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ROLE OF PLANT-MYCORRHIZA SYMBIOSIS IN REVEGETATION OF DISTURBED MINING SITES IN INDIA (NORTH TELANGANA)

Dayakar GOVINDU[1,2], Sheak Rehana BEGUM[1], Srinivas PODETI[1], Borbala BIRO[2]

[1]Kakatiya University, Warangal, India
[2]Szent Istvan University, Budapest, Hungary

Abstract

Extensive mining activities develops with energy requirements of globalizing World. Both underground- and open cast mining are resulting disturbed soil surfaces, which need to revegetate for environmental safety. Coal mining sites at North Telangana region of India was investigated. Soils there are poor habitats for any plant establishment, the growth and survival of revegetated plants are possible only with arbuscular mycorrhiza (AM) fungal inoculation. Two leguminous agroforestry tree species were selected (Acacia nilotica, A.n. and Albizia lebbeck, A.l.). AM fungal spores were isolated and based on their morphological structures, 5 different types of AM species, the Glomus/Rhizophagus-, Gigaspora-, Acaulospora-, Scutellospora- and Sclerocystis sp. were identified. We have investigated the AM colonization rates, efficiency of selected strains in greenhouses and also at coal mine dumps by inoculation experiments on two test plants. AM fungal strains was found to be effective for the selected leguminus tree species both in their natural forests and at disturbed soils. Glomus sp. was the most dominant among the five different AM fungal species, more particularly G. aggregatum, G. fasciculatum showed the best colonization and biomass production with the test plants. The AMF isolates from natural forests are supporting the revegetation of the disturbed mining sites. Preselection is needed for considering the environmental protection and the best soil functioning.

Keywords: *mining sites, revegetation, AM fungi, isolation, inoculation.*

1. INTRODUCTION

Coal is extracted from the earth by opencast and underground mining. Opencast mining is considered one of the most dramatic disturbances in terrestrial ecosystems. The disturbance of green vegetation due to mining it has been reported that there is a difficulty with restoration [1], because of three major macronutrients, namely nitrogen, phosphorus and potassium are generally found to be deficient in overburden dumps [2, 3], heavy metals on the other hand can be accumulating very frequently in mining sites [27].

The AM fungi and other poetential microsymbionts helps the plants to grow in disturbed sites for improvement of environment [4]. By using indigenous AMF from forest systems is essential for reclamation of these mine spoils and overburdens include re-establishment of sustainable plant community [5, 28]. Mycorrhizal symbiosis are very important for survival and growth of plants and plant uptake of nutrient such as phosphorus and nitrogen, especially P deficient soils [6, 27]. In the colonization of arbuscular mycorrhizal (AM) fungi, several species of arbuscular mycorrhizae fungi

occur which helps in plant growth and assists them in their mineral uptake. Plant and microbial growth processes in disturbed mine spoils requires stable nutrient cycles for reclamation [7, 8, 25].

Arbuscular mycorrhizae create the wide spread plant fungi symbiosis that occurs in nature and therefore mycorrhizal fungi are key components of natural ecosystems. They are considered indispensable for ecosystem functioning [9, 27], because they play a fundamental role in soil fertility and in the maintenance of stability and biodiversity within plant communities [10, 11]. AMF play a significant role in the maintenance of ecosystem. Forest soil supports a great diversity of AM fungal flora. Agricultural, disturbed, polluted soils have already lost much of the diversity of AMF. Due to industrialization and mining activities, the forests are fast dwindling. Along with forest flora, the beneficial AM flora is also likely to eliminated. Hence there is a need to identify and conserve these fungi for different forestry programmes. Mining activities result in severe disturbance in the soil and huge mine overburdens.

The coal mine overburden soils are poor in organic carbon, available nitrogen and available phosphorus because of lower measure of microbial functioning in the overburden soils. Soil nutrient and physico-chemical properties also affect plant communities [12, 13, 14, 27].

The pH of the overburden sites is slightly acidic in nature, under these (acidic) conditions of dump materials growth of plants severely affected in various ways. The dump material at all the sampling sites was not found suitable for plant growth. The data reveals that dump soils are deficient in N, P which doesn't support proper plant growth.

Reclamation of these mine spoils and overburdens include re-establishment of plant community. Therefore, a study on possible application of AMF excavated from forest systems is essential.

2. MATERIALS AND METHODS

The AMF cultures were isolated from the native soil samples and identified morphologically [15]. AMF monocultures were mass cultured in the substrate of sterile soil:sand (1:1) mixture for 2 months in green house. P-deficient Hoagland solution was used for the nutrition used on a weakly frequency.

Two test plants *A.n* and *A.l* were treated with five different AM fungal monoculture inoculum treatments. There were 4 grams of inoculums used with root fragmants and spores of the AMF per 1 kg of sterile soil medium. Treated plants were raised in green house for six months and watered accordingly.

Transplantation of AM inoculated seedlings

Inoculum treated seedlings of 2 test plants [*Acacia nilotica A.n) , Albizia lebbeck (A.l)* were transplanted at Ellandhu fresh open cast dump soil (OCD) sites. The Pits were 40x40x30 cm and the distance between each plants was 1.5mx1.5 m. The inoculums treated plants were transplanted on to the site along with farmyard manure in the month of June.

Sampling

After one year of growth in OCD the rhizosphere soil samples and plants were randomly collected from each treatments of two test plants. The rhizosphere soil samples along with the root bits of test plants were collected from the experimental plots at Ellandhu coal mine dumping area. Soil samples from 0-20 cm depth were collected in polyethylene bags and air dried afterwards.

Soil physico chemical characateristics

Soil pH assessment: Air dried soil of 20g was taken in a beaker and 50ml water was added. The mixture (1:2.5 ratio of soil:water) was stirred for 10 min and was allowed to stand for 30 min. The pH was measured by using the electronic digital pH meter.

Organic carbon (OC) content was determined using titration method of Walkley and Black [16]. Total nitrogen (TN) content was determined by Kjeldahl method [17].

A part of the samples was used for spore count and taxonomic analysis. The root samples were cut into 1 cm pieces, washed with tap water, preserved in 70% ethanol and stored at 4^{o}C for further analysis.

The mycorrhizal roots were stained [18], fifty root segments were randomly selected and mounted on microscopic slides and observed under microscope to study the hyphae, vesicles and arbuscules. The length of infection was assessed in mm or cm for each root piece and averaged for ten pieces, and expressed in percentage of colonization. The presence or absence of infection was also recorded in groups of ten each and results expressed in percentage.

There was 50 root fragments observed in each replicate sample. The percentage colonization was calculated by the following formula.

$$\textbf{Percentage of AMF root colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments observed}} \times 100$$

AM fungal spores in the rhizosphere soils were extracted by wet sieving and decanting method [19, 20]. 100g^{-1} of soil was mixed in a water beaker (1000ml) and stirred with a glass rod to make uniform suspension and left for five minutes. The suspension was passed through different sieves. This process is repeated until the top layer of sediment soil become clear. After that we have collected the spores by washing them and mounting spores on a slide. The AMF spores were observed and identified based on morphology [15] and website (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html).

Estimation of plant biomass

Plants were carefully schooped out of the pots at the time of flowering. The shoot and root system were separated and the root system was thoroughly washed in slow running tap water, to remove the adhering soil particles. Then the roots were pressed between dry filter papers to remove the excess water. These were carefully packed in separate papers and are kept in an oven at 80^{o}C for 48 hours until a constant weight is obtained. Later they were weighed on a balance and weights are recorded.

Estimation of Phosphorus

P-content in the oven dried (shoot and root) samples was estimated by molybdate-vanadate method [22]. 1g of the oven dried sample was digested in 10 ml of triacid mixture (750 ml conc. HNO_3 + 150 ml conc. H_2SO_4 + 300 ml HNO_4) for $2_{1/2}$ hours.

The digested mixture was made up to 50ml with distilled water and filtered through whatman No. 1 filter paper. 2ml of the diluted sample solution was taken in a test tube, 2ml of 2N HNO_3 and 5ml of distilled water were added. Then 1 ml of molybdate-vandate solution was added. The yellow color was allowed to develop for 20 min.

The intensity of yellow color was recorded in spectrophotometer at 420 nm against a blank which contained all the reagents except sample whose volume was substituted by distilled water. P content was calculated bye standard graph prepared with KH_2PO_4.

3. RESULTS AND DISCUSSION

The pH of the soil solution is very essential because soil solution carries in it nutrients such as nitrogen, potassium, and phosphorus that plants need in specific amounts to grow, thrive, and fight off diseases. The pH of the present study site is showing less than pH 7 (slightly acidic). Plants were unable to utilize the N, P, K and some micro nutrients if the soil solution pH is acidic.

In the present investigation soil samples with clay loam having very low organic matter (0.14%) as considered it as a depleted soil. Percentage of organic matter range is 1.4%, indicating less accumulation of humus matter in the dump samples of Ellandhu.

Nitrogen was found to be 69.1 kg/ha in selected study site of Ellandhu. Available phosphorus content of the dump materials was recorded in low amount 7.6 kg/ha. This might be due to slightly acidic nature of samples which restricted the microbial functioning resulting in very poor mineralization and organic decomposition process (Table 1).

Table 1. Soil physico chemical characteristics of the studied site

Soil type	**Sample collection area**	**Soil texture**	**pH** (H_2O)	**EC** (mhos /cm)	**Organic matter** (%)	**Available Phosphorus** (kg/ha)	**Available Nitrogen** (kg/ha)
Coalmine open cast soil	Yellandhu (JK-5 ocp)	Clay loam	6.1	0.149	0.14	7.6	69.1

Effect of native AM fungi on the mycorrhizal intensity in terms of root colonization and spore population in rhizospheric soil of *A.l* and *A.n* has been presented in Table 2 & 3 respectively. In the comparative studies all the *Glomus* species showed a significant difference in colonization.

Biomass of treated plants in the form of fresh weight recorded in *Albizia lebbeck* (*A.l)* and *Acacia nilotica* (*A.n)* is ranging from 90.0 to 142.6 g and 72.8 to 168.1 g , respectively. Likewise root/shoot dry weight ranging from 62.4 to 116.3 g and 59.1 to 141.9 g, respectively, at the time of one growth in the transplanted site.

Minimum root/shoot growth was recorded in control plants. In comparison to control, all other treated plants showed a highest root/shoot growth. Maximum root/shoot growth of *Albizia lebbeck* (142.6g) was recorded in *Glomus/Rhizophagus aggregatus* treatment. In *Acacia nilotica* (168.1) the highest growth was observed in *Glomus fasiculatum* and followed by *Gigaspora gigantea.*

In the present study all the five treatments gave best results when compared with control (non inoculated tree species) *Glomus/Rhizophagus aggregatus* supports *Albizia lebbeck* showed highest root colonization and helps the plants to uptake the nutrients such as root/shoot Phosphorus content (0.42/0.38 mg/g).

In *Acacia nilotica* the highest shoot/root phosphorus content (0.46/0.34 mg/g) showed by treatment with *Glomus fasiculatum*.

Among all the five monoculture treatments *Glomus/Rhizophagus fasciculatum* and *Rhizophagus aggregatus* gave best plant growth in all the parameters records plant height, biomass and Phosphorus content. In this study Percentage of AMF root colonization is directly propotional to the biomass and phousphorus content.

The results of this plot experiment is carried only for short period (one year) so there was no significant difference in treatments. Addtion of bioinoculants/helper bacteria along with AM fungi helps the plant growth PGPR [23-25] including N_2-fixing bacteria [26-27] are involved.

Table 2. Screening of Albizia lebbeck for efficient strains of AM fungi

	Treatments	Root colonization (%)	Height of plant (cm)	Biomass (g)		Phosphorus (mg/g)	
				Fresh wt.	Dry wt.	Shoot	Root
1	*Glomus fasciculatum (Rhizophagus fasciculatum)*	72.6	112.1	125.2	108.8	0.36	0.12
2	*Glomus aggregatum (Rhizophagus aggregatus)*	76.4	120.2	142.6	116.3	0.42	0.38
3	*Gigaspora gigantea*	50.2	83.2	111.1	86.2	0.36	0.16
4	*Acaulospora foveata*	52.4	96.6	128.3	110.6	0.26	0.18
5	*Sclerocystis sp.*	39.7	86.5	98.2	65.3	0.14	0.10
6	*Control*	--	76.0	90.0	62.4	0.32	0.15

Table 3. Screening of AM fungal treatments with Acacia nilotica

	Treatments	Root colonization (%)	Height of Plant (cm)	Biomass (in grams)		Phosphorus content (mg/g)	
				Fresh wt.	Dry wt.	Shoot	Root
1	*Glomus fasciculatum (Rhizophagus fasciculatum)*	70.0	160.1	168.1	141.9	0.46	0.34
2	*Glomus aggregatum (Rhizophagus aggregatus)*	62.4	148.9	152.6	133.6	0.32	0.27
3	*Gigaspora gigantea*	69.6	154.2	156.1	139.2	0.41	0.29
4	*Acaulospora foveata*	50.4	120.2	136.2	99.3	0.25	0.16
5	*Sclerocystis sp.*	37.6	97.0	111.2	84.5	0.32	0.22
6	*Control*	--	68.8	72.8	59.1	0.22	0.12

Conclusion

Seedlings inoculated with the indigenous AMF monoculture showed the highest biomass and phosphorus content. When compared to non-mycorrhizal controls, those plants grew very poorly. Within the AM fungi selection of perfect efficient indigenous mycorrhiza inoculations are needed for revegetation of disturbed sites. By the efficient AMF inoculation the agroforesty tree species showed best results in the form of increasing biomass and phosporus uptake.

Inoculation of multi agent bioinoculants such as nitrogen fixers and K solubulizers, as helper bacteria along with AMF inoculations should be also suggested. Fast growing host plants which has more association for mycorrhizal colonization in their roots, are required to be studied for mine site restoration.

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Corresponding author
Dayakar Govindu
Department of Soil Science and Water Management
Faculty of Horticulture
Szent István University
H-1118 Villányi ut 29-43, K building, Room 331.
Budapest, Hungary
Telephone/mobile: +3630-7200-663
E-mail: biro.borbala@kertk.szie.hu