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Studies on vam colonization on selected medicinal plants

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ABSTRACT

An investigation has been made about the vesicular arbuscular mycorrhizal fungi colonization on some medicinal plants. A total number of ten medicinal plants species selected for the study . The predominant VAM spores observed in the soil sample includes Glomus and Gigaspora species. Among the rhizosphere soil examined, a maximum spore count of 80 spores/25 gram soil was reported in Brahmi (Bacopa monneiri) and a lowest of 15 spores/25 gram soil in Neela Amari (Indigofera tinctora). This study reveals the diversity of VAM of temperate medicinal plants.

Keywords: Medicinal plants, Root colonization, VAM fungi.

INTRODUCTION

Medicinal plants are now receiving much attention all over the world for their natural healing power. They are nature's chemical industry for the production of vital medicinal compounds. Growth improvement and quality improvement of medicinal plants is very necessary because it is used for the treatment of many diseases. To avoid the residual toxicity in the raw drugs, it is always advisable to raise the medicinal plants through organic cultivation practices.The cultivation of medicinal and herbal plants has assumed greater importance in recent years due to their tremendous potential in modern and traditional medicine. They are also used as raw materials for pharmaceutical, cosmetic and fragrance industries. Indian system of medicine (ISM) uses 25,000 species belonging to more than 1000 genera. About 25% species are used by the industries [6].

Inoculation of VAM fungi during an early stage of acclimatization process has become an alternative strategy for better establishment by improving the plant growth. The VAM fungi association had not only enhanced the growth of medicinal plants but also improve the productivity of medicinal compounds. Hence there is a need for research in improving the quality and quantity of drugs produced from native medicinal plants in relatively shorter period and at lower expense by using VAM fungi. Though there are reports on beneficial effects of

VAM on other crops, a literature search reveals only a few reports on the VAM association with medicinal plants. Research continues and the practical uses of mycorrhizae continues to expand.

MATERIALS AND METHODS

Medicinal plants used for the study

Ten medicinal plants were used for study. The selected medicinal plants include :1) Black pepper (*Piper nigrum*) 2) Kiriyath (*Andrographis paniculata*) 3) Chittaratha (*Alpinia calcarata Rox*) 4) Neela amari (Indigofera tinctora) 5) Ayyampana (Eupatorium triplinerve) 6) Brahmi (Bacopa monneiri) 7) Ginger (Gingiber officinale) 8) Ramacham (Vettiveria zezanoides) 9) Karinochi (Vitex trifolia) 10) Keezharnelli (Phylantus fratemus).All these ten plants were collected from the medicinal garden of M.S.Swaminathan Research Foundation, Kalpetta, Kerala,India.

Rhizosphere sample collection

Soil samples and roots were collected from the rhizosphere region of ten selected medicinal from two different locations of the medicinal garden. The samples consisting of feeder roots + soil were collected with the help of a soil auger (0-25cm) so as to represent the complex root zone. Root systems of common plant species were excavated taking care to ensure that fine roots predominate in the sample and to exclude entangled roots of other species. Sufficient samples were taken to determine if there is any variation in the constituency and degree of mycorrhizal colonization roots between or within the sampling sites. All soil samples were dried and sealed in polyethylene bags and stored for analysis.

Isolation of VAM spores from soil

Numerous techniques were available to recover VAM spores from soil. The most basis of this is wet sieving and decanting, which remove the clay, sand and organic matter fractions while retaining spores and other similar sized soil particles on sieves of various with stainless steel mesh (50, 125, 180, 300 and 500µm). For the isolation, 25g of soil was weighed and is added to 750ml of water taken in a conical flask. Then the flask was shaken well in a vortex mixture and allowed to sediment for few seconds and was immediately transferred to a series of sieves. The jar was washed twice with water and added in to sieves series. This sieving was collected in respected jars by washing with water. Then transferred the sieving on to a gridded petriplate and observed it under the stereomicroscope. The number of spores were counted and expressed as number of spores/25g of soil sample. These isolated spores were picked up using micropipette and were mounted in Poly Vinyl Lacto Glycerol (PVLG) to make permanent slides.

Preparation of diagnostic slides for observation

A good semi permanent diagnostic slide is critical in making a species determination for specimen of VAM fungi. Semi permanent mountants, such as PVL (Polyvenyl lactophenol) or PVLG (Polyvenyl lactoglycerol) allow slides to remain usable for years.

Place a drop of mountant on one of the corner of a clean dry microscopic slide allowing one end of the slide for labeling. These spores were picked with a micropipette with minimum amount water and were added to the mountant without water. In order to disperse the spores, the mountant along with the spores were mixed gently and is allowed to set for 3-5 minutes to become more viscous. Then a clean dry cover slip was moved at 45^0 angle towards the drop of mountant containing spores until it contacts the mountant. After the contact, the mountant was allowed for few seconds to spread along the coverslip and the cover slip was released gently on to the mountant. Then gentle pressure was applied over the cover slip in order to break the spore walls. This mountant with spores were allowed to dry for several hours in a flat position. After

this, the spores were observed under stereomicroscope. The excess mountant was removed with a cotton swab moistened with solvent such as ethanol. Finally, the edges of the cover slip were sealed and dried.

Clearing and staining root specimens for estimating percentage of mycorrhizal colonization

The roots were collected first by hand sieving and were placed in plastic cassettes and were washed vigorously with water. Then placed them in a beaker containing 10% KOH solution and keep cassettes in water bath at 60^0C for 15 minutes. Then KOH was poured off and rinse the cassettes using at least three complete changes of tap water until no brown colour appears in rinse water. Then the cassettes with roots were covered with alkaline H_2O_2 at room temperature for 15 minutes until the roots were bleached. The cassettes were then thoroughly rinsed to remove H_2O_2 completely. Then covered the cassettes with HCl and soak for 3-4 minutes. After this step, the roots are not washed with water because the specimen must be acidified for proper staining. Then the cassettes were covered with 0.01% acid fuschin or 0.05% trypan blue staining solution and keep them overnight for staining. Then the roots were removed from cassettes and were placed in a gridded petriplate with a grid of 0.5 x 0.5 squares affixed to the base. The stained roots were observed under the stereomicroscope and the number of vesicles and arbuscules were counted. The degree of mycorrhizal colonization were also measured by counting the total number of intersects between lines and roots (R1) and number of intersects where the root is mycorrhizal (R2). The percentage was calculated using the equation

Percentage = R2/R1

Estimation of total root length

The roots were first dispersed against a grid of squares on the bottom of a plastic pan. The roots were spread apart from one another over the grid with 5 x 5cm squares affixed to the base of plastic pan in a 10mm depth of water. Then counted the number of intersections along the vertical and horizontal lines of the grid using a hand clicker counter.

Root length was calculated as:

Total root length = number of counts x (11/14) x size of the grid,

where, (11/14) is a constant, and the size of the grid is the length of one side of one square of the grid, here it is 5 cm.

Table-1. Details of medicinal plants screened for mycorrhizal association

S.No	Name of the plant	Scientific name	Soil type	Location
1	Pepper	*Piper nigrum*	Sandy loam	Farm
2	Kiriyath	*Andrographis paniculata*	Sandy loam	Nursery
3	Chittaratha	*Alpinia calcarata*	Sandy loam	Farm
4	Neela Amari	*Indigofera tinctora*	Sandy loam	Farm
5	Ayyampana	*Eupatorium triplinerve*	Sandy loam	Farm
6	Brahmi	*Bacopa monneiri*	Sandy loam	Nursery
7	Ginger	*Gingiber officinale*	Sandy loam	Plot
8	Ramacham	*Vettiveria zezaniodes*	Sandy loam	Farm
9	Karinochi	*Vitex nigundo*	Sandy loam	Courtyard
10	Keezharnelli	*Phyllanthus amarus*	Sandy loam	Field

RESULTS AND DISCUSSION

In the present investigation, rhizosphere samples collected from ten different medicinal plants were studied Table-1. The natural occurrence of VAM on these ten medicinal plants, percentage of colonization, spore count and occurrence of vesicles and arbuscules were determined. The occurrence of micro organisms especially VAM fungi on the medicinal plants have been reported earlier [5,7,8]. The predominant VAM spores observed in the soil sample includes *Glomus* and *Gigaspora* species. Rarely, *Scutellospora* species were also observed. The results of mycorrhizal spore diversity and predominant spores are listed in the Table- 2.

Table- 2. Mycorrhizal spore count in various medicinal plants

S.No.	Name of the plant	Spore count/ 25 g	Sporocarp	Species of VAM spores	Predominant VAM Spores noted
1	Pepper	41	-	*Glomus Gigaspora Scutellospora*	*Glomus*
2	Kiriyath	54	-	*Glomus Gigaspora*	*Glomus*
3	Chittaratha	79	-	*Glomus Gigaspora*	*Glomus*
4	Neela Amari	15	-	*Glomus Gigaspora*	*Glomus*
5	Ayyampana	69	-	*Glomus Gigaspora*	*Glomus*
6	Brahmi	80		*Glomus Gigaspora*	*Glomus*
7	Ginger	35	-	*Glomus*	*Glomus*
8	Ramacham	42	-	*Glomus Gigaspora*	*Glomus*
9	Karinochi	36	-	*Glomus*	*Glomus*
10	Keezharnelli	58	-	*Glomus Gigaspora*	*Glomus*

Among the ten medicinal plants selected for the work, all showing varying levels of VAM colonization and spore count.The details of VAM colonization on selected medicinal plants are presented in Table-3. Among the rhizosphere soil examined, a maximum spore count of 80 spores/25 gram soil was reported in Brahmi (*Bacopa monneiri*) and a lowest of 15 spores/25 gram soil in Neela Amari (*Indigofera tinctora*). It was also observed that highest root colonization percentage is in Brahmi with more number of arbuscules and vesicles. The results confirm the early reports of VAM colonization on medicinal plants by Bagyaraj and Manjunath [1]. The results are in agreement with the findings of earlier work by Gupta and Janardhanam [4]. Basu and Srivatava [2] have earlier reported that enhanced growth in medicinal plant due to VAM fungal association. Kumar and Murugesh reported mycorrhizal(*G. mossae, G. fasciculatum and G. monosporum*) inoculation was more advantageous in obtaining healthy vigorous seedlings and results in higher biomass of 10 medicinal plants that were found to grow better in the field. Thus, the difference in species composition of endophytes indicated that host specificity was exhibited by certain fungal endophytes [3]. Generally a large number of endophytic fungal genera can be isolated from tropical trees, forest trees and palm [10-12].

However, sampling of these medicinal plants yields only few dominant endophytes others showed low colonization frequency. Presence of few endophytes in this host could be due to the presence of antifungal compounds. As most of the host studied used for ayurvedic medicine preparation. Thus it appears that the occurrence of fungal endophytes are influenced by the type of host tissue and chemicals present in the medicinal plants.

Table-3. Mycorrhizal root colonization of selected medicinal plants

S.No	Name of the plant	Percentage of colonization	Spore count/ 25 g soil	Number of Arbuscules/ 1 cm root bit	Number of Vesicles/ 1 cm root bit
1	Pepper	65	41	1	3
2	Kiriyath	67	54	1	4
3	Chittaratha	80	79	2	6
4	Neela Amari	20	15	-	1
5	Ayyampana	75	69	1	3
6	Brahmi	95	80	1	12
7	Ginger	40	35	-	1
8	Ramacham	65	42	-	2
9	Karinochi	45	36	-	2
10	Keezharnelli	65	58	-	5

REFERRENCES

[1] Bagyaraj, D.J., A. Manjunath,**1980**. *New phytol*. 85, 33.
[2] Basu,M. and N. K. Srivastava,**1998**. *Indian phytopathol*. 64, 110 .
[3] Bills ,G. F. ,**1996**. Isolation and analysis of endophytic fungal communities from woody plants. In. S.C. Redlin &L.M. Carris [eds]. Endophytic fungi in grasses and woody plants: Systematics, ecology, and evolution: 31-65. APS Press, St. Paul, MN.
[4] Gupta, M.L. and K. K. Janardhan, **1991**.*Plant Soil*. 131, 261.
[5] Jaleel ,C.A., R. Gopi, and R. Panneerselvam, **2009**. *Plant Omics J*. **2**, 30.
[6] Jaleel ,C.A., R. Gopi, M. Gomathinayagam, and R. Panneerselvam, **2009**. Process Biochemistry .44, 205.
[7] Jaleel, C.A., P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram, and R. Panneerselvam, **2007**.*Colloids Surf. B: Biointerfaces*. 60,7.
[8] Karthikeyan ,B., C. A. Jaleel, G. M. Alagu Lakshmanan, and M.Deiveekasundaram D., **2008**. *Colloids Surf. B: Biointerfaces*. 62, 143 .
[9] Kumar,G.S. and S. Murugesh, **2002**.*Adv. Plant Sci*. 15, 43.
[10] Rajagopal, K ,2004. Endophytic fungi of a plam, Borassus flabellifer L. – As first report from India. *AJMBES*. Vol. 6(4): 541-544.
[11] Rajagopal, K and Suryanarayanan, T.S. ,**2000**. *Current Science*. 78(11): 1375-1378.
[12] Suryanarayanan, T.S., Kumaresan, V & Johnson, J.A. ,**1998**. *Canadian Journal Microbiology*. 44: 1003-1006.