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Enzyme Profile of AM Fungi and PGPRs Inoculated and Uninoculated *Indigofera* sp

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The morphometric characters and enzyme profiles of two *Indigofera* species were studied in the present investigation. The above parameters of the two plants were discussed under Arbuscular Mycorrhizal (AM) inoculated and uninoculated conditions. The results revealed that the phosphatases, polyphenol oxidase and antioxidant enzyme, peroxidase were higher in plants subjected to triple inoculation of both AM fungi and Plant Growth Promoting Rhizomicroorganisms (PGPRs) (*Bacillus coagulans* and *Trichoderma viride*) when compared to *Glomus aggregatum* and *B. coagulans + T. viride* inoculated plants and uninoculated (control) plants. The study also indicated that these bioinoculants could be used to improve the growth and biochemical parameters of these plants which in turn could improve the bioactive principles of these medicinally important leguminous plants.

Key words: AM fungi, Plant Growth Promoting Rhizomicroorganisms, *Indigofera aspalathoides* and *I. tinctoria.*

Indigofera aspalathoides and *I. tinctoria* are two important plant species well known for their role in curing various diseases of mankind. The leaves, flowers and tender shoots of *I. aspalathoides* are employed in decoction to treat leprosy and malignant tumors. The leaves are applied to abscesses; and oil obtained from the root is used to anoint the head in erysipelas[1]. The whole *I. tinctoria* plant is used to treat intoxication, giddiness, fainting, constipation, hepatomegaly, splenomegaly, blood disorders, oedema and urinary calculi. The root is also useful in treating snake-bite and caries of the teeth. The plant (*I. tinctoria*) also yields a natural dye, indigotin that is useful in dyeing of fabrics[2]. Owing to the resources we acquire from these plants, need has arisen to protect these medicinal treasures from being extinct due to over exploitation. One way is to improve the quality and quantity of the products derived from them by using bioinoculants. Arbuscular Mycorrhizal fungi (AM) fungi, Plant Growth Promoting Rhizobacterias (PGPRs) and biocontrol agents (*Trichoderma viride*) being well known in this aspect, they can well be employed in the process. Inoculation of clover with PSB and *G. mosseae* together significantly increased the dry weight over inoculation with either of these organisms alone[3]. Such positive interactions between PSB and AM fungi on plants have also been reported bymany authors Azcon - Aguilar

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and Barea[4] and Krone *et al*.[5]. Dual inoculation of *G. fasciculatum* with either *B. coagulans* or *T. harzianum* enhanced the plant growth and biomass of *Phyllanthus amarus* [6].

In this study, a pot trial was undertaken to assess the efficiency of these bioinoculants in improving the vigor and growth of the plants.

MATERIAL AND METHODS

Selection of planting materials and inoculation of PGPRs in selected plants

Seeds of *I. aspalathoides* and *I. tinctoria* plants were collected from Marunduvalmalai region, Munchirai hillocks and Veli hills region, southern India. They were soaked in 5% sodium chloride, the floating seeds were discarded and viable seeds were used for further sowing in pots of size 22 cm x 20 cm filled with sand and soil in the ratio 1:3.The seeds were spread on a polythene paper and the liquid based inoculums of both the PGPRs were sprinkled over the seeds and the inoculum coated seeds were then air dried before sowing. The mass multiplied soil and root based inoculum of AM fungi were applied 3 cm below the pot culture soil as thin layer at the rate of 5.0 g/ pot[7] with the following combination.

T_0 - Uninoculated control,
T_1 - *Glomus aggregatum*
T_2 – *B. coagulans + T. viride,*
T_3 - *G. aggregatum + B. coagulans + T. viride*

Estimation of plant biomass and plant height

The dry matter content of the two *Indigofera* species removed 90 days after sowing (DAS) were determined by drying the sample in an oven at 60ºC till constant weight was obtained. Plants were collected from each treatment and the height was measured from the bottom of the root to the tip of the plant and expressed in cm.

Table 1. Effect of native AM fungi and PGPRs on the plant biomass, plant height and total protein content of *Indigofera aspalathoides*

Treatments	Dry weight (g/ plant)	Plant height (cm/plant)	Total protein (mg/g fresh weight)
Control	2.68	19.70	0.8
Glomus aggregatum	3.25*	24.18*	1.14*
B. coagulans +T. viride	2.91*	22.30*	0.98*
G. aggregatum +B. coagulans +T. viride	3.81*	30.19*	1.47*
SEM	0.09	0.87	0.06
CD at 5% level	0.17	1.61	0.15

Each value is a mean of 5 replicates. *Values indicate significance over control.

Table 2. Effect of native AM fungi and PGPRs on the plant biomass, plant height and total protein content of *Indigofera tinctoria*

Treatments	Dry weight (g/ plant)	Plant height (cm/plant)	Total protein (mg/g fresh weight)
Control	4.02	45.06	1.46
Glomus aggregatum	6.54*	60.07*	2.57*
B. coagulans + T. viride	6.11*	56.55*	1.72
G. aggregatum +B. coagulans +T. viride	7.23*	74.20*	3.53*
SEM	1.01	4.52	0.31
CD at 5% level	1.96	9.85	0.63

Each value is a mean of 5 replicates. *Values indicate significance over control.

Protein Estimation and Enzyme assay

The protein content, acid phosphatase and alkaline phosphatase activity of the plants were estimated using standard protocols[8,9,10]. Polyphenol oxidase and peroxidase activity of the plants were also analysed on 90 DAS [11,12].

Analysis of variance

Pot culture study of the test plants were carried out in randomized block design and the data in replicates were subjected to analysis of variance (one way)[13].

RESULTS AND DISCUSSION

The analysis of the test plants, *I. aspalathoides* and *I. tinctoria*, treated with AM fungus *G. aggregatum* and PGPRs, *B. coagulans* and *T. viride* showed improved growth with regard to morphometric characters and enzyme levels.

The present study reports that the plant growth of *I. aspalathoides* and *I. tinctoria* has been influenced positively by AM fungi and PGPRs inoculation. This was evident from the measurement of plant height and total dry matter content as given in Tables 1 and 2. Though dual inoculation of *B. coagulans* and *T. viride* has improved the plant growth when compared to control plants, they were not so effective as that compared with single inoculation of *G. aggregatum* and combined inoculation of the AM fungus with the PGPRs. Dual inoculation with *G. mosseae* plus *T. harzianum* was found to increase the growth, biomass and alkaloid content of *Andrographis paniculata* [14]. *Coleus aromaticus* inoculated with

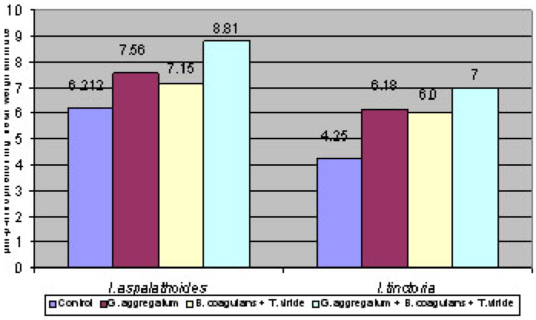

Fig. 1. Effect of AM fungi inoculation on the acid phosphatase activity of the two plants

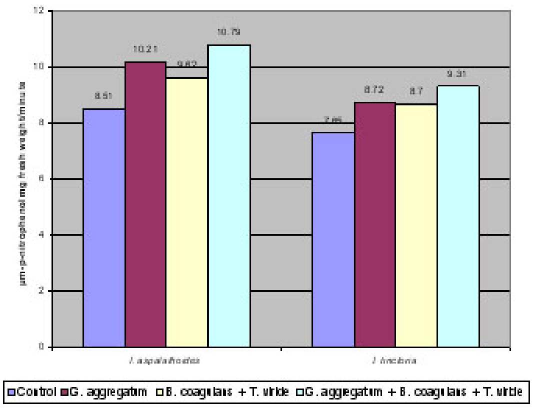

Fig. 2. Effect of AM fungi inoculation on the alkaline phosphatase activity of the test plants

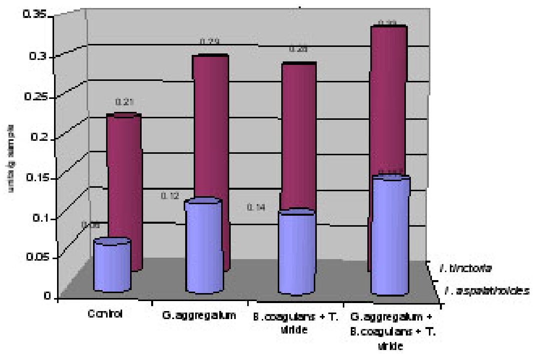

Fig. 3. Polyphenol oxidase activity of AM fungi treated and untreated test plants

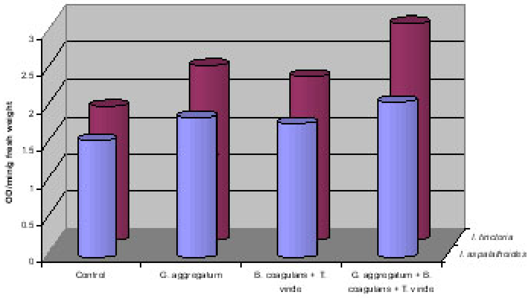

Fig. 4. Peroxidase activity of uninoculated and AM fungi inoculated test plants

G. fasciculatum and PGPRs increased its growth, biomass and P content[15]. Positive correlation between increase in height and AM inoculation in maize was reported[16]. Increase in shoot and root biomass of *Stevia rebaudiana* inoculated with *G. mosseae* and *T. viride* was observed[17]. Significant increase was observed in plant biomass and plant height in *Spilanthes acmella* inoculated with different combinations of *G. mosseae, A. laevis* and *T. viride*[18].

In the present study, the protein contents of the two *Indigofera* sp. were influenced by AM fungal inoculation. In mulberry plants the protein and amino acid contents were high in dual inoculation of plants with *G. fasciculatum* and *Pseudomonas* sp[7]. Increased levels of protein could be attributed to post-infectional stimulation of *de novo* protein synthesis in the mycorrhizal plants. In the present investigation, the combination of the AM fungus and PGPRs has showed better results of acid and alkaline phosphatase activities when compared to other treatments (Fig-1&2). Similar results of increase in acid and alkaline phosphatase activities were reported in wheat and *Vetiveria zizanoides* [19,20].

The antioxidant enzyme peroxidase and polyphenol oxidase activities were higher in AM inoculated Indigofera plants than the control plants (Fig-3&4). Significant increase in peroxidase activity of roots was observed in *G. fasciculatum* and *Pseudomonas* sp. treated mulberry[7]. Peroxidase being an important antioxidant enzyme, is known to be high in mycorrhizal plants than non mycorrhizal plants[21,22]. Mani has also reported the Increase in activities of the enzymes peroxidase and polyphenol oxidase in *Alpinia galanga* and *Coleus amboinicus* plants treated with arbuscular mycorrhizal fungi was also reported[23].

The study therefore concludes that plant growth and enzyme activities have been enhanced as a result of AM inoculation. Since plant biochemical parameters and enzyme activities are considered to be better indicators of plant vigor and as these parameters have been found to be augmented by AM fungal inoculation, the inoculation of the AM species along with PGPRs may very much contribute to improved plant growth which may boost the economic characters of the two plants.

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