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AM fungi: A biological approach towards conservation of endangered plants in Thar desert, India

Jitendra Panwar* and Anil Vyas
Department of Botany, J.N.V. University, Jodhpur 342 001, India

The efficiency of eight arbuscular mycorrhizal species, *Acaulospora mellea* Spain & Schenck, *Gigaspora margarita* Becker & Hall, *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe, *Glomus deserticola* Trappe, Bloss & Menge, *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe, *Sclerocystis rubiformis* Gerd. & Trappe, *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders and *Scutellospora nigra* (Red head) Walker & Sanders, collected from rhizosphere soils of *Moringa concanensis*, was evaluated for nutrient uptake and enhancement of acid phosphatase, nitrate reductase, peroxidase and polyphenol oxidase activities in this endangered tree of the Indian Thar desert. Culturing was done under glasshouse conditions and analyses were performed 180 days after inoculation. All fungi showed beneficial effects, with *G. margarita* being the most efficient in promoting all biochemical parameters.

MORINGA concanensis is a highly endangered multipurpose tree species of the Indian Thar desert, belonging to the family Moringaceae[1]. *M. concanensis* is a source of fodder and food. It helps in sand stabilization and acts as a source of shade. The primary stresses imposed on vegetation by arid environment are lack of water and mineral nutrients[2]. Arbuscular mycorrhizal (AM) fungi are known to help plants in nutrient uptake and disease resistance[3] and offer drought tolerance by tapping water from a large volume of soil[4]. The capacity of AM fungi to act as biofertilizers, bioregulators and bioprotectors[5–7] has repeatedly been demonstrated. Thus, they play a key role in sustainable conservation of tropical gene pool and diversity[8]. AM fungi are well-known to bring about biochemical changes in plants by increasing various enzymatic activities[9]. Acid phosphatase and nitrate reductase are important enzymes of phosphorus and nitrogen metabolism, respectively. Peroxidase (PRO) and polyphenol oxidase (PPO) are important components of the defence mechanism of plants against pathogens. Phenols are also important in plant disease resistance. In view of these benefits, this investigation was undertaken to compare the possible benefits of AM fungal inoculation on growth, nutrient uptake and biochemical enhancement of this endangered tree species of Thar desert.

Eight AM species – *Acaulospora mellea* Spain & Schenck, *Gigaspora margarita* Becker & Hall, *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe, *Glomus deserticola* Trappe, Bloss & Menge, *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe, *Sclerocystis rubiformis* Gerd. & Trappe, *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders and *Scutellospora nigra* (Red head) Walker & Sanders – were examined. Fungal samples were collected by wet sieving and decanting[10] the soil samples from the rhizosphere of *M. concanensis*. Identification of spore type is based on the manual for identification of VA mycorrhizal fungi[11]. All the eight AM species were maintained in pot cultures of *Cenchrus ciliaris*. Soil samples from the pot cultures, along with infected root segments of *C. ciliaris* were used as inoculum. Twenty grams of inoculum containing about 2000 spores and infected root segments was inoculated in 18-cm diameter pots containing sterilized soil. The soil used was sterilized sand–soil mixture (1 : 1), pH 8.3, available P 2.35 ppm, total N 2.31 ppm, and organic carbon 0.13%. One seedling of *M. concanensis* was maintained per pot. There were twenty replicates for each treatment. The pots were placed in a glasshouse at 60% humidity with 12–14 h day length at 24–26°C. The pot without AM inoculation served as control.

*For correspondence. (e-mail: panwarjitendra007@rediffmail.com)

All pots were supplied with half strength Hogland's nutrient solution every 15 days. After six months, the plants were harvested. Phosphorus was estimated by the vanadomolybdate yellow colour method[12] and total nitrogen by microkjeldahl method[13]. NR activity was measured by Wray and Filner's method[14]. Activity of acid phosphates was assayed by the method of Tabatabai and Bremner[15]. Phosphatase activity was expressed in terms of enzyme units (EU), one unit corresponding to the amount of enzyme required to hydrolyse 1.0 µmol of *p*-nitrophenyl phosphate (PNP) at 35°C min^{-1} at pH 5.4. Total phenols was estimated using Folin and Ciocalteu's reagent and absorbance of blue colour developed was read at 670 nm (ref. 16). PRO activity was measured by incubating the samples with guaiacol and hydrogen peroxidase[17]. PPO activity was measured at 420 nm, using the method of Mahadevan[18].

Observations regarding changes in acid phosphatase NR activities are presented in Table 1. AM-colonized plants showed higher acid phosphates activities than non-mycorrhizal ones. Among the eight AM species used during the present investigation, *G. margarita* increases the acid phosphatase activity most efficiently, while *A. mellea* responded least effectively. Thus increase in acid phosphatase activity was similar to the earlier findings of AM-inoculated wheat plants[19]. NR activity was in the range of 0.17–0.41 m mol h^{-1} g^{-1} fresh weight. However, the AM species varied in their effects on this enzyme. Inoculated *G. margarita* plants showed a more than two-fold increase in NR activity. A similar enhancement of NR activity was reported in *Z. numularia*[20]. The results in the present investigation suggest that with a capacity for reducing nitrate, it is likely that the symbiotic effectiveness of AM fungi is enhanced in terms of nitrogen assimilation and translocation to the host plant.

An increase in uptake of both the nutrients, i.e. phosphorus and nitrogen was observed in the AM-inoculated plants (Table 2). *G. margarita* resulted in more than a two-fold increase in uptake of both the nutrients, followed by *G. gigantea*. Least response was noticed in *A. mellea*-inoculated plants compared to control. The increased P-uptake by AM endophytes has been well recognized[21,22]. Cliquet and Stewart[23] observed increased nitrogen uptake by AM fungi by changing nitrogen metabolism. Mathur and Vyas[9] reported changes in enzymes of nitrogen metabolism by AM fungi. The increased nitrogen content in our study might be due to such a phenomenon.

AM inoculation also increased PRO and PPO activities in roots of *M. concanensis* (Table 3). The increased PRO activity by AM fungi might be due to an increased P-uptake resulting from the symbiosis. McArthur and Knowles[24] reported lower peroxidase activity in low-P roots than high-P roots.

A positive correlation was observed between total phenol accumulation and PPO activity in AM-inoculated *M. concanensis* roots (Table 3). *G. margarita* also was the most efficient in enhancing phenolic accumulation. The increased phenolic accumulation might have resulted from an increased PPO activity. Accumulation of phenols in AM plants has been reported[25]. PRO and PPO are important in the defence mechanism against pathogens. Both enzymes are involved in the oxidation of phenolic compounds to quinones, which are presumably toxic to pathogens[26]. The considerable increase in PRO and PPO by *G. margarita* may be significant in protecting this endangered tree from attacking pathogens.

It can be concluded that inoculation with *G. margarita* will cause elevation of assimilating enzymes most efficiently in *M. concanensis*, which collectively will lead to

Table 1. Acid phosphatase and nitrate reductase activities in *M. concanensis* resulting from infection by different AM fungi

Treatment	Acid phosphatase (EU × 10^{-4})	NR (mmol h^{-1} g^{-1} fw)
Control	1.26	0.17
A. mellea	1.38	0.19
G. gigantea	1.82	0.37
G. margarita	1.88	0.41
G. deserticola	1.61	0.27
G. fasciculatum	1.52	0.23
S. rubiformis	1.46	0.21
S. calospora	1.76	0.34
S. nigra	1.68	0.30
C.D. ($P = 0.05$)	0.45	0.15

Table 2. Influence of AM fungi on nutrient uptake in *M. concanensis*

Treatment	Phosphorus (mg $plant^{-1}$)	NR (mmol h^{-1} g^{-1} fw)
Control	3.5	4.5
A. mellea	3.8	4.8
G. gigantea	6.4	8.2
G. margarita	7.2	9.4
G. deserticola	4.8	6.0
G. fasciculatum	4.2	5.4
S. rubiformis	4.0	5.0
S. calospora	5.2	6.8
S. nigra	5.8	7.3
C.D. ($P = 0.05$)	0.16	0.12

Table 3. Changes in PRO and PPO activities in roots of *M. concanensis* as a result of infection by different AM fungi

Treatment	PRO activity (units mg^{-1} protein)	PPO activity (ΔA_{420}/100 mg fw)	Total phenol (% dry weight)
Control	92.4	98.4	10.0
A. mellea	108.2	118.3	10.5
G. gigantea	150.0	154.0	13.0
G. margarita	161.4	162.0	13.4
G. deserticola	128.4	134.4	11.8
G. fasciculatum	121.8	128.6	11.5
S. rubiformis	116.6	123.4	11.0
S. calospora	143.4	144.2	12.6
S. nigra	138.5	140.5	12.2
C.D. ($P = 0.05$)	12.6	14.2	0.42

increased nutrient uptake. It will also make the plant more resistant to pathogens as a result of increased PRO and PPO activities. The present investigation suggests that AM inoculation would help in re-establishment and conservation of this endangered tree of the Indian Thar desert. Use of AM fungi as a fertilizer helps in the establishment of plants in arid and semiarid regions and also in the increase of fertility of soil by making available phosphorus and nitrogen to the plant. Use of AM fungi as a biofertilizer is a low-cost technique, but results are surprisingly better than the use of chemical fertilizers. So, these results open new prospects on the utilization of AM fungi.

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Meiotic XY association could be both chiasmate and achiasmate in the Indian pygmy field mouse, *Mus terricolor*

A. Bardhan[†] and T. Sharma*

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India
[†]Present address: Department of Genetics, Institute of Life Sciences, The Hebrew University of Jerusalem, Givat Ram 91904, Jerusalem, Israel

Between the two alternative hypotheses explaining the nature of meiotic XY association in male mammals, chiasmate and achiasmate, only the former has gained credence. XY bivalents at diakinesis/metaphase-I in the Indian pygmy field mouse *Mus terricolor* make cytological visualization of chiasma feasible, due to the accretion of a block of heterochromatin distal to the pseudoautosomal region. The results revealed that meiotic XY association in *M. terricolor* could be either chiasmate or achiasmate, suggesting that the latter could also ensure successful meiotic progression.

MEIOSIS is the process whereby ploidy is maintained over generations in sexually reproducing organisms. In mammals with XY sex determination system in the males, maintenance of the association between the X and Y chromosomes is essential for survival of the meiocytes to form gametes[1], barring exceptional species where the X and Y chromosomes do not associate in meiotic prophase-I. The X and Y chromosomes share a segment of genetic homology, the pseudoautosomal region (PAR), that has the potential to undergo homologous synapsis and reciprocal recombination. However, the cytological manifestation of reciprocal exchange, i.e. chiasma, is generally not seen in a majority of mammals, including the mouse; and the X and Y chromosomes are seen associated end-to-end at diakinesis/metaphase-I. This association is variously interpreted as 'obligate chiasmate'[2,3] or 'achiasmate'[4] association. Presently, there is a strong

*For correspondence. (e-mail: tsharma@banaras.ernet.in)