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DIVERSITY IN ARBUSCULAR MYCORRHIZAL MORPHOLOGY IN SOME MEDICINAL PLANTS OF FAMILY LAMIACEAE

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Abstract

Present study was designed to find out the mycorrhizal status of some selected medicinal plants of family Lamiaceae growing in KPK (Khyber Pakhtunkhawa). The mycorrhizal infections were present in all studied species. Typical Arum and Paris type mycorrhiza are recorded for the first time from the family Lamiaceae. The number of studied plants with Arum type was higher than Paris type while two plants were recorded with intermediate type. Variations in the percentage of general VAM infection ranged from 55-100%.

Introduction

Arbuscular Mycorrhizal (AM) symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomes (Smith *et al.,* 2010). Arbuscular mycorrhizal fungi (AMF) are of great ecological importance, since arbuscular mycorrhizae is the most widespread plant symbiosis that often improves plant productivity (Fedderman *et al.,* 2010). The main advantage of mycorrhizae to the host plants is the extension of the penetration zone of the root fungus system. The interconnected networks of external hyphae act as an additional catchment and absorbing surface in the soil (Sharma, 2004). The increased efficiency of mycorrhizal roots versus non mycorrhizal roots is caused by the active uptake and transport of nutrients especially immobile minerals like P, Zn and Cu (Phiri *et al.,* 2003; Jamal *et al.,* 2002).

Medicinal herbs are sources of phytochemically active compounds throughout the world (Toussaint *et al.,* 2007). Plants of the family Lamiaceae are medicinally important due to variety of chemical constituents and uses. There had been few reports on AM studies of family Lamiaceae. including *Ajuga pyramidalis* (Eriksen *et al.,* 2002), *Betonica officinale* (Fuchs & Hasselwandter, 2004), *Clinopodium gracile* (Yamato, 2004), *Lavendula angustifolia* (Linderman & Davis, 2003), *Thymus polytrichus* (Whitefield *et al.,* 2004), *Ocimum basilicum* (Dickson, 2004), *Salvia azurea* (Wilson *et al.,* 2001), *Mentha species* (Moremmani *et al.,* 2003; Gupta *et al.,* 2002; Freitas *et al.,* 2004; Silveira *et al*., 2006). Mycorrhizal inoculation not only promoted the growth of medicinal plants but also improved the productivity and quantity of chemicals. Hence, there is an upcoming demand for research in improving the quality and quantity of drugs produced from native medicinal plants in relatively less time with application of AM fungi (Karthikeyan *et al.,* 2009). AM morphology is distinguished into Arum-type and Paris-type. The Arum-type association is characterized by intercellular hyphal growth in the root cortex, with short lateral branches into cortical cells forming arbuscules. Intracellular hyphal coils frequently having intercallary arbuscules spreading cell to cell in the cortex characterize the Paris-type association (Smith & Smith, 1997).

Recently mycorrhizal status of weeds (Burni *et al*., 2009) and other plants from various sites of Khyber Pakhtunkhawa (Burni *et al*., 2008; Sharif *et al*., 2005) have been reported but there is lack of information about the mycorrhizal status of medicinal plants of Khyber Pakhtunkhawa. Therefore, present study was undertaken to examine the mycorrhizal status, colonization and morphology of AM in some selected medicinal plants of Family Lamiaceae.

Materials and Methods

Plant roots and soil samples of 15 medicinal plants of family Lamiaceae were randomly sampled during January 2007 to May 2008 from Peshawar parts of Manshera and Haripur. Plants were taken out carefully avoid to damage secondary and tertiary rootlets which were the sites of endophyte development.

Roots were thoroughly washed with distilled water to remove soil particles and preserved in 70% alcohol. Two procedures of Phillips & Hayman (1970) and Kormanik (1982) were used for staining fungal structures in roots. Percent colonization was measured by the method of Giovannetti & Mosse (1980), AM morphology was studied by the method devised by Dickson (2004) and Muthukumar & Parkash (2009) AM morphology was classified as Arum or Paris-type based on whether the fungal hyphae were present mainly as hyphae running through intercellular spaces or within cells as coils, respectively.

Results and Discussion

Fifteen composite samples of roots and soil were collected from various parts of Peshawar and Hazara. All the studied medicinal plants were mycorrhiza (Table 1). Our results agree with those of Karagiannidis *et al*., (2010), who also reported the occurrence of mycorrhiza in some members of the family Lamiaceae ranged from 55-100%. Colonization was 55% in *Ocimum americanum* and highest (100%) in *Mentha arvensis* and *Salvia* spp. Khaliq & Janardhanan, (1997) reported AM colonization levels in different species of *Mentha,* which ranged from 32–85.5%, *Mentha arvensis* had heavy AM infection (85.5%) while lowest (32%) was observed in *M. viridis.* on the contrary Muthukumar *et al*., (2006, 2009) found no infection in *Mentha arvensis.* Gupta *et al*., (2002) reported that AM inoculation could significantly increase the root colonization, growth and essential oil yield and nutrient acquisition of mint.

Fuchs & Haselwandter (2004) reported the mycorrhizal status of *Betonica officinalis* from Lamiaceae. Furthermore, Zubeck & Blaszkowski (2009) reported the mycorrhiza status of *Mentha citrata, Origanum majoroma. Salvia officinalis* and *Thymus vulgaris* found to be colonized by AMF (67-100%) which were mostly of Arum type. During present studies AM morphology was recorded in the investigated species of Lamiaceae for the first time from Khyber Pakhtunkhawa. The Arum-type mycorrhiza is characterized by the presence of intracellular arbuscules intercellular hyphae and vesicles (Table 2 Figs. 2, 4, 5). While intracellular hyphal coils, intracellular vesicles and arbuscular were the characteristics of Paris-type (Figs. 1, 3, 8, 9). Both types were prevalent in studied plants while few could be placed under intermediate category (Figs. 5, 6, 7). Our results are similar with those of Muthukumar & Parkash (2009), who also reported the occurrence of Arum type in *Ocimum basilicum* and *O. americanum in*Lamiaceae while Arum and intermediate was recorded by Dickson, (2004).

Table 1. AM morphologies and mycorrhizal status of plants of family Lamiaceae.

S.#.	Taxon	Collection sites	Morphology Types	General infection (%)
1.	*Mentha arvensis* L.	Peshawar	Intermediate	100 ± 0.78
2.	*M. longifolia* (L.) L.	Peshawar	Paris	80 ± 0.56
3.	*M. piperata* L.	Peshawar	Arum	70 ± 0.30
4.	*M. royleana* Benth.	Manshera	Arum	76 ± 0.64
5.	*M. spicata* L.	Haripur	Arum	60 ± 0.65
6.	*Rasmarinus officinals* L.	Peshawar	Paris	66 ± 0.50
7.	*Ocimum basilicum* L.	Peshawar	Paris	60 ± 0.80
8.	*O. americanum* L.	Peshawar	Arum	55 ± 6.30
9.	*Origanum vulgare* L.	Manshera	Arum	78 ± 0.38
10.	*O. majorana* L.	Manshera	Arum	50 ± 4.45
11.	*Coleus forskohlii* L.	Peshawar	Arum	70 ± 0.6
12.	*Coleus aromaticus* Benth.	Manshera	Arum	60 ± 0.79
13.	*Lallementia rovelena* Benth.	Peshawar	Arum	60 ± 1.85
14.	*Salvialanata* Roxb.	Galliat	Intermediate	95 ± 1.25
15.	*S. nubicola* Wall. ex Sweet	Manshera	Intermediate	100 ± 2.40

Mean ± Standard error

Table 2. Arbuscular mycorrhizal structural colonization % in some plants of family Lamiaceae.

S.#.	Taxon	E.M[1]	I.M[2]	AB[3]	VS[4]
1.	*Mentha arvensis* L.	9.1 ± 0.54	19.8 ± 0.25	49.9 ± 1.24	21.2 ± 2.24
2.	*M. longifolia* L.	9.5 ± 0.46	38.8 ± 0.44	18.5 ± 0.48	13.2 ± 0.50
3.	*M. piperata* L.	18.3 ± 0.25	12.2 ± 0.15	17.4 ± 0.38	22.1 ± 0.19
4.	*M. royleana* Benth.	10.4 ± 0.54	29.6 ± 0.55	28.2 ± 0.60	7.8 ± 0.60
5.	*M. spicata* L.	14.3 ± 0.57	10.7 ± 0.45	20.5 ± 0.55	14.5 ± 0.64
6.	*Rosmarinus officinals* L.	13.2 ± 0.63	22.5 ± 0.52	23.5 ± 0.61	6.8 ± 0.58
7.	*Ocimum basilicum* L.	11.2 ± 0.48	19.8 ± 0.39	17.2 ± 0.47	11.80 ± 0.36
8.	*O. americanum* L.	8.7 ± 0.76	14.6 ± 0.60	12.2 ± 0.72	19.50 ± 0.70
9.	*Origanum vulgare* L.	18.3 ± 0.25	16.5 ± 0.19	32.8 ± 0.16	10.40 ± 0.20
10.	*O. majorana* L.	8.9 ± 0.35	14.7 ± 0.39	20.3 ± 0.30	6.10 ± 0.29
11.	*Coleus forskohlii* L.	14.8 ± 4.45	10.2 ± 2.11	30.6 ± 0.99	14.4 ± 0.90
12.	*Coleus aromaticus* Benth.	10.3 ± 0.51	18.5 ± 0.41	15.3 ± 0.39	15.9 ± 2.25
13.	*Lallementia royleana* Benth.	8.6 ± 0.65	19.4 ± 0.61	13.7 ± 0.71	18. 3 ± 0.48
14.	*Salvialanata* Roxb.	14.6 ± 0.98	40.6 ± 0.76	20.5 ± 0.56	19.3 ± 0.57
15.	*S. nubicola* Wall ex Sweet	6.7 ± 1.22	12.2 ± 1.98	13.6 ± 0.80	17.5 ± 0.46

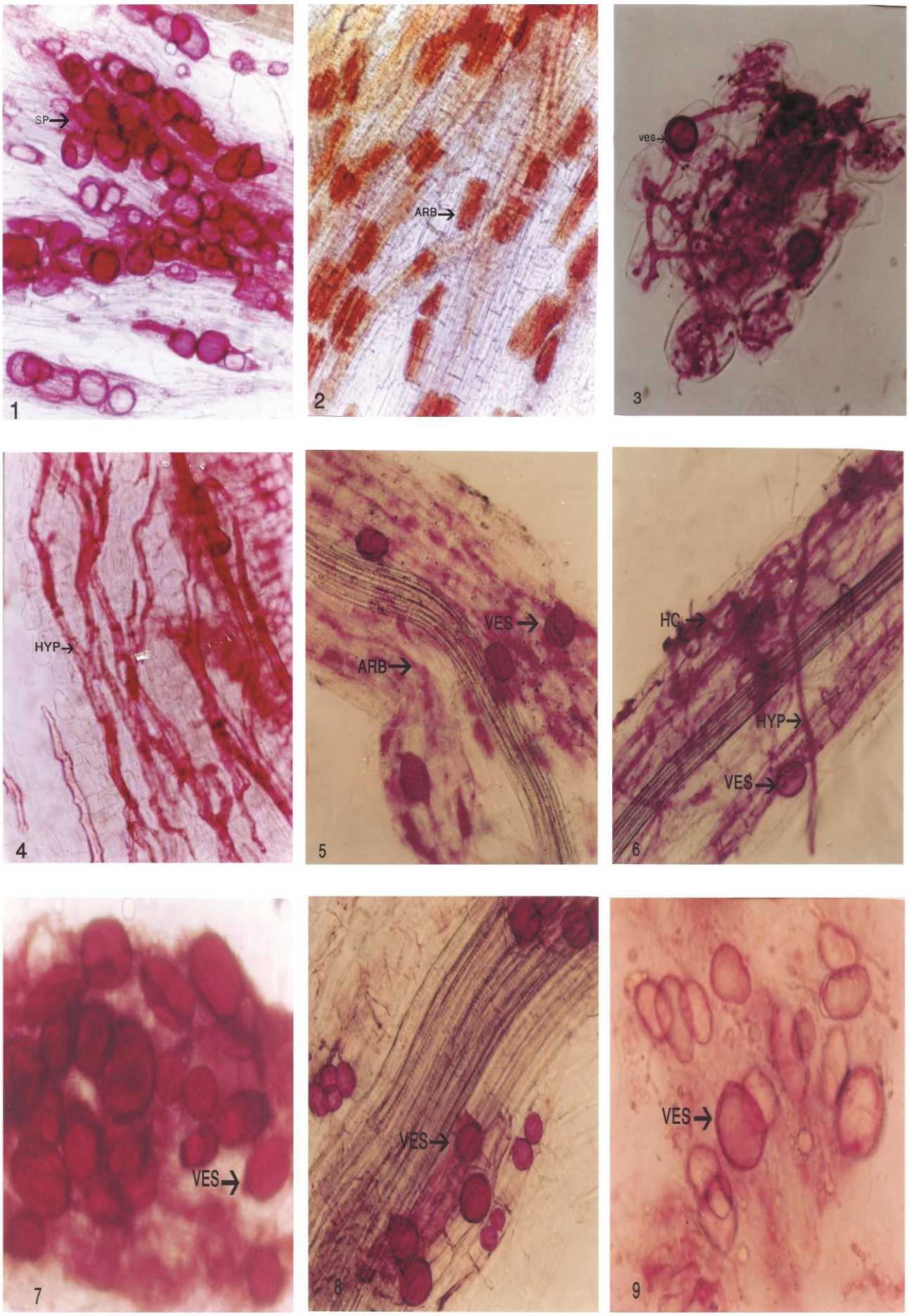
Mean: ± Standard error 1: External mycelium 2: Internal mycelium 3: Arbuscules 4: Vesicles

It has been shown that morphological type is controlled by the host plant and the results suggest that plant identity strongly influences AM morphology. It may happen that different morphological types are found in the same genus (Muthukumar & Parkash, 2009). Moreover, the co-occurrence of both types in families like Arecaceae, Poaceae, Euphorbiaceae, Fabaceae, Rubiaceae, Solanaceae and Lamiaceae has also been reported by Smith & Smith, (1997). Muthukumar *et al*., (2006) reported Arum-type in *Leucas aspera* and *Ocimum tenuiflorum.* The physiological and functional disparity between Arum type and Paris type is still not clear but it has been reported that the development of Arum-type is faster than that of Paris type (Brundrett & Kendrick, 1990; Cavagnaro *et al.,* 2001). Anatomical characters of host roots might influence the AM morphology (Brundrett & Kendrick, 1988, 1990).

Recently, Kubato *et al*., (2005) described that the morphology of AM type is the result of interaction between both the plant and fungal species. It is, therefore, essential to examine a wider range of plants growing in different habitats to understand those variation in the morphology of AM. Associated fungal species may be a reason for this inconsistency. Cavagnaro *et al*., (2001) found that both AM morphological types in *Lycopersicon esculentum* (wild type tomato) depended on the fungal species. As mentioned by Yamato (2004) AM morphology type is determined by both plant and fungal identity, but many plants tend to form one morphology type and those plants from the studied location were dominated by AM fungi which tend to form Arum type. Moreover environmental factors such as temperature, light intensity and soil moisture may affect AM morphology as mentioned by Cavagnaro *et al*., (2001). These factors affect the growth and morphology of roots.

In the present investigation varieties in the morphology of vesicles and different hyphal width were observed in roots of studied plants. This variations might be due to the infection by multiple endophytes (Iqbal & Naseem, 1986; Burni *et al.,* 1993, 2008). Variations in the infection levels may also be due to the reason that host response can differ with fungal species and seasonal development (Bethylenfalvay & Ames, 1987). Moreover, environmental factors have strong and sometimes unpredictable effects on infection as well as functioning of the AM endophytes. Host genome also affect the establishment and morphology of AM infection (Lackie *et al.,* 1987).

The present investigation might create the possibility of AMF applications in herbal industry in order to improve herb production and quality considering the impact of mycorrhizal fungi. It seems to be crucial that in the case of species dependence on an AMF for their performance eg., growth, secondary metabolite production more attention should be paid on soil monitoring and mycorrhizal development during the process of cultivation (Zubek & Blaszkowski, 2009). The present investigation would enable us to select plant species highly colonized by AMF. These findings would be helpful to find out the mycorrhizal dependency and the influence of AMF on the production of secondary metabolites.



Figs. 2, 4, 5: Arum type mycorrhiza in the root cortex of *Mentha piperata, M. royleana* and *M. spicata,* respectively.
Figs. 1, 3, 8, 9*: Paris type mycorrhiza in the root cortex of M. longifolia, Rosmarinus officinlalis* and *Ocimum basillicum,* respectively.
Figs. 5, 6, 7*:* Intermediate type mycorrhiza in the root cortex of *M. arvensis. Salvia lanata* and *S. nubicola,* respectively.
SP: Spores: ARB: Arbuscules: VES: Vesicles: HYP: Hyphae: HP: Hyphal coils.

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