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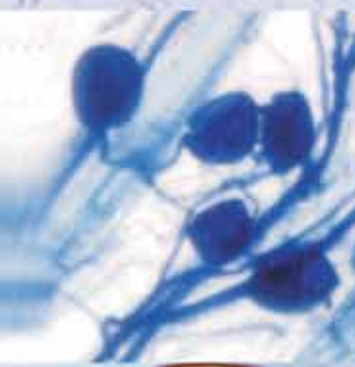


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Mycorrhiza News

The Mycorrhiza News provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers compiled from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.



Contents

Research finding papers

Growth in *Vigna mungo* as influenced by *Glomus fasciculatum*, *Pseudomonas fluorescens* Os25, and *Trichoderma viride*

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Introduction

Black gram originated in India and has been in cultivation since ancient times as one of the most highly priced pulses. The seeds are commonly consumed and comprise 17–34 per cent protein (Gaur, 1993). It is affected by a number of diseases caused by fungi, bacteria, and viruses, but the use of chemicals may pose danger to the environment by polluting the ecosystem. Further, seed treatment with fungicides does not protect the crop for a longer period. Additionally, in rain-fed conditions, soil drenching with fungicides is uneconomical.

The treatment of non-pathogenic, Plant Growth Promoting Rhizobacteria (PGPR) has been suggested as an alternative strategy for the control of plant diseases. Fluorescent pseudomonads have been widely tested for biocontrol against fungal pathogens because of their rapid growth rate and their ability to colonize the rhizosphere to a large extent besides their ability to suppress soil borne pathogens (Fukui *et al.*, 1994).

Arbuscular Mycorrhizal (AM) fungi are of special interest in the tropics because of their association with a large number of agricultural crop plants. AM fungi either directly or indirectly benefit the host plants. Chanway and Holl (1991) found that the PGPR are synergistic with mycorrhizae in stimulating plant growth and may stimulate root colonization by mycorrhizal fungi. Dual inoculation studies of AM fungi and PGPR, especially fluorescent *Pseudomonas* on crop plants are very few.

Several fungal (*Trichoderma* spp.) and bacterial (*Pseudomonas* spp. and *Bacillus* spp.) antagonists have been successfully used as biocontrol agents in the control of seed and soil borne pathogens like *Sclerotium rolfsii*, *Rhizoctonia solani, Fusarium oxysporum,* and *Sclerotinia sclerotiorum* in various crops. In recent years, biological control has become a promising alternative to chemical control in the management of soil borne diseases.

Materials and methods

The growth of *Vigna mungo* in phytopathogen amended soil as influenced by biocontrols *Pseudomonas fluorescens* Os25 and *Trichoderma viride* and mycorrhiza *Glomus fasciculatum* was studied. The following 18 different combinations of treatments of phytopathogens, biocontrols, and mycorrhiza were evaluated:

- Uninoculated seeds
- *Pseudomonas fluorescens* Os25
- *Glomus fasciculatum*
- *Trichoderma viride*
- *P. fluorescens* Os25 + *Glomus fasciculatum*
- *P. fluorescens* Os25 + *Trichoderma viride*
- *Trichoderma viride* + *Glomus fasciculatum*
- *Fusarium oxysporum*
- *Rhizoctonia bataticola*
- *P. fluorescens* Os25 + *F. oxysporum*

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- *P. fluorescens* Os25 + *R. bataticola*
- *G. fasciculatum* + *F. oxysporum*
- *G. fasciculatum* + *R. bataticola*
- *T. viride* + *F. oxysporum*
- *T. viride* + *R. bataticola*
- *P. fluorescens* Os25 + *G. fasciculatum* + *T. viride* + *F. oxysporum*
- *P. fluorescens* Os25 + *G. fasciculatum* + *T. viride* + *R. bataticola*
- *P. fluorescens* Os25 + *G. fasciculatum* + *T. viride*

With respect to the above mentioned treatments, the following parameters were studied.

Per cent germination of seeds of Vigna mungo

A fixed number of ten seeds were sown in each pot. Seven days after sowing, the seeds were scored for their germination. The per cent germination of seeds was calculated by the following formula:

$$\text{Per cent germination} \left\{100 - \left(\frac{\text{Total no. of seeds sown - No. of seeds germinated}}{\text{Total no. of seeds sown}}\right) X100\right\}$$

Root and shoot length of Vigna mungo at 35 DAI

The length of roots and shoot was measured with a metre scale.

Statistical analysis

The data were subjected to statistical analysis using IRRI STAT package for one way Analysis of Variance (ANOVA) (Lindman, 1974).

Determination of dry weight of Vigna mungo at 35 DAI

The plant materials were cut into bits, dried in an oven at 90 °C for three days, and then dry weight was determined.

Statistical analysis

The data were subjected to statistical analysis by using IRRI STAT package for one way ANOVA.

Estimation of total nitrogen in Vigna mungo at 35 DAI

The dried plant material was ground in a porcelain mortar and pestle and total nitrogen content was estimated according to the modified micro-Kjeldahl method (Umbriet *et al.*, 1972).

The dried and pulverized plant materials (10 mg) were put in a micro-Kjeldahl flask. A pinch of catalyst and 0.5 ml of concentrated H_2SO_4 were introduced into the Kjeldahl flask. The flask was gently heated in a digestion rack until the digest in the flask turned to an apple green colour. After cooling, the digest was made up to 20 ml with distilled water. To 2 ml of the diluted digest, 2 ml of water, and 2 ml of colour reagent (KI+HgI2) of 2N NaOH were added in series. After 15 minutes, the absorbance of the solution was read at 490 nm against a reagent blank.

Quantities of nitrogen in the sample were determined with reference to a standard graph prepared using NH_4Cl.

Catalyst (Humphries, 1956)

1 g of Copper sulphate, 8 g of Potassium sulphate, and 1 g of Selenium dioxide were powdered separately and mixed together.

Colour reagent

To 4 g of KI, 4 g of HgI_2 was added and dissolved in 25 ml of distilled water. About 1.75 g of light coloured gum ghatti was pulverized and dissolved in 750 ml of boiling distilled water. Then the KI and HgI_2 solution was mixed with the gum ghatti solution. This solution was made up to 1000 ml with distilled water and then filtered through Whitman No. 1 filter paper.

Statistical analysis

The data were subjected to statistical analysis by using IRRI STAT package for one way ANOVA.

Estimation of total phosphorus in Vigna mungo at 35 DAI

Acid-soluble total phosphorus was estimated by the method of Fiski-Subba Rao as modified by Bartlett (1959). The plant material was dried, powdered, and passed through a 2 mm sieve. An aliquot (100 mg) of the powder was homogenized in ice cold, 0.2 N perchloric acid and the extract was held in an ice box for 15 minutes prior to clarifying it by centrifugation. The extraction by perchloric acid was repeated at least three times and the extracts were pooled and analysed for total phosphorus.

To 1 ml of the extract, 1ml of 60 per cent trichloroacetic acid (TCA) was added and the contents were digested at 160–180 °C. After digestion, each sample received 4.5 ml of 2.5 per cent ammonium molybdate (2.5 g ammonium molybdate in 100 ml of 5N H_2SO_4) and 0.2 ml/kg of 0.25 per cent 1-amino 2-naphthol 4-sulfonic acid (ANSA). The contents of the test tube were mixed well and heated in a boiling water bath for 10 minutes. After cooling, the volume was adjusted to 10 ml with distilled water. The blue colour developed was measured against reagent blank at 650 nm in a spectrophotometer. Potassium dihydrogen phosphate was used as standard.

Statistical analysis

The data were subjected to statistical analysis by using IRRI STAT package for one way ANOVA.

Assessment of colonization of G. fasciculatum in Vigna mungo roots

The root materials were cleared and stained using the improved procedure of Phillips and Hayman (1970). The root segments in 10 per cent KOH were incubated at 90 °C for two hours and washed well with distilled water. Then the segments were immersed in 30 per cent hydrogen peroxide for 10–15 minutes for bleaching. They were then thoroughly rinsed in water to remove hydrogen peroxide and acidified in 5N HCl. They were stained by simmering for 30 minutes in 0.05 per cent tryphan blue in lactophenol and mounted. Then the root segments were squashed gently on slides containing a few drops of PVLG (polyvinyl alcohol 3.0 g + lactic acid 25 ml + phenol 20 ml + glycerol 50 ml) mixture and the coverslip was sealed with nail polish.

The per cent infection of AM fungi for each plant was calculated by grid-line intersect method (Giovannetti and Mosse, 1980).

$$\text{Per cent germination} = \left\{ \frac{\text{Total no. of infected intersecting grid-lines}}{\text{Total no. of roots intersecting grid-lines}} \right\} \text{X100}$$

Statistical analysis

The data were subjected to statistical analysis by using IRRI STAT package for one way ANOVA (Lindman, 1974).

Seedling Vigour Index

Plant growth-promoting activity by fluorescent pseudomonads in *Vigna mungo* was assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). The vigour index was calculated by using the formula described by Abdul Baki and Anderson (1973):

Seedling Vigour Index = [Root length + Shoot length] × Per cent germination

Results and discussion

Per cent germination of seeds of Vigna mungo

The seeds of *V. mungo* treated with *P. fluorescens* Os25 or *G. fasciculatum* or *T. viride* expressed 100 per cent germination. The uninoculated control seeds also expressed 100 per cent germination. The per cent seed germination in *V. mungo* upon inoculation with *F. oxysporum* and *R. bataticola* was only 40.18 per cent and 51.12 per cent, respectively. In the presence of *P. fluorescens* Os25 or *G. fasciculatum,* the pathogen effect was significantly reduced. The percentage of germination with *T. viride* treated seeds and challenged with *F. oxysporum* and *R. bataticola* was 65.13 per cent and 70.12 per cent, respectively. The data is represented in Table 1 and graphically in Figure 1.

Table 1. Per cent germination of seeds of *Vigna mungo*

S. No.	Treatments	per cent Germination
1	Uninoculated seeds	100
2	*P. fluorescens* Os25	100
3	*G. fasciculatum*	100
4	*T. viride*	100
5	*P. fluorescens* Os25 + *G. fasciculatum*	100
6	*P. fluorescens* Os25 + *T. viride*	100
7	*T. viride* + *G. fasciculatum*	100
8	*F. oxysporum*	40.18
9	R. *bataticola*	51.12
10	*P. fluorescens* Os25 + *F. oxysporum*	100
11	*P. fluorescens* Os25 + *R. bataticola*	100
12	*G. fasciculatum* + *F. oxysporum*	56.71
13	*G. fasciculatum* + *R. bataticola*	61.73
14	*T. viride* + *F. oxysporum*	65.13
15	*T. viride* + *R. bataticola*	70.12
16	*P. fluorescens* Os25 + *G. fasciculatum* + *T. viride* + *F. oxysporum*	100
17	*P. fluorescens* Os25 + *G. fasciculatum* + *T. viride* + *R. bataticola*	100
18	*P. fluorescens* Os25 + *G. fasciculatum* + *T. viride*	100

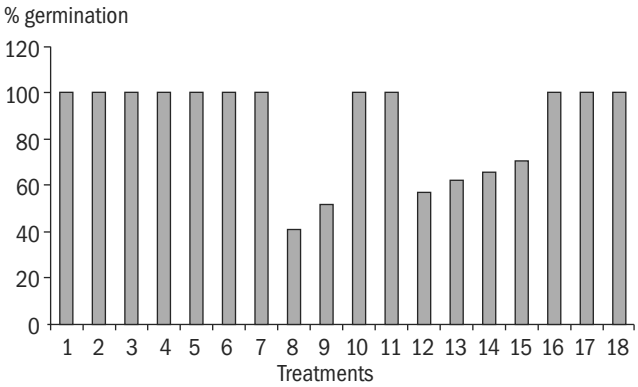

Figure 1. Per cent germination of seeds of *Vigna mungo*

In the present study, *P. fluorescens* strain Os25 was proved to be the best biocontrol agent with the seeds showing 100 per cent germination in various treatments including phytopathogen challenged treatments. Its biological efficiency can be attributed

to its various growth promoting activities, including phosphate solubilizing capacity, production of phytohormones, production of secondary metabolites, and induction of systemic resistance. Similarly, Babita *et al*. (2008) indicated that due to the potential of solubilizing the mineral phosphates, production of phytohormones and broad-spectrum antifungal activity against phytopathogenic fungi strains of fluorescent pseudomonads, FP12 and FP13 may be considered as inoculants for soil fertility, plant growth, and plant protection.

In the present study, *T. viride* is used as an additional biocontrol agent, besides *G. fasciculatum*, for control of phytopathogens, viz., *F. oxysporum* and *R. bataticola*. Windham *et al*. (1986) concluded that *Trichoderma* species produced a growth regulation factor that increases the rate of seed germination and dry weight of the seeds. Jetiyanon and Kloepper (2002) proposed a combinational use of different biocontrol agents for improved and stable biocontrol agents against a complex of diseases.

The results of the present study support the earlier observations that a combination of biocontrol agents with different mechanisms of disease control will have an additive effect and result in enhanced growth compared to their individual application.

Root length of Vigna mungo

Single inoculation with either *P. fluorescens* Os25 or *G. fasciculatum* induced an increase in root length of *V. mungo* as compared with the control. The increase in root length was very pronounced in plants inoculated with the *P. fluorescens* Os25 + *G. fasciculatum* combination, measuring 30.57 ± 0.58 cm plant^{-1} as compared to the root length of the control which was only 22.53 ± 0.55 cm plant^{-1} and the percentage increase was 35.69 per cent.

Significant increase in root length of the plants were recorded, particularly in the triple inoculations compared with single or dual inoculations. Maximum root length of 32.53 ± 0.65 cm plant^{-1} was observed in tripartite association of the plants with *P. fluorescens, G. fasciculatum,* and *T. viride* where a 44.38 per cent increase over the control was observed.

In contrast, the root length was lower in plants inoculated with *F. oxysporum* and *R. bataticola* with values of 11.66 ± 0.28 cm plant^{-1} and 14.36 ± 0.63 cm plant^{-1}, respectively. Improvement in root length was observed in *F. oxysporum* challenged plants in the presence of *P. fluorescens* Os25, *G. fasciculatum,* and *T. viride* measuring 18.46 ± 0.45 cm plant^{-1}, 16.66 ± 0.28 cm plant^{-1} and 16.56 ± 0.15 cm plant^{-1}, respectively. In addition, in triple inoculations of the plants grown in pathogen amended soils, the adverse effect of the pathogen was significantly reduced. In *R. bataticola* amended soils treated with tripartite association of *P. fluorescens* Os25, *G. fasciculatum,* and *T. viride*, the root length of the plant was observed to be 23 ± 0.5 cm plant^{-1} which was an increase of 2.08 per cent over the control. The ANOVA results are documented in Table 2.

Table 2. Root length and shoot length of *Vigna mungo* at 35 DAI

Treatment	Root Length (cm/plant)	Shoot Length (cm/plant)
Uninoculated seeds	23.53±0.55 ^{a}	25.14±0.08 ^{a}
P. fluorescens Os25	28.35±0.11 ^{cd}	33.28±0.31 ^{a}
G. fasciculatum	26.10±0.73 ^{a}	30.01±0.02 ^{d}
T. viride	26.05±0.08 ^{a}	29.71±0.45 ^{d}
P. fluorescens Os25 + *G. fasciculatum*	30.57±0.58 ^{b}	39.04±0.08 ^{d}
P. fluorescens Os25 + *T. viride*	30.83±0.28 ^{d}	38.87±0.12 ^{c}
T. viride + *G. fasciculatum*	29.05±0.68 ^{c}	37.03±0.10 ^{a}
F. oxysporum	11.66±0.28 ^{d}	8.2±0.28 ^{g}
R. bataticola	14.36±0.63 ^{g}	9.33±0.53 ^{h}
P. fluorescens Os25 + *F. oxysporum*	18.46±0.45 ^{a}	20.26±1.11 ^{a}
P. fluorescens Os25 + *R. bataticola*	19.38±0.68 ^{ad}	21.32±1.24 ^{b}
G. fasciculatum + *F. oxysporum*	16.66±0.28 ^{de}	17.56±0.25 ^{c}
G. fasciculatum + *R. bataticola*	17.10±0.1 ^{a}	18.04±0.10 ^{g}
T. viride + *F. oxysporum*	16.56±0.15 ^{b}	17.07±0.13 ^{ab}
T. viride + *R. bataticola*	18.03±0.05 ^{e}	18.4±0.12 ^{d}
P. fluorescens Os25 + *G. fasciculatum* + *T. viride* + *F. oxysporum*	20.66±0.28 ^{c}	32.44±0.41 ^{a}
P. fluorescens Os25 + *G. fasciculatum* + *T. viride* + *R. bataticola*	23.00±0.5 ^{d}	33.84±0.25 ^{a}
P. fluorescens Os25 + *G. fasciculatum* + *T. viride*	32.53±0.65 ^{a}	43.43±0.66 ^{a}
LSD	0.098	0.456

± Standard deviation

$p \leq 0.05$

Values suffixed with different letters on the same column indicate significant differences

In the present study, the biological efficiency of the biocontrols including *P. fluorescens* and *Trichoderma* in counteracting the deleterious effects of the phytopathogens and thereby enhancing the growth of *V. mungo* was proved. Beneficial microbes associated with pathogens in several ways and were effective in plant growth promotion and disease control in a wide range of crops.

Pseudomonas spp. are effective root colonizers and biocontrol agents through production of antibiotics and other antifungal metabolites including hydrogen cyanide and siderophores. In the present study, the production of phenazine antibiotic by *P. fluorescens* Os25 strain was reported. Suppression of take-all of wheat, caused by *Gaeumannomyces graminis* var. *tritici*, was correlated with the production *in situ* of PCA by *Pseudomonas fluorescens* 2-79 (Thomashow *et al*., 1990) and with the production of 2,4-DAPG by *P. fluorescens* CHAO (Keel *et al*., 1992). Similarly, Robert *et al*. (1993) reported that seed treatment with *T. harzianum* and *T. viride* was more effective in controlling *R. solani* in *Phaseolus vulgaris*, both under greenhouse and field conditions.

The improvement of root length in *V. mungo* grown in pathogen amended soils and treated with biocontrols can be attributed to antibiotic production.

Shoot length of Vigna mungo

Dual inoculation of *P. fluorescens* Os25 with *T. viride* or *G. fasciculatum* exhibited higher increase in shoot length of *V. mungo* when compared with control or single inoculation with either. Single inoculation with *P. fluorescens* Os25 or *G. fasciculatum* or *T. viride* also increased the shoot length of the plant over the uninoculated control. Higher shoot length (33.28 ± 0.31 cm) was observed in plants treated with *P. fluorescens* Os25 when compared to *T. viride* treated plants which recorded shoot length of 29.71 ± 0.45 and the increase was by 12.07 per cent.

Jagadish (2006) also observed the efficiency of *Pseudomonas* in enhancing plant growth. He found that at 60 DAT, *Pseudomonas* B-25 treated tomato plants recorded the highest plant height (51.13 cm), number of branches (11.33/plant), number of leaves (59.00/plant), total biomass (39.72 g/plant), chlorophyll content (2.040 mg/g tissue), and fruit yield (0.690 kg/plant). All these parameters increased by 44.8, 69.9, 59.5, 52.2, and 102.0 per cent, respectively.

In the present study, *V. mungo* plants treated with *F. oxysporum* showed a substantial decrease in shoot length, measuring 8.2 ± 0.28 cm plant^{-1} as compared to the uninoculated control which was 25.14 ± 0.08 cm, amounting to a decrease of 67.38 per cent. Similarly, the plants treated with *R. bataticola* showed a substantial decrease in shoot length, measuring 9.33 ± 0.53, which was a 62.89 per cent decrease over the control. In *R. bataticola* amended soils, the shoot length was highest in *P. fluorescens* Os25 treated plants (21.32 ± 1.24 cm plant^{-1}) followed by *T. viride* (18.40 ± 0.10 cm plant^{-1}) compared to *G. fasciculatum* treated plants which recorded the lowest shoot length of 18.04 ± 0.12 cm plant^{-1}. The ANOVA results are documented in Table 2.

These results clearly suggest the efficiency of the isolated strain of *P. fluorescens* when compared to *G. fasciculatum* and *T. viride* in the biocontrol of phytopathogens *F. oxysporum* and *R. bataticola.*

The antagonistic ability of *P. fluorescens* Os25 can also be attributed to its chitinase production. Similarly, Singh *et al.* (1999) reported that the main mode of antagonistic activity of microbes is production of lytic enzymes including chitinase and β-1,3-glucanase which act on cell walls of organisms which have chitin and glucan as their cell wall component.

A positive relationship was observed between the antifungal activity of chitinolytic *P. fluorescens* isolates and their level of chitinase production (Velazhahan *et al*., 1999). Yedidia *et al*. (1999) reported that the root inoculation of *T. harzianum* induced increased chitinase activity in leaves of cucumber seedlings. *P. fluorescens* and *Trichoderma* can act synergistically in controlling the phytopathogens as the dual inoculation of *P. fluorescens* Os25 and *T. viride* exhibited an increase in shoot length of *V. mungo* when compared with control or single inoculation with either.

Dry weight of Vigna mungo

Dual inoculation among the biocontrols, viz., *P. fluorescens* Os25, *T. viride* and mycorrhiza *G. fasciculatum* exhibited an increase in dry weight in roots and shoots of *V. mungo* when compared with control or single inoculation. Maximum dry weight of 2.07 ± 0.03 g was observed in triple inoculations of the plants.

In addition, Akkopru and Demir (2005) reported that the rhizobacteria and *Glomus intraradices* enhanced dry root weight effectively. The increased growth response induced by *Trichoderma* spp. has been reported for many crops, such as beans *Phaseolus vulgaris, Cucumis sativus*, *Capsicum annuum*, *Dianthus caryophyllus*, *Zea mays*, and *Tritichum aestivum* (Lo and Lin, 2002). Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. were competition for space and nutrients, production of diffusible and volatile antibiotics, and hydrolytic enzymes like chitinase and β-1,3-glucanase (Kubicek *et al.,* 2001). These hydrolytic enzymes partially degrade the pathogen cell wall and lead to its parasitization.

In this study, single inoculation with any one of the biocontrols also increased the dry weight over the

uninoculated control. Total dry weight was found maximum in plants treated with *P. fluorescens* Os25 (1.19 ± 0.03 g) followed by *G. fasciculatum* (1.11 ± 0.03 g) with least total dry weight observed in *T. viride* inoculated plants (1.13 ± 0.03 g) and the increases over the control were 105.17 per cent, 91.38 per cent and 94.82 per cent respectively. In *R. bataticola* amended soils, the dry weight of the plants was increased by 9.52 per cent with *P. fluorescens* Os25 inoculated plants when compared to *G. fasciculatum* inoculated plants. The ANOVA results are documented in Table 3.

The results obtained show that in the presence of *F. oxysporum*, higher dry weight was observed in plants co-inoculated with *P. fluorescens* Os25, *G. fasciculatum,* and *T. viride* (0.75 ± 0.02 g) amounting to 27.12 per cent increase compared to only *P. fluorescens* Os25 treated plants which recorded dry weight of 0.59 ± 0.01 g. This was due to the increase in colonization of *P. fluorescens* Os25 when the plants were colonized with *G. fasciculatum* besides the growth promoting activities of *Trichoderma*. Similarly, Sood (2003) reported that *Pseudomonas fluorescens* were attracted towards tomato roots colonized by *Glomus fasciculatum*

Table 3. Dry weight of *Vigna mungo* at 35 DAI

Treatment	Root Dry Weight (g/plant)	Shoot Dry Weight (g/plant)	Total Dry Weight (g/plant)
Uninoculated seeds	0.17±0.02 ^{ab}	0.40±0.005 ^{a}	0.58±0.02 ^{c}
P. fluorescens Os25	0.25±0.01 ^{cd}	0.90±0.01 ^{a}	1.19±0.01 ^{c}
G. fasciculatum	0.23±0.02 ^{def}	0.87±0.01 ^{a}	1.11±0.03 ^{c}
T. viride	0.23±0.02 ^{ad}	0.89±0.01 ^{a}	1.13±0.03 ^{b}
P. fluorescens Os25 + *G. fasciculatum*	0.36±0.01 ^{ac}	1.52±0.03 ^{d}	1.89±0.03 ^{b}
P. fluorescens Os25 + *T. viride*	0.35±0.02 ^{cdf}	1.30±0.01 ^{d}	1.66±0.03 ^{b}
T. viride + *G. fasciculatum*	0.27±0.01 ^{dde}	0.98±0.01 ^{d}	1.26±0.02 ^{a}
F. oxysporum	0.06±0.005 ^{fgh}	0.22±0.02 ^{d}	0.28±0.01 ^{a}
R. bataticola	0.07±0.01 ^{fgh}	0.30±0.01 ^{de}	0.37±0.006 ^{a}
P. fluorescens Os25 + *F. oxysporum*	0.16±0.01 ^{a}	0.43±0.02 ^{efg}	0.59±0.01 ^{a}
P. fluorescens Os25 + *R. bataticola*	0.18±0.01 ^{b}	0.49±0.01 ^{efg}	0.69±0.01 ^{adef}
G. fasciculatum + *F. oxysporum*	0.13±0.01 ^{ab}	0.41±0.03 ^{b}	0.54±0.01 ^{affg}
G. fasciculatum + *R. bataticola*	0.16±0.01 ^{bc}	0.47±0.02 ^{bc}	0.63±0.02 ^{ed}
T. viride + *F. oxysporum*	0.11±0.005 ^{dfg}	0.42±0.01 ^{a}	0.56±0.01 ^{de}
T. viride + *R. bataticola*	0.14±0.01 ^{ab}	0.42±0.006 ^{ab}	0.56±0.01 ^{de}
P. fluorescens Os25 + *G. fasciculatum* + *T. viride* + *F. oxysporum*	0.23±0.01 ^{ad}	0.51±0.006 ^{cd}	0.75±0.02 ^{de}
P. fluorescens Os25 + *G. fasciculatum* + *T. viride* + *R. bataticola*	0.25±0.005 ^{ac}	0.57±0.02 ^{de}	0.83±0.02 ^{de}
P. fluorescens Os25 + *G. fasciculatum* + *T. viride*	0.44±0.007 ^{ad}	1.64±0.01 ^{fg}	2.07±0.03 ^{sd}
LSD	1.67	0.456	1.34

± Standard deviation
$p \leq 0.05$
Values suffixed with different letters on the same column indicate significant differences

Similar results were obtained by Jagadish (2006). He found that *Pseudomonas* spp. B25 inoculation in tomato increased root length by 132 per cent, root biomass by 69 per cent, shoot biomass by 114 per cent, and fruit yield by 350 per cent when compared to the check.

In the present study, *F. oxysporum* and *R. bataticola* inoculation reduced the dry weight in *V. mungo* over the control by 51.73 per cent and 36.21 per cent respectively. Increase in dry weight of the plant was observed when various biocontrol treatment schedules in phytopathogen amended soils were employed.

compared to non-vesicular-arbuscular mycorrhizal tomato roots. Bianciotto *et al.* (2001) showed the involvement of the extracellular polysaccharides in the attachment of *Pseudomonas fluorescens* bacteria to the structures of AM fungi.

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Nitrogen Acquisition by Ectomycorrhiza: A mini review

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Nitrogen (N) is the most important element for all living organisms after carbon, hydrogen, and oxygen. The acquisition and assimilation of nitrogen is second only to photosynthesis in terms of importance for plant growth and development (Vance, 1997). On an average, 15 per cent of proteins and 13 per cent of nucleic acids are constituted by this element (Inokuchi *et al.*, 2002). Despite its inexhaustible natural reserves, nitrogen availability is one of the key factors limiting plant yield (Smil, 1999; Socolow, 1999; Graham and Vance, 2000). Even though soil N normally exceeds plant N by many times, N deficiencies in natural ecosystems are common because only a small fraction of the total soil N is available to plants at any given time. Most of the soil N is sequestered in organic compounds (Tamm, 1991) which range from simple amino acids and amino sugars to complex polypeptides and chitin (Leake and Read, 1997). The ability of forest trees to utilize organic N sources is limited to simple amino acids (Näsholm and Persson, 2001). Only a very small fraction (3–7 per cent) gets converted into accessible inorganic forms (nitrate and ammonium) after mineralization (Johnson, 1994). Competition for this nutrient among decomposers, nitrifying bacteria, non-biological processes, and plants is intense and plants have, therefore, adapted strategies for efficient uptake and recycling of nitrogen (Dickson, 1989). One of these is the formation of ectomycorrhizae which allows woody plants to compete efficiently with soil microorganisms (Smith and Read, 1997). The suggested pathways available in literature by which ectomycorrhizae help plants in acquiring nitrogen are fixation of atmospheric elemental nitrogen and acquisition of organic and inorganic (mineral) forms from soil.

Ectomycorrhizae are formed by fungi belonging to the higher Basidiomycetes and Ascomycetes. The host plants of these fungi are predominately trees belonging to the *Pinaceae* (pines, fir, larch, spruce, hemlock), *Fagaceae* (oak, chestnut, beech), *Betulaceae* (alder, birch), *Ericaceae* (arbutus), and other families (Meyer, 1973). These fungi are symbiotically associated with the roots of woody plants and can dramatically enhance plant growth. Typically, the fungi form a mycelial mantle around the short lateral roots of their hosts and penetrate between epidermal and cortical cells, surrounding them with a highly branched structure, the Hartig net (Peterson *et al.*, 2004). Significant variation exists in the morphology of ECM root tips that are infected by different fungal taxa, and analysis of macroscopic and microscopic characteristics of ECM roots is used widely for identification of the ECM fungi (Agerer, 1987–2002). The fungus–plant interface formed by the Hartig net in the ECM root is functionally critical to mutualism as it represents the interface across which nutrients and carbon are transferred between the partners (Smith and Read, 1997; Simard and Durall, 2004; Selosse *et al.*, 2006). In addition to the structures formed at the host root, ECM fungi produce mycelia that extend from the mantle into the surrounding soil, though the extent and structure of this extramatrical mycelium is thought to differ between ECM fungal taxa (Agerer, 2001). At the ecosystem level, these mycelia are of further significance in the mobilization of nutrients from organic and recalcitrant inorganic sources — both in competition and in cooperation with soil-dwelling saprotrophs — in the relocation of nutrients within the soil–plant continuum, and in the delivery and distribution of carbon below ground (Leake *et al.*, 2002; Wallander, 2006).

The role of mycorrhiza in nitrogen fixation has been suggested by many workers (Stevenson, 1959; Richards and Voigt, 1964). Nitrogenase activity (acetylene reduction activity) of mycorrhizal systems was estimated in *Pinus caribaea* and *P. patula* by Thapar and Pokhriyal (1993). They also assayed the nitrogenase activity of six ECM fungi — *Pisolithus tinctorius, Laccaria laccata, Cenococcum geophilum, Rhizopogon luteolus, Thelephora terrestris,* and *Suillus breviceps* — in pure cultures. *L. laccata* showed the highest activity followed by *S. breviceps*. Paul *et al.* (2007) also measured significant nitrogenase activity associated with *S. tomentosus* / *P. contorta* tuberculate ectomycorrhizae whereas no activity was found with non-tuberculate mycorrhizae or secondary roots without mycorrhizae. Maximum nitrogenase activity estimated was 25098.8 nmol C_2H_4 g^{-1} tubercle day^{-1}. Nitrogenase activity was significantly higher in young stands than in old stands of *P. contorta*. It was concluded that *S. tomentosus* inoculated into *P. contorta* are sites of significant nitrogenase activity

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which could be an important contribution to the nitrogen budget of *P. contorta* stands. Nitrogen-15 technique was used to test for fixation in *P. radiata* by Stevenson (1959). He found that mycorrhizal seedlings of *P. radiata* grew successfully in nitrogen free culture where non-mycorrhizal plants died. Also, when such plants were exposed to ^{15}N enriched atmosphere, an uptake of molecular nitrogen was detected. Richards and Voigt (1964) also reported subsequent accumulation of nitrogen in ectomycorrhizal pine seedlings grown in N-free substrates. Mikola (1986) objected to their inferences and postulated that since these experiments were not conducted in bacteria free conditions, N-fixation could have been caused by free living bacteria rather than by ectomycorrhizal fungi. André *et al.* (2005) inoculated bradyrhizobial strains along with *Pisolithus albus* into *Acacia holosericea* and concluded that ectomycorrhizal and biological nitrogen-fixing symbioses were very dependent on each other.

George and Marschner (1996) suggested that in forest soils with high organic matter content, the much better acquisition of organically bound nitrogen by ectomycorrhizal roots may be the reason for improved nitrogen nutrition, rather than the increased uptake of mineral nitrogen. Experiments by Read and his co-workers during the past 12 years unequivocally support Frank's organic nitrogen theory postulating that mycorrhizal colonization might provide access to the nitrogenous reserves contained in organic horizons (Read, 1987). The fungal symbionts of ectomycorrhizal plants excrete significant quantities of enzymes such as chitinases, phosphatases, and proteases. Depolymerization of N-containing polymers by these extracellular enzymes releases organic N-containing monomers that may be used by either plants or microbes and, thereby, allow the organic residues to be tapped directly for nutrients (Leake and Read, 1997; Perez-Moreno and Read, 2000; Tibbett and Sanders, 2002; Nygren *et al.*, 2007). Studies have shown that some ECM fungi can utilize a wide range of amino acids as sources of both N and carbon (Plassard *et al.*, 2000) and that a proportion of the assimilated N is transferred to the host plant (Abuzinadah *et al.*, 1986; Finlay *et al.*, 1992; Taylor *et al.*, 2004). The potential role of the ectomycorrhizal fungi in plant nutrition and biogeochemical cycles has been highlighted by Wallander (2006) and Van Scholl *et al.* (2008). So, a completely new conceptual model of the soil N cycle needs to incorporate recent research on plant–microbe competition and micro site processes to explain the dynamics of N across the wide range of N availability found in terrestrial ecosystems (Schimel and Bennett, 2004).

The uptake of 15NH4+ by external mycelia in intact mycorrhizal systems has been studied by Rygiewcz et al. (1984), Finlay et al. (1988), and Ek et al. (1994). Their experiments demonstrated significantly greater rates of 15NH4+ uptake in ectomycorrhizal plants. Chalot et al. (2006) highlighted the potential for direct transfer of ammonia from mycorrhiza to plant cells. However, few reports propose a direct effect of ectomycorrhizae on the uptake of NO3– (Wu et al., 1999). The recently sequenced genome of Laccaria bicolor shows an excess of enzymes used in ammonia uptake, and lack of enzymes that would be needed to degrade plant wall material. Thus, it is assumed the fungus is providing nitrogen (and perhaps other nutrients), and a guarantee not to attack the tree in return for glucose (Martin, 2008). Lucic et al. (2008) showed that L. bicolor has the genetic potential for both mineral and organic N compound utilization. The expression of a part of this genetic potential will further define the functions of the different mycelia types and will emphasize the dual trophic abilities (symbiotic versus saprobic) of L. bicolor.

Conclusion

The ability of fungal hyphae to exploit resources is far greater than that of roots due to their innate physiological and morphological plasticity. Mycorrhizal fungi extend the effective root length by 100 times or more. Not only do they increase the surface area over which nutrients and water can be taken up by the plant, fungal hyphae can also reach into smaller pores than roots can. Association between plants and mycorrhiza is special in the sense that the latter is capable of unlocking another growth limiting factor, phosphorous (Sylvia *et al.*, 2005), besides nitrogen, both being unavailable to autotrophs. Low availability of N is a characteristic feature of many soil ecosystems dominated by ectomycorrhizal plants. In some forests of the northern hemisphere, N is the most important determinant of productivity. Though the possibility of a direct involvement of ectomycorrhizal fungi in N acquisition by plants was suggested as early as in 1894 by Frank, until recently, less attention was paid to the role of mycorrhizae in N nutrition than to their role in the P nutrition of plants. These fungi contribute to tree nutrition by means of mineral weathering (Landeweert *et al.*, 2001) and mobilization of nutrients from organic complexes (Read and Perez-Moreno, 2003). ECM fungi are, thus, regarded as key elements of forest nutrient cycles and as strong drivers of forest ecosystem processes (Read *et al.*, 2004).

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Five simple steps for perpetuation of AMF culture

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It's always a delightful experience to receive requests of diverse Arbuscular Mycorrhizal Fungi (AMF) and Ectomycorrhizal fungi cultures from researchers across the globe, which subsists in the basket of Centre for Mycorrhizal Culture Collection (CMCC). However, quite often we have encountered a simple question from our young researcher friends, how to maintain the cultures and perpetuate them further? In this edition we have taken an attempt to explain the basic method of doing so for AMF. On receiving a culture request, CMCC processes the material by mixing desired spores (often ranging from 800 to 1000 in numbers) with hundred grams of soil-based substrate. For the convenience of our readers, we will split up the protocol into five simple steps.

STEP 1: Checking and storing of culture

After receiving the culture (inoculum) from CMCC, the first obligatory step is to preserve the inoculum at 4 °C in the refrigerator so that the necessary arrangements for its initiation as trap culture can be done in the meantime. In case someone is interested to cross check the status as well as the count of spores, the wet sieving and decanting method as described by Gerdemann and Nicolson (1963) can be followed. A small quantity of soil sample of a definite measurement can be reserved, washed, and sieved through 53 micron mesh. The spores collected in the mesh can then be analysed by preparing voucher specimens and accordingly can be quantified. However, it is not recommended as a mandatory step.

STEP 2: Surface sterilization of host seeds

A wide range of host plants can be used for multiplication of AMF as trap cultures. For example, *Allium cepa, Sorghum vulgare, Zea mays, Lycopersicum esculentum, Daucus carota, Tagetus sp., Gossypium sp., Vigna mungo, Vetiver, Plantago lanceolata,* etc. We recommend the usage of *Sorghum vulgare, Zea mays* or *Allium cepa* as bait plants since they have been found to be performing very well under our growth conditions. In order to prevent the perpetuation of unwanted fungal growth, surface sterilization of host seeds is preferred. A short duration treatment (less than a minute) of ethanol is given since it is a powerful sterilizing agent and is extremely phytotoxic, followed by sodium hypochlorite, the most common choice for surface sterilization. A balance between concentration and time must be determined empirically for each type of explant used considering the phytotoxic effect. To enhance the effectiveness of sterilization procedure, surfactants (for example, Tween 20) is frequently added to sodium hypochlorite. The solutions that the seeds are dipped in are often continuously stirred so that the entire surface area is disinfected. Once the disinfection process is completed, the

STEP 1 : Checking and storing of culture

STEP 2 : Surfactants (for example, Tween 20) is frequently added to sodium hypochlorite. The solutions that the seeds are dipped in are often continuously stirred so that the entire surface area is disinfected. Once the disinfection process is completed, the

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seeds must be rinsed thoroughly with sterile water. Typically three to four separate rinses are performed to completely remove the traces of chemicals. These seeds should then be left for two to three days in the incubator for germination.

STEP 3: Pots and sand

Substrate should be prepared by mixing loamy soil and sand in a one is to one proportion (1:1 v/v). This homogenous sand soil mixture should be autoclaved and cooled before use. Autoclaving aids in killing the growth of unwanted saprophytic fungi as well as pests. Trap culture can be initiated in three replicates by using 500 gm, capacity pots or root trainer. Smaller pot sizes should be avoided, because roots occupy the volume very quickly, similarly larger pots should also be avoided since optimal sporulation takes longer time in them. Three forth of the root trainer is usually filled with the soil and sand mixture (substrate). A soil profiling test is then carried out by adding water into the root trainer till it starts dripping from the bottom. These are then placed in the polyhouse/green house/ growth rooms at 20 °C to 25 °C.

STEP 4: Culture initiation

The inoculum is divided into three parts and each part is added into a three-fourth filled root trainer. Large numbers of pre germinated seeds are added on the inoculum; this over seeding is done for several reasons; to prevent exposure of soil surface, to prevent the chance of contamination; to minimize splashing and movement of surface particulates during watering; higher root biomass generation for higher inoculum production. After placing the seeds, they are gently covered with remaining substrate. Watering is done at the interval of two to three times a week and fertilization (half strength Hoagland solution) only when plants show signs of phosphorus deficiency (purpling of leaves).

STEP 5: Duration and maintenance

Cultures are allowed to grow in the greenhouse till the completion of first life cycle of sorghum plants. It has been observed that sporulation is initiated after the completion of three months. Towards the end of plants' life cycle, there is a boost in the sporulation rate, as justified by INVAM. During the fourth month, when plant shoots (and roots) have ceased growth, carbon could seem to be repartitioned to sporulation rather than Mycorrhizal development. In case the researcher desires to extract all the spores for trial experiments, the root trainers can be dried leaving it undisturbed in a shaded place without adding any water or nutrient solution to it for at least two weeks, after which the spores can be extracted using the wet sieving and decanting method as described by Gerdemann and Nicolson (1963). In case storing of the culture is required, the desiccation process should be for a longer duration, that is, till all moisture is removed from the soil. This desiccated soil should then be stored in zip lock bags under refrigeration.

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STEP 3 : Pots and sand

STEP 4 : Culture initiation

STEP 5 : Duration and maintenance

Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

- *Acta Oecologica*
- *Applied Soil Ecology*
- *Chemosphere*
- *Ecological Engineering*
- *European Journal of Soil Biology*
- *Fungal Biology*
- *Fungal Ecology*
- *Geoderma*
- *Journal of Plant Physiology*
- *Mycoscience*
- *Plant Science*
- *Science of The Total Environment*
- *Scientia Horticulturae*
- *Soil Biology and Biochemistry*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
Aroca R*, Ruiz-Lozano J M, Zamarreño A M, Paz J A, García-Mina, Pozo M J, López-Ráez J A. 2013	**Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants** *Journal of Plant Physiology* **170**: 47–55 [*Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas (EEZ-CSIC), Profesor Albareda 1, 18008 Granada, Spain]
Cseresnyés I*, Takács T, Végh K R, Anton A, Rajkai K. 2013	**Electrical impedance and capacitance method: A new approach for detection of functional aspects of arbuscular mycorrhizal colonization in maize** *European Journal of Soil Biology* **54***:* 25–31 [*Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, H-1022 Budapest, Herman Ottó út 15, Hungary]
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Heydarizadeh P*, Zahedi M, Sabzalian M R, Ataii E. 2013	**Mycorrhizal infection, essential oil content and morpho-phenological characteristics variability in three mint species** Scientia Horticulturae **153**: 136–142 [*Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran]
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Liu Y*, Gianinazzi-Pearson V, Arnould C, Wipf D, Zhao B, Tuinen D v	**Fungal genes related to calcium homeostasis and signalling are upregulated in symbiotic arbuscular mycorrhiza interactions** *Fungal Biology* **117**(1): 22–31 [*College of Life Science and Technology, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China]

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Ogura-Tsujita Y*, Umata H, Yukawa T. 2013	**High mycorrhizal specificity in the mycoheterotrophic *Burmannia nepalensis* and *B. itoana* (Burmanniaceae)** *Mycoscience*, ***In Press, Corrected Proof***, *Available online 9 May 2013* [Botanical Gardens, Tohoku University, 12-2 Kawauchi, Aoba-ku, Sendai, Miyagi 980-0862, Japan [This article is not included in your organization's subscription. However, you may be able to under your organization's agreement with Elsevier.]
Oztekin G B*, Tuzel Y, Tuzel I H	**Does mycorrhiza improve salinity tolerance in grafted plants?** *Scientia Horticulturae* **149**: 55-60 [Ege University, Faculty of Agriculture, Department of Horticulture, 35100 Bornova-Izmir, Turkey]
Pagano M C, Zandavalli R B, Araújo F S. 2013	**Biodiversity of arbuscular mycorrhizas in three vegetational types from the semiarid of Ceará State, Brazil** *Applied Soil Ecology* **67**: 37–46 [Universidade Federal do Ceará, Campus do Pici, Centro de Ciências, Biology Department, Bloco 906, Av. Mister Hull, s/n CEP: 60455-760, Fortaleza, Ceará, Brazil]
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Salomón M E S*, Barroetaveña C, Rajchenberg M. 2013	***Pseudotsuga menziesii* invasion in native forests of Patagonia, Argentina: What about mycorrhizas?** *Acta Oecologica* **49**: 5–11 [*Ingeniería Forestal Universidad Nacional de la Patagonia S J Bosco, Sarmiento 849, Esquel, Chubut, Argentina]
Salvioli A and Bonfante P. 2013	**Systems biology and "omics" tools: A cooperation for next-generation mycorrhizal studies** *Plant Science* **203–204**: 107–114 [*Department of Life Sciences and Systems Biology, Viale Mattioli 25 – 10125 Torino, Italy]
Seleiman M F*, Santanen A, Kleemola J, Stoddard F L, Mäkelä P S A. 2013	**Improved sustainability of feedstock production with sludge and interacting mycorrhiza** *Chemosphere* **91**(9): 1236–1242 [Department of Agricultural Sciences, University of Helsinki, P.O. Box 27, FIN-00014, Helsinki, Finland]
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Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
Twanabasu B R*, Stevens K J, Venables B J. 2013	**The effects of triclosan on spore germination and hyphal growth of the arbuscular mycorrhizal fungus *Glomus intraradices*** *Science of The Total Environment* **454–455**: 51-60 [*Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA]
Vohník M* Mrnka L, Lukešová T, Bruzone M C, Kohout P, Fehrer J. 2013	**The cultivable endophytic community of Norway spruce ectomycorrhizas from microhabitats lacking ericaceous hosts is dominated by ericoid mycorrhizal *Meliniomyces variabilis*** *Fungal Ecology*, ***In Press, Corrected Proof***, *Available online 14 May 2013* [*Department of Mycorrhizal Symbioses, Institute of Botany ASCR, Průhonice 252 43, Czech Republic]
Zubek S*, Stefanowicz A M, Błaszkowski J, Niklińska M, Seidler-Łożykowska K. 2012	**Arbuscular mycorrhizal fungi and soil microbial communities under contrasting fertilization of three medicinal plants** *Applied Soil Ecology* **59**: 106–115 [*Laboratory of Mycology, Institute of Botany, Jagiellonian University, Lubicz 46, 31-512 Kraków, Poland]

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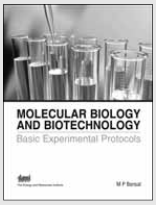

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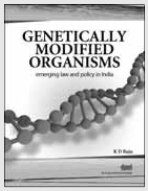

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