International Journal of Agricultural Science and Research (IJASR)
ISSN 2250-0057
Vol. 3, Issue 1, Mar 2013, 151-156
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ISOLATION AND CHARACTERIZATION OF VESICULAR ARBUSCULAR MYCORRHIZA FROM BARLEY FIELDS OF JAIPUR DISTRICT

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ABSTRACT

An intensive survey of barley fields in Jaipur district was done to investigate distribution, population and occurrence of VAM fungi in association with *Hordeum vulgare*. Different species of VAM were isolated from soil collected. The percentage occurrence of different VAM species varies to a different extent. The two main genera were identified viz. *Glomus*, *Gigaspora* and *Scutellospora.* Data collected from the farmer revealed that the barley fields showing association with VA mycorrhizal fungi had better growth comparatively to fields lacking VA mycorrhizal infection.

KEYWORDS: *Gigaspora, Glomus*, *Hordeum Vulgare, Scutellospora,* VAM Fungi

INTRODUCTION

Mycorrhizal fungi differ from other plant–fungus associations because of their ability to create an interface for nutrient exchange which occurs within living cells of the plant (Brundrett, 2004 and Brundrett, 2002). Mycorrhizal fungi interact with plants at different levels and can be grouped into obligately mycorrhizal, facultatively mycorrhizal and non-mycorrhizal plants (Brundrett, 2004).

Along with accessing soil nutrients, the hyphae of VAM fungi allows greater access to water through mechanisms such as stomatal regulations, increased root hydraulic conductivity, osmotic adjustments and maintenance of cellular water pressure and cell wall elasticity changes (Augé, 2000).

Other recent studies have shown the capability of AM fungi to influence plant growth, crop quality and adaptability to stress conditions (Mena- Violante *et al*., 2006; Fagbola *et al*., 2001). VAM can be used to determine AM hyphal growth and activity in the soil (Lovelock *et al*., 2004 and Wright and Anderson, 2000).

VAM fungi alleviate plant stunting caused by toxic metals by binding to these metals in the root zone with the aid of the extraradical mycelium and altering the plant cells ability to capture the metals. The polyphosphates produced by AM fungi are proposed to be the reason behind this sequestration though this has not been confirmed (Smith and Smith, 1997; Turnau *et al*., 1993). The potential use of AM fungi in detoxification of environments polluted with heavy metals and in phytoremediation (Khan, 2006).

Barley (*Hordeum vulgare* L.), a member of the grass family, is a major cereal grain of India. Important uses include use as a cereal crop, animal fodder, as a source of fermentable material for beer and certain distilled beverages. In a 2007 ranking of cereal crops in the world, barley was fourth both in terms of quantity produced (136 million tons) and in area of cultivation (566,000 km²). Industrial demand of barley has been increased rapidly in last few years.

A number of studies have been done on other cereal crops in different areas which initiated the present study of VAM in barley fields at Jaipur district. Rajasthan is the leading state in quantity production and area cultivated for barley.

MATERIALS AND METHODS

So the Objectives of the present Study include the Survey and Collection of soil samples from the local barley fields and Isolation and Characterization of VAM fungi from the soil. Barley is the major cereal crop of Rajasthan, as rabi crop .Soil samples were collected from different barley fields of Jaipur district of Rajasthan (India) in the months of July and august' 2012. Soil sample were collected from 22 different location of jaipur district. In most of the localities only barley was cultivated. Soil sample were collected from the rhizosphere of *Hordeum vulgare* plants which showed high vigour and also from plants which showed less vigour. For each field surveyed, a composite soil sample comprising of five to ten subsample were collected. Soil from all the subsample was mixed thoroughly with hands and about 500g soil was filled in bags, tagged with relevant information and tied. To characterize the VAM spores, spore size, spore colour, hyphal attatchment, presence/absence of cell wall, suspensor size, etc. was taken into consideration (Schenck and Perez, 1990). Different species of VAM were isolated from fields by using wet sieving and decanting technique followed by Geredemann and Nicolson (1963), which was modified accordingly in our laboratory depending on the availability of same, and is as follows

- A portion of soil (50g) was mixed by hand in water (200ml) in large beaker until all soil aggregates have dispersed to leave a uniform suspension.
- Beakers of soil samples were placed under white light (table lamp was used as a source of white light) and floating spores were observed under white light.
- Visible spores were isolated with the help of paint brush of size zero, in petriplates containing water.
- These spores were observed under microscope.
- Then spores were placed on slides in lactoglycerol and covered with coverslip in order to take photographs of spores.

OBSERVATION AND RESULTS

After intensive survey of barley fields in Jaipur district, Data collected from the farmer revealed that the barley fields containing VA mycorrhizal fungi had better growth comparatively to fields lacking VA mycorrhizal infection. Interaction with the farmers also revealed that they are not aware of benefits of VAM infection.

The genera are differentiated by their various spore and other characteristics and relationship of the spore to the associated hyphal attachments. The two main genera were identified viz. Glomus, Gigaspora and *Scutellospora* with following species: *Glomus fasciculatum* (Gerdmann and Trappe 1974; Walker and Koske. 1987), *Gigaspora margarita* (Bentivenga and Morton, 1995) (figure.5 and figure.6), *Gigaspora decipiens* (figure.7 and figure.8) and *Scutellospora gregaria* (Bentivenga, and Morton, 1995) (figure.9 and figure.10).

Table 1: Following Genera of VAM Fungi Were Identified in Different Areas of Jaipur District Surveyed

S.No.	Locality Surveyed	VAM Species Isolated
1	Rambas	*Glomus fasciculatum, Gigaspora margarita*
2	Dsarnachaur	*Glomus fasciculatum ,Glomus mosseae*
3	Jodhpura	*Gigaspora margarita, Glomus fasciculatum*
4	Kalyanpura	*Glomus mossseae, Gigaspora margarita, Glomus fasciculatum*
5	Ramsingpura	*Glomus fasciculatum, Gigaspora margarita*
6	Sardarpura	*Scutelospora gregaria, Gigaspora decipiens, Gigaspora margita*
7	Boyta	*Glomus mossseae, Glomus fasciculatum, Gigaspora margarita*
8	Bhankari	*Glomus mosseae, Gigaspora margarita, Glomus fasciculatum*

Table 1: Condt.,

S.No.	Locality Surveyed	VAM Species Isolated
9	Paota	*Scutelospora gregaria, Glomus fasciculatum*
10	Pragpura	*Glomus fasciculatum*
11	Rampura	*Glomus mossseae , Gigaspora decipiens*
12	Nivai	*Scutelospora gregaria, Gigaspora margarita*
13	Haripura	*Glomus fasciculatum ,Glomus mosseae*
14	Hasampura	*Glomus fasciculatum ,Glomus mosseae*
15	Tankarda	*Glomus fasciculatum, Gigaspora margarita*
16	Sanganer	*Scutelospora gregaria, Glomus fasciculatum*
17	Durgapura	*Glomus mosseae,Gigaspora decipiens, Glomus fasciculatum, Scutelospora gregaria*
18	Phuchalavali	*Gigaspora margita, Gigaspora decipiens, Glomus fasciculatum*
19	Kurbda	*Gigaspora margarita, Glomus fasciculatum*
20	Kaladera	*Gigaspora decipiens, Glomus fasciculatum, Gigaspora margarita*
21	Bhojraj ki kothi	*Glomus fasciculatum, Gigaspora margarita*
22	Krishnapura	*Scutelospora gregaria, Gigaspora margarita*

Glomus was abundantly observed in Rambas, Dsarnachaur, Jodhpura, Kalyanpura, Ramsinghpura, Boyta, Bhankari, Paota, Pragpura, Haripura, Hasampura, Tankarda, Sanganer, Durgapura, Phuchalavali, Kurbda, Kaladera, Bhojraj ki kothi, Krishnapura, Rampura.*Gigaspora* presence was also available in Rambas, Jodhpura, Kaylanpura, Ramsinghpura, Boyta, Bhankari, Tankarda, Durgapura, Phuchalavali, Kurbda, Kaladera, Bhojraj ki kothi, Krishnapura, as well as Sardarpura, Nivai. *Scutellospora* presnce was observed in soil of Krishnapura, Durgapura, Sanganer, Nivai, Paota, Sardarpura.

Table 2: Characteristics of Identified Species of *Glomus*

S.No.	VAM Species	Suspensor Size (µm)	Spore Size (µm)	Auxillary Cells	Cell Wall	Composite Spore Wall Width or Range (µm)	Cell Wall Group	Spore Colour H Y	Y Br	Br B
1.	(S) *Glomus mosseae*	16-32	105-310	Absent	Present	2-7	1	-	-	#
2.	(S,R)*Glomus fasciculatum*	8-10	50-149	Absent	Present	7-12	1	-	#	-

H→Y = Hyaline white to light yellow in colour, Y→Br= Yellow to brown in colour, Br →B = Dark brown to black in colour, #= Usual colour , - = Usually not found, S=Sporocarpic roots, R= in roots

Table 3: Characteristics of Identified Species of *Gigaspora*

S.No.	VAM Species	Suspensor Size (µm)	Spore Size (µm)	Auxillary Cells	Cell Wall	Composite Spore Wall Width or Range (µm)	Cell Wall Group	Spore Colour H Y	Y G	YBr Br
1.	*Gigaspora Margarita*	27-58	260-480	Present	Present	5-24	1	#	-	-
2.	*Gigaspora decipiens*	<65	320-490	Present	Present	20-47	1	-	-	#

H→Y = Hyaline white to light yellow in colour, Y→G= Yellow to golden in colour, YBr→Br = yellow brown to brown, #= Usual colour, - = usually not found.

Table 4: Characteristics of Identified Species of *Scutellospora*

S.No	VAM Species	Suspensor Size (µm)	Spore Size (µm)	Auxillary Cells	Cell wall	Composite Spore Wall Width or Range (µm)	Cell Wall Group	Spore Colour H w	Y G	YBr Br
1.	*Scutellospo--ra gregaria*	39-80	250-480	Present	Present	10-23	2	-	#	-

H→W=hyaline to white, Y→G = yellow to gold, YBr→B r = yellow brown to brown to red brown,

#= Usual colour, - = usually not found.

Figures: For different types of VAM fungi spores isolated and charachterized from soil sample.

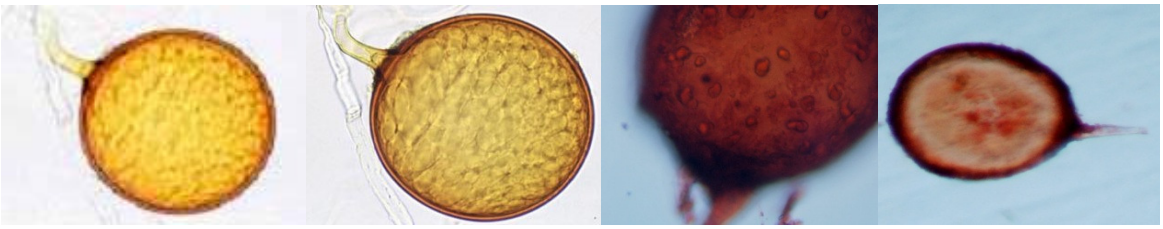

Figure 1: Spores of *Glomus fasciculatum* Figure 1: Spore of *Glomus mosseae* Figure 2: Spore of *Glomus mosseae* Figure 4:Spore of *Glomus mosseae*

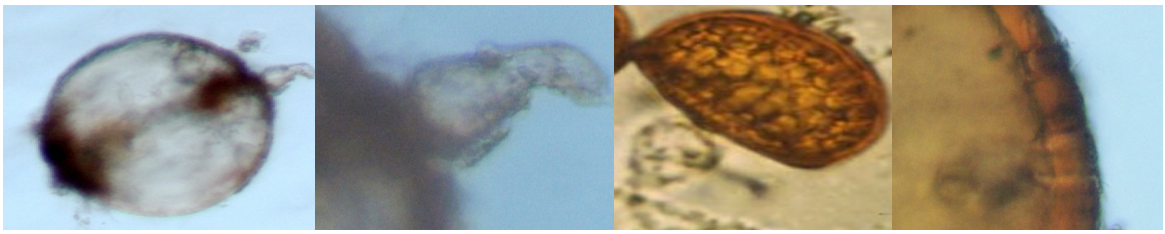

Figure 5: Spore of *Gigaspora margarita* Figure 6: Sporeof *Gigaspora margarita* Figure 7: Spore of *Gigaspora decipiens* Figure 8: Spore wall of *Gigaspora decipien*

DISCUSSIONS

From the present study of barley fields of different areas of Jaipur district it is concluded that the barley fields containing Vesicular Arbuscular Mycorrhiza has better growth comparatively to fields lacking Vesicular Arbuscular Mycorrhizal infection. They are reported by various workers from time to time in diverse land areas such as calcareous grasslands, arid/semi arid grasslands, several temperate forests, tropical rain forests and shrub lands in diverse parts of the world (Oehl *et al*., 2003). Root colonization by *Glomus* could be an adequate strategy to alleviate the deleterious effects of drought stress and retard the senescence syndrome in wheat (Jose and Marta, 2008). In comparison to non-mycorrhizal plants, mycorrhizal plants had higher phosphorus use efficiency at low Pi concentrations (Jianchang N. and Jonnathan R.C., 2001). *Glomus* play an important role in the overall nutrient cycling of ecosystems. *Glomus* may inhabitat the roots of 70-90% of vascular plants. It is important ecologically by providing nutrients to it host. Phosphorous, nitrogen and water may be provided to host plants through the intracellular interface (Schubler 2001). They are reported to be found in diverse land areas such as calcareous grasslands, arid/semi arid grasslands, several temperate forests, tropical rain forests and shrub lands in diverse parts of the world (Oehl *et al*., 2003). Recently, AM fungi have received more attention especially in African countries such as Namibia, Cameroon, Kenya, Morocco, Nigeria, Senegal, Zambia and South Africa. These studies

have concentrated on AM fungal diversity in various regions and soil types or the mycorrhizal status of indigenous crop and plant species (Bouamri *et al*., 2006 and B*â et al*., 2000). Results from these studies reveal that different species of AM fungi are obtained depending on plant species and geographic location. Amongst AM fungal species, *Glomus* sp. were consistently isolated while others species belonging to the genera *Acaulospora*, *Gigaspora* and *Scutellospora* were either absent or found in few numbers (Bouamri *et al*., 2006).

Vesicular Arbuscular Mycorrhiza has symbiotic association with crops. Vesicular Arbuscular Mycorrhiza helps in capture of nutrients mainly Phosphorus from soils of all types, provides greater access to water, protects crops from pathogens, helps in soil aggregation, helps in reclamation, increase crop resistance towards metal toxicity. It was observed that out of 22 areas surveyed, VAM was present in all 22 areas. The *Glomus mosseae* was present in 36%, *Glomus fasciculatum* was in 85%, *Gigaspora decipiens* was in 22.6%, *Gigaspora margarita* was in 55%, *Scutelospora gregaria* was in 25% of the fields surveyed. Due to the potential of mycorrhizal fungi to enhance nutrient uptake, this benefit has however brought about the suggested use of AM inoculum instead of some chemical fertilisers for plant productivity, growth and restoration of polluted soils or in revegetation. (Cardoso and Kuyper, 2006; Khan, 2006; Quilambo, 2003).

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