fold increase in the concentration of P in the plant tissue, the level varying with the VA fungus. Highest tissue P concentration occured with Glomus fasciculatum (0.13%) and least with G.fasciculatum E3 (0.076%).

In case of sorghum, inoculation with G. fasciculatum, G. mossea or Gigaspora calospora resulted in two fold increase in dry weight over control (Table 2). G. calospora which ranked highest for pearl millet did not increase dry weight of sorghum significantly ($P<0.05$). G.fasciculatum E3 which depressed the growth of pearl millet significantly

increased the dry weight of sorghum. Percetage P concentration and total uptake of P was also highest in plants inoculated with G. fasciculatum. Phosphorus estimated in the bleeding sap showed significant positive correlation with the dry weight ($r=0.65; P<0.05$).

Increases in the total dry matter and grain yield of the two genotypes of pearl millet, BJ 104 and MBH 110 was not significant (Table 9). Rankings of VAM fungi for BJ 104 was similar to one found in pot culture trials but for MBH 110 there was a slight difference. Since, the differences in dry matter and grain yield were not significant, the rankings need careful consideration.

There again was an increase in P concentration in plant tissue of pearl millet following inoculation with Glomus fasciculatum; and a 3.6 fold increase in P uptake (Table 4). Gigaspora calopsora also stimulated plant growth and P uptake over that of uninoculated controls with or without P fertilizer addition. The shoot dry-matter produced by inoculated plants and those given P fertilizer was similar and significantly greater than for the uninoculated control.

DISCUSSION

There are differences between the three major genera of VAM fungi in their ability to stimulate plant growth and P uptake of pearl millet. Similar variations among VAM fungal

isolates have been shown for soybean . There may be specific interactions between the plant species and the mycorrhizal isolate. G.fasciculatum E3, an isolate known to increase growth and P uptake of barley and many other crops actually decreased growth and P uptake of pearl millet compared with the uninoculated plants. Such negative effects on plant growth due to VAM inoculation have also been recorded for maize, oats[14] and soybeans[2]. Hence selection of an efficient VAM strain needs to be made for each crop but little work has been done in this direction.

Some important criteria for screening efficient plant VAM symbiosis combinations could be plant dry matter, final yield, total P uptake, percentage P concentration in the tissues concentration of P translocated (i.e. in bleeding sap) and P translocated per unit time in the bleeding sap.

Selection based on the final dry-matter production is a time and space consuming process when a large number of isolates are to be tested; thus there is a need for a technique which measures the efficiencies of VAM fungi at an early stage in the plant-VAM symbiosis which correlates well with the enhancement of final yield and P uptake.

The differences in P concentration in the tissue due to VAM inoculation are not usually significant. Often due to plants inherent ability to maintain a threshold P concentration the VAM effects on tissue P concentration gets masked. The total P uptake seems to be single relevant

criterian to decide on the efficiency of symbiosis, when it is solely mediated by P uptake. However, this system has its own draw back in that when we compare diverse genotypes with wide ranged growth rates and duration to maturity the total P uptake as a differentiating characteristic gets overwhelmed by the factor of dry matter i.e., plant growth rate.

In the present investigation, for sorghum, the bleeding sap method when standardized seems a sound method to distinguish VAM fungal efficiency for P uptake and translocation provided all the VAM fungi are tested on a single plant genotype and in this case CSH 5. It would be worthwhile to select the genotype having highest affinity (percentage VAM colonization) for such studies.

A recent report on peanut, shows a significant positive correlation between 'excess' alkaline phosphatase activity and 'excess' drymatter due to VAM inoculation . Similar results have been reported for onion although no such correlation have been calculated[12]. This method although sensitive is time consuming and needs sophistication.

Highly significant increases obtained in the pot experiments usually breakdown when tested in field. Perhaps mainly due to back ground VAM flora and other inhibitory organisms if any or due to soil nutrient status and environmental conditions.

Three main factors in VAM symbiosis are the plant, fungus and environment. Unfortunately not many have recognised the first factor as important. Some plant species have been designated as least responding (e.g., grasses) while legumes such as Stylosanthis and Trifolium as highly responsive to VAM fungal inoculation. However considering the wide diversity with which we can encounter among genotypes of a particular crop, it is felt that such a conclusion is premature. At ICRISAT we have obtained evidences to show that there is wide range in the degree of responsiveness of pearl millet genotypes to VAM fungi. Hence, this necessitates screening of plant genotype for higher affinity to VAM colonization. This has a great bearing on breeding programs of major mandate crops of the world, when percantage VAM colonization as an attribute is shown to be inheritable by plant species.

A second arm on the screening chart (Fig.1) would be the selection based on beneficial characteristics of VAM fungi- P uptake, translocation and plant growth increase. Having selected suitable plant genotype and the VAM fungal combination a major step would be to get repeated optimal growth increases in different situations and this needs screening under different soil and environmental conditions. Untill this stage it is easier to work in controlled pot culture conditions but further screenig needs to be done in field situation at different locations. Obtaining repeatable growth and yield increase due to VAM inoculation

in the field still remains an aim and not an observed fact.

Most investigators have attributed the increased plant growth and yield to increased P uptake resulting for VAM inoculation. Unfortunately very few have compared the VAM effect with P fertility response. For pearl millet, the increase in the plant growth due to VAM inoculation was equivalent to the addition of 8 kg P/ha as triple super phosphate fertilizer. Earlier studies showed that the growth increases due to VAM inoculation were equivalent to the addition of 50 kg P/ha for maize[10], wheat[11] and barley[12]. For Abelmoscus, a dicot, the VAM contribution was equivalent to 16.5 Kg P/ha[12]. The contribution of VAM in terms of P fertilizer equivalent would be expected to vary with the crop species and demand for P, plant age, VAM species, soil P status and growth conditions.

Hence, it is strongly felt that screening and selection will not only help in identifying the most efficient VAM isolate for a particular genotype of the crop but also work for optimising the VAM benefits.

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Table 1 Effect of VAM inoculation on growth and P uptake of pearl millet hybrid BJ104 grown in sterile alfisol soil

Mycorrhizal cultures	Shoot dry weight (g/plant)	Phosphorus uptake Concentration (% dry wt)	Phosphorus uptake Total (mg/plant)
Gigaspora calospora	21.07	0.11	23.2
Glomus fasciculatus	19.49	0.13	25.2
Gigaspora margarita	19.05	0.11	21.4
Glomus mosseae	17.16	0.09	15.9
Acaulospora laevis	16.46	0.09	15.2
G.fasciculatus(E3)	7.46	0.08	5.4
Control	14.51	0.05	8.0
SE	1.19	0.006	1.42
CV(%)	12	15	20

Values are means of 5 replicates; growth period 54 days.

Table 2: Influence of VAM fungi on phosphorus uptake and dry matter production of sorghum

VAM fungi	Percentage colonisation	Shoot dry matter (g/plant)	P uptake		P in bleeding sap	
			Conc. (% dry wt)	Total (mg/plant)	Conc. (µg/plant)	Total (µg/plant)
G. fasciculatum	66	1.93	0.51	9.8	83	25
G. mosseae	52	2.20	0.35	7.7	59	18
Gig. margarita	48	2.07	0.49	10.1	50	23
G. fasciculatum E3	40	1.43	0.10	2.5	77	17
Gig. calospora	36	1.14	0.31	3.6	28	7
Acaulospora sp	32	1.33	0.22	2.7	40	13
Control	25	0.98	0.17	1.7	20	5
SE ±		0.15	0.02	0.6	5	3
CV %	11	21	13	23	21	45

Table 3. Effects of inoculation of VAM fungi on two genotypes of pearl millet in the field

VAM fungus	BJ 104		MBH 110	
	Total dry matter	Grain yield	Total dry matter	Grain yield
	(kg/12 m^2 plot)			
Gig.calospora	6.55	3.14	7.07	3.64
Gl.fasciculatum	6.40	3.15	7.10	3.47
Gl.mosseae	6.33	3.08	6.66	3.64
Gig.margarita	6.84	3.18	6.19	3.05
Control	6.49	3.03	6.61	3.27

SE TDM: 0.51
GY: 0.20

CV% TDM: 15
GY: 18

Table 4 Comparison of mycorrhizal inoculation and phosphorous fertilization on pearl millet cv BJ 104

Treatment	Percentage colonization	Plant height (cm)	Shoot dry-matter (g/plant)	Phosphorous uptake	
				conc. (% drywt)	Total (mg/plant)
Glomus fasciculatum	60 A	72	7.2	0.14	9.8
Gigaspora calospora	57 A	76	9.1	0.14	13.2
Phosphorous	0 B	96	8.0	0.09	7.2
Control	0 B	56	5.8	0.08	4.2
SE	-	4.0	0.64	0.009	0.817
CV (%)	13	15	24	21	26

a Values are means of eight replicates; growth period 43 days.

b Data for per cent root colonization by mycorrhiza were analysed after (x+0.5) square root transformations and values with different superscript are significantly different at P#0.05; CV(%) calculated on transformed da

c 230 mg P/pot applied as triple super phosphate; uninoculated.

Screening for response to VAM inoculation - a flow chart

Plant genotypes

* High affinity
* Marked response

VAM Fungi

* Percentage colonization
* Phosphorus uptake & growth
* Spore production & viability

↓

Plant Genotype x Fungal isolate interaction

* Soil type
* Fertility condition
* Environmental condition
* Competitive ability

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* Field location trials