Philippine Journal of Science
129 (1): 51-55, June 2000
ISSN 0031 - 7683

RESEARCH

Arbuscular Mycorrhizal Fungi Associated with Some Economically Important Spices and Aromatic Plants

G. VENKATESHWAR RAO[1], C. MANOHARACHARY*[1],
I.K. KUNWAR[1] and B.R RAJESHWAR RAO[2]

[1]Department of Botany, Osmania University, Hyderabad – 500 007, India
[2]Central Institute of Medicinal and Aromatic Plants Field Station,
Boduppal, Uppal, Hyderabad – 500 039, India

Economically important spice crops viz. cinnamon, coriander, curry leaf, fenugreek, onion and aromatic crops viz. citronella, cornmint, east Indian jamrosa, lemon grass, lemon scented gum, palmarosa, rose scented geranium and vetiver were screened for arbuscular mycorrhizal (AM) fungal association. 18 AM fungal species represented by eight species of *Acaulospora*, four of *Glomus*, three of *Gigaspora,* two of *Scutellospora* and one of *Sclerocystis* were found associated with the rhizosphere soils of these plants. Among all the fungal species, *Glomus fasciculatum* was the most predominant species found associated with 11 crops. This is the first report on the occurrence of these many genera and species of AM fungi in these crops. The number of AM fungal propagules ranged from 40-120 / 10g soil. The AM fungal colonization percentage in the roots ranged from 0-59%. Maximum colonization was observed in the roots of rose scented geranium (59%) followed by cornmint (46%) and vetiver (42%).

Key words: Root colonization, propagule number, phosphorus, soil.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in nature and form obligate symbiotic association with the roots and other underground parts of most of the angiospermic and some other plants. They have gained considerable importance in recent years owing to their beneficial response in improving crop productivity and disease resistance. There are reports on the association of AMF with medicinal, spice and aromatic crops (Barthakur & Bordoloi 1990, Boerner 1990, Gupta & Janardhanan 1991, Khaliq & Janardhanan 1994, Kothari & Singh 1996, Prasad & Sailaja 1995, Chee *et al.* 1998, Saleh *et al.* 1998, Kunwar et al. 1999, 2000). For our investigations we selected thirteen economically important spice and aromatic crops, and studied the qualitative and quantitative composition of AMF associated with selected spice and aromatic crops and various physico-chemical characterstics of the soil in relation to number of propagules of AMF.

Materials and Methods

Five spice crops, namely, cinnamon (*Cinnamomum zeylanicum* Blume), coriander (*Coriandrum sativum* L.), curry leaf (*Murraya koenigii* (L.) Spreng.), fenugreek (*Trigonella foenum-grecum* L.) onion (*Allium cepa* L.) and eight aromatic crops, namely, citronella (*Cymbopogon winterianus* Jowitt.), cornmint (*Mentha arvensis* L.f. *piperascens* Malinvaad ex Holmes), jamrosa (*Cymbopogon* sp.), east Indian lemongrass (*Cymbopogon flexuosus* (Nees ex Steud) Wats.), lemon-scented gum (*Eucalyptus citriodora* Hook.), palmarosa/Rosha grass (*Cymbopogon martinii* (Roxb.)

*Corresponding author: kunwar@iict.ap.nic.in

Wats. var. *motia* Burk.), rose-scented geranium (*Pelargonium* sp.) and vetiver/cuscus (*Vetiveria zizanioides* (L.) Nash) growing in the experimental farm of Central Institute of Medicinal and Aromatic Plants. Field Station, Hyderabad, India were selected for this study. The soil of the experimental farm is red sandy loam (alfisol) and is deficient in N, P and Zn. The experimental location experiences semi-arid tropical climate. All the test crops were being cultivated following standard agricultural practices.

Rhizosphere soil samples of all the 13 crops were selected at random up to a depth of 10" from 10 plants of each crop at different locations. A composite sample was made by pooling the rhizosphere soils from 10 plants located at different places. From the composite sample, three sub samples were analyzed for number of propagules per 10 gm of soil by wet sieving and decanting method (Gerdemann and Nicolson 1963). Physico-chemical characterstics of the soils viz pH, moisture content, available phosphorus (Jackson, 1973) were determined. The AMF were reidentified with the help of relevant literature (Walker 1987, Morton 1988 & Schenck & Perez 1990).

For observing colonization of roots by AMF, five plants of each crop (except cinnamon, where representative root samples were collected from the well grown trees) from different locations in the field were carefully dug out with almost complete root system and surrounding soils. The roots were carefully washed in water to remove adhering soil particles and 1 cm segments of terminal feeder roots were fixed in formalin: acetic acid : ethanol : water (FAA) (10:5:50:35) (V:V) mixture. Twentyfive representative root bits were cleared in 10% KOH for 20 min at 80°C, washed with several changes of water, kept in 0.5N HCl for five to ten min to neutralize the alkali and washed three to four times with water, cleared bits were stained in 0.05% trypan blue in lactophenol (lactic acid : phenol : glycerol : water) (1:1:2:1) (Phillips & Hayman 1970) (procedure modified). Before observation, the mounted root bits were gently squashed under cover slip. Absence and presence of colonization by the hyphae, arbuscules, vesicles and spores of AMF in each segment of the root was expressed as percentage of colonization in accordance with the slide method of Giovannetti and Mosse (1980).

Results and Discussion

Number of Propagules

AMF were of widespread occurrence in all the soils investigated but with variation both in number and type of spores and sporocarps (Table 1). The number of propagules / 10 g soil ranged from 40-1 20. The highest number of propagules were observed in rhizosphere soil collected from curry leaf and rose scented geranium (120/10g soil). By contrast, the lowest were observed in cinnamon (40/10g soil). Srivastava and Basu (1995) reported 9 spores/10g soil in fenugreek. In this study, the number of spores was 80/10g soil. Khaliq & Janardhanan (1994) reported 416-707 spores/100g soil (42-71 spores/10g soil) in mint species.

Root Colonization

Colonization was not observed in cinnamon, citronella and jamrosa roots. Khaliq and Janardhanan (1994) studied six cultivated species of mint. They reported 37.2-56.0% colonization by AMF in the roots. Srivastava and Basu (1995) also studied AMF colonization in fenugreek, cultivated in yellow loamy soil with pH 6.5, but they observed very high colonization (95%) whereas we recorded only 29%. Both spice and aromatic crops having greater number of AM fungal propagules in their rhizosphere soils exhibited higher percentage of root colonization because of significant positive correlation (r=0.69) between these two parameters.

Relationship between moisture content and number of propagules

Moisture content and spore numbers were positively correlated (r=0.06). There are contradictory reports on the effect of moisture content on (AM) fungal propagules. A negative effect of increasing moisture content on spore numbers was observed by Reid and Bowen (1979). Durga (1995) also reported significant positive correlation between moisture content and AM fungal spores in *Terminalia* rhizosphere soil indicating a positive influence. Similar was the observation of Kunwar *et al.* (1999, 2000) in garlic and ginger rhizosphere soil.

Relationship between pH and number of propagules

The pH of the soil and number of propagules were negatively correlated (r=0.21). Kunwar *et al.* (1999, 2000) reported them to be unrelated in garlic and ginger rhizosphere soil.

Relationship between phosphorus and number of propagules

It is a well known fact that the nutritionally deficient soils and phosphorus deficient soils in particular harbour more AMF. In the present investigation a range of 0.8 – 3.6 ppm of phosphorus has been reported indicating that the soils have low 'P' content. However, AM fungal number distribution is a decisive factor as it depends

Table 1. Arbuscular mycorrhizal fungal (AMF) colonization in the roots, no. of AMF propagules/10g soil and physico-chemical characteristics of rhizosphere soils of spice and aromatic crops.

Crop	Moisture %	pH	Available phosphorus mg/kg	No. of AMF propagules 10g soil	Root colonization (%)
Spice crops					
Cinnamon	3.5 ± 0.1	7.3 ± 0.06	2.0 ± 0.1	40 ± 2.08	0
Coriander	3.6 ± 0.15	7.2 ± 0.06	1.7 ± 0.1	70 ± 3.06	32 ± 1.15
Curry leaf	2.7 ± 0.06	7.4 ± 0.1	2.0 ± 0.06	120 ± 4.04	36 ± 1.53
Fenugreek	1.8 ± 0.06	6.9 ± 0.07	1.8 ± 0.06	80 ± 2.08	29 ± 2.08
Onion	3.1 ± 0.07	7.0 ± 0	2.0 ± 0.12	110 ± 2.65	38 ± 2.52
Aromatic crops					
Citronella	1.7 ± 0.15	6.9 ± 0.06	1.8 ± 0.06	50 ± 2.65	0
Cornmint	1.8 ± 0.11	6.9 ± 0.1	2.8 ± 0.10	100 ± 3.79	46 ± 1.53
Jamrosa	1.6 ± 0.12	7.3 ± 0.06	1.8 ± 0.2	60 ± 4.16	0
Lemongrass	3.5 ± 0.12	6.8 ± 0.17	2.0 ± 0.15	50 ± 2.65	21 ± 1.53
Lemon-scented gum	3.4 ± 0.1	7.2 ± 0.1	2.0 ± 0.06	60 ± 2.08	38 ± 1.15
Palmarosa	3.8 ± 0.15	7.1 ± 0.0	0.8	100 ± 4.81	41 ± 1.73
Rose-scented geranium	3.3 ± 0.14	6.6 ± 0.12	3.6 ± 0.06	120 ± 2.65	59 ± 1.73
Vetiver	3.2 ± 0.06	6.9 ± 0.1	1.8 ± 0.03	90 ± 2.65	42 ± 3.1
r values	+0.06	-0.21	+0.94	+0.69	+0.81

upon many factors including the type of phosphorus available to the plant. In our studies, the evaluated P is the available P content only and much of the P is in the form of non-available state.

The tropical soils are usually of complex nature hence role of a particular element can not be specifically defined. However, in the present investigations statistically a positive correlation (r=0.94) has been obtained between P and AM fungal propagule numbers. Further the P content is below 10 ppm hence the number of AM fungal propagules will increase till the P content is 10 ppm. The decline of AM fungal propagules will start only after 10 ppm of 'P' content. Hence in the present investigations a direct correlation has been noticed as the P content had not reached the limiting stage. Janaki and Manoharachary (1994), Kothari and Singh (1996) and Kunwar *et al.* (1999, 2000) reported P content and no. of AMF propagules to be negatively correlated.

Prevalence of AM fungal species

Eighteen species of AMF were found associated with the spice and aromatic crops (Table 2). Among these, eight were of *Acaulospora*, three were of *Gigaspora*, four were of *Glomus*, one of *Sclerocystis* and two were of *Scutellospora*. *Glomus fasciculatum* was the most abundant species followed by *Acaulospora foveata*. *A. laevis* and *Glomus mosseae*. *Gigaspora margarita* was least abundant. The abundance of AM fungal species varied from plant to plant. It is observed that some fungal species were host specific. Not a single AM fungal species was found associated either with all the spice crops or with all the aromatic crops. *Glomus aggregatum* found in the rhizosphere soil of palmarosa by Gupta and Janardhanan (1991) was not observed with this crop in our study. Khaliq and Janardhanan (1994) reported *Glomus fasciculatum*, *G.mosseae* and *Sclerocystis* species from mint.

This field investigation revealed that all the spice and aromatic crops examined harboured 18 AM fungal species in their rhizosphere soils. Maximum fungal species were found associated with rose scented geranium followed by cornmint and vetiver. From the colonization point of view except cinnamon, citronella and jamrosa all the other plants were found colonized by AM fungi in the roots (21-59%). The soils supporting arbuscular mycorrhizal fungi were found to be deficient in phosphorus. Root colonization of the crops by more than one mycorrhiza was reported by Daft and Hogarth (1983) who observed more consistent benefits to crop growth from a combination of AM fungal species than from a single species. The absence of roots colonization in certain crop species inspite of the presence of good number of AM propagules in the rhizosphere soils needs further studies to elucidate reasons for the observed results. The presence or absence of certain AM fungal species in specific crops provides a broad indication of crop AM fungal species association specificity under the prevailing conditions. Khaliq and Janardhanan (1994) also suggested some degree of host preference by mycorrhizas. However, this aspect needs further indepth investigation.

Table 2. Arbuscular mycorrhizal fungi (AMF) and the number of arbuscular mycorrhizal (AM) fungal propagules/10gm rhizosphere soil isolated from spice and aromatic crops with their relative frequency and abundance.

AM fungi/parameters	No. of AM fungal propagules/10g soil													AM fungal species		
	Cinnamon	Coriander	Curry leaf	Fenugreek	Onion	Citronella	Cornmint	Jamrosa	Lemongrass	Lemon-scented	Palmarosa	Rose-scented geranium	Vetiver	No. of crops AMF present	Relative Frequency	Abundance
Acaulospora appendicula Spain, Siever. & Schenck	–	–	7	–	–	–	3	–	12	5	–	3	–	5	5.2	6.6
A. bireticulata Rothwell & Trappe	–	–	–	8	–	–	5	–	–	3	–	–	4	4	4.2	5
A. delicata Walker, Pfieffer & Bioss	–	2	11	–	11	–	–	–	–	–	–	7	–	4	4.2	7.8
A. foveata Trappe & Janos	9	13	19	–	16	–	16	12	–	11	19	9	15	10	10.4	13.9
A. lacunosa Morton	–	3	–	–	22	15	5	–	9	–	–	4	7	7	7.3	9.3
A. laevis Gerdemann & Trappe	7	–	5	11	26	12	–	9	7	6	17	–	9	10	10.4	10.9
A. morrowae Spain & Schenck	–	–	–	–	–	–	4	–	–	–	12	10	–	3	3.1	8.7
A. scrobiculata Trappe	–	7	–	6	5	–	–	–	–	–	–	8	–	4	4.2	6.5
Gigaspora decipiens Hall & Abbott	–	3	2	–	6	5	–	–	–	–	–	4	5	6	6.3	4.2
G. gagantea (Nicol. & Gerd.) Gerd. & Trappe	–	–	–	4	–	–	–	3	–	4	2	5	3	5	6.3	3.5
G. margarita Becker & Hall	–	4	–	–	–	–	3	–	–	–	–	–	3	3	3.1	3.3
Glomous epigaeum Daniels & Trappe	–	–	–	9	18	–	6	–	–	–	–	9	6	5	5.2	9.6
G. fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	18	32	62	42	–	–	32	36	18	24	37	33	27	11	11.4	32.8
G. mosseae (Nicol. & Gerd) Gerd. & Trappe	6	6	14	–	–	18	11	–	–	7	13	9	5	9	9.3	9.9
Glomous sp.	–	–	–	–	6	–	–	–	–	–	–	9	–	2	2.1	7.5
Sclerocystis Sinuosa Gerdemann & Bakshi	–	–	–	–	–	–	7	–	–	–	–	–	6	2	2.1	6.5
Scutellospora gregaria (Sch. & Nicol.) Walker & Sanders	–	–	–	–	–	–	5	–	4	–	–	6	–	3	3.1	5
S. nigra (Redhead) Walker & Sanders	–	–	–	–	–	–	3	–	–	–	–	4	–	2	2.1	3.5
Total no. of AM fungal species for each crop soil	4	8	7	6	8	4	12	4	5	7	6	14	11			
No. of AM fungal propagules/10g soil	40	70	120	80	110	50	100	60	50	60	100	120	90			
Relative frequency (%) of AM fungi in the crop soil	4.2	8.4	7.4	6.3	8.4	4.2	12.6	4.2	5.3	7.4	6.3	14.8	10.5			
Abundance of AM fungi in the crop soil	10	8.8	17.1	13.3	13.8	12.5	8.3	15	10	8.6	16.7	8.6	8.2			

Acknowledgements

The authors are thankful to UGC, New Delhi for providing financial assistance under UGC-SAP Programme and to the Director, CIMAP, Lucknow and the scientist-in-charge, CIMAP Field Station, Hyderabad for facilities and encouragement. I.K. Kunwar is grateful to UGC for financial support.

References

Barthakur M P & Bordoloi DN. 1990. Occurrence of VAM fungi in some *Cymbopogon* species of northern India. Curr. Sci. 59:470-471.

Boerner REJ. 1990. Role of mycorrhizal fungus origin in growth and nutrient uptake by *Geranium robertianum.* Am. J. Bot. 77:483-489.

Chee Le, Garcia CR & Ferrera CR. 1998. Onion biofertilization with vermicompost and arbuscular mycorrhizae. ASAE, Annual Meeting. 12-16 July, Orlando, Florida, USA.

Daft MJ & Hogarth BG. 1983. Comparative interactions amongst four species of *Glomus* on maize and onion. Trans. Br. Mycol. Soc. 80:339-345.

Durga VVK. 1995. Studies on VAM fungi associated with *Tectona grandis* Linn. F and *Terminalia arjuna* (Roxb.) Wight & Arn. Ph.D. Thesis, Osmania University, Hyderabad.

Gerdemann JW & Nicolson TH. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. Trans. Br. Mycol. Soc. 46:235-246.

Giovannetti M & Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infections in roots. New Phytol. 84: 489-500.

Gupta ML & Janardhanan KK. 1991. Mycorrhizal association of *Glomus aggregatum* with palmarosa enhances growth and biomass. Plant and Soil. 131: 261–263.

Jackson ML. 1973. Soil Chemical Analysis. Prentice Hall, New Delhi, India.

Janaki R & Manoharachary C. 1994. Occurrence and distribution of VAM fungi associated with safflower. Indian Phytopathol. 47:263–265.

Khaliq, Abdul & Janardhanan KK. 1994. Variation of native vesicular-arbuscular mycorrhizal association on cultivated species of mint. Symbiosis – Rehovot. 16:75-82.

Kothari SK & Singh UB. 1996. Response of Citronella Java (*Cymbopogon winterianus* Jowitt) to vesicular arbuscular mycorrhizal fungi and soil compaction in relation to phosphorus supply. Pl. & Soil. 178:231-237.

Kunwar IK, Reddy PJM & Manoharachary C. 1999. Occurrence and distribution of AMF associated with garlic rhizosphere soil. Mycorrhiza News. 11:4-6.

Kunwar IK, Reddy PJM & Manoharachary C. 2000. Arbuscular mycorrhizal fungi associated with ginger rhizosphere soil : Occurrence and distribution. In "Frontiers in Biotechnology and Plant-Pathogen Interactions." Proc. Int'l Conf. Jan. 16-18, Osmania University, Hyderabad. (in press).

Morton JB. (1988). Taxonomy of vesicular-arbuscular mycorrhizal fungi; classification, nomenclature and identification. Mycotaxon 32:267–324.

Phillips JM & Hayman DS. 1970. Improved procedure for clearing root and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158–161.

Prasad KS & Sailaja K. 1995. Effect of VAM inoculation on periwinkle. In: "Mycorrhiza : Biofertilizers for the future." Eds. Adholeya, A. and Singh, S. pp.403-406. Proc. Third Nat'l Conf. on Mycorrhiza, March 13-15, TERI, India.

Reid CPP & Bowen GD. 1979. Effects of soil moisture on VA mycorrhiza formation and root development in *Medicago.* In "The Soil-Root Interface." Eds. Harley, J.L. and Russel, R.S. pp. 211. Academic Press, London.

Saleh EA, Nokhal TH, EL Borollosy MA, Fendrik I, Sharaf MS & EL Sawy M. 1998. Effectiveness of dual inoculation with diazotrophs and VAM on growth and medicinal compounds of *Datura stramonium.* Arab Universities Jr. of Agr. Sci. 6:343–355.

Schenck NC & Perez Y. 1990. Manual for the identification of VAM fungi. 3rd ed. Synergistic Publ. Gainsville, Florida, USA. pp.286.

Srivastava NK & Basu M. 1995. Occurrence of vesicular arbuscular mycorrhizal fungi in some medicinal plants. In "Mycorrhizae : biofertilizers for the future." Eds. Adholeya, A. and Singh, S. pp.58-61. Third Nat'l Conf. On Mycorrhiza, TERI, India.

Walker C. 1987. Current concepts in the taxonomy of endogonaceae. In "Mycorrhizae in the next decade, practical applications and research priorities." Eds. Sylvia, D.M., Hung, L.L. and Graham, J.H. pp.300-302. Proc. Seventh NACOM, Florida, USA.