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Effect of *Glomus aggregatum* and plant growth promoting rhizomicroorganisms on growth, nutrition and content of secondary metabolites in *Glycyrrhiza glabra* L.

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Abstract

Glycyrrhiza glabra L. is one of the important medicinal plant belonging to the family *Fabaceae (Leguminosae)* and commonly called as 'liquorice'. The main ingredient of *G. glabra* is 'glycyrrhizin', a substance 50 times sweeter than sucrose with cortisone -like effects. It is a very sweet, moist, soothing herb that is anti-inflammatory and expectorant, with controls coughing. Externally liquorice is used for eczema, herpes and shingles. Economically, the roots are boiled to extract the familiar black substance used in liquorice confectionery and this is solid dried to eat. A study was under taken to determine the effect of AM fungus, *Glomus aggregatum* and some plant growth promoting rhizomicroorganisms(PGPR's) on the growth, biomass, nutrition and content of secondary metabolites of *G. glabra* under green house conditions. Various plant growth parameters like total plant biomass, mycorrhizal parameters, shoot and root phosphorus, potassium, iron, zinc, copper content and polyphenolic compounds viz., total phenols, ortho-dihydroxy phenols, tannins, flavonoids and alkaloids were analyzed and found to vary with different treatments. Among all the treatments, plants inoculated with 'microbial consortium' consisting of *Glomus aggregatum* + *Trichoderma harzianum* + *Bacillus coagulans* performed better than other treatments and uninoculated control plants. The results of this experiment clearly indicated that inoculation of *G. glabra* with *Glomus aggregatum* along with PGPR's enhanced its growth, biomass ,yield, nutrition and secondary metabolites.

Utilization of biofertilizers in the cultivation of medicinal and aromatic plants is

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of recent interest. Introduction with Arbuscular Mycorrhizal fungi (AMF) is known to increase the growth of many plant species including medicinal plants. This is attributed to increased uptake of nutrients, production of growth promoting substances and phytochemical constituents, tolerance to drought, salinity, transplant shock, resistance to plant pathogens and synergistic interaction with other beneficial soil microorganisms such as N_2-fixers, P-solubilizers etc., [1,14,16]. It has been established that mycorrhizal plants grow better in infertile soils because of improved mineral nutrition through hyphae which help in exploring greater volume of soil, beyond root hairs[24].

With the advent of innovative technologies and the importance being given to sustainable agriculture, AM fungal association is of great economic significance on growth of agricultural and medicinal crops. Certain plant growth promoting rhizomicroorganisms (PGPR's) have been reported to enhance the activity of mycorrhizal fungi and consequently plant growth[6,7,10,15,22,25,27]. Therefore microbial inoculants would be helping to maintain good soil health and fertility that contribute a greater extent for a sustainable yield and quality of products. However, the information available on the use of these beneficial microorganisms in medicinal plants is meager.

Glycyrrhiza glabra L. is one of the important medicinal plant, belonging to the family *Fabaceae (Leguminosae)* and commonly called as 'liquorice'. The main substance of *G. glabra* is 'glycyrrhizin', which is 50 times sweeter than sucrose, with cortisone-like effects. It is a very sweet, moist, soothing herb that is anti-inflammatory and expectorant, controls coughing and has hormonal effects. It detoxifies and protects the liver. Medicinally, it is used internally for Addison's disease, asthma, bronchitis, peptic ulcer, arthritis and allergic complaints. Externally liquorice is used for eczema, herpes and shingles. Economically, the roots are boiled to extract the familiar black substance used in liquorice confectionery; and this is solid to eat. Liquorice is also used as foaming agent in beers and fire estinguishers[2,13]. It is found to grow in lateritic and acidic soils. Lateritic soils are, in general, poor in nutritional status and are especially deficient in phosphorus. The present study was undertaken to study the effect of AM fungus, *Glomus aggregatum* and the PGPR's, *Bacillus coagulans* and *Trichoderma harzianum*, singly and in combination, on the growth, biomass, nutrition and the content of secondary metabolites of *Glycyrrhiza glabra* raised under glass house condition.

Pot culture experiment:

Liquorice (*Glycyrrhiza glabra* L.) seedlings were raised in seed pans containing sand:soil mix (1:1 v/v). The seedlings after germination were maintained for four weeks. *Glomus aggregatum* maintained as a pot culture using sterilized sand:soil mix (1:1 v/v) as the substrate and onion (*Allium cepa* L.) as the host was used in the present study. The substrate along with the roots was air dried. The hyphae, spores and root segments in the dried substrate served as mycorrhizal inoculum. *Bacillus coagulans*, which is not only a PGPR but also a mycorrhiza helper bacterium (MHB) was grown in nutrient broth and *Trichoderma harzianum* in potato dextrose broth in 2-lit flask containing 800 ml medium. After 3 days growth of *B. coagulans* and 7 days growth of *T. harzianum*, the cultures were used for inoculation

along with *G. aggregatum* at the time of sowing and the plants were maintained in a glass house for 90 days. The microbial cultures were separately mixed in sterile lignite powder and their populations were determined by serial dilution plate method.

Pots of 4.5 kg capacity were filled with sandy loam soil:sand (1:1 by volume) potting mix. The soil used as an alfisol type kaolinitic isohyperthemictypic kanhaplustafs. The pH of the potting mixture was 6.2 and contained 2.7 ppm available phosphate (NH_4F+ HCl extractable), a planting hole was made of the centre of the pot. 10 g of *G.aggregatum* (1400 IP g^{-1}), *B. coagulans* (2.8 X 10^8 cfu g^{-1}) and *T. harzianum* (3.4 X 10^8 cfu g^{-1}) inocula were added as per the treatment allocation shown in Table-1. One seedling was maintained per pot with 5 replications for each treatment. The plants were kept in a glass house and watered regularly.

Experimental analysis :

The plants were harvested 90 days after planting (DAP). Growth parameters viz., plant height, number of leaves and branches were recorded at harvest. Dry weight of shoot and root was recorded after drying the samples at 60°C to constant weight in a hot air oven. The phosphorus and potassium content of the plants were estimated by vanadomolybdate phosphoric acid yellow colour and Flame photometric methods respectively[12]. Atomic absorption spectrophotometer was employed to estimate zinc, copper and iron content of the plant root samples, using respective hollow cathode lamps. Acid phosphatase activity was estimated in the root zone soil as per the procedure given by Tabatabai[28]. The content of secondary metabolites like total phenols (Mc Donald, 2001), ortho di-hydroxy phenols[18]; flavonoids[3], alkaloids[11] and tannins[30] were assayed in the plant root samples.

Mycorrhizal root colonization was determined by grid line intersect method[9] after staining the root samples[23] with acid fuchsin (0.2%). Extrametrical chlamydospore numbers in the root zone soil were enumerated by wet-sieving and decantation method[8]. The data thus generated were subjected to statistical analysis of completely randomized block design and the means were separated by Duncan's Multiple Range Test[17].

Growth, nutrition and secondary metabolites:

In general, inoculated plants appreciably enhanced plant height than uninoculated control plants but the increase was significant in the *B. coagulans* treatment (29.2 cm) (Table-1). It was significantly superior over other treatments. This was followed by *Glomus aggregatum* + *Bacillus coagulans* + *Trichoderma harzianum* (28.5 cm). There was no significant difference in the number of leaves and branches due to inoculation with PGPR's alone and uninoculated control plants. But maximum number of leaves on 90 DAT(days after treatment) were recorded in plants inoculated with *Glomus aggregatum* + *Bacillus coagulans* + *Trichoderma harzianum* (33.4/ plant) and maximum number of branches were recorded in the plants inoculated with *G. aggregatum* + *B. coagulans* + *T. harzianum* (5.7 / plant) which was significant over all other treatments and least number of leaves and branches were recorded in control plants. Such

a response of improved plant growth was also elucidated in the investigation of Earanna *et al*.,[5] in *Coleus aromaticus* and Sivakumar *et al*.,[25] in *Pelargonium graveolens*, inoculated with *Glomus fasciculatum* and some PGPR's.

Single inoculation with *G. aggregatum* also significantly enhanced the total dry weight of liqourice plants followed by dual inoculation with *G. aggregatum* + *B. coagulans*. Plants similarly inoculated with *G. aggregatum* + *B. coagulans* + *T. harzianum* (9.7g/plant) showed maximum shoot and root dry weight was recorded and least biomass was recorded in control. This may be due to synergistic interaction of AM and PGPR's in the rhizosphere of plants[10,15,25].

Maximum percent root colonization were recorded in plants inoculated with *G. aggregatum* + *T. harzianum* + *B. coagulans* (94.6 %). Similarly, spore number was maximum when plants inoculated with *G. aggregatum* + *B. coagulans* (580.2/100g soil) and *G. aggregatum* + *B. coagulans* + *T. harzianum* (563.6/100 g soil), least number was recoded in uninoculated control plants. Synergistic interactions have been reported between the free-living rhizosphere bacteria, N_2 fixing organisms and mycorrhizal fungi[6,21] with respect

Table 1. Effect of AM fungus and PGPR's on growth and biomass of *Glycyrrhiza glabra*

S. No	Treatment	90 DAT			Plant biomass (g/plant)		
		Plant height (cm)	No. of leaves	No. of branches	Shoot	Root	Total
1.	Uninoculated control	17.2^{e}	16.2^{e}	3.2^{e}	1.3^{d}	1.4^{f}	2.7^{e}
2.	*Glomus aggregatum (G.a)*	26.8^{c}	25.6^{e}	4.4^{c}	5.4^{c}	4.2^{b}	9.6^{a}
3.	*Bacillus coagulans (B.c)*	29.2^{b}	21.6^{d}	3.5^{d}	5.5^{a}	2.2^{d}	7.7^{c}
4.	*Trichoderma harzianum (T.h)*	18.5^{e}	21.8^{d}	3.6^{d}	1.4^{d}	1.8^{e}	3.2^{d}
5.	*G.a + B.c*	28.5^{b}	30.1^{b}	4.8^{b}	5.4^{b}	4.2^{b}	9.6^{a}
6.	*G.a + T.h*	26.5^{c}	29.6^{c}	4.6^{c}	4.8^{c}	4.2^{c}	9.0^{b}
7.	*B.c + T.h*	21.5^{d}	28.9^{c}	4.8^{b}	4.6^{c}	4.0^{c}	8.6^{b}
8.	*G.a + B.c + T.h*	28.5^{a}	33.4^{a}	5.7^{a}	5.6^{a}	4.9^{a}	9.7^{a}
	LSD (5 %)	4.8	6.2	1.2	0.8	0.8	1.2

NOTE :
DAT: days after transplanting
Means in the same column followed by the same superscript do not differ significantly according to Duncan'sMultiple Range Test ($P<0.05$).

Table 2. Influence of AM fungus and PGPR's on % root colonization, spore number in the root zone soil, and nutrient status in the roots of *G. glabra*

S. No.	Treatment	Percent root colonization	Spore number/ 100 g of soil	RootP (mg/ plant)	RootK (mg/ plant)	Root Zn (µg/g)	Root Cu (µg/g)	Root Fe (µg/g)	Acid phosphatase activity*
1.	Uninoculated control	28.9^{e}	124.0^{e}	1.58^{e}	2.2^{f}	38.6^{f}	18.6^{e}	22.4^{e}	5.06^{e}
2.	*Glomus aggregatum (G.a)*	86.5^{b}	431.8^{b}	15.20^{c}	10.5^{c}	160.5^{d}	53.6^{c}	60.5^{c}	14.40^{c}
3.	*Bacillus coagulans (B.c)*	30.5^{d}	160.5^{d}	3.40^{d}	2.8^{e}	56.2^{e}	42.5^{d}	48.2^{d}	6.02^{d}
4.	*Trichoderma harzianum*	31.2^{d}	140.6^{d}	3.08^{d}	2.9^{e}	62.0^{e}	40.2^{d}	41.5^{d}	6.08^{d}
5.	*(T.h)*	82.6^{b}	580.2^{a}	20.22^{b}	12.5^{b}	394.5^{b}	60.8^{b}	92.5^{b}	23.03^{b}
6.	*G.a + B.c*	62.8^{c}	320.5^{c}	16.56^{c}	11.4^{b}	251.8^{c}	56.8^{c}	90.5^{b}	13.03^{c}
7.	*G.a + T.h*	45.2^{d}	285.0^{cd}	13.45^{bc}	8.2^{d}	120.2^{d}	38.4^{d}	85.6^{b}	18.05^{c}
8.	*B.c + T.h* *G.a + B.c + T.h*	94.6^{a}	563.6^{a}	27.14^{a}	15.2^{a}	507.2^{a}	89.2^{a}	94.0^{a}	33.5^{a}
	LSD (5 %)	5.25	12.6	2.8	1.45	32.8	4.2	5.7	3.2

Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P< 0.05$).

NOTE:

* Enzyme activity expressed in µg/g soil/hr.

to the percent root colonization and spore number.

The root phosphorus, potassium, zinc, copper and iron contents were maximum in the plants treated with *G. aggregatum* + *B. coagulans* + *T. harzianum* (27.14 mg/plant; 15.2 mg/plant, 501.2µg/plant, 89.2 µg/g and 94.2 µg/g respectively) and with the plants inoculated with *G.aggregatum* alone (15.20 mg/plants; 10.5 mg/plant; 160.5 µg/g, 530.6 µg/g and 60.5 µg/g respectively). This is probably due to the enhanced mycorrhizal colonization. The phosphorus, potassium, zinc, copper and iron contents were least in the un inoculated control plant. Such an increased P, K, Zn, Cu and Fe uptake due to mycorrhizal inoculation with PGPR's was reported by earlier workers[16,29].

Acid phosphatase activity in the root zone soil of all the inoculated seedlings was significantly more compared to the root zone

Table 3. Influence of AM fungus and PGPR's on the content of secondary metabolites in the roots of *G. glabra*

S. No.	Treatment	Total phenol (µg/g fresh wt.)	OD phenol (µg/g fresh wt.)	Flavonoids (µg/g fresh wt.)	Alkaloids µg/g	Tannins µg/g
1.	Uninoculated control	94.0^{e}	63.5^{e}	3.12^{e}	4.25^{e}	0.285^{e}
2.	*Glomus aggregatum (G.a)*	124.5^{b}	75.2^{b}	3.34^{b}	4.48^{b}	0.380^{b}
3.	*Bacillus coagulans (B.c)*	118.2^{c}	70.4^{d}	3.21^{c}	4.26^{d}	0.286^{d}
4.	*Trichoderma harzianum (T.h)*	110.6^{d}	69.2^{d}	3.16^{d}	4.32^{c}	0.285^{d}
5.	*G.a + B.c*	125.5^{b}	76.8^{b}	3.36^{b}	4.48^{b}	0.385^{b}
6.	*G.a + T.h*	112.4^{d}	73.2^{c}	3.24^{c}	4.21^{d}	0.365^{c}
7.	*B.c + T.h*	110.5^{d}	70.6^{d}	3.18^{d}	4.23^{d}	0.314^{d}
8.	*G.a + B.c + T.h*	130.2^{a}	84.5^{a}	3.78^{a}	5.12^{a}	0.454^{a}
	LSD (5 %)					

Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P<0.05$).

soil of uninoculated control plants but it was highest in the root zone of plants inoculated with *G. aggregatum* + *B. coagulans* + *T. harzianum* (33.5 µ/g soil/hr) and followed with the *G. aggregatum* + *B. coagulans* (23.03 µ/g soil/hr) inoculated plants. Enhanced acid phosphatase activity in the root zone soil of neem because of inoculation with AM fungi has been reported earlier[26].

The root secondary metabolites like total phenols, ortho di-hydroxy phenols, flavonoids, alkaloids and tannins were maximum in the plants treated with *G. aggregatum* + *B. coagulans* + *T. harzianum* (130.2 µg/g; 84.5 µg/g; 3.78 µg/g; 5.12 µg/g and 0.454 µg/g) and with plants dually inoculated with *G. aggregatum* + *B. coagulans* (125.5 µg/g; 76.8 µg/g; 3.36 µg/g; 4.48 µg/g; 0.388 µg/g). This is also probably due to the enhanced mycorrhizal colonization and nutrient status of plants. Such an increased content of secondary metabolites due to mycorrhizal inoculation with PGPR's were reported by earlier workers[4,19]. Hence it can be concluded that "Microbial Consortium" consisting of *Glomus aggregatum*, *Bacillus coagulans* and *Trichoderma harzianum* are best suited for *Glycyrrhiza glabra*. The results of these experiments clearly indicate that inoculating *G. aggregatum* along with plant growth promoting rhizosphere microorganisms encourages the ability of *G. aggregatum* and

enhances the growth, biomass, nutrition and the content of secondary metabolites of *Glycyrrhiza glabra*.

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