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ESTIMATION OF VESICULAR *Arbuscular Mycorrhizal* (VAM) FUNGI STATUS FROM *Rhizospheric Soil* OF *Zea mays* IN SOME REGIONS OF PATNA DISTRICT (BIHAR)

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Key words : VAM, Rhizospheric Soil of *Zea mays*, Patna

Mycorrhiza is the keystone among microorganisms that form a critical linkage between the plant root and soil. This symbiotic association is characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant. The present study aims at isolation and identification of some mycorrhizal fungal spores from maize plant in some regions of Patna district of Bihar during 2009-2011. Random rhizospheric soil samples were collected from Patna, Mithapur ,Danapur, Maner and Didarganj regions of Patna district. Wet sieving and decanting method and sucrose centrifugation technique were employed for the isolation and mycorrhizal spores. Spores concentrations of different size, shape, colour and hyphal attachment were examined under compound microscope. The spore density seemed to be dominated mainly by species of *Glomus*. However, *Gigaspor*a, *Acaulospora* and *Scutellospora* too were identified at a comparatively lesser percentage.

INTRODUCTION

The term mycorrhiza was coined by A.B. Frank, a scientist in Germany, more than 100 years ago (Habte, 2000). It literally means Fungus- root and describes the mutualistic association existing between a group of soil fungi and higher plant (Habte, 2000). AM fungi are considered to be obligate biotrophs as they are unable to grow and reproduce in regions of the soil where the host plant roots are absent (Meyer, 2007). Mycorrhiza refers to an association or symbiosis between plants and fungi that colonize the cortical tissue of roots during periods of active growth (Sylvia et al., 1998). Depending on the individual AM fungi and conditions, many plant species show large positive growth responses to AM colonization. These responses are usually due to more efficient acquisition of soil nutrients, especially P, from the relatively extensive mycorrhizosphere compared with the rhizosphere of non - mycorrhizal (NM) control plants (Smith et al., 1979; Facelli et al., 1999). However, some plant species show little or no growth increase; other - including wheat - can show growth depression at least during vegetative stage when colonized (Graham & Abbott, 2000, Zhu et al., 2001a). In nature, more than eighty percent of angiosperms and almost all gymnosperms are known to have mycorrhizal associations.Arbuscular-mycorrhizal fungi are obligate biotrophic symbionts with a life cycle divided into two distinct stage. On the one hand, the resting and reproductive stage, (spores, sporocarps and possibly also vesicles) are independent of the plant. On the other hand, vegetative stages are involved in complex interaction, colonization and nutrient exchange. The stages are represented by development of external hyphae in soil and hyphae and soil, arbuscles and vesicles within the root. Mycorrhiza represents one of the nature's best gift to mankind in addressing the constraints of enhanced quality productivity with sustainability.

MATERIAL AND METHOD

Field survey was conducted during the year 2008, in the different regions of Patna district to determine the soil sample collection sites and status of vesicular arbuscular mycorrhizal (VAM) fungal spores' concentration in the soil of those regions. Patna, Mithapur ,Danapur, Maner and Didarganj regions of Patna district were selected for soil sample collection sites. Random collection of rhizosphere soil samples of Maize from Patna, Mithapur ,Danapur, Maner and Didarganj regions of Patna district was done during 2009-2011. Plant roots and rhizosphere soil samples were collected from various selected locations of different regions of Patna district. For isolation of Arbuscular mycorrhizal (AM) fungal spores wet sieving and decanting (Gerdemann and Nicolson, 1963) and sucrose centrifugation technique (Daniel and Skipper, 1982) were carried out.

WET SEIVING AND DECANTING TECHNIQUE

The wet sieving and decanting method are one of the easiest techniques when compare to other techniques. This technique is used for sieving the coarse particles of the soil and retaining AMF spores and organic particles on the sieves of different sizes.

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100 gm of soil was mixed with 1000 ml of water. The soil mixture was agitated vigorously to free the AMF spores from soil and allowed to settle for 15-30 minutes and the supernatant was decanted through 500 µm, 250 µm, 100 µm and 38 µm sieves. By using a dissecting microscope, spores were picked by mean of pipette or needle.

Sucrose centrifugation Technique

Spores were purified by re-suspending the sieve in the 40% sucrose solution and centrifugation was carried out. Centrifugation is carried out at 1750 rpm for 5 minutes. The supernatant was removed and poured into the sieves. The spores that hold on the sieves are carefully rinsed with tap water. The spores were collected by using dissecting microscope.

COLLECTION AND STORAGE OF ROOT SAMPLES

- Roots were taken from the regions between 50 cm and 100 cm of root material for each plant species.
- Care was taken to collect as many of the fine lateral roots as possible along with the main system.
- Roots were not collected if they were enlarged with the roots of other species in order to avoid incorrect assessment. Root samples were placed into labelled vials containing distilled water in order to wash the sand from them. They were generally processed the following day. However, if processing was to be delayed, they were transferred to vials containing 50% ethanol. Ethanol was chosen as the fixative over FAA (Formalin Acetic Acid Alcohol) due to the caustic nature of the latter.

Root Staining Technique

Root staining procedure target arbuscules, vesicles and hyphal structures within the root cortex. Staining of target structures is performed for rapid, simple and cost-effective assessment of fungal symbiosis. Using light microscopy, the required skill sets are lower and the procedure can be performed with case. Lactophenol cotton blue (C37 H27 N3 Na2 O9 S3) is one of the several stains that has been widely utilized for many years (Abdel Fattah, 2001). However, a move to the use of trypan blue (C34H28N6 O14S4), originally developed by Phillips and Hayman (1970) has improved the clarity of characteristic AM fungal components.

CHARACTERIZATION OF MYCORRHIZAL FUNGAL SPORES

Extracted spores were mounted using Polyvinyl Alcohol Lactic Acid Glycerol (PVLG) and then morphologically characterized with the help of Manuals (Schneck and Prez, 1990) under Compound Microscope (40 X - 100 X). Major and minor details regarding Shape, Color, Hyphal attachment were used, for identification upto generic level. The characteristics used for identification include size, shape, colour and hyphal attachment of spores.

PREPARATION OF POLYVINYL ALCOHOL LACTIC ACID GLYCEROL (PVLG)

PROCEDURE

Polyvinyl Alcohol Lactic Acid Glycerol (PVLG) is used to prepare permanent slides with unbroken and crushed spores, as well as with fragments of Mycorrhizal roots. Its viscosity enables to manipulate the position of the specimen examined and hence accurately determine their properties.

ESTIMATION OF VAM ROOT COLONIZATION

VAM root colonization of host plant was studied after processing the roots according to Kaske and Gemma (1989). The total percentage of root colonization was determined by using the formula

$$\text{Root Colonization} = \frac{\text{No. of root segment colonized}}{\text{Total No. of root segments observed}} \times 100$$

RESULTS

Periodical survey of various places such as Patna, Mithapur, Danapur, Maner and Didarganj region of Patna district was undertaken to collect and identify different AM fungi genera and species association with maize Plant. Rhizosphere soil sample collected from various localities revealed presence of several species of different genera on the basis of resemblances the AM fungi as *Glomus spp.*, *Acaulospora spp.* shown in plate.

Figures-I (a, b and c) showing survey of maize field.

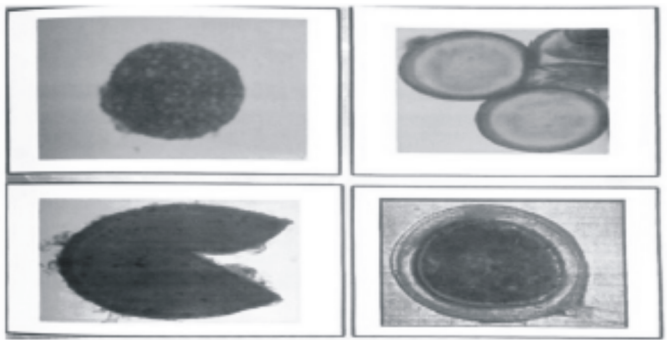

Figure-2 Showing different types of Acautospora spp spore collected from rhizospheric soil samples

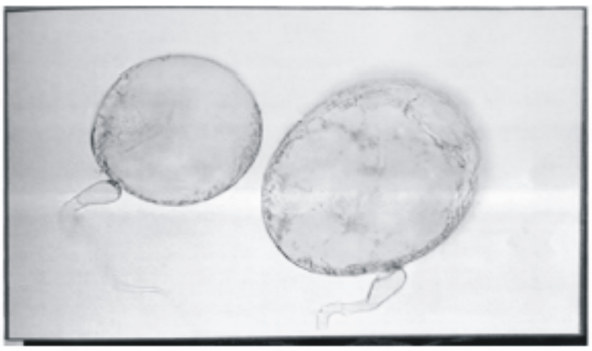

Figure-3 Showing spore of *Sculellospora* spp.

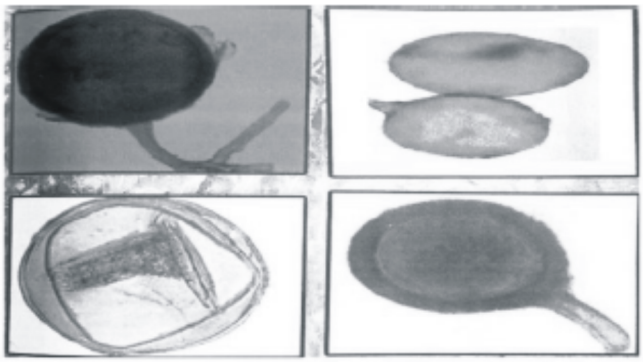

Figure-4 Showing different types of *Glomus* spp from collected soil samples

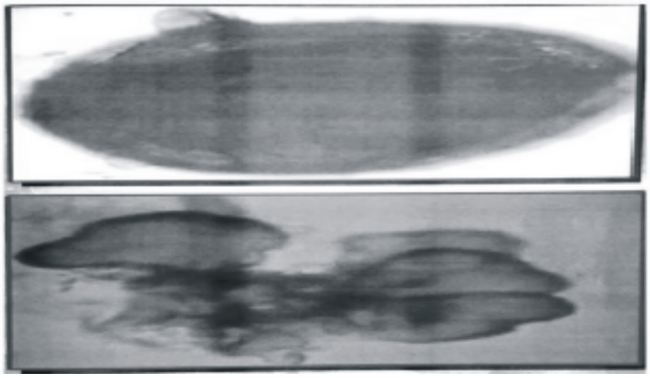

Figure-5 Showing different types of Sigaspora spp. spore collected from rhizospheric soil samples

The number of AM fungal spores isolated from different sites of Maize crop was given in Table-I and Figs. 1-5. The number of AM fungal spores ranged from 80 - 140 per 100 gm of soil. This study describes the distribution of AM fungi in the rhizosphere soil of Maize plant. Both plant and rhizosphere soils were collected during three-year period (2009 - 2011) at different site and during different seasons.

TABLE-1 AVERAGE NUMBER OF SPORES AREA WISE AND YEAR WISE

YEAR	PATNA	MITHAPUR	DANAPUR	MANER	DIDARGANJ	AVERAGE SPORE YEAR WISE
2009	110	110	118	105	108	110.2
2010	122	90	141	120	98	114.2
2011	116	112	134	112	110	118
AVERAGE SPORES AREA WISE	116	106	131	112.33	105.3	

Root Infection Assessment:

In order to find out the potential of AM fungi infection in roots of Maize, the root samples collected were cleaned, chopped into small pieces and then subjected to fixation, Cleaning, Rinsing and Bleaching in KOH solution following standard techniques were used for Microscopic observations. In case of infected roots, the presence of AM fungi infection was observed on the basis of root cuts (1 cm size), infected and uninfected, the degree of root infectivity was worked out in term of percentage (Table-2).

TABLE-2. R.C. VALUE OF MAIZE

YEAR	MAIZE PLANT R.C. VALUE
2009	20
2010	24
2011	30

DISCUSSION

In the present study *Glomus* was the most common genera and dominant in shifting system. The finding corroborates with the finding of Morton (1988) that the genus *Glomus* is predominantly distributed genus in the soil all over the world. *Glomus* were common and made up for more than 75% of total isolates followed by *Acaulospora* and *Gigaspora*. Dominancy of *Glomus* in the present study is in the agreement with the finding of (Panawar and Tarafdar, 2006; Pande and Tarafdar, 2004; Burni and Illahi 2004; Mirtha and Dhar, 2007; Sharma et al, 2009; Burni et al., 2009).In Indian context our findings corroborate with the finding of Rani and Manoharachary (1994) that the most frequently identified VAM fungi were *Glomus* spp. (7 species).Singh and Adholeya (2002) also observed that the genus *Glomus* was ubiquitous. The predominance of *Glomus* species under varying soil conditions might be due to the fact that they were widely adaptable to the varied soil conditions and survive in acidic as well as in alkaline soils (Pande and Tarafdar, 2004). In the present study the maximum spores were observed from undisturbed natural vegetation of Danapur of Patna district. The potential reason for maximum number of spore's availability in undisturbed natural vegetation is that spores keep multiplying in association with plant whereas, in cultivated habitat the top soil is disturbed each time as some fresh crop was sown. Previously several researchers like Gaur and Kaushik (2011), also reported that quantitative spore population differed in cultivated and uncultivated soil. Mycorrhizas are an essential below-ground component in the establishment and sustainability of plant communities, but thorough knowledge is required to achieve maximum benefits from these microorganisms and their associations.

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