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Effect of Native AM Fungus *Glomus fasciculatum* and Plant Growth Promoting Rhizomicroorganisms on Growth and Productivity of *Alpinia galanga* Willd

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Medicinal plants are potential renewable natural resources, they are generally considered to play beneficial role in human health care. Arbuscular Mycorrhizal Fungi(AMF) and Plant Growth Promoting Rhizomicroorganims(PGPRs) are known to improve plant growth through better uptake of nutrients, water and increased resistance to drought and root pathogens. The use of these beneficial microorganisms in medicinal plants particularly *Alpinia galanga* is limited in Tamil Nadu. The present study undertaken to examine the species richness, density, diversity and percentage of root colonization of AMF and PGPR associated *Alpinia galanga.* Native AM fungi were multiplied in the roots of *Sorghum vulgare* Pers in pot culture. A pot trial was conducted to study the influence of native AM fungus *Glomus fasciculatum* and some PGPRs(*Bacillus coagulans* and *Trichoderma harzianum*) on growth, nutrition and rhizome productivity of *A.galanga*.

Key words: Medicinal plants, *Alpinia galanga, Glomus fasciculatum,* AM fungus.

Mycorrhizae show widespread occurrence in natural ecosystem[1]. Importance of AMF in growth and development of plants has been increasingly realized. Medicinal plants play a vital role in human health care; about 80 per cent of the world populations rely on the use of traditional medicine, which is concomitantly based on plant materials. In recent years due to over exploitation of natural resources, biofertilizers have emerged as an important component of integrated nutrient supply system (INSS) and hold a promise for reducing the production costs, improve the crop yields, quality, nutrient supplies and sustaining the productivity over a longer period[2]. The role of soil microorganisms in sustainable productivity has been reviewed[3]. AMF represent one of the nature's best gifts to mankind in the conservation of arid soil to productive and fertile soil[4]. PGPRs are of great potential importance, the possible mechanism by which the PGPR and plant growth includes suppression of root pathogens through production and chelating of iron, production of antibiotics[5], fixation of nitrogen[6] and production of plant hormones[7]. Role of AM fungi and PGPRs in improving plant growth is well documented[8,9]. PGPRs are synergistic with mycorrhizae in stimulation of plant growth[6].

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MATERIAL AND METHODS

Two different areas of Thanjavur district, namely herbal gardens of Kattuthottam and Tamil University, Tamil Nadu, India with varying physico chemical characteristics were selected for the study. The average annual atmospheric temperature varied from 32.5°C to 24.2°C. Annual rainfall was 300 mm-350 mm. Soil sample was analysed for texture,pH,EC, N,P,K,Zn,Ca,Mn and Fe at the Soil Testing Laboratory, Tamil Nadu Rice Research Institute, Aduthrurai by using following standard methods[10,11]. The feeder roots and soil samples were collected from 0 to 40 cm depth in polythene bags and stored at 5-10°C. As the roots were washed thoroughly free of attached soil particles and cut into1 cm and fixed in formalin acetic alcohol (FAA) in the field itself. The roots were cleared in 10 per cent KOH and then stained with 0.08 per cent trypan blue[12]. The stained roots were observed for mycorrhizal infection. Presence of mycorrhizal association was confirmed by observing vesicles and arbuscles. The root colorization percentage of infection was calculated by using the following formula[13]

$$\% \text{ of root colonization} = \frac{\text{Total No. of Am positive segments}}{\text{Total No. of root segments observed}} \times 100$$

AM resting spores in rhizosphere soils were extracted by wet sieving and decanting method[14]and later identified with the key provided[15]. Spores and sporocarps of AM fungi were identified using the synoptic keys[16]. Maize plants were used for AM fungal inoculum preparation. Inoculum potential of AM fungi was done by using MPN method. To examine the synergistic effect of *Glomus fasciculatum* and PGPRs, the pot trial was conducted. The experiment was undertaken in brown sandy loam soil with 168.33 kg ha-1 available nitrogen, 18.68 kg ha-1 potassium. Soil pH was neutral to alkaline about 7.4. the experiment was laid out in randomized complete block design(RCBD), the treatments were no inoculation(control), inoculated with *Glomus fasciculatum*(Gf) alone, *Bacillus coagulans*(Bc)alone, *Trichoderma harzianum*(Th) alone, Gf and Bc, Gf and Th, Bc and Th,Gf with Bc and Th. Uniform rhizome bits about 5 cm length of *Alpinia galanga* sown in nursery beds and watered whenever necessary. Seedlings were also raised in uninoculated beds and all beds were maintained for five weeks. PGPRs namely *B.coagulans* and *T.harzianum* were multiplied in nutrient broth and potato dextrose broth respectively for ten days. They were mixed with sterile lignite powder separately to form a thick slurry which was used for dipping roots of *Alpinia* seedlings for 10 mins before transplanting to the pot. On 75th day of transplantation all growth observations like plant height, rhizome length and diameter, shoot, rhizome, root biomass and leaf yield were recorded. Mycorrhizal root colonization and chlamydospore number in root zone soils were assessed by grid line intersection method[13]. Phosphorous, and nitrogen contents in the roots, rhizome and leaves were estimated by vanadomolybdate yellow colour and microkjeldahl methods respectively[10,11]. Zinc, copper, manganese and iron contents were estimated by atomic absorption spectrophotometer[10].

RESULTS AND DISCUSSION

The diversity of AM fungi were observed from rhizosphere soils and the roots of *Alpinia galanga* were taken from two different localities. The soil types were red sandy loam and black sandy loam soils. Generally, the soils were nutrient deficient. *A.galanga* roots were recorded for AM fungal colonization in both localities. Percentage of root colonization was as low as 74.5 in *A.galanga* collected from Tamil University locality, as high as 100 per cent in Kattuthottam area. The presence of high degree of AM colonization with various AM fungal structures such as infection pegs, hyphal coils, hyphal dimorphism, intracellular arbuscules, inter and intracellular vesicles were observed. In the roots of *A.galanga,* twelve AM fungal species were isolated. *Glomus fasciculatum* was the predominant colonizing fungal species was identified. Based on the frequency of occurrence, the AM fungal species identified were grouped as

Table 1. Effect of *Glomus fasciculatum* and PGPRs on mycorrhizal effect percentage and morphological parameters of *A. galanga*

Treatments	Plant parts height(cm/plant)				Plant dry biomass(g/plant)			Mean
	Shoot Length	Root Length	Rhizom Length	Rhizome diameter	Shoot	Rhizome	Root	mycorrihal effect(%)
Control (without AM and Bc and Th)	26.5	24.6	13.2	2.4	3.41	4.35	1.02	-
Glomus fasciculatum (Gf)	33.4	28.5	14.8	3.2	5.82	4.62	1.27	159.86
Bacillus coagulans (Bc)	28.4	25.4	14.2	2.8	5.43	4.43	1.13	140.25
Trichoderma harzianum (Th)	27.2	25.2	13.8	2.6	4.13	4.36	1.12	138.26
Gf+Bc	34.5	30.2	14.6	3.4	5.93	4.46	1.35	160.25
Gf+Th	33.8	30.1	14.4	3.3	5.83	4.46	1.35	160.15
Bc+Th	29.4	26.2	14.1	3.2	4.22	4.41	1.25	140.15
Gf+Bc+Th	32.5	28.4	15.8	3.6	4.93	5.15	1.27	160.25

dominant (100 %), common(above 60 %) and rare(below 50 %) forms. *Gigaspora margarita, Glomus aggregatum, G. fasciculatum*, *G.geoporum* and *Scutelospora heterogama* were dominant forms. *Acaulospora bireticulata, A. scrobiculata, Glomus macrocarpum, G. mossae, Sclerocystis rubiformis, S. sinuosa* and *Scutellospora calospora* constitutes common forms.

All inoculation treatments were showed increase plant growth response. Among the 6 AM fungal isolates screened, *Glomus fasciculatum* was most efficient in improving growth, biomass and mean mycorrhizal effect (%) of *Alpinia* (Table 1). *G.fasciculatum* inoculated plants were showed significant increase in plant height and biomasss compared with control plants.

Table 2. Influence of *Glomus fasciculatum* and PGPRs on Phosphorous, Nitrogen and Potassium contents in *A.galanga* (mg/plant)

Treatments	Phosphorous			Nitrogen			Potassium		
	Shoot	Rhizome	Root	Shoot	Rhizome	Root	Shoot	Rhizome	Root
Control (without AM and PGPRs)	42.93	64.2	17.50	89.39	90.5	52.24	72.52	76.2	60.40
Glomus fasciculatum (Gf)	82.75	85.6	38.08	105.25	110.5	64.25	85.45	89.2	75.22
Bacillus coagulans (Bc)	62.56	72.2	22.0	92.62	94.5	60.25	77.42	80.4	70.45
Trichoderma harzianum (Th)	45.25	65.2	19.2	91.23	93.2	54.25	74.50	80.2	70.12
Gf+Bc	98.55	84.2	42.50	115.06	96.4	66.45	87.50	90.1	78.42
Gf+Th	97.50	80.5	41.25	113.25	96.2	65.26	85.25	91.2	76.42
Bc+Th	52.25	70.5	34.05	90.28	95.1	85.25	74.50	88.4	73.25
Gf+Bc+Th	74.50	90.4	35.56	102.48	115.2	62.48	72.60	92.4	76.20
SEM±	3.32	4.2	2.21	3.46	4.1	4.46	3.2	4.0	3.46
CD at 5% level	12.94	14.2	5.64	12.82	14.2	12.92	12.4	16.0	12.82

Dual inoculation of *G.fasciculatum* with either *B.coagulans* or *T.harzianum* and triple inoculation with all the three (Gf+Bc+Th) organisms also enhanced significantly greater plant growth. Dual inoculations, *G.fasciculatum* with *B.coagulans* and *G.fasciculatum* with *Trichoderma harzianum* enhanced shoot and root growth, potassium, nitrogen and phosphorous contents significantly when compared to inoculation with *G.fasciculatum* alone (Table 2). Mycorrhizal root colonization and spore numbers in the root zone soils were significantly more in all treatments as compared with control (Table 3).

Table 3. Percentage of root colonization and number of AM fungal spores root zone of *Alpinia galanga* as influenced by various treatments

Treatments	Root colonization (%)	Total number of AMfungal spores/100 g soil
Control	45.25 ± 1.2	120 ± 1.6
Glomus fasciculatum (Gf)	85.65 ± 1.6	450 ± 2.8
Bacillus coagulans (Bc) Inoculated with	52.48 ± 1.2	145 ± 1.2
Trichoderma harzianum (Th)	50.42 ± 1.4	140 ± 1.6
Gf + Bc	90.62 ± 1.4	565 ± 3.6
Gf + Th	88.45 ± 1.2	560 ± 3.2
Bc + Th	50.25 ± 1.4	220 ± 1.4
Gf + Bc + Th	60.25 ± 1.4	520 ± 3.2

Value are mean ± indicates S.D

The role of AM fungi in the improvement of plant growth and yield of crop plants in agriculture and horticulture is well documented[17]. The selections of sampling areas for *Alpinia* plants were based on soil characteristics as suggested[18]. Differences in AM colonization and spore number in the present study could be influenced by soil edaphic factors[19]. The role of AM fungi and PGPRs in improving plant growth is well documented[8]. Plant growth promotion by rhizomicroorganisms may be due to the production of growth hormone and vitamin production, nutrient release from soil organic matter or increased uptake and translocation of minerals[20,21]. *B.coagulans* is known to secrete enzymes which help in easy penetration of the intracellular spaces by dilating the host cell wall in the cortex region *Trichoderma* spp. producing volatile compounds promoting the growth of AM hyphae has also been documented[22]. It could be concluded that PGPRs have a synergistic effect with AM fungi. In the present study there was a positive correlation between mycorrhizal root colonization and plant growth. This finding support the earlier observations made by the other workers[23].

REFERENCES

1. Bergelson, J.M. and Crawley, J.M. Mycorrhizal infection and plant species Diversity, *Nature*, 1988; **334**: 202-204.
2. Gill, M.S., Singh, K. and Walia,S.S. Use of N_2 fertilizers in Agricultuure. *Kisan World*, 2000; **3**: 32-33.
3. Lee, K.E. and Panchurst, C.E. Soil organisms and sustainable productivity. *Aust. J. Soil Res.* 1992; **30**: 855-892.
4. Mukerji, K.G. and Dixon, R.K. Mycorrhizae in Reforestation. *In:* Rehabitation of Tropical Rainforest Ecosystems, Research and Development Priorities, Malaysia, Sclangar. Malaysia, 1992.
5. Kloepper, J.W. Zablotowicz, R.M. Tipping, E.M. and Lifshitz, R. Plant growth promotion mediated by bacterial rhizosphere colonizers. *In:* Beltsville Symposia in Agricultural Research No.14 [D.L. Keister and P.Cregan(eds)] 1991.
6. Chanway,C.P. and Holl, F.B. Biomass increase and associative nitrogen fixation of mycorrhizae. *Canadian J. Botany,* 1991; **69**: 507-511.
7. Holl,F.B., Chanway, C.P., Turkington,R., and Radley, R.A., Response of crested wheat grass *Agropyron cristatum* L. perennial ryegrass *Lolium perenne* and White clover *Trifolium repens* L. to inoculation with *Bacillus polymyxa*, *Soil Biol. Biochem.*, 1988; 19-24.
8. Lakshman, H.C. Development and response of vesicular arbuscular mycorrhizal Fungi in *Termanalia bellirichia* Roxb. 1992: 179-182.

9. Murthy, N.K., Srinivasan, S. and Warrier, R.K. Effect of *Azospirillum* and *Phosphobacterium* in improving seed germination and vigour of amla. *J. Non Timber Forest Products*. 1998; **5**: 34-36.
10. Piper C.S. *Soil and Plant Analysis*. International Incorporation, New York, U.S.A., 1950.
11. Jackson, M.L. *Soil Chemical Analyses*. Prentice Hall, New Delhi. 1973.
12. Phillips, J.F. and Hayman, D.S. Improved procedures for clearing root parasitic and staining vesicular arbuscular mycorrhizal fungi for rapid assessment infection. *Trans. Br. Mycol.Soc*., 1970; **55**: 158-160.
13. Giovannetti, M. and Mosse, B. An evaluation of techniques for measuring Vesicular and arbuscular mycorrhizal infection in roots. *New Phytol*. 1980; **84**: 489-500.
14. Gerdemann., J.W. and Nicolson, T.H. Spores of mycorrhizal Endogone species Extracted from soils by Wet sieving and decanting methods. *Trans. Br. Mycl. Soc*. 1963; **46**: 235-244.
15. Schenck, N.C., and Perez, Y. Manual for the identification of VA Mycorrhizal Fungi, INVAM, Gainsville, Fla. 1990.
16. Morton, J.B., and Benny, G.L. Revised classification of Arbuscular Mycorrhizal Fungi (Zygomycetes). *Mycotaxon*. 1990; **37**: 471-491.
17. Bagyaraj, D.J. Handbook of Applied Mycology, Soil and plants. Marcel Dekker Inc., New York, 1991.
18. St. John, T.V. and Coleman, D.C. The role of Mycorrhizae in plant ecology, *Canadian J.Bot*. 1983; **61**: 1005-1014.
19. Giovannetti, M. Seasonal variations of Vesicular Arbuscular Mycorrhizae and Endogonaceons spores in a maritime sand dunes. *Trans.Brit. Mycol. Soc*. 1985; **84**: 679-684.
20. Chang, K.P., Hu, H.T. and Kao, D.C. Effect of endomycorrhizal fungi and *Rhizobium* inoculation on growth of *Acacia auriculiformis* Nitrogen fixing tree, *Res.Report.* 1986; **4**: 40-41.
21. Azcon, R. Selective interaction between free living rhizosphere bacteria and VAM fungi. *Soil Biol. Biochem.* 1989; **21**: 639-644.
22. Calvet, C. Barea, J.M. and Pera, J. *In vitro* interactions between the Vesicular Arbuscular fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates. *Soil Biol. Biochem.,* 1992; **24**: 775-780.
23. Raj, M., Acharya, D., Singh, A., and Varma, A. Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhizae.* 2001; **11:** 123-128.