Isolation of AM Fungal Spores from Rhizospheric and Non-Rhizospheric Soil :Lablab Purpurens (L)

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Abstract

Isolation of AM fungal spores(**Zygomycetes**) was carried out from soil sample of rhizosphoric and non–rhizosphoric soil sample of **Lablab purpurens**(L) from four different localities of mahad taluka of Raigad District[**Shinde, B. P. and L. N. Nair 1995**].During the study 16 different species were isolated belongs to four genera.In non-rhizosphoric soil sample eight species were isolated belongs to three genera. During the study it was observed rhizosporic soil sample show more no of fungal spores where non-rhizospheric soil sample show less numbers.AM fungi useful to plants because it provides water and minerals which accelerates the nodulation as well as nitrogen fixing.

Introduction: The AM fungi have received very less attention from any of its members in the group Zygomycetes[**Abdul Mallik, M. 2000**]. The AM fungi were studied with respect to their morphotaxonomy, distribution, physicochemical properties of soil[**Abbott, L.K. and Robson, A. D 1991**], percentage root colonization, number of AM Fungipropagules and their species per locality and morphology of AM fungal spores, to study the effect AM fungi on the various growth parameters of Sweet bean plant [**Abdul Mallik, 2000**]during drought and salt stress, also the various changes in the biochemical contents of the leaves and seeds of Sweet bean plant during water and salt stress with respect to mycorrhiza. As Sweet bean is a leguminous plant and shows symbiotic association with Rhizobium, the study evaluated the response of Sweet bean (**Lablab purpureusL**) to arbuscularmycorrhizal fungi and Rhizobium regarding the growth, nodulation and yield.

KEYWORDS:AM fungi ,Rhizosphere, leguminous plant, Soil etc

Material and method:

The isolation of spores of AM fungi [**Hosamani, P. A. 2005**]was carried out by wet sieving and decanting method from 100 g rhizosphere and non rhizosphere soil. 100gram soil was suspended in 1 liter of tap water. The mixture was stirred well and the coarse particles were allowed to settle down 15-20 minutes. The sufernent was decanted through a series of sieves arranged in descending order of mesh size (400, 350, 210, 150 and 75 µm). The spores from each sieve were collected in a beaker containing tap water separately. The supernent from each beaker was then separately filtered through What man No.1 filter paper. The filter papers were placed in the Petri-plate, care being taken to ensure that they remain moist. The contents of the filter papers were examined for spores and sporocarps under Leica upright Trinocular Research microscope (Model DM750) with EC3 digital camera.

Picking and Mounting of spores AM fungi

The isolated spores both crushed and intact were mounted on a clean glass slide in polyvinyl alcohol lacto glycerol . Slides were prepared in such a way that the spores mounted in polyvinyl alcohol lacto glycerol (PVLG) were centrally located and properly labelled in the space towards the right side of the slide[**Kadlag, P. R. et.al 2013**]. After transfer of the spores, PVLG was allowed to set for 3-5minutes to become more viscous. Intact spores were crushed carefully by applying slight pressure on the cover slip using the blunt end of a needle. This allowed easy and accurate identification and characterization of the spore wall layers. For removing the unwanted air bubbles and oil drops, slides were placed in an oven at 60°C for 24 hours. All spores mounted in PVLG were used to study the gross spore morphology. After removing slides from the oven, the cover slip was sealed with colourless nail polish and labelled slides were stored in a flat slide box. Data pertaining to the name of the identified species, locality of occurrence were recorded on data sheet[**Shinde, S. K. et.al ,2014**]

Identification of AM fungi

All AMl fungal spores were mounted in PVLG and observed under trinocular research microscope. And photograph with digital camera. The morphological characters such as spore size, shape, colour, number of wall layers, ornamentation, mantle on the spores, visible spore contents, shape of the subtending hyphal attachment, germination shield, sporiferoussaccule etc. were observed.[**Khade, S. W. et.al, 2003**]. These characteristics were compared with the characters given in the manual for identification of VAM fungi.Estimation of AM fungal spore count were carried out by filter paper method.

Result :

AM fungi from rhizospheric soil of Sweet bean on 30th and 60th day from four localities.

S. N.	Name of genus and species	L1		L2		L3		L4	
		30	60	30	60	30	60	30	60
1.	AcaulosporadilatataMorton	+	+	-	+	+	+	+	+
2.	AcaulosporafoveataTrappe and Janos	–	+	+	+	+	+	-	+
3.	AcaulosporanicolsoniiWalker,Reed and Sanders	+	+	-	-	-	+	+	-
4.	AcaulosporarehmiiSieverdingandToro	+	-	+	+	+	+	+	-
5.	AcaulosporaspinosaWalker andTrappe	+	-	+	+	+	-	-	-
6.	AcaulosporaundulataSieverding	+	+	+	+	-	+	-	+

7.	GigasporaroseaNicolson and Schenck	+	+	-	-	+	+	-	+
8	Glomus. botryoidesRothwell and Victor	+	+	-	-	+	-	+	+
9.	G. fasciculatum(Thaxter) Gerdemann and Trappe emend. Walker and Koske	+	-	+	+	+	+	+	+
10.	G. fistulosumSkou and Jakobsen	+	+	+	+	-	+	-	-
11.	G. macrocarpumTulasne and Tulasne	+	+	-	+	-	+	+	-
12.	G. maculosumMiller and Walker	-	+	+	-	-	+	-	+
13.	G. reticulatumBhattacharjee and Mukerji	+	+	-	+	-	-	-	+
14.	ScutellosporaarenicolaKoske and Halvorson	+	+	-	+	-	+	+	+
15.	S. biornataSpain,SieverdingandToro	+	-	+	-	+	+	+	-
16.	S. minuta(Ferrer and Herrera)Walker and Sanders	+	+	-	-	+	-	-	-
	Total number of species	26		18		21		17	

AM fungi from non- rhizospheric soil of sweet bean on 30th & 60th day from four localities.

S. N.	Name of Genus and species	L- 1		L- 2		L- 3		L-4	
		30	60	30	60	30	60	30	60
1.	AcaulosporadilatataMorton	+	+	-	-	+	+	-	-
2.	AcaulosporaspinosaWalker andTrappe	+	+	-	-	+	+	+	+
3.	A. undulataSieverding	_	+	+	+	+	+	-	-
4.	GlomusfistulosumSkou and Jakobsen	+	+	+	+	-	-		-
5.	G. maculosumMiller and Walker	+	+	-	-	+	+		-
6.	G. reticulatumBhattacharjee and Mukerji	+	-	+	+	-	+	+	+
7.	ScutellosporaarenicolaKoske and Halvorson	-	+	-	-	+	+	-	+
8.	S. biornataSpain,Sieverding and Toro	+	+	-	+	-	+	-	-
	Total number of species	13		7		12		05	

Abb: + Present, - Absent, L-1 Chochinde, L-2 Dasgaon , L-3 Kondivate and L-4 Kol

Discussion:

The spore count was taken on 30th and 60th day after the germination of Sweet bean seeds. It provides minerals which accelerates the nodulation as well as nitrogen fixing.Sixteen species of four genera of AM fungi were isolated from the rhizosphere soil of sweet bean[**Rhatwal, S. M. et.al 2011**] plant from four selected localities. It incudes genus**Acaulospora**with six species**[Bagyaraj, D. J. et.al 1979]Acaulosporadilatata**Morton ,**Acaulosporafoveata**Trappe and Janos ,**Acaulosporanicolsonii**Walker,Reed and Sanders ,**Acaulosporarehmii**SieverdingandToro,**Acaulosporaspinosa**Walker andTrappe**Acaulosporaundulata**Sieverding,The genus Acaulospora was recorded with 6 species. The genus **Glomus** also recorded with 6 species[**Berch, S. M. et.al 1983**]i,e.,**Glomus. botryoides**Rothwell and Victor, **G. fasciculatum**(Thaxter) Gerdemann and Trappe emend. Walker and Kosk, **G. fistulosum**Skou and Jakobsen, **G. macrocarpum**Tulasne and Tulasne,**G. maculosum**Miller and Walker,**G. reticulatum**Bhattacharjee and Mukerji; One species belonged to**Gigasporarosea**Nicolson and Schenck. The genus **Scutellospora** showed they are**Scutellosporaarenicola**Koske and Halvorson ,**S. biornata**Spain,SieverdingandToro,**S. minuta**(Ferrer and Herrera)Walker and Sanders. Maximum number of AM fungi spores was found at L1(Chochinde locality) was 26 followed by L3 (Kondivate) 21,L2 (Dasgaon) 18 and L4 (Kol) 17

The non-rhizosphere soil of Sweet bean plant showed eight species belonging to three genera. Out of them, three species belonged to **Acaulospora** was**Acaulosporadilatata**Morton,**Acaulosporaspinosa**Walker andTrappe, **A. undulata**Sieverdingand genus **Glomus**was observed with three species**[Berch, S. M. et.al 1986]**each **Glomusfistulosum**Skou and Jakobsen,**G. maculosum**Miller and Walker,**G. reticulatum**Bhattacharjee and Mukerjiand two belonged to **Scutellospora**I,e.**Scutellosporaarenicola**Koske and Halvorson,**S. biornata**Spain,Sieverding and Toro,. Locality-1 (Chochinde) showed 13 species, followed by L3(kondivate) 12 species,L3 (Dasgaon)07 species and L4(Kol) only 05 species.

Ocurrance of these Zygomycetes fungi are terrestrial.They live close to the plants,usually to soil and on decaying to the plant material [**Benjamin, R. K. 1979**].Because they decompose soil,plant matter it is utilised as organic fertilizer by plants for the normal grwth and development and ultimately the production of crop increased .These fungi play major in carbon cycle.

Conclusion:AM fungi which are present in rhizosphoric region are useful to plants because it provides water and minerals which accelerates the nodulation as well as nitrogen fixing leads to better growth and productivity.It also helps to increase the resistance to root infecting pathogen.

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