RESEARCH ARTICLE

Soil Trap Culture of strawberry associated AM fungi from Melghat (M.S.) India

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ABSTRACT

Present world demands the production of high quality food in a most sustainable way causing least damage possible to the ecosystem. Today, fertilizers and pesticides are being used at high levels in the intensive production of plants. A cheap and non-destructive means of achieving high productivity rests on the establishment of a viable low input farming system. However, in order to implement such a plan, we must develop plant systems that can efficiently scavenge and utilize soil nutrients present at low levels. The symbiotic arbuscular mycorrhizal fungi (AMF) have a major impact on the functioning and stability of any ecosystem. An attempt has been made in the present research to isolate and identify AM fungi of strawberry fields in the Melghat area of Maharashtra, India. The rhizosphere soil was then used for soil trap culture of dominant AMF species. Various practices like use of waste substrates along with the traditional substrate (soil-sand mixture) are being tried for mass culture of AM fungi these days. Addition of any substrate into soil provides minerals in addition to beneficial elements that ultimately enhance the growth of AM fungi as well as plant.

Since strawberry is the most economically competent crop of the area, the focus has been to isolate, characterize and identify its most dominant AM fungal species to prepare trap culture. The two varieties of Strawberry (*Fragaria* Sp.) namely winter down and tissue culture were collected for the study. Assessment of rhizosphere soil samples and roots was carried out. All the samples showed presence of AMF propagules. The genus *Glomus* was found to be the most frequent morpho-taxonomically identified AMF. The preparation of trap cultures is the only viable technique to increase spore number and to recover intact, fresh and healthy spores which may be used in future for initiation of monospecific cultures. The research finds its extension in the industrial produce on large scale by mass multiplication of these native AM fungal strains and its wider application in the farming. The findings of the present study are significant in upbringing of the local tribal community using nature's own resource.

Keywords: AM fungi, strawberry, Melghat forest area.

INTRODUCTION

Arbuscular Mycorrhizal fungi (AMF) are natural and integral part of healthy soil ecosystems which contribute to efficient utilization of soil resources namely nutrients and water. AM fungi belonging to the phylum *Glomeromycota* are geographically ubiquitous occur over a broad ecological range including associated agriculture, horticulture, pasture grasses, tropical plants and cereals. Mycorrhizal plants are known to alter nutritional status, altered photosynthetic rates, regulating substances and altered patterns of root exudation due to change in membrane permeability. Further, AM fungi play siginificant role in fruit production by transporting slowly mobile nutrients is specially P, Mn, Zn, Fe, and Cu from bulk of soil beyond the depletion zone surrounding active roots. AMF are said to reduce the impact of environmental stress. Thus, mycorrhiza can be considered as biological agents in maintaining the quality and sustainability of fast degrading environment and managing these symbiotic organisms in agriculture and flori-horticultural systems of world for the benefits of society and soil welfare.

Melghat is among one of the nine tiger reserves of India. It is located at 21 26' 45" to 770 11' 55" E in northern part of Amravati district of Maharashtra in India. The inhabitants are mainly tribal, largely of the Korku tribe. There is a deep connection between the tribal economy and minor forest produce. To protect the biodiversity of melghat forest there is ban to collect forest produce from this area so directly effect on their self-reliance. So, there is urgent need to develop new agriculture technology to sustainable livelihood of farmers of this region.

The strawberry, *Fragaria*, is one of the most popular berry fruits in the world and it offers a wide range of health benefits. Strawberries are an excellent source of vitamins C and K as well as providing a good dose of fibre, folic acid, manganese and potassium. They contain powerful antioxidants and are thought to protect against inflammation, cancer and heart disease. Therefore the objectives of this study are to determine the diversity of dominant AM fungi associated with strawberry plant to develop soil trap culture.

MATERIALS AND METHODS

Site selection and sampling

Amravati is a district in the state Maharashtra, India. It is the seventh most popular metropolitan area in Maharashtra. At the northern extreme of the Amravati district on the border of Madhya Pradesh, lies the Melghat in the South-western Satpura mountain ranges. Melghat means "meeting of the ghats". Motha is a small village near the Chikhaladara from which samples of strawberry plants were collected. Two varieties of strawberry namely tissue culture (Kamaroja) and Winter dawn variety samples were collected in the month of December 2015 along with their roots and rhizosphere soil in triplicates. The plants were carefully uprooted and roots were washed under tap water. A part of fresh root system of each variety of strawberry was cut and after careful rinsing with tap water, the root samples were stored in FAA. The rhizosphere soils from each plant was dug out up to the depth of 15-20 cm and about 500 gm soil sample was collected in polythene bags. These soil samples were brought to the laboratory and after shade drying the soil was stored in clean polythene bag. Each bag was labeled with sampling site number, name of the host and sampling date, etc. The composite sample was used for physico-chemical analysis by standard methods and the individual samples were used for isolation of AMF spores to find out the most dominant AM fungal spores for soil trap culture. physico-chemical analysis of soil samples was done by standard methods Jackson ML (1967).

Processing of Roots

The preserved root samples were used for the further analysis by the process given by Phillips and Hayman (1970). The AM percent root colonization was calculated by using the Grid line intersect method (Giovannetti and Mosse, 1980). The isolation of AM spores was carried out by following method of Gerdemann and Nicolson (1963). The method given by Gaur and Adholeya (1994) was used for counting AMF spores.

The isolated spores were given a thorough microscopic examination to record their morpho-taxonomic features. The AM fungi were identified by using the manual of Schenck and Perez (1990). Soil trap culture were prepared by the method by Rodrigues and Muthukumar,2009.

RESULTS AND DISCUSSION

As far as the current agricultural practices and socio-economic growth of target area is concerned this project is a unique attempt for Melghat region to explore belowground interactions, focusing on the studies of Arbuscular Mycorrhizal fungi associated with strawberry plants. On this account, Melghat soils attracted the attention for detailed studies of AMF and utilization of these in future for the welfare of mankind. Keeping this view in mind the present study was made and the following results were obtained.

physico-chemical parameters of rhizosphere soil samples-

The pH of soil samples tested was moderately alkaline (7.68) in nature. Electrical Conductivity (Ec) was in normal range 0.65 ds/m. Review suggested that nutritionally deficient soils especially p-deficient harbours more AMF. Available P of soil samples is low as compared to the normal range(2.95Kg/ha). Potassium was found to be very high (390.78 Kg/ha). From the field of strawberry, the Organic Carbon was very low (0.19%). The major role in plant growth is also played by micronutrients in soil particularly as per previous research work the Cu and Zn uptake is enhanced by mycorrhizal association. In the present investigation, it was found to be 2.00ppm and 2.01ppm respectively (Table 1).

The % colonization and spore count

The whole root system and rhizosphere soils of all the plants of study area were collected and screened for their mycorrhizal status. The young feeder roots are primary sites for initiation and infection of AMF. Hence all those were collected and stored separately and assessed to know the percent root colonization. The observations are recorded in Table 2.

All the plant roots showed the typical inter and intracellular coenocytic mycelium, Hyphal coils, H-shaped connections, Arbuscules and Vesicles which are the characteristic features and thus confirmation of AM colonization.

The variation in number of spore propagules in each rhizospheric soil and site were observed. Density of AM fungal spores or the resting spores of AM fungi in the rhizosphere soils of both the host plants from all the sites were studied and the results are tabulated in Table-II. The total number of spores in per 100g of all samples of rhizosphere soils found in the range of 1210 to 1558. The highest spore density was observed in the rhizosphere soil of Kamaroza at S2.

Table 1: Correlation between AMF colonization, Spore count and edaphic factors

	pH	EC	Organic C	N	P	K	Cu	Zn	% Col	Spore
pH	1									
EC	-0.3546152	1								
Organic C	-0.8356752	0.01572	1							
N	-0.2060736	-0.2084	-0.00585	1						
P	-0.5292297	0.451344	0.605094	-0.02172	1					
K	-0.0099562	0.211047	0.299936	-0.3438	0.669718	1				
Cu	-0.3760053	-0.28757	0.512941	0.084242	-0.09246	0.222814	1			
Zn	0.30621716	-0.52368	-0.41361	0.209987	-0.89818	-0.89449	-0.3542	1		
%Col	0.74924162	-0.2575	-0.41961	-0.16519	0.075592	0.601924	-0.17556	-0.32363	1	
Spore	0.53562858	-.15937	-0.19505	-0.13956	0.40043	0.637173	-0.38182	-0.49638	0.902148	1

Table 2: AMF % colonization and spore count

Sr. No.	Variety	% colonization	Spore/ 100g
1.	Kamaroza		
	S1.	64%	1499
	S2.	58%	1558
	S3.	54%	1210
2.	Winterdawn		
	S1	56%	1308
	S2	60%	1498
	S3	58%	1411

Table 3: AM fungal species isolated from rhizosphere soil of strawberry

Sr. No.	Variety	AMF species
1.	Kamaroza	
	1.	*Glomus leptotichum*
	2.	*Glomus botryoides*
	3.	*Glomusfecundiosporum*
	4.	*Glomus fistulosum*
	5.	*Glomus australe*
	6.	*Glomus deserticola*
	7.	*Glomus dimorphicum*
2.	Winterdawn	
	1.	*Glomus albidum*
	2.	*Glomus tortuosum*
	3.	*Glomus reticulatum*
	4.	*Glomus etunicatum*
	5.	*Glomus fasciculatum*
	6.	*Glomus aggregatum*
	7.	*Glomus ambisporum*

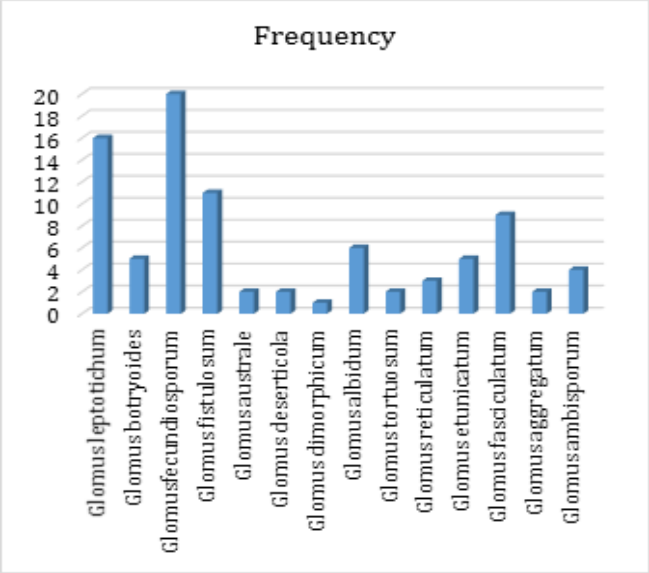

Fig. Frequency of AM fungal species isolated from rhizosphere soil of strawberry

Strawberry Field at Motha (Melghat)

Soil Trap Culture

Identification of AMF Spores

A total of fourteen AMF species of *Glomus* were isolated during the course of this investigation from the sites considered for studies from the field of Strawberry in two varieties. The data is recorded in the Table-III (Graph- I). Some of the interesting observations, which were made during this study, were of spore syndrome. Development of many small spores inside the dead spore of other species is called spore syndrome. Such spores were observed by the author in the present study. Most of the spore syndrome were of *Glomus* species. Formation of spore

syndrome could be a protective mechanism from the parasitic attack by soil microorganism or predatory larvae. The present study is the first report on inventory of AMF species associated with Strawberry from Melghat forest of Maharashtra for its soil trap culture. Similarly Norman et al. (1996) observed colonization rates of 55.4%-70.8% in strawberry plants when inoculated with *Glomus fasciculatum and Glomus etunicatum*.

Conflicts of interest: The authors stated that no conflicts of interest.

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