Screening of Leguminous Plants for VAM Association and Their Role in Restoration of Degraded Lands

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Abstract: In present study, 50 leguminous plant species were assessed for association of Vesicular-Arbuscular Mycorrhizal fungi. For this, fine roots of these plants were carefully dug out, washed and stained using root clearing methods and observed under microscope. Out of 50 species screened, 5 showed no VAM association, 2 species showed very low level of colonization (> 20%), 17 species showed 20 to 49 % colonization, 24 species showed 50 to 69 % colonization and only 2 species showed very high level of colonization i.e. <70%. Most of the plant showed hyphae with vesicle/arbuscles. However in five species viz. *Bahunia retusa, Crotolaria albida, Desmodium elegans*, *D. heterocarpon* and *Vicia rigidula* only hyphae of mycorrhizal fungi is present. Thus, the legumes with high to very level of VAM colonization can be use in restoration of degraded lands. [Journal of American Science. 2011;7(1):7-11]. (ISSN: 1545-1003).

Keywords: Legumes, roots, vesicles, arbuscles, colonization

1. Introduction

Restoration is defined as a tactic employed to return degraded lands to its original condition. Of the total earth's surface 78% of the land area is unsuitable for agriculture. Out of the remaining land, about 9% suffers from physical, chemical and biological constraints requiring special management practices. Since, land degradation process involve loss of vegetation and accelerated run off and soil loss, they require assistance for their restoration and regeneration. Mycorrhizal fungi and nitrogen-fixing bacteria are among the major beneficial components of soil microbial community, which contribute to plant growth and survival by reducing stresses through symbiosis (Sylvia and Williams 1992). These plants have special nutritional relationship between the two symbionts: the high phosphorus requirement of the nitrogen fixing root nodule and the high nitrogen requirement of the chitin walled VAM fungi and the high carbon requirement of both. Since each symbiont can supply the other's need in excess, the endophytes can bring about when the association is grown in nutrient-deficient soil (Norris et al 1994). In addition, VAM mycelia can extend to a long distance and can link the rhizosphere and mycorrhizosphere of different plant species; it can make common pool of the available nutrients of the other plant species (Norris et al 1994). This way, the nutrients released into the overlapping mycorrhizosphere by plant root exudation or by root and nodule decay become available for non-N_2 fixing plants (Simard et al 1997), which can play important role and facilitates the survival and growth of other plant species. Such observations led to a view of the legume microsymbionts as biological substitutes for fertilizers.

The present study assessed VAM colonization in leguminous plants of Kumaun Himalayan region. This study has greater implications as results of present study can be used to stabilize many degraded areas in the region, which is a frequent phenomenon all over the Himalaya.

2. Material and Methods

This study was conducted in Kumaun Himalayan region of Central Himalaya, India. Kumaun is situated between the latitude of 28^{o}44' - 33^{o}49' N and longitudes of 78^o 45' - 81^{o}05' E covering an area of about 21,033 km^2 ranging from 300 m to the 5400 m elevation. Broadly this region can be differentiated into four physiographic domins viz. the outer Himalaya with Terai and Bhabhar belts and Siwalik ranges; the Lesser Himalaya; the Great Himalaya and the Trans Himalaya (Jalal 1988). The climate of the region is governed by the monsoon and the year can be divisible into summer (April- mid June), rainy (mid-June- September) and winter (October - Feburary). The annual rainfall is about 2500 mm in most of the places, of which about three-fourth occur during rainy season (Singh and Singh 1992).

3. Material and Methods

The field surveys were conducted in different forest sites and leguminous plants growing in the region were identified. For each species fine terminal roots were collected from different places of root system selecting five plants at random and collected in separate polythene and brought to the laboratory. Roots were gently washed, cut into one cm segments and preserved in formalin-acetone alcohol (FAA) in the ratio of 90:5(v: v: v). These preserved root samples were used for studying the level of VAM colonization. Root clearing method (Phillips and Haymann 1970) was used to stain the roots by heating in KOH. The root sample stored in FAA were washed thoroughly with tape water and placed in test tubes and 10% KOH solution was added. These test tubes were heated at 90^{o} C for one hour in a water bath. After heating, the KOH solution was poured off from the test tube and roots were rinsed with several changes of distilled water. Then roots were placed in 1% HCL solution for 5 minute to acidify the roots. The roots were again rinsed several times. After rinsing, the roots were stained with 0.1% trypan blue staining solution and heated again for 5 minutes. Extra stain was poured off and lactophynol solution was added. Heavily pigmented roots were bleached prior to staining with alkaline H_2O_2 (Phillips and Haymann 1970). The root segment were then pressed gently and observed under a microscope for VAM colonization. Percent VAM colonization was calculated using Nicolson 'simple formula (1955; see; Gupta and Mukerji 1999):

$$\text{Colonization (\%)} = \frac{\text{No. of root segments colonized with VAM}}{\text{Total number of root segments observed}} \times 100$$

The colonization was categorized in the following groups based on the intensity of infection:

1. **Excellent:** Mycelia/vesicle/arbuscules present on whole surface of the root bits (1 cm length) in very large number.
2. **Good:** Mycelia/vesicle/arbuscules present on whole surface of the root bits.
3. **Moderate:** Mycelia/vesicle/arbuscules present sparsely on the surface of the root bits.
4. **Poor:** Mycelia/vesicle/arbuscules present on root surface only in few numbers.
5. **Nil:** Mycelia/vesicle/arbuscules totally abscent.

3. Results

A total of 50 plant species belonging to 3 families of legumes (Papilionaceae, Caesalpiniaceae and Mimosaceae) were surveyed for VAM association. Out of 50 plants sampled, 5 plants namely *Crotolaria madicaginea, Lathyrus aphaca, Vicia pallida, V. tetrasperma* and *Pterocarpus marsupium* did not possess any VAM association. Two plant species viz. *Crotolaria albida* and *Desmodium heteropogon* possessed very low level of association (less than 20%). The sparse infection in these species could be attributed to the continuous cover of root hairs. Baylis (1975) suggested that plants with greater number of root hairs would have less dependence on mycorrhiza. Intermediate level of association (20-49%) was recorded in 17 plant species. Some of the important species in this range were *Astragalus leucocephalus, Lespedeza gerardiana*, *Bahunia variegata* etc. High level of VAM colonization (50-69%) was recorded in 24 plants including *Indigofera dousa, Cassia floribunda, Albezia lebbeck* etc. Two plants *Indigofera heterantha* and *Trifolium repens* possessed very high (> 70%) level of colonization (Table 1). There were differences between the different species of same genus in the percentage of root infected. For example, in *Indigofera dousa* the infection was 64% while in *I.heterantha* the infection was 72% (Table 1).

Five plants showed presence of only VAM hyphae in their roots where neither arbuscules nor vesicles were recorded. They were *Bahunia retusa, Crotolaria albida, Desmodium elegans, D. heterocarpon* and *Vicia rigidula.* Rest of the species contained hyphae along with vesicles and or arbuscles (Table 1). Although many species (25%) of the legume in the present study possessed arbuscules, the number of plants possessing vesicles was higher than plants bearing arbuscules. These results suggested that roots of majority of the plants colonized were mature as vesicles are storage organs and generally produced in the older region of the infection.

Table 1.Vesicular Arbuscular Mycorrhizal colonization in some leguminous plants of Kumaun Himalaya.

Species	**Habit**	**Distribution**	**Habitat**	**Percent of root segment colonized**			**Intensity of colonization***
				A	**B**	**C**	
Family Papilionaceae							
Astragalus chlorostachys Lindl	Erect Shrub	2000-3600	As an undergrowth of birch-rhododendron forest	48	55	50	3
A. leucocephalus Garh.ex Benth	Herb	1600-2500	Shady and dry places	24	45	32	3
Argyrolobium flaccidum (Royle) Joub.	Prostrate herb	Upto 2800	On the edges of miscellaneous forest and open grassy localities	66	70	55	1
A. roseum (Camb.) Joub.	Prostrate herb	Upto 2300	Common on open grassy localities	52	55	32	3
Cajanus mollis (Benth.)Van der Maessen	Herb	Upto 2000	Shady dry and sunny places	56	62	55	3
Crotolaria albida Heyne.ex Reth	Herb	Upto 2000	Open grassy slopes	10	15	-	4
C. madicaginea Lamk.	Erect herb	Upto 1300	Open grassy places	-	-	-	5
Dalbergia sissoo Roxb	Tree	Upto 1500	Often planted roadsides	25	36	16	3
Desmodium elegans DC	Shrub	1500-2000	In oak forests and scrub jungles	0	46	0	4
D. floribundum D. Don	Erect undershrub	Upto 2600	Common in oak-Rhododendron forest and grassy localities	32	40	15	2
D. heterocarpon (Linn) DC.	Suberect or prostrate under shrub	Upto 1600	Common in grassy localities and forest clearing	-	14	-	4
D. microphyllum (Thumb) DC	Diffused perennial herb	Upto 2000	Grassy localities, roadsides and forest edges	48	56	47	2
D. gangeticum Linn	Shrub	Upto 1500	Common in sal forest, roadsides grassy localities	45	47	38	3
Dolichos fulcatus Klein	Herb	1800-3200	In grassy localities	26	44	26	2
Erythrina arborescens Roxb.	Tree	1500-3000	Throughout the hills	45	56	55	3
Flemingia bracteata Wight.	Undershrub	1500	Common in sal forest	34	48	28	3
F. strobilifera R.Br.ex Ait	Shrub	Upto 2500	In oak forest and grassy slopes in chir pine forest	32	36	35	4
F. vestita Benth ex Baker	Trailing herb	1800-3000	Grassy localities throughout the hills	48	56	45	2
Indigofera cassioides Rottl. Ex DC	Shrub	300-1600	Common in sal, chir and miscellaneous forests	32	56	48	2
I. dosua Buch-Hm. Ex Don	Diffused shrub or under shrub	1300-3600	In open chir and oak forests	30	64	56	1
I. heterantha Wall ex Brandis	Shrub	Upto 3000	Throughout the hills along way sides and vacant plots in oak forest	60	72	50	1
Lathyrus aphaca Linn	Annual trailing herb	Upto 2200	Common in fields, roadsides and field borders	-	-	-	5
Lespedeza gerardiana Garh.ex Baker	Undershrub	1500-2000	Open grassy localities	35	47	40	2
Medicago denticulata Willd.	Annual procumbent herb	Upto 1500	Common in gardens, agricultural fields and waysides	-	-	-	5
M. lupulina Linn	Decumbent ascending herb	Upto 1500	Common in grassy localities and roadsides	26	32	12	4
Parochetus communis Buch.-Ham ex D. Don	Herb	Upto 3800	Common on damp shaded plces and grassy localities	45	55	50	3
Pterocarpus marsupium Roxb.	Tree	500-1300	In open miscellaneous forests	-	-	-	5
Smithia ciliata Royal	Diffused herb	800-1900	In marshy and sandy localities	25	54	28	3
Trifolium repens Linn	Perennial herb	1400-3200	In waste places, waysides and fields	47	58	36	3
Trigonella. emodi Benth	Herb	1500-3600	Common on grassy fields and ground vegetation in oak forest	47	58	50	3
T. gracilis Benth	Diffused herb	2000-3000	In forest edges and grassy slopes	35	40	20	2
Vicia pallida Turoz.	Climber	1000-2500	In waste places and agricultural fields	-	-	-	5

V. rigidula Royal	Herb	2000-2800	Forest edges, scrub jungles and roadsides	0	54	0	4
V. tetrasperma (Linn) Moench	Prostrate climbing annual herb	300-1800	Grassy localities	-	-	-	5
V.vexillata (Linn) A Richard	Trailing herb	Upto 2500	In pine forest and open grasslands	42	56	45	2
Zornia gibbosa Span.	Herb	Upto 1500	In grassy localities, river beds, roadsides and borders of agricultural field	36	42	40	3
Family Caesalpiniaceae							
Bahunia retusa Buch-Ham ex Roxb.	Tree	300-1800	Common in miscellaceous forests	48	56	-	4
B variegata Linn.	Tree	300-1900	In chir pine and miscellaceous forest	36	36	47	2
Caesalpinia bonduc (Linn) Roxb.	Rambling climber	Upto 1000	Common in miscellaneous forest	48	50	32	2
Cassia floribunda Cav.Descr.	Shruby herb	Upto 1800	Throughout the hills in waste lands	40	67	54	2
C. mimosoides Linn.	Prostrate herb	Upto 1600	In grassy open localities, roadside and wayside	38	36	28	2
C. occidentalis Linn	Erect herb or undershrub	Upto 1300	In waste lands, roadsides and sometimes near river banks	29	54	27	3
C. tora Linn.	Erect herb or undershrub	Upto 1200	In waste lands, roadsides and agricultural field	34	46	21	3
Delonix regia (Hook.) Raf.	Tree	Upto 1000	Planted roadside; and as ornamental plant	48	51	36	4
Family Mimosaceae							
Acacia farnesiana (Linn.) Willd	Shrub or small tree	Upto 1800	Throughout the area	47	55	15	3
A. nilotica (Linn) Del	Small tree	Upto 1400	In valleys	27	35	18	3
Albizia chinensis (Osbeck) Merr.	Tree	300-1400	Scattered in open and miscellaneous forests	40	46	24	3
Albezia lebbeck (Linn.) Willd	Large tree	Upto 1500	In miscellaneous forests	43	56	42	2
Leucaena leucocephala (Lam.) De. Wit.	Tree	Upto 1400	Planted	26	48	25	2
Mimosa himalayana Gamble	Small tree or straggling shrub	Upto 1600	Along the water courses and scrub jungles	46	58	42	1
M. pudica Linn.	Under shrub	Upto 1500	Common on roadsides and waste places	37	40	12	3

A =percent external colonization; B= percent internal colonization C= percent vesicles

- 1= Excellent; 2= Good; 3= Moderate; 4= Poor; 5= Nil

4. Discussions

Degradation of natural resource and environment has been a global problem. In India, more than half of its geographical area faces problem of land degradation of one or the other kind. In the Himalayan region, degradation of forests has become a wide spread feature and restoration of such degraded land has become a major challenging conservational problem. Many efforts have been made to check the natural resource but have not yielded the desired sustainability. Microbial populations are key components of the soil-plant system where they are immersed in a framework of interactions affecting plant development (Lynch 1990).Perhaps the most widespread and certainly significant mutualism between plants and fungi is the root symbiosis, termed as arbuscular mycorrhiza(AM). These AM fungi are the most common natural association makers with the nodulated nitrogen-fixing legumes and other plants (Kothamasi et al 2001).

Woody legumes are useful for revegetation of water- deficient ecosystems that have low availability of N, P and other nutrients (Danso 1992). The scarcity of available P and the imbalance of trace elements in degraded ecosystems actually limit establishment of legumes and nitrogen fixation. But when associated with mycorrhizae it was found to increase the establishment of legumes (Barea et al 1992). In addition, woody legumes exhibit a considerable degree of dependence on mycorrhizae to thrive in stressed conditions (Osonubi et al 1991). After forming symbiotic association with legume roots, AM fungi develops an extraradical mycelium that links the roots and soil environment and help the plants to use soil nutrients more efficiently (Barea et al 1992).

Sustainable management of any degraded ecosystem involves practices that are equally concerned with productivity and soil conservation. In this situation, the Vesicular-arbuscular mycorrhizal legumes could reduce the amount of fertilizer needed for the establishment of vegetation and also increase the rate at which the desired vegetation becomes established by stimulating the development of beneficial microorganisms in the rhizosphere. Since, the cell walls of VAM hyphae are composed of amino-sugar chitin, the soil mycelium may be one of the most important vehicles for nitrogen and carbon input into the soil. Owing to their role, the VAM legumes can be used for revegetation of eroded, desertified or degraded ecosystems. In addition, the dual inoculation (Vesicular- Arbuscular Mycorrhiza along with *Rhizobium*) can be a biological tool for the management of N_2- fixing plants in restoring and maintaining soil fertility.

The capacity of VAM fungi to act as biofertilizers, bioregulators and bioprotectors has repeatedly been demonstrated. These associations help to maintain the general plant vigour under a variety of adverse and inhospitable ecological conditions. Present investigation is an attempt to enhance our knowledge of the ecology and applicability of VA Mycorrhizal legumes in successful reclamation and restoration practices in degraded ecosystems. On degraded sites where original soil has deteriorated markedly, these VAM associated nitrogen- fixing species can be planted. They can improve soil condition and growth of associated plant species through nutrient-rich leaf litter and biological nitrogen-fixation and therefore, useful in restoration of degraded lands.

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References

1. Sylvia DM, Willams SE. Vesicular-arbuscular mycorrhizae and environmental stress. In: Bethlenfalvay GJ, Linderman RG eds. Mycorrhizae and sustainable Agriculture. ASA Special Publication 54. Madison, QWI,1992: 101-124.
2 Norris JR, Read D, Verma AK. *Techniques for mycorrhizal research : methods in microboilogy*. Academic Press, Harcourt Brace and Company, Publishers, London, 1994.
3 Simard SW, Perry DA, Jones MD, Myrolds DD, Durall DM, Molina R. Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 1997; 388: 579-582.
4. Jalal DS. Geographical perspectives of Kumaun. In Valdiya KS, ed. Kumaun Land and People. Gyanodaya Prakash ,Nainital 1988.
5 Singh JS, Singh SP. Forest of Himalaya: Structure, Functioning and Impact of man. Gyanodaya Prakashan, Nainital, India, 1992
6. Philips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 1970;55:158-161
7. Gupta R, Mukerji KG. Effect of VAM fungus on the growth and flowering of *Helianthus annuus* L. Var. Peredovik EC-68414. *Phytomorphology* 1999;49 37-42
8. Baylis G T S The magnoloid Mycorrhiza and mycotrophy in root systems derived from it; In Sanders FE, Mosse B, Tinker PB, eds. Endomycorrhizas. Academic Press, New York USA 1975:373-389
9. Lynch, J.M. The rhizosphere. John Wiley, New York1990.
10. Kothamasi D, Kuhad RC, Babu CR Arbuscular mycorrhizae in plant survival strategies; Tropical Ecology 2001;42(1) 1-13.
11. Danso SKA, Bowen GD, Sanginga N. Biological nitrogen fixation in trees in agro ecosystems. Plant and Soil 1992; 141: 177-196.
12. Barea JM, Azcon R, Azcon Aguilar C. Vesicular arbuscular mycorrhizal fungi in nitrogen fixing systems. Methods Microbiology. 1992;24:391-416.
13. Osonubi O, Mulongoy K, Auotoye OO, Atayese MO, Okali D. Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. Plant and Soil. 1991;136: 131-143.