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Response of *Andrographis paniculata* to different arbuscular mycorrhizal fungi.

Tharun Chiramel[1], D.J. Bagyaraj [2*] and C.S.P. Patil [1]

[1]Department of Silviculture and Forest Biology, College of Forestry, University of Agricultural Sciences, Ponnampet 571 216, Karnataka, India
[2]Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK campus, Bangalore, India 560 065

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A glass house study was conducted under nursery conditions to study the efficacy of eleven arbuscular mycorrhizal (AM) fungi on kalmegh (*Andrographis paniculata*). Seedlings were raised in polythene bags containing soil inoculated with isolates of different AM fungi. Kalmegh seedlings raised in presence of AM fungi generally showed an increase in plant growth and andrographolide (active ingredient) concentration over those grown in the absence of the inoculation of soil with AM fungi. The extent of improvement by AM fungi varied with the species of AM fungi inhabiting the roots of kalmegh seedlings. Considering the various parameters and andrographolide concentration of the plants, it was observed that *Glomus leptotichum* and *Glomus intraradices* are the two best AM symbionts for kalmegh compared to others used under this experiment.

Keywords*:* Arbuscular mycorrhiza; *Andrographis paniculata*; andrographolide

Introduction

Tropical forests are an abode of medicinal plants. They are in great demand due to the increased acceptance of ayurveda and traditional medicines, because of their property of less or no side effects. In fact, large scale collection of medicinal plants from forests has accelerated the depletion of tropical forests, thus making them endangered and threatened group of plant species. In order to maintain a sustained supply of raw materials to the drug industry, these plants are encouraged to be cultivated outside the forest ecosystem in recent years. Kalmegh (*Andrographis paniculata* Nees.) is one such medicinal plant with numerous medicinal properties. It is an erect branched annual herb of height 0.3–0.9 m, with branches sharply quadrangular, leaves lanceolate, flowers small, white, solitary with yellowish brown seeds.

[*]Corresponding author: D.J.Bagyaraj; e-mail: djbagyaraj@vsnl.com

The fresh and dried leaves of kalmegh and juice extracted from the herb is an official drug of Indian pharmacopoeia. It is a source of several diterpinoids of which andrographolide is important. The drug is used for general debility, certain forms of dyspepsia, chronic malaria, jaundice and dysentry. Some scientists have observed that andrographolide has the potential to be included in the cocktail vaccine against AIDS by virtue of its antagonistic property with HIV II virus (Weibo, 1995). It is already being used in treating cancer as it promotes cell differentiation in tumour cells (Matsuda *et al.*, 1994). Though leaves contain maximum andrographolide the entire plant is used for extracting the active ingredient.

The current day emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus, use of microbial inoculants play an important role in sustainable agriculture. AM fungi are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens and offer resistance to drought and salinity (Jeffries, 1987). Though these fungi are not host specific, recent studies have clearly brought out host preference in AM fungi thus emphasizing the need for selecting efficient AM fungi for inoculating a particular host. Host preference has been reported in many forest tree species like *Casuarina equisetifolia* (Vasanthakrishna *et al*., 1995); *Tectona grandis* (Rajan *et al*., 2000); *Garcinia indica* (Lakshmipathy *et al*., 2003) and a few medicinal plant species like *Phyllanthus* a*marus* and *Withania somnifera* (Earanna, 2001), and *Coleus forskohlii* (Gracy and Bagyaraj, 2005). Hence, in the present investigation, it was envisaged to screen and select an efficient AM fungus for inoculating kalmegh for its cultivation.

Materials and methods

The investigation was carried out under nursery conditions in a glass house. The substrate used was sand: soil: vermicompost mixture in 1:1:0.25 v/v/v ratio. The soil used for this study has been classified as fine, kaolinitic, isohyperthermic, kanhaplustalfs. The substrate pH was 6.93, and it contained 11.3 ppm available phosphorus and an indigenous AM mycorrhizal population of 78 spores per 50 g.

The AM fungal species used in the study were either isolated or obtained from different places as mentioned in Table 1. These fungi were multiplied using sterilized sand – soil mix (1:1 v/v) as the substrate and Rhodes grass as the host. After six weeks of growth, shoots of Rhodes grass were severed and the substrate containing hyphae, spores and root bits was air dried and used as inoculum. The inoculum potential (IP) of each culture was calculated adopting the most probable number (MPN) method as outlined by Porter (1979). The

mycorrhizal inoculum with 12, 500 IP was applied to the planting hole at a depth of about 5 cm just before transplanting.

Each polythene bag containing the substrate, with or without AM inoculum, as per the treatment be, was planted with one kalmegh seedling. Fourteen days old kalmegh seedlings raised in the substrate described above, were transplanted to polythene bags of size 25 × 12 cm holding 2.2 kg of the substrate. Each treatment with 10 replications was maintained in a glass house and watered regularly. Plant height, number of leaves and number of branches were measured and recorded at 30 days interval. However, only observations recorded on day 90 are presented in this paper. The plants were harvested at 90 days after planting (DAP). Dry biomass of shoot and root portions were recorded separately after drying the plant sample at 60°C to a constant dry weight in a hot air oven. Phosphorus concentration of the plant tissue was determined by employing the vanadomolybdate phosphoric acid yellow colour method (Jackson, 1973). The andrographolide concentration was estimated using ultra violet spectrophotometric analysis after soxhlet extraction (Gaind *et al*., 1963).

Fine roots were stained using 0.02% trypan blue as described by Philips and Hayman (1970) and the per cent root colonization was estimated by adopting the gridline intersect method (Giovanetti and Mosse, 1980). Soil samples (50 ml) were collected from each polythene bag and subjected to wet sieving and decantation method as outlined by Gerdemann and Nicolson (1963) to estimate the population of spores.

The results were subjected to two way analysis of variance suitable for CRD for the test of significance and the means were separated using Duncan's Multiple Range Test (DMRT) (Little and Hills, 1978).

Results

In general, plants inoculated with AM fungi grew taller than the uninoculated plants. Plants inoculated with *Glomus leptotichum, G. etunicatum* and *G. mosseae* showed significantly higher plant height compared to uninoculated plants and the treatment with *G. macrocarpum* (Table 1). Plants inoculated with *Gigaspora margarita* and *G. etunicatum* showed the highest number of leaves closely followed by *Scutellospora calospora*, *G. leptotichum* and *G. intraradices,* all the five being statistically on par with each other but differing significantly from the uninoculated plants (Table 1). Highest number of branches was present in plants inoculated with *G. leptotichum* and *G. intraradices* both being statistically on par with each other but differing from other treatments. All other inoculated treatments except *G. macrocarpum* and

S. calospora, did not differ significantly from the uninoculated treatment (Table 1).

Only plants inoculated with *G. intraradices* and *G. leptotichum* showed significantly higher shoot weights, compared to the uninoculated plants. Significantly higher root weight was observed only in *G. intraradices* treated plants, compared to the uninoculated control treatment (Table 1).

Glonum intraradices and *G. leptotichum* treated plants had higher P concentration in shoot which differed significantly from other treatments except *G. macrocarpum* and *S. calospora*. Root phosphorus concentration was found to be highest in plants inoculated with *G. leptotichum* and *G. intraradices*, which did not differ significantly from other treatments except *A. laevis* and uninoculated control (Table 2).

Regarding the andrographolide concentration of the plant, only in plants inoculated with *G. leptotichum* and *G. intraradices* there was significant enhancement of the active ingredient compared to all other treatments (Table 2).

In general, mycorrhizal inoculation increased the per cent mycorrhizal root colonization and spore numbers in soil. The highest per cent root colonization was observed in *G. intraradices* treated plants followed by those treated with *G. monosporum* and *G. leptotichum*. Highest number of AM spores per 50 g root zone soil was found in plants treated with *G. leptotichum* which did not differ significantly from other inoculation treatments except *G.mosseae.* The lowest spore numbers was observed in the root zone soil of uninoculated plants (Table 2).

Discussion

In general, plant height, number of branches, shoot and root dry weight were significantly greater in plant inoculated with *Glomus leptotichum* and *G. intraradices* compared to the other treatments. Increase in the shoot dry weight, because of inoculation with *G. intraradices* and *G. leptotichum* compared to uninoculated plants was 37.5 and 36.4% respectively.

The increase in root biomass because of inoculation with *Glomus leptotichum* and *G. intraradices* was 51.4 and 91.4% respectively compared with uninoculated plants. Improvement of plant growth with inoculation of AM fungi has been reported in a number of forest tree species (Rajan *et al*., 2000; Vasanthakrishna *et al*., 1995) and medicinal plants.

Table 1. Effect of soil inoculation with arbuscular mycorrhizal fungi on plant height, number of leaves, number of branches, shoot and root dry weight of *Andrographis paniculata*

Treatment [a]	Plant height (cm)	No. of leaves/ plant	No. of branches/plant	Shoot dry weight (g)	Root dry weight (g)
Uninoculated	52.4 [c]	70.4 [c]	12.4 [c]	10.72 [b]	2.22 [bc]
Acaulospora laevis (Ned)	59.5 [abc]	87.6 [abc]	13.5 [bc]	13.81 [ab]	2.98 [abc]
Gigaspora margarita (Hyd)	61.2 [abc]	95.6 [a]	13.6 [bc]	13.62 [ab]	2.02 [c]
Glomus bagyarajii (All)	57.0 [abc]	83.4 [abc]	13.8 [bc]	13.74 [ab]	3.78 [ab]
Glomus etunicatum (Slc)	66.0 [a]	94.3 [a]	13.5 [bc]	14.24 [ab]	3.70 [ab]
Glomus fasciculatum (Riv)	57.0 [abc]	74.2 [bc]	14.0 [bc]	13.20 [ab]	2.64 [bc]
Glomus intraradices (Ban)	61.5 [abc]	91.0 [ab]	16.4 [a]	14.74 [a]	4.25 [a]
Glomus leptotichum (Ban)	66.5 [a]	91.0 [ab]	17.2 [a]	14.62 [a]	3.36 [abc]
Glomus macrocarpum (Ban)	54.8 [bc]	86.2 [abc]	14.5 [b]	13.47 [ab]	3.17 [abc]
Glomus monosporum (Ned)	60.3 [abc]	87.0 [abc]	13.4 [bc]	14.01 [ab]	3.42 [abc]
Glomus mosseae (Ban)	64.0 [ab]	77.2 [abc]	14.0 [bc]	12.26 [ab]	2.51 [bc]
Scutellospora calospora (Hyd)	62.9 [abc]	91.8 [ab]	14.3 [b]	13.25 [ab]	3.05 [bc]

[a] Ned – Isolate from University of Western Australia, Nedlands, Australia; Ban – Isolate from University of Agricultural Sciences, Bangalore, India; Hyd – Isolate from ICRISAT, Hyderabad, India; Riv – Isolate from University of California, Riverside, USA; Slc – Isolate from Native Plants Institute, Salt Lake city, USA; All – Isolate from University of Allahabad, India.

Means with same superscript in each column do not differ significantly at P = 0.05 level by Duncan's Multiple Range Test

(Earanna 2001; Boby and Bagyaraj, 2003). Generally, treatments with higher plant dry weight also had higher phosphorus concentration. Highest dry matter production and tissue P concentration was observed in plants treated with *G. intraradices* and *G. leptotichum*. The increase in shoot and root P concentration was 10.6 and 4.4% respectively in *Glomus leptotichum* inoculated plants and 12.8 and 3.6% in *Glomus intraradices* inoculated plants compared with uninoculated plants.

The main effect of mycorrhizal fungi in improving plant growth is through improved uptake of nutrients, especially phosphorus due to the exploration by the external hyphae of the soil beyond root hair zone when

Table 2. Effect of soil inoculation with arbuscular mycorrhizal fungi on shoot and root P, plant andrographolide concentration, mycorrhizal root colonization and spore number in root zone soil of *Andrographis paniculata*

Treatment a	Shoot P (%)	Root P (%)	Andrographolide concentration (%)	Colonisation (%)	Spore No /50g soil
Uninoculated	0.243 d	0.613 c	1.895 c	30.67 b	84.0 c
Acaulospora laevis (Ned)	0.396 bc	0.713 bc	1.875 c	49.00 a	170.0 a
Gigaspora margarita (Hyd)	0.370 bcd	0.827 ab	2.400 abc	46.33 a	153.7 ab
Glomus bagyarajii (All)	0.320 cd	0.770 abc	2.025 c	44.67 ab	159.0 ab
Glomus etunicatum (Slc)	0.336 cd	0.777 ab	2.137 abc	51.00 a	161.3 ab
Glomus fasciculatum (Riv)	0.326 cd	0.733 abc	1.875 c	48.67 a	184.0 a
Glomus intraradices (Ban)	0.553 a	0.833 ab	2.738 ab	57.67 a	184.0 a
Glomus leptotichum (Ban)	0.500 a	0.880 a	2.775 a	54.67 a	191.7 a
Glomus macrocarpum (Ban)	0.470 abc	0.770 abc	2.400 abc	48.67 a	186.7 a
Glomus monosporum (Ned)	0.356 bcd	0.780 ab	2.100 bc	55.00 a	148.0 ab
Glomus mosseae (Ban)	0.323 cd	0.733 abc	2.063 c	44.00 ab	121.0 bc
Scutellospora calospora (Hyd)	0.490 ab	0.797 ab	2.325 abc	48.67 a	157.0 ab

[a]Legend same as Table 1
Means with same superscript in each column do not differ significantly at P = 0.05 level by Duncan's Multiple Range Test

phosphorus is depleted (Gerdemann, 1975). Increased phosphorus uptake has been attributed not only to increased surface area of absorption (Sanders and Tinker, 1971) but also to enhanced hyphal translocation (Hattingh *et al*., 1973). Enhanced plant biomass and P uptake because of AM fungal inoculation has been reported by earlier workers in forest tree species and a few medicinal plants (Vasanthakrishna *et al.*, 1995; Gracy Sailo and Bagyaraj, 2005).

The andrographolide concentration was also highest in *Glomus leptotichum* treated plants followed by those treated with *Glomus intraradices*. Though the mechanism of this enhancement is unknown, this is the first report on AM fungal inoculation increasing andrographolide concentration in

kalmegh. However, an increase in forskolin (active ingredient) content of *Coleus forskohlii* after AM inoculation has been reported recently (Boby and Bagyaraj, 2003).

Species and strains of AM fungi have differed to the extent by which they increase nutrient uptake and plant growth (McGraw and Schenck, 1981, Gracy Sailo and Bagyaraj, 2005). Hence, the need for selecting efficient AM fungi that can be used for inoculating different mycotrophic plants has been stressed (Abbott and Robson, 1982, Jeffries, 1987, Bagyaraj and Varma, 1995). The efficiency refers to ability of the fungus to increase plant growth in a phosphate- deficient soil (Abbott and Robson, 1982). This depends on the ability to form extensive and well – distributed hyphae in soil, to form extensive colonization in the root system, and to absorb P from soil.

In the present study, mycorrhizal parameters, such as percent root colonization and extrametrical spores, were considerably higher in all the inoculated treatments compared to the uninoculated treatment. The extent of colonization and the spore count varied with different AM fungi. Higher root colonization and the sporulation allows more fungal-host contact and exchange of nutrients, hence better plant growth.

By giving emphasis to parameters like andrographolide concentration and plant biomass, and not neglecting the other characteristics *Glomus leptotichum* and *Glomus intraradices* are considered to be the most promising symbionts for inoculating kalmegh. This technology being simple can easily be adapted by farmers cultivating this medicinal crop.

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References

Abbott, L.K. and Robson, A.D. (1982). The role of vesicular-arbuscular mycorrhizal fungi and selection of fungi for inoculation. Australian Journal of Agriculture Research 33: 389-408.

Bagyaraj, D.J. and Varma, A. (1995). Interactions between arbuscular mycorrhizal fungi and plants: their importance in sustainable agriculture in arid and semiarid tropics. Advances in Microbial Ecology 14: 119-142.

Boby, V.U. and Bagyaraj, D.J. (2003). Biological control of root-rot of *Coleus forskholii* Briq. using microbial inoculants. World Journal of Microbiology and Biotechnology 19: 175-180.

Earanna, N. (2001). VA mycorrhizal association in medicinal plants of Southeastern dry zone of Karnataka and response of *Phyllanthus amarus* and *Withania somnifera* to inoculation with VAM fungi and plant growth promoting rhizomicroorganisms. Ph.D thesis. Submitted to University of Agricultural Sciences, Bangalore.

Gaind, K.N., Dar, R.N. and Kaul, R.N. (1963). Spectrophotometric method of assay of andrographolide in Kalmegh. Indian Journal of Pharmacy 25: 225–226.
Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. Transactions of the British Mycological Society 46: 235-244.
Gerdemann, J.W. (1975). Vesicular – arbuscular mycorrhizae. In: *The Development and Function of Roots*. (eds. J. G. Torrey and D. T. Clarkson). Academic Press: London: 575-591.
Giovannetti, M. and Mosse, B. (1980). An evaluation of technologies for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84: 489-500.
Gracy. L. Sailo. and Bagyaraj, D.J. (2005). Influence of different AM fungi on growth, nutrition and forskholin content of *Coleus forskohlii*. Mycological Research 109: 795-798.
Hattingh, M.J., Gray, L.E. and Gerdemann, L.W. (1973). Uptake and translocation of ^{32}P labeled phosphate to onion roots by mycorrhizal fungi. Soil Science 116: 383-387.
Jackson, M.L. (1973). Soil Chemical Analysis. pp 239-241. New Delhi: Prentice Hall (India) Pvt.Ltd.
Jeffries, P. (1987). Use of mycorrhizae in agriculture, CRC Critical Review of Biotechnology 5: 319-357.
Lakshmipathy, R., Balakrishna Gowda, Chandrika, K. and Bagyaraj, D.J. (2003). Symbiotic response of *Garcinia indica* to VA mycorrhizal inoculation. Indian Journal of Forestry 26: 143-146.
Little, T.M. and Hills, F.J. (1978). Agricultural Experimentation: Design and Analysis. John Wiley and Sons.Inc. USA.
Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A. and Nishi, K. (1994). Cell differentiation-inducing diterpenes from *Andrographis paniculata* Nees. Chemical Pharmaceutical Bulletin. 42: 1216-25.
McGraw, and Schenck, N.C. (1981). Effects of two species of vesicular arbuscular mycorrhizal fungi on the development of *Fusarium* wilt of tomato. Phytopathology 7: 894-897.
Philips, J.H. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular – arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-161.
Porter, W.M. (1979). The most probable number method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Australian Journal of Soil Research 17: 515-519.
Rajan, S.K., Reddy, B.J.D. and Bagyaraj, D.J. (2000). Screening of mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. Forest Ecology and Management 126: 91-95.
Sanders, F.E. and Tinker, P.B. (1971). Mechanism of absorption of phosphate from soil by *Endogone* Mycorrhizas. Nature 233: 278–279.
Vasanthakrishna, M., Bagyaraj, D.J. and Nirmalnath, J.P. (1995). Selection of efficient VA mycorrhizal fungi for *Casuarina equisetifolia* – Second screening. New Forests 9: 157-162.
Weibo, L. (1995). Prospect for study on treatment of AIDS with traditional Chinese medicine. Traditional Chinese Medicine 15: 3-9.

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