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Pakistan Journal of Biological Sciences, 2 (3): 906-910 *1999* **Research Article**

Status of Vesicular Arbuscular Mycorrhiza (VAM) in Medicinal Plants of the Salt Range (Pothowar) and Margalla Hills Islamabad

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Abstract

Medicinally important wild plants (17) of Margalla hills Islamabad and Salt range of Pothowar were compared based on th vesicular arbuscular mycorrhizal (VAM) status. Statistical interpretation of the data indicated a significant difference betwe the results of vesicles, arbuscules and hyphal infection in plants of Margalla hills and Salt range area. The number of VA spores recovered from the rhizospheric soils of both the areas was also significantly different. Soils analyses further reveal a significant difference for values of sand, silt, phosphorus, potassium, calcium carbonate and soil pH while clay a nitrogen contents were non-significant. The study concludes that wild plant have enormous ability to establish mycorrhiz association under stressed conditions particularly in the saline environment of Salt range, VAM root colonizati emphatically reflects an adaptive mechanism of plants.

Introduction

Mycorrhiza is a kind of mutualistic symbiotic association. This is an association between two living partners where both the members get benefit from each other. Many research workers have reported that VA mycorrhizal fungi have a profound influence on the availability of host nutrients. Marschner and Dell (1992) while performing the experiments observed that external hyphae can deliver up to 80 per cent plant P, 25 per cent plant N, 10 per cent plant K, 20 per cent Zn and 60 per cent of plant Cu. Thus fungal hyphae may provide a significant delivery system for N, K, Zn, Cu, in addition to P in many soils.

So far as the medicinal plants are concerned a little work has been done. However, Waheed (1982) surveyed medicinal plants of Murree hills and Kaghan valley. Later on Nazar (1992) worked on VAM infection of medicinal plants of Muzzaffarabad. The occurrence of VAM in medicinal plants such as coffee tree, *Capsicum annum* and pineapple has been reported by many workers (Sreenivasa, 1992; Aziz *et al.*, 1990). More recently it has been reported that VAM fungus has a wide range of host so far as medicinal plants are concerned (Tetsuya Ud *et al.*, 1992). In this context, it seems that mycorrhizal association has a definite role in growth of medicinal plants. In Pakistan, the natural reserves have enormous plant diversity in which many species possessing medicinal importance, flourishing under wild conditions. The contributing factors regarding their growth are no doubt manifold besides mycorrhizal dependence.

Keeping in view the probable role of mycorrhizal fungi in the establishment of plant species in different environmental conditions, the study was designed to investigate the VAM status in some medicinal plants of Salt range and Margalla hills.

Materials and Methods

A total number of 17 important medicinal plants of vario families were collected from Salt range area of Pothow region, which includes Chakwal, Choa Saiden Shah a Pind Dadan Khan. For comparison, the same plant speci were collected from Margalla hills, Islamabad. Plant speci included in the study were *Acacia nilotica, Acacia modes Adhatoda vasica, Broussonetia papyrifera, Canabis sati Chrozophora tinctoria, Calotropis procera, Carissa opa Cassia occidentalis, Datura stramonium, Dodonaea visco Fiscus racemosa, Otostegia limbata, Ricinus commun Saccharum bengalense, Xanthium strumarium and Ziziph jujuba.*

Roots were collected carefully by excavating the whole r system. Fine root-lets were fixed in F.A.A. (Formali Acetic acid, Alcohol). Approximately 2-kg soil from rhizosphere of each plant was also collected and stored the polythene bags at 20°C. The fixed roots were stai by modified method of Koske and Gemma (1989). Stai root samples were cut into pieces (1 cm each). Ten pie of each root sample were carefully placed on the slide gently cover with cover slip and observed un microscope. For the assessment of colonized roots, s length method was used (Giovannetti and Mosse, 19 The mycorrhizal spores from the soil were recovered by sieving and decanting technique (Gerdeman and Nicol 1963).

Soil texture was determined by using the method descri by Piper (1942). Soil pH was measured by glass electr in soil saturation extracts (Soil Salinity Mannual, 19 IWASRI). Amount of phosphorus was determine saturation extracts by Molybdenum Blue method (Alle *al.*, 1974). The percentage of total nitrogen of the samples was find out by semi-micro Kjeldhals me (Metson, 1956). Potassium was determined directly soil saturation extract by flame photometer (Price, 19

Amount of calcium carbonate was determined by titrating unused acid (HCl) against sodium hydroxide using Bromothymol blue as an indicator. The paired sample t-test was applied for the statistical analyses of the data.

Results

The number of VAM spores observed, was between the range of 3 to 240 per 50g soil. There was a significant difference between the hyphal infection of the root samples of Salt range and Margalla hills (Table 1). Comparatively, hyphae were thick walled in the root samples of Salt range while they were thin walled in the root samples of Margalla hills. Statistical interpretation of soil analyses showed a significant difference in the values of sand, silt, soil pH, phosphorus, potassium and calcium carbonate between the two areas (Table 1). However, there was no significant difference between clay and nitrogen contents in the soil samples of both the sites.

Table 1: Statistical analyses of data (obtained for Margalla hills and Salt range area) subjected to paired sample t-test by design ($P < 0.05$).

Variables	Standard deviation	Critical value	t. value	Difference (mean)
Sand	13.807	0.100	1.74	5.841*
Silt	9.126	0.022	2.54	5.611*
Clay	5.727	0.884	0.15	0.250
$CaCo_3$	45.092	0.250	1.19	13.058*
Nitrogen	0.030	0.000	8.21	0.0597
Phosphorus	10.553	0.44	2.19	5.94*
Potassium	16.826	0.135	8.21	6.145*
PH	2.198	0.013	2.81	1.496*
Vesicles	1.623	0.485	0.73	0.3826*
Spore No.	81.612	0.357	0.95	18.764*
Arbuscules	0.923	0.191	1.37	0.3059*
Hyphal infection (%)	4.108	0.820	0.23	0.230*
Total infection (%)	11.97	0.842	0.20	0.588

* = Significant difference

Table 2: Number of spores and percentage of VA mycorrhizal infection in cleared and stained roots of medicinal plants of Salt Range and Margalla Hills.

Plant species	Family	Site	Ves. (%)	Arb. (%)	Spore No. 50g [1]
Acacia nilotica	Minosaceae	M.H	4.50	3.5	-
		S.R.	5.25	0.9	60
Acacia modesta	Minosaceae	M.H	0.80	-	-
		S.R.	0.55	-	20
Adhatoda vasica	Acanthaceae	M.H	0.37	0.25	59
		S.R.	5.90	1.7	3*
Broussonetia papyrifera	Moraceae	M.H	0.82	4.00**	104
		S.R.	0.50	0.65	15
Cannabis sativa	Canabinaceae	M.H	1.10	0.175	-
		S.R.	2.12	0.225	80
Crozophora tinctoria	Euphorbiaceae	M.H	10.92	0.95	100
		S.R.	12.5**	1.2	20
Dictropis procera	Asclepiadaceae	M.H	0.25	0.275	-
		S.R.	0.25	-	15
Dodonaea opaca	Apocynaceae	M.H	2.3	1.35	-
		S.R.	0.2	0.25	100
Gleditsia occidentalis	Leguminosae	M.H	2.55	0.9	50
		S.R.	0.55	0.4	25
Datura stramonium	Solanaceae	M.H	-	0.5	-
		S.R.	0.25	0.5	240**
Melia azedarach	Sapindaceae	M.H	0.42	0.62	-
		S.R.	0.45	0.37	100
Morus racemosa	Moraceae	M.H	0.37	0.77	-
		S.R.	0.40	-	-
Otostegia limbata	Lamiaceae	M.H	0.087*	0.06*	-
		S.R.	0.36	0.15	15
Ricinus communis	Euphorbiaceae	M.H	0.25	0.14	50
		S.R.	0.30	-	-
Saccharum bengalense	Poaceae	M.H	0.33	0.7	100
		S.R.	0.25	-	40
Xanthium strumarium	Asteraceae	M.H	0.52	0.1	-
		S.R.	0.35	-	25
Zizyphus jujuba	Rhamnaceae	M.H	0.50	-	-
		S.R.	2.75	-	15

Vesicles; * = Lowest percentage; Arb = Arbuscules; ** = Highest percentage; MH = Margalla Hills; SR = Salt Range

Table 3: Amount of sand, silt and clay in the rhizospheric soil samples of the plants collected from Salt Range and Margalla Hills.

Plant species	Site	Sand(%)	Silt (%)	Clay (%)
Acacia nilotica	M.H	78.2	16.0	5.8
	S.R.	97.2	-	-
Acacia modesta	M.H	85.0	8.0	8.8
	S.R.	73.2	20.0**	6.8
Adhatoda vasica	M.H	99.2	-	-
	S.R.	93.2	20.0**	6.8
Broussonetia papyrifera	M.H	89.2	10.0	0.8
	S.R.	71.2*	20.0**	8.8
Canabis sativa	M.H	85.2	10.0	4.8
	S.R.	84.2	12.0	3.8
Chrozophora tinctoria	M.H	77.2	13.8	4.5
	S.R.	91.2	8.0	0.8*
Calotropis procera	M.H	93.2	2.0*	4.8
	S.R.	91.3	8.0	0.8*
Carissa opaca	M.H	99.6**	-	-
	S.R.	79.2	17.0	3.8
Cassia occidentalis	M.H	91.2	8.0	0.8*
	S.R.	85.2	10.0	4.8
Datura stramonium	M.H	87.2	8.0	4.8
	S.R.	75.2	16.0	8.1
Dodonaea viscosa	M.H	99.2	-	-
	S.R.	83.2	16.0	8.1
Ficus racemosa	M.H	85.2	4.0	10.8**
	S.R.	96.2	3.0	0.8*
Otostegia limbata	M.H	98.2	-	-
	S.R.	74.2	15.0	10.8**
Ricinus communis	M.H	85.2	8.0	6.8
	S.R.	73.2	20.0**	6.8
Saccharum bengalense	M.H	87.2	8.0	4.8
	S.R.	93.2	6.0	0.8*
Xanthium strumarium	M.H	85.0	8.2	6.8
	S.R.	75.2	16.0	8.8
Ziziphus jujuba	M.H	83.4	7.6	0.9
	S.R.	93.2	6.0	0.8*

* = Lowest percentage; M.H. = Margalla Hills; ** = Highest percentage; S.R. = Salt Range

All roots samples showed vesicular infection. Statistically a significant ($P<0.05$) difference was found in the vesicular infection in the roots of two areas (Table 1). Lowest percentage (0.087 per cent) of vesicular infection was found in *Otostegian limbata* sampled from Margalla hills and highest percentage (12.5%) was found in *Chrozophora tinctoria* collected from Salt range area. Vesicular infection was absent in *Datura stramonium* sampled from Margalla hills (Table 2).

Arbuscular infection was present in almost all root samples except in the roots of *Ziziphus jujuba* sampled from Margalla hills and *Acacia modesta, Calotropis procera, Ficus racemosa, Ricinus communis, Saccharum bengalense and Xanthium strumarium* collected from Salt range area (Table 2). Statistical analysis revealed a significant difference in the arbuscular infection of both the areas (Table 1). Lowest range (0.06 per cent) was found in *Otostegia limbata* and highest (4 per cent) in *Broussonetia papyrifera* both were sampled from Margalla hills (Table 2). When data was analysed statistically, a significant difference was noted in the number of spores per 50g of soil between the areas (Table 1). Higher number of spores was observed in Salt range area as compared to Margalla hills (Table 2).

Discussion

Statistical analyses indicated a significant difference in hyphal, vesicular and arbuscular infection between the plants of Salt range and Margalla hills (Table 1). The present study indicated that if the phosphorus concentration is higher in the soil, higher will be the vesicular infection (Table 2 and 4). Many field experiments have shown that fertilizer application decrease the quantities of mycorrhizae (Hayman, 1975; Jensen and Jacobsen, 1980; Plenchette and Corporon, 1987; Vivekanandan and Fixen, 1991). This is in contrary to the findings of many other workers who suggested that VAM infection was higher with higher P concentrations (Porter *et al.*, 1978; Sylvia and Schenck, 1983; Lamar and Davey 1988; Douds and Schenck, 1990; Deneh, 1987; Gryndler *et al.*, 1990). Thus difference in VAM infection in the root samples collected from salt range and Margalla hills may be attributed by unequal status of N-P-K in soil at both the sites. Hayman (1983) suggested that genotype of the plants may be one of the factors in such contradictory findings. However, there may be many other factors that do take part in the establishment of VAM infection.

It has been reported by some workers that eroded soils have reduced number of mycorrhizal propagules (Powell 1980; Day *et al.*, 1987; Habate, 1989; Rashid *et al.*, 1997). This might be one of the reasons that lower number of VAM spores were recovered from soil of Margalla hills which are under severe erosion stress (Rashid and Ahmad 1996). Menge (1984) has demonstrated the effect of hydrogen ion concentration (pH) on VA mycorrhizae. Mosse (1972) has shown that certain endophytes (VAM) do not grow in low soil pH, where as other developed poorly after liming. In our study, high pH levels in the rhizospheric soil samples of *Ficus racemosa* from Salt range area with low vesicular and arbuscular infection indicates the influence of pH on VAM establishment (Table 2 and 4). It seems reasonable to believe that plants and VAM fungi have adapted to tolerate high saline environment of this area. The *Saccharum bengalanse* collected from the roof top of the salt mines in 'Khewra' is a good example of such behaviour as in this plant, vesicular infection was found good even under high salt concentration (Table 4). These results clearly indicate that VAM fungi have the ability to withstand saline environments.

In conclusion, it is evident that in both the localities the medicinal plants are growing under a certain type of stress. However, saline condition at Salt range has resulted in comparatively harsher environment than Margalla hills.

Table 4: Concentration of N-P-K, calcium carbonate and pH in the soil samples of the plants collected from Salt Range and Margalla Hills.

Plant species	Site	pH	P (ppm)	N (%)	K (ppm)	$CaCO_3$ (%)
Acacia nilotica	M.H	8.04	1.25	0.072	3.590	66
	S.R.	12.09	8.75	0.039	16.00	44
Acacia modesta	M.H	8.04	1.22	0.128	3.725	25
	S.R.	11.25	6.00	0.106	17.605	60
Adhatoda vasica	M.H	8.02	1.17	0.106	3.370	12
	S.R.	11.00	6.00	0.100	18.105	93**
Broussonetia papyrifera	M.H	8.06	0.2*	0.123	1.746*	43
	S.R.	12.15	6.25	0.028	7.30	05
Canabis sativa	M.H	8.04	11.17	0.117	2.528	20
	S.R.	8.25	1.2	0.078	12.640	52
Chrozophora tinctoria	M.H	10.02	15.75	0.106	8.410	20
	S.R.	8.04	5.87	0.016*	18.800	70
Calotropis procera	M.H	8.04	0.53	0.050	3.568	69
	S.R.	8.07	25.62	0.022	16.300	41
Carissa opaca	M.H	8.03	1.26	0.173	2.275	20
	S.R.	8.05	1.37	0.078	3.359	55
Cassia occidentalis	M.H	8.03	1.26	0.134	3.690	48
	S.R.	8.00	12.5	0.067	4.170	24
Datura stramonium	M.H	8.03	1.22	0.128	34.470	40
	S.R.	8.16	1.25	0.078	4.160	05
Dodonaea viscosa	M.H	8.00	1.2	0.179**	2.624	04
	S.R.	11.85	6.12	0.061	18.605	97
Ficus racemosa	M.H	12.00	6.06	0.145	8.710	15
	S.R.	12.25**	2.62	0.062	16.110	05
Otostegia limbata	M.H	8.01	1.2	0.112	8.710	01*
	S.R.	12.01	7.75	0.039	17.605	20
Ricinus communis	M.H	8.07	1.62	0.112	9.857	58
	S.R.	8.58	12.65	0.072	4.180	05
Saccharum bengalense	M.H	8.00	1.23	0.112	3.632	35
	S.R.	7.81*	14.75	0.044	34:150	85
Xanthium strumarium	M.H	8.55	17.25	0.117	35.00**	30
	S.R.	8.69	12.25	0.067	4.15	08
Ziziphus jujuba	M.H	8.07	1.37	0.100	3.536	25
	S.R.	11.12	29.68**	0.039	35.00**	84

* = Lowest percentage; M.H. = Margalla Hills; ** = Highest percentage; S.R. = Salt Range

spite of that stress, plants were found to have considerable amount of VAM infectivity. This on one hand reflects an inherent ability of these plants to establish mycorrhizal association and on the other, it manifested the behaviour of VAM fungi to colonize and proliferate in different soil environments. It seems appropriate to believe that plants of such areas can be used an important tool for studying physiological aspects of vesicular arbuscular mycorrhizae in wild and stressed conditions.

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