Comparison of suitable hosts of ArbascularMycorrhizal (AM) Fungi between Maize (*Zea mays L.)* and Wheat(Triticum aestivum L.*)*

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ABSTRACT

Mycorrhiza is symbiotic mutualism relation between special soil and fungi and fine plant root. They form a fundamental link between biotic and abiotic components of the soil system. In the present investigation during 2008 field survey was conducted to determine sample collection sites and the status of AM fungal spores concentration in the soil of those regions. Collection of rhizosphere soil samples of Maize and wheat from Naubatpur, Mokama, Bidupur, Diyara and Jadua regions of Patna and Vaishali district was done during 2009 to 2011. Rhizospheric soil samples were collected at various interval of time a time . This study was designed to AM fungi in study localities by isolation, identification, enumeration of spores found in field rhizosphere soil samples.Wet sieving and decanting method were employed for the isolation of mycorrhizal spores. Collected rhizospheric soil was suspended in water and passed through sieves of different sizes of 500 µm, 250 µm, 100 µm and 38 µm. Spores concentration of different size, shape, colour and hyphal attachment were examined under Stereo microscope. The spore density seemed to be dominated mainly by species of *Glomus*. However, *Gigaspora, Acaulospora, Scutellospora* too were identified at comparatively lesser percentage.Two host plant species viz. maize (*Zea*mays*)*and wheat plant(*Triticum aestivum*) was tried for selection of suitable host of AM fungi.

INTRODUTION

Research in the field of arbascularMycorrhizal symbiosis has been a giant leap in the past three decades as demonstrated by the vast amount of literature being published every year. The success of Mycorrhizal evolution has been attributed to the role of Mycorrhizal fungi play in capture of nutrients from the soil of all ecosystem (Bonfante and Perotto, 2000). Literally "mycorrhiza" means fungus root and is derived from the Greek word "Mykes" meaning Fungus and "Rhiza" meaning root (Friberg, 2001). This term was first used by Frank, a German plant pathologist in 1885 to describe the symbiotic relationship between plant, roots and fungi. The symbiosis is characterized by the exchange

of nutrients where carbon in the form of hexose sugar flows to the fungus and inorganic nutrients are passed to the plaint, thereby providing a linkage between the plant root and the soil (Sylvia etat., 1998). Mycorrhizal fungi differ from other plant-fungus associations because of their ability to create and interface of nutrient exchange, which occurs within living cells of the plant (Brundrett, 2004, Brundrett, 2002). Mycorrhizal colonization changes the metabolism of the roots and modifies the amount and composition on compounds released by the roots into the soil. The few results available on the effect of Mycorrhizal colonization on the root exudates show that the exudation patterns vary depending on plant species, mycorrhizal fungus, plant age and nutritional condition (Laheurte etat., 1990).

Arbascular-Mycorrhizal fungi are obligate biotropic symbionts with a life cycle divided into two distinct stage. On the one hand, the restiny 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 hyphociin soil and hyphoc 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 METHODS: -

In the present investigation during 2008 field survey was conducted to determine sample collection sites and the status of AM fungal spores concentration in the soil of those regions. Collection of rhizosphere soil samples of Maize and wheat from Naubatpur, Mokama, Bidupur, Diyara and Jadua regions of Patna and Vaishali district was done during 2009 to 2011. Rhizospheric soil samples were collected at various interval of time a time described in methodology. This study was designed to AM fungi in study localities by isolation, identification, enumeration of spores found in field rhizosphere soil samples.

SOIL SAMPLING BEFORE CULTIVATION: -

Before sowing of maize, soil samples were randomly collected from the respected crop field of each region of Patna andVaishali district. From each site of the collection, Soil was collected by a small showed from an area of 15 cm diameter of 10 cm depth after the elimination of organic debris and humus particles. For each sample 200 gm soil was collected in transparent polythene bags of 30 cm × 20 cm size and brought into the laboratory and stored at temperature of 4˚ C, for the estimation of mycorrhizal infection rates. Two host plant species viz. maize (*Zea*mays)and wheat plant(*Triticum aestivum*) was tried for selection of suitable host of AM fungi. Both species were selected on the basis of their suitability to the agro climate conditions of the areas ,having thick root system for sizeable sporulation and annual habit.

SOIL SAMPLING ON CULTIVATION: -

When above mentioned cereals plants reached at the flowering stage, soil samples were collected from rhizospheric regions of the plants. The collection of rhizosphere soil samples, a whole root system was dug out carefully so as not to lose the terminal and lateral fine feeder roots. A whole root system was shaken off gently and the compact excess mass of soil from the excavated root system was discarded and soil adhering to the root system was retained. 200 gm soil was collected from the soil mass still adhering to a root system in transparent polyethylene bags.

Wet sieving and decanting method is the commonly used method for spore isolation (Gerdemann and Nicolson, 1963; Walker et. al., 1982). Wet sieving and decanting is a simple method which used sieve of various size to separate spores and other similar size particles from sand and clay (Daniels and Skipper, 1982). The various steps involved in wet sieving and decanting method were as follows: -

1. First 10 g soil sample was taken and dissolved in 100 ml distilled water in conical flask.
2. Then conical flask was shaken for 30 min.
3. After that the conical flask was kept in undistributed condition for 30 min.
4. The heavier particles were allowed to settle down.

5. Suspension was decanted through a 500 μm sieve to remove organic matter and roots.
6. This suspension was decanted through 250 μm, 100μm and 38 μm sieve consequently.
7. The entire residue was collected on 38μm sieve.
8. After settlement residue was dissolved in distilled water and filtered through filter paper.
9. This paper was spread in Petridis and a residue present in filter paper was taken and mounted on a slide and was examined.

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, for identification unto generic level. The characteristics used for identification include Spore Color, Shape, Size, Wall structure, Ornamental hyphal attachment and Occultation.

PREPARATION OF POLYVINYL ALCOHOL LACTIC ACID GLYCEROL (PVLG): -

Poly vinyl alcohol lactic acid glycerol used in study of vesicular – arbuscular mycorrhizal fungi consists of the ingredients listed below: -

INGREDIENT	**QUANTITY**
Distilled Water	100 ml
Lactic Acid	100 ml
Glycerol	10 ml
Polyvinyl Alcohol	16.6 g

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.

ROOT STAINING TECHNIQUE: -

Root colonization was observed by rapid clearing and staining technique (Philip & Hayman, 1970). For AM colonization assessment root samples were cleared with 10% KOH, acidified with 1N Hcl and stained with Lacto Glycerol Trypan Blue (.05%).

The stained roots were mounted on Microscopic slides and the segment were examined by light Microscope (40 X – 100 X).

ESTIMATION OF VAM ROOT COLONIZATION

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

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

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.

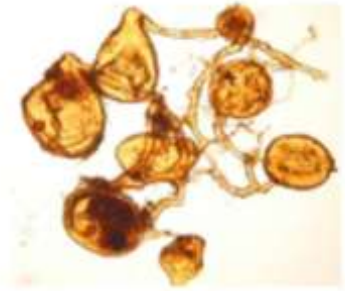
Root samples were placed into labelled vials containing distilled water in order to wash the sand from them. They were generally processed the following day. However, if processing was to be delayed, they were transferred to vials containing 50% ethanol. Ethanol was chosen as the fixative over FAA (Formalin Acetic Acid Alcohol) due to the caustic nature of the latter.

Observation:

In the present investigation during 2008 field survey was conducted to determine sample collection sites and the status of AM fungal spores concentration in the soil of those regions. Collection of rhizosphere soil samples of Maize and wheat from Naubatpur, Mokama, Bidupur, Diyara and Jadua regions of Patna and Vaishali district was done during 2009 to 2011. Rhizospheric soil samples were collected at various interval of time a time described in methodology. This study was designed to AM fungi in study localities by isolation, identification, enumeration of spores found in field rhizosphere soil samples. Wet sieving and decanting method (Gerdemann and Nicolson, 1963) for the isolation of AM fungal spores was used. Direct counts of AM fungal spores were made from rhizosphere soil samples of study sites. Estimation of spore number and identification was achieved by recovering spores from 100 g samples of rhizosphere soil sample. Isolated AM fungal spores were identified to genus level according to criteria of Schenk and Perez (1990) and Morton (1988 and 1997). The distinguishing characteristics for the identification of genera were described in genus description. Rhizospheric soil samples collected from various localities revealed presence of several species of 4 genera on the basis of resemblances the AM species identified as *Glomus, Acaulospora, Gigaspora*and *Scutellospora.* The number of AM fungal spores isolated from different sites of maize and wheat crops are give in table (2).

Fig. *Gigaspora spp.*

Fig. Acaulospora spp.



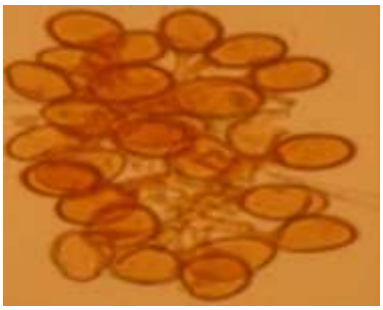


Fig. *Glomus spp.* Fig. *Glomus spp.*

The no. of AM fungal spores ranged from 80-150 per 100 gm of soil considering both plants individually under study.

Table 2

Average number of AM spores per 100 gm of soil for host plant maize and wheat

Locality	Average No. of spores in maize plant	Average No. of spores in wheat plant	Overall Average No. of spores
Naubatpur	108	98	103
Mokama	110	82	96
Bidupur	98	81	89.5
Diyara	151	93	122
Jadua	104	90	97

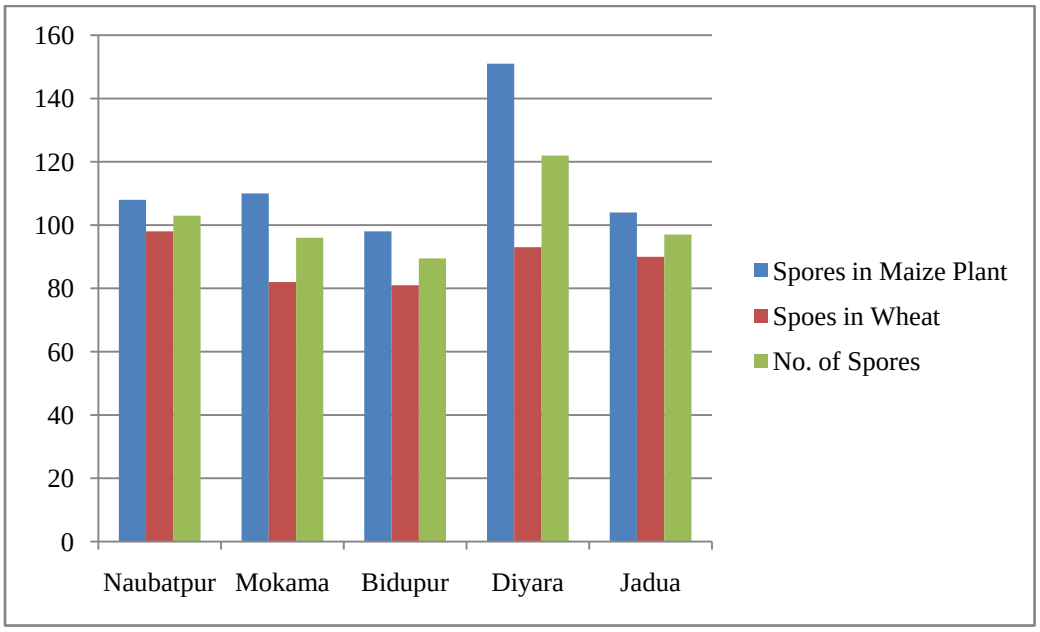


Fig.-1 : Average number of AM spores per 100 gm of soil for host plant maize and wheat

AM fungal spores are well disturbed throughout Patna and Vaishali district. Maximum members of spores were isolated from Diyara region of Vaishali. Soil collected from non-cultivated such as Diyara and Jadua had large number of spores in both hosts in comparison to cultivated site such as Bidupur, Mokama and Naubatpur. Maximum number of AM spores in maize were isolated from Diyara region (non-cultivated). Maximum number of AM spores in wheat were isolated from Naubatpur. In Naubatpur wheat is widely cultivated. The presence of maximum number of AM spores in wheat from Naubatpur may be due to host specificity of these spores. In maize plant's rhizosphere soil Gomus spp. Were dominated followed by Acoulospora, Gigaspora and Scatellospora. Glomus spp. AM fungal spores were totally dominated in wheat crop, more than 90% where observed.

This study depicted the distribution of AM fungi in the rhizosphere soil of both maize and wheat plant. Both plants and rhizosphere soils were collected during a year period (2009-2011), it different sites and during different seasons. The average number of

spores isolated from maize and collectively from year 2009-2011 from diverse sites had been show in table (3).

Table 3

Average number of Spore Area and year wise

Year	Naubatpur	Mokama	Bidupur	Diyara	Jadua	Area year wise
2009	98.5	105	101	124	110	107.7
2010	132	99	98	132	118	115
2011	110	112	112	136	107	115.4
Average Area wise	113.5	105.33	103.66	130.66	111.66	

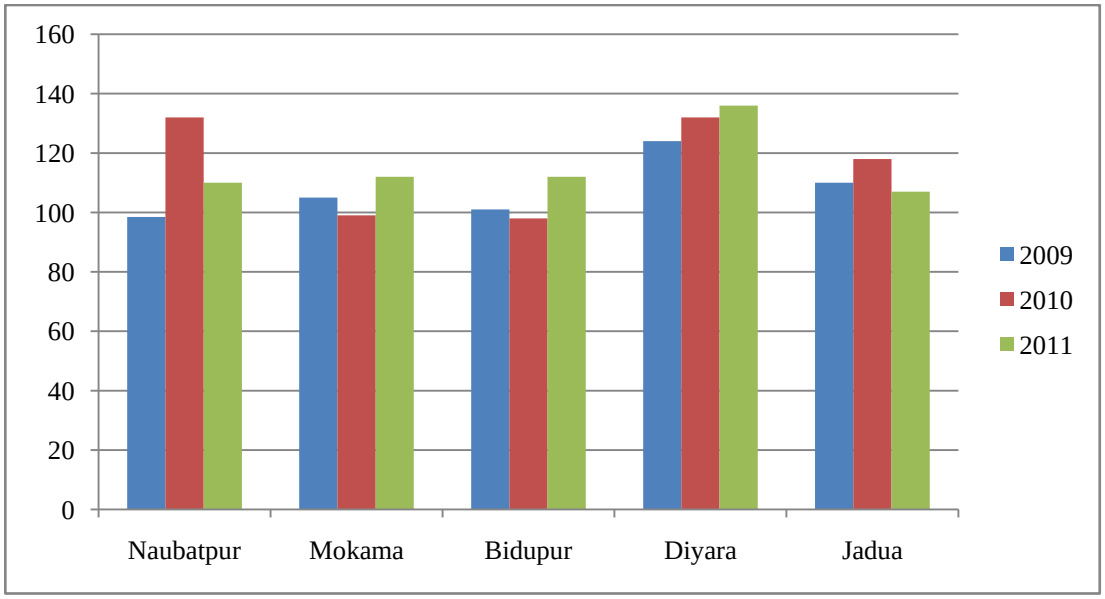


Fig.-2 : Average number of Spore Area and year wise

Root infection assessment

In order to find out the potential of AM fungi infection in roots of maize and wheat, the root samples collected were clean, chopped into small pieces and then subjected to

fixation, cleaning, rinsing and bleaching in KOH solution following standard techniques for microscopic observations. In case of infected roots, the presence of AM fungi fornication 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. The Root colonization value of both plant was given in table (3) year wise during 2009-2014.

Table 4

R.C. value of maize and wheat year wise

Year	Maize plant RC value	Wheat plant RC value
2009	16	12
2010	28	15
2011	20	10

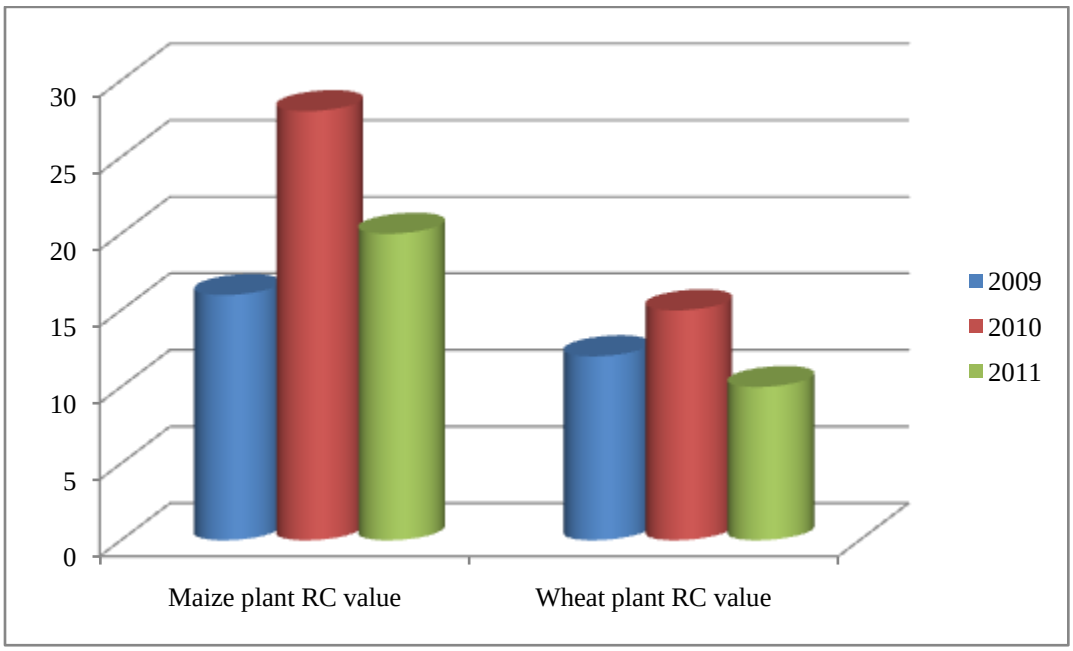


Fig.–3 : R.C. value of maize and wheat year wise

RESULT AND DISCUSSION

In the present study *Glomus* were the most common Genera and dominant in shifting system. My 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; Burns and Illahi 2004; Mirtha and Dhar, 2007; Sharma et al, 2009; Burni et al., 2009).In Indian context my finding is 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 acedic as well as in alkaline soils (Pande and Tarafdar, 2004).In the present study the maximum spores were observed from undisturbed natural vegetation of Diyara of Vaishali district. The potential reason for maximum number of spores 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.

The host must be highly susceptible to AM fungi colonization, produce rapidly growing fibrous root systems. It plays an important role in distribution, predominance and AM fungi infectivity of the host plant. The number of AM fungal spores present in study sites of Patna and Vaishali district was higher as compared with both hosts, maximum spores are observed in maize followed by wheat.Maximum spores in maize plant is in the agreement with finding of Singh and Pandya,1995. They reported maximum spore density in maize followed by pearl millet, pigeon pea, chick pea and wheat in Madhya Pradesh.

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