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BIODIVERSITY OF VAM AND RHIZOSPHERE FUNGI ASSOCIATED WITH WHEAT GROWN IN NORMAL AND DISTURBED FIELDS

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Abstract

The agriculture community is facing a great challenge to enhance the production. To meet the challenge, the focus is on to reclaim the degraded land and understands the complexities and interactions of soil biological system and agro-ecosystem as a whole. Soil microorganisms play very important role in reclamation of such degraded lands and have important influence on soil fertility and plant health. The importance of AM association in restoration and improvement of revegetation of disturbed mined lands is well recognized. In the present studies, the indigenous species of fungal associates and vesicular arbuscular (VAM) fungi of *Triticum aestivum* plant were isolated from the disturbed field (cement mining area) and normal field (10 km from cement mining) in Darlaghat, Himachal Pradesh, India. Rhizospheric soil samples of *Triticum aestivum* revealed the presence of eighteen species of fungi. *Aspergillus*, *Fusarium* and *Absidia* were found to be the predominant genera and maximum genera isolated belong to the subdivision Deuteromycotina in both the fields. 15 species of VAM fungal spores belonging to 6 genera (*Acaulospora*, *Glomus*, *Claroideoglomus*, *Dentisculata*, *Scutellospora* and *Gigaspora*) with *Glomus* as most dominant genus were isolated in present investigation from root adhering soil samples of *Triticum aestivum* from Normal (9 sp.) and Disturbed field (13 sp.).

Key words : Wastelands, mining, VAM, mycorrhizosphere.

Introduction

Land sources are limited and population size is increasing day by day. In India, 14.91% of total geographic area are wastelands under different categories. 40.36% of total geographic area in Himachal Pradesh falls under different categories of wasteland. Out of which 7.46% is mining wasteland. Wastelands are synonymous with drastically disturb lands, where the native vegetation and animal communities have been removed and the top soil has been lost, altered or buried. Such lands will not become naturally rehabilitated within the life time of man through normal successional process (Chandra, 1992). Due to lack of proper planning and negligence of mining regulations, an appreciable amount of environmental degradation and ecological damage to soil has occurred (Dhar, 1993).

To fulfill the requirements of ever increasing population, the demands are placed on agriculture to supply future food and fibre needs. The agriculture community is facing a great challenge to enhance the production. To meet the challenge, the focus is on to reclaim the degraded land and understands the complexities and interactions of soil biological system and agro-ecosystem as a whole. Soil microorganisms play very important role in reclamation of such degraded lands and have important influence on soil fertility and plant health. The importance of AM association in restoration and improvement of revegetation of disturbed mined lands is well recognised (Mukhopadhyay and Maiti, 2009). Mycorrhizae are highly evolved, mutualistic association between soil fungi and plants roots. VAM (vesicular arbuscular mycorrhizae) ameliorate and increase the tolerance to adverse soil conditions and they increase the plant productivity (Brundrett *et al*., 2013 and Bennett, 2013). There is an urgent need to explore, isolate and identify the indigenous soil microorganisms so that such marginal lands can also be used for agriculture purpose to meet the demands of food requirements. Present studies were conducted to investigate the biodiversity of VAM and rhizosphere fungi associated with wheat grown in normal and disturbed fields.

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Table 1 : List of fungi isolated from Mycorrhizosphere soil samples of *Triticum aestivum* (wheat) from Disturbed Field (Kashlog Cement Mining Area) and Normal Field (Kararaghat).

S. no.	Name of fungus isolated (Disturbed field)	Name of fungus isolated (Normal field)
1.	*Absidia* sp.	*Absidia* sp.
2.	*Absidia ramose*	*Aspergillus niger*
3.	*Cephalosporium* sp.	*Aspergillus terreus*
4.	*Cladosporium* sp.	*Aspergillus ustus*
5.	*Fusarium oxysporium*	*Fusarium solani*
6.	*Fusarium* sp.	*Gliocladium roseum*
7.	*Penicillium funiculosum*	*Rhizoctonia solani*
8.	*Rhizopus oryzae*	*Trichoderma* sp.
9.	*Trichoderma viride*	*Sterile mycelium*
10	*Sterile mycelium*	

Table 2 : Comparison of occurrence of different VAM fungal spores isolated from the rhizosphere soil of *Triticum aestivum* (Wheat) taken from Normal Field (Kararaghat) and Disturbed Field (Kashlog Cement Mining Area).

S. no.	VAM fungal spores isolated	Normal field	Disturbed field
1.	*Acaulospora bireticulata*	+	+
2.	*Acaulospora denticulata*	+	+
3.	*Acaulospora longula*	+	+
4.	*Acaulospora scrobiculata*	+	+
5.	*Claroideoglomus etunicatum*	+	+
6.	*Dentisculata nigerita*	+	–
7.	*Gigaspora gigantea*	–	+
8.	*Glomus aggregatum*	+	+
9.	*Glomus ambisporium*	–	+
10.	*Glomus clarum*	+	+
11.	*Glomus fasciculatum*	+	–
12.	*Glomus occultum*	–	+
13.	*Glomus rubiforme*	–	+
14.	*Glomus spercum*	–	+
15.	*Scutellospora minuta*	–	+

+ = Present, - = Absent.

Materials and Methods

Sampling

Study materials (Rhizospheric soils samples and roots of wheat (*Triticum aestivum*) were collected from selected site of Kashlog Mining Areas, Darlaghat, distt Solan, Himachal Pradesh (India) adjoining agricultural fields of Chakru village (Disturbed field) and 10 km from mining site (Control) at National highway no. 88 (Kararaghat) in the April (premonsoon season) month of 2014.

Isolation of rhizosphere fungi from soil samples

For the isolation of rhizosphere fungi, dilution plate method of Wakesman (1927) and Warcup (1950) was followed. The media used for culturing rhizosphere fungi were Czapeks Dox (Raper and Thom, 1949) and potato Dextrose Agar (Rawling, 1933).

Assessment of VAM fungal colonization in roots

Root pieces were thoroughly washed with sterilized water and boiled at 90^0 C for 1-2 hours in 10% KOH, acidified in 5N, HCl, stained in lactophenol trypan blue. The segments were mounted on slides containing acetic acid: glycerol (1:1v/v).

Isolation of VAM fungal spores

For isolation of VAM spores, wet sieving and decanting technique (Gerdemann and Nicolson, 1963) was followed. Spores were identified according to, size, shape, wall characteristics (Schench and Prez, 1988).

Results and Discussion

18 species of rhizosphere fungi were isolated from the mycorrhizosphere of *Triticum aestivum* (Normal and Disturbed Field). The fungi isolated from the normal field were *Absidia* sp., *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus ustus*, *Fusarium solani*, *Gliocladium roseum*, *Rhizoctonia solani* and *Trichoderma* sp. Fungi isolated from rhizosphere soil of *Triticum aestivum* from the disturbed field were *Absidia* sp., *Absidia ramose*, *Cephalosporium* sp., *Cladosporium* sp., *Fusarium* sp. *Fusarium oxysporum*, *Penicillium funiculosum*, *Rhizopus oryzae* and *Trichoderma viridae* (table 1). *Aspergillus*, *Fusarium* and *Absidia* were found to be the predominant genera in mycorrhizosphere of *Triticum aestivum* in both the fields. These fungal isolates were further grouped into subdivisions *viz.*, Zygomycotina, Ascomycotina and Deuteromycotina (fig. 1). Sagar *et al.* (2007) studied the soil mycoflora of cold desert of H.P. (Lahaul and Spiti and Kinnaur) and isolated 44 species of fungi belonging to 18 genera *viz. Alternaria*, *Aspergillus*, *Cephalosporium*, *Cunninghamella*, *Dreschlara*, *Epicoccum*, *Fusarium*, *Glicocladium*, *Mucor*, *Nigrospora*, *Paecilomyces*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Stachylomyces*, *Stachylotrys* and *Tricoderma*.

In the present investigation, it was observed that maximum genera isolated from both the fields belongs to the subdivision Deuteromycotina. The possible explanation of this is attributed to the reason that fungi imperfecti can tolerate wider environmental conditions as compared to other fungal population (Behera and

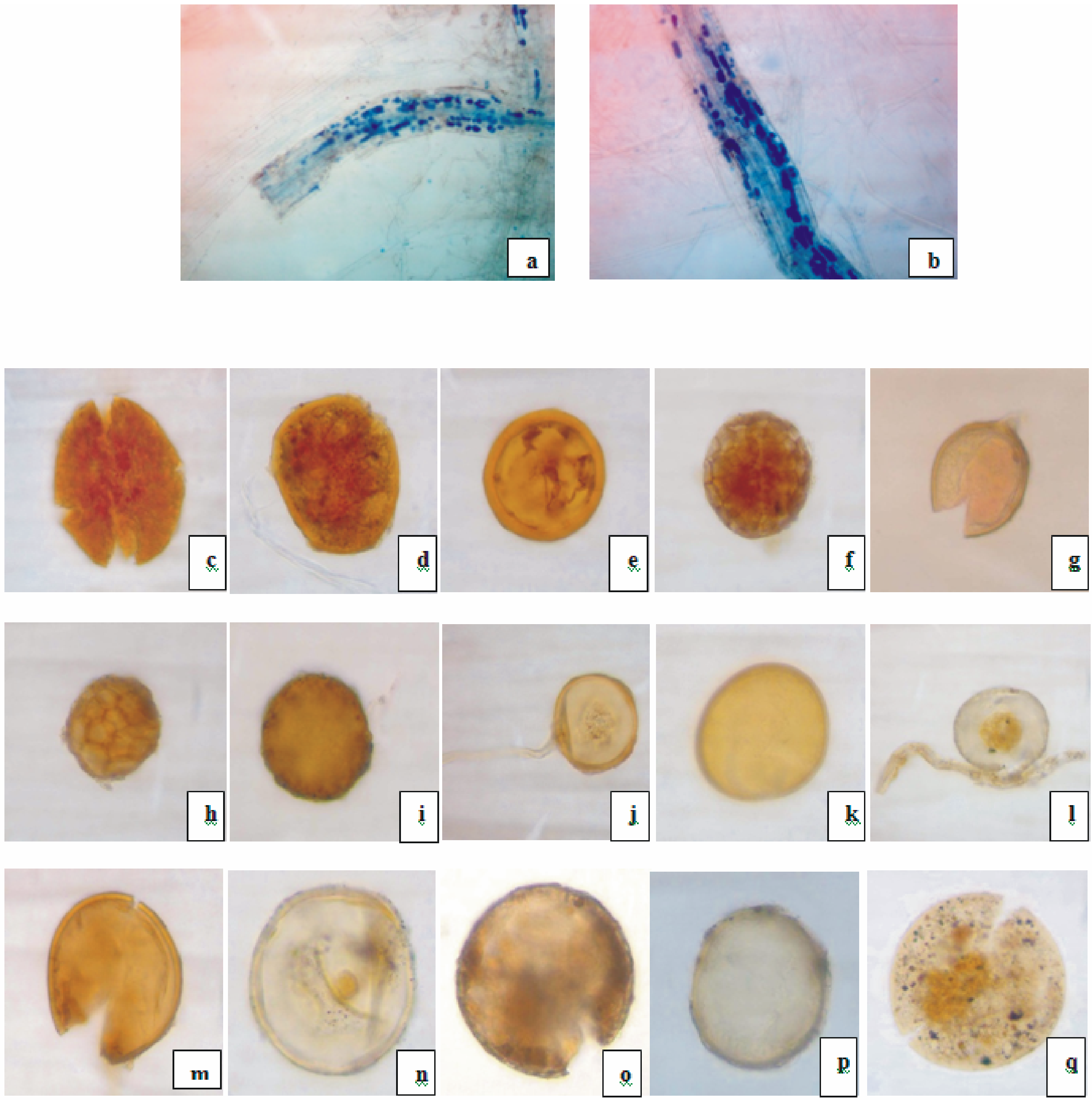

Plate : VAM infection in roots of *Triticum aestivum* in normal field **(a)** and in disturbed field **(b)**; VAM spores from both fields: *Acaulospora bireticulata* **(c)**, *Acaulospora denticulata* **(d)**, *Acaulospora longula* **(e)**, *Acaulospora scrobiculata* **(f)**, *Claroideoglomus etunicatum* **(g)**, *Dentisculata nigerita* **(h)**, *Gigaspora gigantean* **(i)**, *Glomus aggregatum* **(j)**, *Glomus ambisporium* **(k)**, *Glomus clarum* **(l)**, *Glomus fasciculatum* **(m)**, *Glomus occultum* **(n)**, *Glomus rubiforme* **(o)**, *Glomus spercum* **(p)**, *Scutellospora minuta* **(q)**.

Mukherji, 1984; Sagar, 2012). Thomas and Parkinson (1967) have reported that the rhizosphere activity of the host may selectively favour the growth of some microorganism than others and observed that maximum isolated genera belong to subdivision Deuteromycotina followed by Zygomycotina and Ascomycotina. A comparison of two experimental field's distribution of mycorrhizosphere fungi of *Triticum aestivum* revealed that maximum number of fungal isolates (10 sp.) were recorded in disturbed field (Kashlog cement mining area) and minimum number of fungal isolates were recorded in Normal field (9 sp.) (table 1). In arid and semi-arid regions, generally low soil fertility makes plants highly dependent on mycorrhization (Tarafdar and Kumar, 1996).

In the present study, 15 species of VAM fungal spores belonging to 6 genera (*Acaulospora*, *Glomus*, *Claroideoglomus*, *Dentisculata*, *Scutellospora*, *Gigaspora*) were isolated from root adhering soils of *Triticum aestivum* from Normal (9 sp.) and Disturbed

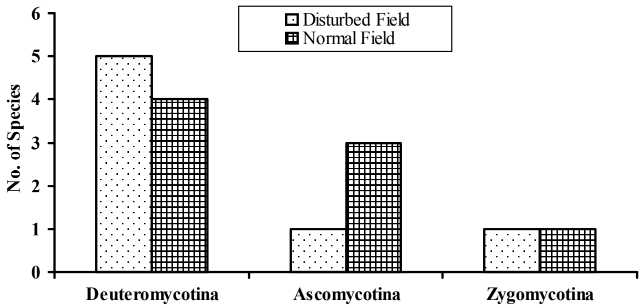

Fig. 1 : Distribution of fungal species (in different fungal subdivisions) isolated from the rhizosphere soil samples of *Triticum aestivum* from Disturbed Field (Kashlog Cement Mining Area) and Normal Field (Kararaghat).

Field (13 sp). The fungal spores isolated were *Acaulospora denticulate*, *Acaulospora bireticulata*, *Acaulospora scrobiculata*, *Claroideoglomus etunicatum*, *Dentisculata nigerita*, *Glomus aggregatum*, *Glomus clarum*, *Glomus fasciculatum* from normal fields and *Acaulospora bireticulata*, *Acaulospora denticulate*, *Acaulospora longula*, *Acaulospora scrobiculata*, *Claroideoglomus etunicatum*, *Gigaspora gigantea*, *Glomus aggregatum*, *Glomus ambisporium*, *Glomus clarum*, *Glomus occultum*, *Glomus rubiforme*, *Glomus spercum* and *Scutellospora minuta* were from disturbed fields (table 2). *Glomus* was found most dominant genus in present investigation. Sagar *et al.* (1993) and Kaur *et al.* (1997) while studying VAM associates of *Celtis australis* and *Grewia optiva* reported the genus *Glomus* to be more dominant in Himachal Pradesh soils. Predominance of *Glomus* in semi arid areas, showed the tolerance of these species to stressful environments. AM fungal species richness and Glomerospore production remains unaffected due to mining activity (Rios *et al*., 2013 and Kehri *et al.*, 2013). Dugaya *et al.* (1996) has isolated and identified nine species of VAM fungi belonging to the 3 major genera *viz. Acaulospora*, *Glomus* and *Gigaspora* from a rehabilitated coal mine spoil at Bisrampur, Madhya Pradesh, India. *Acaulospora scrobiculata* and *Paraglomus occultum* species were more dominant in six identified species in reclaimed areas after Bauxite mining (Melloni *et al*., 2003).

VAM fungi adapted in waste dumps can be used for successful reclamation programme. Kullu and Behera (2012) studied the diversity of VAM fungi in different age series sponge iron solid waste dump. A total of 35 VAM species belonging to 5 genera namely *Glomus, Gigaspora*, *Acaulospora*, *Scutellospora* and *Sclerocystis* were recorded of which 30 were noted in waste dumps. AM fungi native to limestone mine spoils may play a critical role in revegetating the mine spoils in arid areas by improving the water uptake and transport in plants, enhancing the uptake of various nutrients especially P and the ability of plants to withstand high temperature. AM fungi improve the biological productivity of these mine spoils for sustainable plant production and to create a better ecosystem (Rao and Tak, 2001; Silva *et al.*, 2005).

Present investigations have established a base for future exploitation of these rhizospheric as well as VAM fungi for reclamation of wastelands in the form of biofertilizers.

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