



Biodiversity of Endophytic Mycorrhizal fungi associated with some medicinal plants of Himachal Pradesh

Aditya Kumar*, Chhavi Mangla** and Ashok Aggarwal***

*Department of Botany, Dayanand P.G. College, Hisar, Haryana, INDIA-125001

Email ID: adityagohar@yahoo.com

** Department of Botany, F.C. College for Women, Hisar, Haryana, INDIA -125001

*** Department of Botany, Kurukshetra University, Haryana, INDIA -136119

(Received 10 June, 2013, Accepted 31 July, 2013)

ABSTRACT: Mycorrhiza is the most important member of functional soil microbial community in natural ecosystem and creates an intimate link between plant roots and soil. In the present investigation, attempts were made to screen out endomycorrhizal biodiversity from different medicinal plants. A total of twelve plants from six different families were screened for arbuscular mycorrhizal (AM) spore number and root colonization. The mycorrhizal root colonization ranged from 0.0 to 67.50±10.60 percent. *Adhatoda vesica* showed maximum root colonization and *Achyranthus aspera* lack mycorrhizal root colonization. The spore number ranged from 25.66±3.00 to 388.33±8.50. The highest mycorrhizal spore count was found in *Aster thomsonii* and lowest in *Aloe vera*. It was found that number of spores in the rhizosphere of plant was not related to the intensity of AM root colonization. The study confirmed that the biodiversity of AM fungi differ in different plants.

Keywords: medicinal plants; arbuscular mycorrhizal fungi; vesicles.

INTRODUCTION

Many microorganisms form symbiosis with plants that range, on a continuous scale, from parasitic to mutualistic. Among these, arbuscular mycorrhizal (AM) fungi are ubiquitous plant root symbionts that can be considered as 'keystone mutualists' in terrestrial ecosystem, forming a link between biotic and abiotic ecosystem components via carbon and nutrient fluxes that pass between plant and fungi in the soil (O'Neill *et al.*, 1991). Medicinal and aromatic plants (MAPs) are used in various systems of medicines in different parts of the globe. The demand of MAPs has been increasing rapidly with the consumption of herbal drugs. This led to an increase in the cultivation of MAPs in order to maintain a steady supply to support the increasing demand due to decline in their natural population. During recent years the importance of conserving and managing the biological wealth growing on the earth has been assessed in many folds for sustainable development of the society. In addition to conventional cultivation of MAPs, recent emphasis is on exploiting useful and appropriate soil microorganisms present in the rhizosphere of medicinal plants. Knowledge about the presence and diversity of AMF is an essential first step to utilizing these fungi in any application.

Keeping in view the above facts, the study of endomycorrhizal biodiversity on some medicinal plants is, therefore, necessary from efficient utilization and conservation point of view. Considering the importance and status of medicinal plants in Himachal Pradesh, the present investigation was carried out to study the endomycorrhizal status of these medicinal plants and to select the predominant AM fungi for future inoculation studies for production of quality seedling of important plants in nurseries and their better survival in adverse conditions.

MATERIAL AND METHODS

Study Site: The study was undertaken at different sites located in Himachal Pradesh. Wild differences in geo-physical features accounts for considerable variation in climate and rainfall in different sub-regions of the state. Physiographically, the state is a part of Himalayan system.

Field Sampling: Roots and soil samples were collected from three individuals for twelve medicinal plants at different stages of growth (vegetative and reproductive) during course of investigation from 2007-2008. The samples of each plant were collected for further processing for the isolation of AM spores and studying mycorrhizal root colonization.

1. Estimates of AM root colonization: Root samples were rinsed with tap water and then staining was performed by 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970). Assessment of root colonization was done by estimating total percentage of root colonization by root slide technique (Giovannetti and Mosse, 1980). The percentage of mycorrhizal root colonization was determined by the following equation:

$$\text{Percentage AM root colonization} = \frac{\text{Total number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

2. Extraction and Quantification of AM fungal Spores:

Rhizospheric soil samples were wet-sieved for AM spores using the technique of Gerdemann and Nicolson (1963). The quantification of AM spores was done by 'Grid Line Intersect Method' (Adholeya and Gaur, 1994).

RESULTS AND DISCUSSION

AM fungi are ecologically important root symbionts of most terrestrial plants. Basic knowledge of the fungal diversity associated with medicinal and aromatic plants may be useful for the conservation point of view. In the present investigation, status of AM fungi associated with twelve medicinal plants was studied.

Root samples of all the plant species showed a wide range of variation in terms of AM root colonization. Table I shows the total AM colonization in different plant species collected from various localities of Himachal Pradesh. The mycorrhizal structures present in the roots included mycelium, vesicles and arbuscules. Mycelia of various type like Y-shaped, H-shaped and parallel mycelia were reported in the roots of different plants. In some cases extensive mycelial growth was also observed. Vesicles of different shapes like elliptical, round, globose, oval, beaked and elongated were observed. *Allium coralinianum* was found to have only mycelial infection. Both vesicular and arbuscular type of colonization was also observed in four plant species. A single plant was found to be without mycorrhizal root colonization. It is evident from the results that eleven plants were colonized by AM fungi and showed variation in mean percent mycorrhizal root colonization. AMF root colonization ranged from 0.0±0.0 to 67.50±10.60 percent. The plant lacking mycorrhizal infection was *Achyranthes aspera* (0.0±0.0).

The results of the soil assessment of different plant species have been presented in the Table I. Total spore population of AM fungi in the rhizosphere soil of different medicinal plants varied significantly as depicted in table. The AM spore density ranged from 25.66±3.00 to 388.33±8.50. The highest spore population was recorded in the rhizospheric soil of *Aster thomsonii* (388.33±8.50) and it was followed by *Ageratum conyzoides* (209.33±5.50) and *Arnebia hispidissima* (153.00±6.24). The lowest was recorded in *Aloe vera* (25.66±3.00). Among the families, Asteraceae followed by Boraginaceae were found to possess higher spore population while Liliaceae was observed with least spore count. A variation in AM development in roots of different medicinal plant species has been observed.

The different medicinal plant species from Himachal Pradesh showed AM colonization and spore population in their respective rhizospheric soils. The variation in root colonization may be due to the exudation of toxic metabolites resulting in substances in proximity to the roots which attracts the AM fungi such as production of easily oxidisable compounds resulting in increased colonization, physiological difference between species (Koske, 1987; Albert and Sathianesan, 2009). In this study, high levels of AMF colonization and low sporulation were found in some plant species. This may have been due to inadequate fungal biomass development and poor root development (Gazey *et al.*, 1992) or high rates of spore degradation or predation by other soil organisms (Gryndler, 2000). Such a high degree of

colonization could be due to the fact that the study areas were essentially phosphate deficient which has been shown to induce high levels of AM fungal species (Muthukumar and Udaiyan, 2000). Zangaro *et al.* (2005) reported that the presence of fine root system as found in most of the investigated plant species possibly facilitates higher AM fungal colonization. Similarly, a high range of AM sporulation and root colonization was also reported in some ornamental plants of Himachal Pradesh (Kumar *et al.*, 2012). A wide range of variation in spore population was observed in current study. The high spore number in the rhizosphere soils of studied medicinal plants supported the conclusion of Wang *et al.* (2004), that the host species apparently had direct effects on spore density and colonization of AM fungi. Patterns of spore production, spore quantity etc. are closely related to the plant phenology, root phenology and root production (Brundrett, 1991). In the present investigation AM spore numbers are not always correlated with the extent of root colonization. It is generally assumed that AMF do not show host specificity and are randomly distributed in natural ecosystems (Eom *et al.*, 2000).

Table 1: Endomycorrhizal studies of some medicinal plants of Himachal Pradesh.

* Mean of three replicates

| Sr.No. | Botanical Name | Common name | Family | Type of Infection | | | AM spore count / 50gm. of soil | % AM Root Colonization |
|--------|-----------------------------|-------------------|----------------|-------------------|----|----|--------------------------------|------------------------|
| | | | | #M | #V | #A | | |
| 1. | <i>Achillea millefolium</i> | Gandana, Millifol | Asteraceae | + | + | - | *74.00 ± 5.2 | 44.12 ± 7.40 |
| 2. | <i>Achyranthes aspera</i> | Puthkanda | Amaranthaceae | - | - | - | 79.00±7.00 | 00.00 |
| 3. | <i>Adhatoda vesica</i> | Basuti | Acanthaceae | + | - | + | 115.66±1.52 | 67.50±10.60 |
| 4. | <i>Agave americana</i> | Ramban | Amaryllidaceae | + | + | + | 85.00±5.29 | 40.05±8.78 |
| 5. | <i>Ageratum conyzoides</i> | Gumdrya, Ujadu | Asteraceae | + | + | - | 209.33±5.50 | 44.44±1.11 |
| 6. | <i>Allium coralinianum</i> | Ladam | Liliaceae | + | - | - | 105.00±3.0 | 36.00±5.83 |
| 7. | <i>Aloe vera</i> | Kumar patha | Liliaceae | + | - | + | 25.66±3.00 | 61.85±7.40 |
| 8. | <i>Arnebia benthamii</i> | Laljari | Boraginaceae | + | + | + | 52.33±4.93 | 61.66±4.40 |
| 9. | <i>Arnebia hispidissima</i> | Ratanjot | Boraginaceae | + | + | + | 153.00±6.24 | 27.60±5.56 |
| 10. | <i>Artemisia annua</i> | Maleria buti | Asteraceae | + | - | + | 70.00±2.64 | 42.12±4.00 |
| 11. | <i>Asparagus filicinus</i> | Satavari | Liliaceae | + | - | + | 112.6 ± 5.0 | 66.92 ± 11.7 |
| 12. | <i>Aster thomsonii</i> | Thomson's aster | Asteraceae | + | + | + | 388.33±8.50 | 42.27±2.62 |

M-Mycelium, V-Vesicles, A-Arbuscules

+ : Present

- : Absent

± : Standard deviation

Arbuscular mycorrhizal colonization and spore population varied significantly in different plant species as showed by results. The study confirmed the widespread occurrence of AM fungi in the soils of different sites of Himachal Pradesh.

ACKNOWLEDGEMENT

The authors (AK and CM) are grateful to Kurukshetra University, Kurukshetra for providing financial assistance and facilities to carry out the research work.

REFERENCES

- [1] A. Adholeya and A.Gaur, *Myco. News*, 6(1), 10, (1994).
- [2] E.S.R. Albert and M.S. Sathianesan, *Trop. Life Sci. Res.* 20(1), 99, (2009).
- [3] M. Brundrett, *Adv. Ecol. Res.* 21, 271, (1991).
- [4] V. Gazez, L.K. Abbott and A.D. Robson, *Mycol. Res.* 96, 643, (1992).
- [5] J.W. Gerdemann and Y.H. Nicolson, *Trans. Brit. Mycol. Soc.* 46, 235, (1963).
- [6] M. Giovannetti and B.Mosse, *New Phytol.* 84, 489, (1980).
- [7] M. Gryndler, *Physiology and Function. (Eds.)*, (Dordrecht, 239, 2000).
- [8] R.E. Koske, *Mycologia.* 79, 55, (1987).
- [9] A. Kumar, S.K. Bhatti, and A. Aggarwal, *Biol. Forum-An Int. J.* 4(2), 45, (2012).
- [10] T. Muthukumar and K., *Mycorrhiza.* 9, 297, (2000).
- [11] E.G. O'Neill, R. V. O'Neill and R.J. Norby, *Environ. Pollut.* 73: 271, (1991).
- [12] J.M. Philips and D.S. Hayman, *Trans. Brit. Mycol. Soc.* 55: 158, (1970).
- [13] F.Y. Wang, R.J. Liu, X.G. Lin, and J.M. Zhou, *Mycorrhiza.* 14, 133, (2004).
- [14] W. Zangaro, F.R. Nishidate, F.R.S. Camargo, G.G. Romagnoli, and, J. Vandressen, *J. Trop. Ecol.* 21, 529, (2005).