J. Mycopathol. Res. **42(2)** : *241-244 (2004)*

Selection of efficient arbuscular mycorrhizal species for growth and nutrition of sweet orange (*Citrus sinensis*) in red laterite soil of West Bengal

B. N. PANJA AND S. CHAUDHURI
Department of Plant Pathology, BCKV Research Complex Building, Kalyani 741235, Nadia, West Bengal

Sweet orange seedlings for experimentation were grown in pot containing highly porous acidic, low nutrient (particularly phosphorus) and arbuscular mycorrhizal status of red laterire soil under glass house condition. At the time of transplanting seedlings were inoculated separately with four different arbuscular mycorhizal fungal (AMF) isolates viz. *Glomus mosseae* (Kalyani), *Glomus mossage* (Bangalore), *Glomus fasciculatum* (Kalyani) and *Gigaspora margarita* (Kalyani) belong to three species and their mixed consortium. Plant height, root and shoot dry weight, phosphorus concentration and uptake by AM inoculated plants were significantly increased over non — mycorrhizal control plants. *G. fasciculatum* exhibited higher values for the said parameters including leaf member and leaf area as compred to other isolates tested as well as to their mixed consortium. The growth and nutrition promotion performance of the mixed consortium were found to be lower for the parameters tested from the performance of the single species/isolate inoculated sweet orange plants. *G. fasciculatum* appeared to be the best performing among the AM species/isolate tested for growth and phosphorus nutrition improvement of sweet orange plant grown in red laterite soil of West Bengal.

Key words : Mycorrhiza, phosphours, sweet orange plant

INTRODUCTION

Sweet orange (*Citus sinensis* Osbeck) cultivation is gaining importance in homestead and commercial gardens in the lateriate soil of West Bengal. Sustainable growth and productivity of this crop through nutritional management has become a serious concern owing to high soil acidity, deficiency of major plant nutrients particularly phosphorus, low water holding capacity resulting scarcity of soil moisture during drought cycle and inherently poor arbuscular mycorrhizal fungal (AMF) status. AM fungi are known to support growth and productivity of crop grown in nutrient dificient soil by augmenting the sone of nutrient acquisition and their efficient transport (Hattingh *et al.*, 1973), to help the plant to nutrient acquisition and their efficient transport (Hattingh *et al.*, 1973), to help the plant to withstand drought by improving water relation (Levy and Krikun, 1980) and also help the plant to tolerate the disease. Citrus crop with sparingly branched magniliod type of root system is considered foremost among the mycorrhiza dependent crops (Menge *et al.*, 1978). The application of mycorrhiza to such nutritionally stressed and poorly mycorrhizal laterite soil hold promise for growth, productivityand nutritional management of AM dependent sweet orange plant. Since AM fungi differ in their capacity to form efficient mycorrhiza, so before their application in agricultural application require the selection of efficient and appropriate fungus (Menge, 1983). Inoculation of *Citrus* spp. with efficient mycorrhizal spcies is known to improve their growth and nutrition (Chang and Chien, 1990).

Any attempt has hardly been made to take care of the improvement of growth and nutrition of sweet

orange plant in red laterite soil of West Bengal by using mycorrhizal technology specially with the introduction of efficient AMF species/isolates or their mixed consortium. An experiment was conducted with four different species/ isolates of AM fungi along with their mixed consortium with a view to screen out the best performing one with respect to the improvement of growth and nutrition of sweet orange plant in red laterite soil.

MATERIALS AND METHODS

An experiment was conducted during March — June in the glass house nursery of University Research Farm at Kalyani with sweet orange (*Cirus sinensis* Osbeck) seedlings following six treatments in four replicates arranged in complete randomized design. Four out of six treatments consist of four different arbuscular mycorrhizal fungal species/ isolates viz. *Glomus mosseae* (Nicol. and Gerd.) Gerdmann and Trappe (Kalyani isolate), *Glomus mosseae* (Bangalore isolate), *Glomus fasciculatum* (Thax. sensu Gerd.) Gerdemann and Trappe (Kalyani isolate), *Gigaspora margarita* Becker and Hall (Bangalore isolate). Mixed consortium of these isolates/ species and a suitable control constitute the rest two treatments of the experiment.

The inoculum of four different AMF species/ isolates as well as their mixture was prepared by inoculating the pot grown maize plants separately with these species/isolates and their mixture at the time of sowing and maintained them in the maize rhizosphere for 60 days. Maize plants root with 85% mycorrhizal infection were harvested separately, air-dried, finely powdered, mixed with their respective air-dried soil and stored in cold room for future experimental use.

The number of infective propagules per g of the mycorrhizal inoculum as well as the experimental soil was determined following a ten fold dilution series (Powell, 1980) using sterile soil as diluents and *Cajanus cajan* as test plant ant the thereby comparing the number of positive vials of the last three higher dilutions with the most probable number (MPN) table (Alexander, 1965). The number of infective propagules per g was 60 for *Glomus mosseae* (Kalyani), 56 for *G. mosseae* (Bangalore), 65 for *G. fasciculatum* (Kalyani), 50 for *G. margarita* (Bangalore), 59 for mixed culture and 4.3 for experimental soil.

Transplanting of one month's old sweet orange seedlings was done singly in 2.5 L earthen pot containing 3 kg coarsely sieved solarised red laterite soil transported from the Jhargram Sub-Division of Midnapore district in West Bengal. Seedlings were inoculated separately with four diferent mycorrhizal species or their mixed inoculum equivalent to 300 numbers of infective propagules. The seedlings under control set were givenequal amount of heat-killed inoculum. A toal of twelve plants per treatment were maintained for 90 days. During the entire period of experimentation no other treatment except normal watering is given to the sweet orange plant. Data on growth parameters were recorded after harvest i.e. at the 90 days of transplanting. The dry weight of plant was recorded after reaching to constant weight. Physico-chemical analysis of soil and chemical analysis of the plant samples were done according to the methods proposed by Dewis and Freitas (1984). Mycorrhizal roots were cleared and stained following the methods proposed by Philips and Hayman (1970) and its colonization was assessed as per slide micrometric method (Kormanik and McGraw, 1984). AM spore were isolated from soil by wet sieving and decanting method proposed by Gerdemann and Nicolson (1963). Spores with subtending hyphae were identified as per standard spore morphological description proposed by Schenck and Perez (1990). All data recorded were analyzed statistically following procedure laid down in Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Results of the physico-chemical analysis of the experimental red laterite soil exhibited the nature of soil as acidic, sandy loam with poor water holding capacity, low in organic carbon, nitrogen and phosphorus content (Table 1). Mycorrhizae are known to perform well under soil stress (Mosse, 1977). So, the red laterite soil stress may provide the ideal ecological niche for arbuscular mycorrhizal fungal growth, multiplication and

efficient function. Mycorrhizal analysis of the experimental soil revealed very lower number of propagules per 100 g and number infetive propagules per g. species diversity was also found low as evident from the record of only five mycorrhizal species belongs to three genera (Table 2). Based on the quantitative distribution, *Glomus fasciculatum* was found to be dominant followed by *G. mosseae*, *G. margarita* and other. Dominance of mycorrhizal species specific to certain crop — soil combination indicated its better suitability and adoptability as compared to others. As the quantity of these mycorrhizal species was very low in the experimental soil, the population of such species could be augmented wither by selecting suitable mycorrhizal susceptible crops as per-crop (Panja and Chaudhuri, 1998) or by the application of AMF single species inoculum from external sources.

Table 1 : Physico-chemical properties of the experimental red laterite soil

Mechanical composition (%)			pH	Organic carbon (%)	Total N (%)	Available (ppm)		Water holding capacity (%)
Sand	Silt	Clay				P	K	
52.2	20.0	25.8	5.5	0.18	0.025	5.1	98	31.3

Table 2 : Mycorrhizal status of the red laterite soil

AMF spores (per 100 g dry soil)	MPN of soil (No. per g soil)	Dominant species
162	4.3	*Glomus fasciculatum* > *Glomus mosseae* > *Gigaspora margarita* > *Gigaspora* spp. > *Acaulospora* spp.

Sweet orange seedlings were inoculated separately with four AM fungal species/isloate cultures and their consortium. Seedlings inoculated with either single species culture of AMF of their mixed consortium have singificant growth improvement in terms of plant height, leaf number, root — shoot dry weight as well as nutrient status especially phosphorus concentration and phosphorus uptake as compared to non — inoculated control (Table 3). Shoot nitrogen content of the inoculated plants did not differ significantly over control but phosphorus nutrition in terms of shoot's P concentraion and uptake of AMF inoculated plants was demonstrably increased. Improved growth of sweet orange plants appeared tobe associated with incremental P uptake and tissue P concentration (Hayman and Mosse, 1971 ; Hughes *et al.*, 1979).

Table 3 : Effect of arbuscular mycorrhizal fungi (AMF) inoculation on the growth performance of sweet orange in red laterite soil.

AMF species	Plant height (cm)	Leaf number	Average leaf area (cm^2)	Root dry weight (g)	Shoot dry weight (g)
Glomus fasciculatum Kalyani	15.50	12	7.45	0.96	0.86
Glomus mosseae Kalyani	14.90	10	5.48	0.72	0.63
Glomus mosseae Bangalore	13.50	10	5.37	0.84	0.64
Gigaspora margarita Bangalore	13.40	9	5.16	0.57	0.43
Mixed consortium of inocula	12.20	9	4.58	0.54	0.41
Control (Heat killed inoculum)	10.20	7	4.71	0.46	0.28
SEm ±	0.52	0.6	0.32	0.01	0.02
CD 0.05	1.59	1.8	0.95	0.03	0.06

Table 4 : Effect of arbuscular mycorrhizal fungi (AMF) inoculation on phosphorus and nitrogen nutrition of sweet orange in red laterite soil.

AMF species	P concentration (mg per g) shoot dry matter	P uptake (mg) per plant shoot matter	N content (g) per 100 g shoot dry matter	N uptake g per plant dry
Glomus fasciculatum Kalyani	4.45	3.83	1.83	0.016
Glomus mosseae Kalyani	3.95	2.49	1.60	0.010
Glomus mosseae Bangalore	4.05	2.59	1.76	0.010
Gigaspora margarita Bangalore	2.80	1.20	1.65	0.007
Mixed consortium of inocula	2.55	1.05	1.60	0.006
Control (Heat killed inoculum)	1.97	0.55	1.75	0.007
SEm ±	0.11	0.09	NS	0.005
CD 0.05	0.33	0.27		0.0015

Out of four AMF Species/isolates tested against sweet orange plant, *G. fasciculatum* (Kalyani) caused the highest stimulation of all growth and nutrient parameters recorded followed by *G. mosseae* (Kalyani) whereas *G. margarita* (Bangalore) and mixed consortium exhibited the least. Though the growth parameters viz. plant height, leaf number and leaf area of *G. mosseae* (Kalyani) whereas *G. margarita* (Bangalore) and

mixed consortium exhibited the least. Though the growth parameters viz. plant height, leaf number an leaf area of *G. mosseae* (Kalyani), *G. mooseae* (Bangalore) and *G. margarita* (Banglore) inoculated plant remained at par but they differed significantly with each other with respect to their dry biomass, P uptake and P concentration. Based onthe growth stimulation and nutrient uptake efficiency of different single species cultures/ isolates, *G. fasciculatum* (Kalyani) could be selected as the best performint AM fungus for sweet orange grown under red laterite soil (Table 4). Similar selection for identification of efficient AM fungus for nutrition improvement of citrus crop was made earlier by Vinayak and Bagyaraj (1990). Growth and P nutrition of mixed consortium inoculated sweet orange plant was also stimulated significantly over control but the same except plant height, leaf number and leaf area parameters were found significantly lower from the orange plants inoculated with single species cultures. So, the inoculation of orange plants for growth and nutrition improvement with mixed consortium instead of single species would not derive any additional benefit.

Therefore, it may be concluded from the abovementioned experimental results that *G. fasciculatum* appeared to be promising and screen out as the best AM fungus among the four species/ isolates tested for growth and P nutrition improvement of lemon under red laterite soil of West Bengal.

REFERENCES

Alexander, M. (1965). Most probable number method for microbial populations. In *Methods of Soil Analysis Part — II,* eds C. A. Black, D. D. Evans, J. L. White, L. E. Ensminger and F. E. Clark, Amer. Soc. Agron., Wisconsin, USA, pp 1467-1472.

Chang, B. K. and Chien, K. S. (1990). VA — mycorrhizae of cirtus seedlings inoculated with *Glomus epigaeum* and its growth effects. *Agriculture, Ecosystems and Environment.* **29**: 35-38.

Dewis, J. and Freitas, F. (1984). *Physical and Chemical Methods of soil and Water Analysis.* FAO of the United Nations, Rome, p 275.

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of mycorrhizal fungi isolated from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46** : 235-244.

Gomez, K. A. and Gomez, A. A. (1984). *Statistical Procedures for Agricultural Research,* 2nd Edition, New York, USA, John Willy and Sons, p. 680.

Hattingh, M. J. ; Gray, L. E. and Gerdemann, J. W. (1973). Uptake and translocation of ^{32}P leveled phosphate to onion roots by endomycorrhizal fungi. *Soil Sci.* **116** : 383-387.

Hayman, D. S. and Mosse, B. (1971). Plant growth response to vesicular — arbuscular mycorrhizal I. Growth of *Endogone* inoculated plants in phosphate deficient soils. *New Phytol.* **70** : 19-27.

Hughes, M., Chaplin, M. H. and Martin, L. W. (1979). Influence of mycorrhiza on nutrition of red raspberries. *Hort. Sci.* **14** : 521-523.

Kormanik, P. P. and McGraw, A. C. (1984). Quantification of vesicular arbuscular mycorrhizae in plant roots. In : *Methods and Principles of Mycorrhizal Research,* ed. N. C. Schenck, Amer. Phytopathol. Soc., USA, 37-45.

Levy, Y. and Krikun, J. (1980). Effect of vesicular — arbuscular mycorrhiza on *Citrus jambhiri* water relations. *New Phytol.* B **85** : 25-31.

Menge, J. A. ; Johnson, E. L. V. and Platt, R. G. (1978). Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. *New Phytol.* **81** : 553-559.

Menge, J. A. (1983). Utilization of vesicular arbuscular mycorrhizal fungi in agriculture. *Can. J. Bot.* **61** : 1015-1026.

Mosse, B. (1977). The role of mycorrhiza in legume nutrition on marginal soils. In : *Exploiting the Legume Rhizobium Symbiosis inTropical Agriculture,* eds J. A. Vicent, A. S. Wintet and J. Bose, College Trop. Agric. Univ, Hawaii, iscellaneous Publication, pp. 275-295.

Panja, B. N. and Chaudhuri, S. (1998). Effect of monoculature of some plants of inoculum build up and quantitative distribution of different VA-mycorrhizal species in the rhizosphere. *Mycorrhiza News.* **10** : 13-15.

Philips, J. M. and Hayman, D. S. (1970). Improved procedure for clearing roots and staining parasitic and VA-mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55** : 158-161.

Powell, C. L. (1980). Mycorrhizal infectivity of eroded soil. *Soil Biol. Biochem.* **12** : 247-250.

Schenck, N. C. and Perez, Y. (1990). *Manual for the Identification of VA — Mycorrhizal fungi,* 3rd edition, Gainesville, USA, Synergistic Publications, p. 286.

Vinayak, K. and Bagyaraj, D. J. (1990). Selection of efficient VA-mycorrhizal fungi for trifoliate orange. *Biol. Agril. Hort.* **6** : 305-311.

(Accepted for publication August 14, 2004)