Research Article

VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH RHIZOSPHERE OF *HORDEUM VULGARE* L. IN SIKAR DISTRICT

*Ameeta Sharma and Savita Yadav

Department of Biotechnology - The IIS University, Jaipur- 302020, Rajasthan, India *Author for Correspondence

ABSTRACT

Mycorrhiza is a mutualistic relationship connecting plant roots and fungal hyphae, mediating the transfer of nutrients between them. A survey of Vesicular arbuscular mycorrhizal fungi (VAMF) variety and Barley (*Hordeum vulgare* L.) root colonization in semi-arid areas was undertaken in fifteen barley fields located in the Sikar district (Rajasthan). The plants were collected with their roots and their respective rhizospheric soil from different areas. The microscopic analysis of the soils revealed that all samples had VAM. The number of VAM fungal spores detected in diverse field soils was relatively high and it varied to different extend. The study of morphological characters uncovered the existence of six's generas and nine species, namely *Glomus, Gigaspora, Acaulospora, Scutellospora, Entrophospora* and *Sclerocystis*

Key Words: VAM Fungi, Rhizosphere, Hordeum Vulgare L., Nutrient Uptake

INTRODUCTION

A mycorrhiza is a ubiquitous symbiotic association between fungi along with the roots of a vascular plant. The word mycorrhiza is has come from the conformist Greek words used for 'mushroom' and 'root'. Arbuscular mycorrhizal fungi are considered as obligate bio-trophic symbionts and are associated with the Rhizosphere of over 80% terrestrial plants and crops. The main function of AM fungi is of phosphorus transportation. Extra-radiscal mycelium of AM fungi easily access P from soil and deliver to root cortical cells as polyphosphate which at last translocate to host plant after solubilization and it is sketchy that external hyphae deliver up to 80% of P requirement of the plant. Population of AM fungi is highly partial by several environmental factors including climatic surroundings, soil physico-chemical status, age and variety of host plant and numerous agricultural practices etc.

Moreover, various species of VAM fungi vary in their tolerance to hard physical and chemical condition in soil (Kumar and Ghose, 2008). It is by now reported that, AMF is naturally take place in salty environment and their spore density is improved as saline tension stimulates sporulation. The occurrence of mycorrhizas in water plants was firstly reported in 1977. Since then mycorrhiza have been originate in marine macrophytes colonizing lakes and stream (Beck-Nilsen and Madsen, 2001).VAM fungi have gained much magnitude in the field of agriculture as this play an inner role in the capture of nutrients from the soil of all ecosystems. The main added two of mycorrhiza is its finer soil examination and increasing uptake of P, N, K, Zn, Cu, S, Fe, Mg, Ca and Mn and the elevate of these nutrients to the crowd roots (Sundar et al., 2010). Mycorrhiza association of fungi and plant and plant root, is a general knowledge from side to side out of the plant kingdom and is valuable and even necessary for healthy increase of the host plants. Among the different types of mycorrhizae, arbuscular mycorrhiza is one of the most general and the most recurrent endomycorrhiza all over the world. Arbuscular mycorrhizal (AM) fungi are non-pathogenic oblige symbionts. The institution of symbiotic organization of AM fungi with plant roots has many similarities to nodule configuration in legumes by non-pathogenic rhizobia (Kawaguchi and Minamisawa, 2010). Mycorrhiza is a mutualistic symbiosis between plants and fungus localized in a root or root like composition in which energy moves mainly from plant to fungus and inorganic resources move from fungus to plant.

Research Article

MATERIALS AND METHODS

Sampling Sites

The Preliminary survey done in the arid zones of Sikar district of Rajasthan (INDIA) revealed the association of VAM fungi in the roots of barley. Fifteen soil sampling sites were retained. Collection of sample for each field surveyed, a composite soil sample comprising of ten sub sample were collected. Root samples were harvested around the crop plant from 10 to 40 cm depth, and mixed together. Soil samples were bought to the laboratory and were stored in the refrigerator at 4 degree Celsius until their further processing, which was done within a week. Soil samples were collected from fields in localities of Rampura, Dalptpura, Karjo, Meenakanangal, Balupura, Jhamash, Raipur, Ryakabasha, Haripura, Rajpura, Motuka, Ranasar, Patan, Ganesvr, Khurdiya areas.

Physical and Chemical Analysis of Soil

Soil sub-samples from each location were analyzed for various properties. The soil parameters which were measured are: the pH in 1:1 water, available phosphorus (Olsen *et al.*, 1954), nitrogen N- NH4 N- NO3 (Bremner and Keeny, 1966), soil texture and organic matter. This soil texture and organic matter was indirectly measured by comparing the dry weight after 6 hrs at 105°C with the dry weight after 4 hrs at 550°C, and soil texture.

Spore Extraction

Several techniques for the extraction out of AM fungi spores from soil have been studied by Hayman (1970) and Schenck and Perez (1990). The wet sieving and decanting as described by Gerdeman and Nicolson (1963), after that sucrose density gradient centrifugation technique was found to be the most appropriate and useful technique. An additional method of separating spores from debris was by use of series of sieves of various pore sizes for seiving. 100 g of soil was suspended into water. Vigorous mixing was done in the suspension to free the spores from the soil and roots for 1 min in 300 ml of water to free the spores from roots. After that, heavier particles in suspension is certified to settle for 15 to 45 (times vary depending on soil texture) and the supernatant decanted through benchmark sieves. Sieves were selected so as to capture the spores of interest then soil samples were rinsed through 850, 500, 250, 100 and 50 mm Sieves followed by recovering of soil material from each sieve. After recovery, from each sieve, samples were again suspended in water, and then centrifuged at 3000 RPM for 3 min. The supernatant was removed and the soil material was re-suspended in a sucrose solution (60%) and centrifuge at 1000 RPM for 2 min. The supernatant containing spores was filtered on filter paper (Whatman # 2). The spores were observed and recovered under the dissecting microscope, were separated and characterized and finally evaluated for their abundance in barley fields.

Characterization of Spores

To characterize the VAM spores, various features like spore size, spore colour, hyphae attachment, presence/absence of cell wall, suspensor size, etc. was taken into consideration. To calculate the spore size, occular micrometer used which was calibrated .Identification of VAM species Studies of spores adhering to roots were prepared for further examination and identification of VAM species were done under the compound microscope. Standard procedure used (Phillips and Hayman, 1970). Mycorrhizal spores were obtained by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). For quantification of spores 50 g of soil was added to 300 ml of water and supernatant was decanted through a set of sieves and mycorrhizal spores were retained on the finest sieve. The material on the finest sieve was washed with water and spore suspension was then taken on a slide in 1cm area and examined under $100 \times$ magnification of the spores, different VAM fungal genera in the present study were identified up to generic level using the taxonomic keys by Schenck and Perez (1990).

Research Article





c. Acaulospora delicate



d. Scutellospora gilmorei e. Entrophospora colombiana f. Sclerocystis Figures 1: For different species of VAM isolated from Barley Fields of Sikar district



Figure 2: For occurrence of VAM in barley fields Undertaken for present study

RESULTS AND DISCUSSION

The present research has been done for the study of identification and occurrence of VA mycorrhiza in soils sample of District Sikar. Most of the kharif and rabi stage crops have been showing the lobby group of VAM fungi up to a range of different levels. However, the percentage of VAM fungal colonization of plant roots differs appreciably in kharif and Rabi period crops, within a field. VAM

Research Article

fungal root immigration varied from crop to crop, term to season and field to field. The analysis of the soil samples revealed strapping alkaline soil with low amount of nitrogen contemplation less than 0.26 mg/kg for N-NH4 and 3.47 mg/kg for N-NO3, very much weakly to be had P concentrations of 12-60 μ g/g except for the site Ranasar where medium deliberation of 220.5 μ g/g, and a squat level of organic matter flanked by 0.42 and 3.33%. So if VAM fungi are functional to such crop fields then it can augment output.

The present investigation reported following species of VAM viz. Glomus mosseae, Glomus fasciculatum, Glomus rosea, Scutellospora gilmorei, Entrophospora colombiana, Gigaspora margarita, Gigaspora gigantea, Acaulospora delicate and Sclerocystis. Similarly In semi-arid zones of Africa, America, and India various VAM like G.mosseae, G. aggregatum, G. constrictum and G.macrocarpum have been reported (Mohammad et al., 2003; Muthukumar and Udaiyan, 2002) simultaneously with a variety of Acaulospora and Scutellospora species by various workers (Duponnois et al., 2001; McGee, 1989). The Glomus species were most common in all soils which confirm the findings of Kendricks and Berch (1985). They add to phosphorus uptake, make better uptake of other plant nutrients by root system and are advantageous in the life nitrogen fascination of Rhizobium, years be in charge of root pathogens and dearth encounter (Dela Cruz, 1987)

Many researchers have reported that vesicular-arbuscular mycorrhiza fungi not only increases phosphorus uptake, but also plays an important role in the uptake of other plant nutrients and water. Colonisation by VAM fungi improves the drought resistance of plants (Puppi and Bras, 1990). Mycorrhiza mediated plant-to-plant transfer of nutrients can significantly affect competitive relationships is related to the quantity of nutrients that can be transported through the hyphae to nutrient gradients between sources and sinks and to the relative sink strengths of the associated plants (Bethlenfalvay et al., 1996). Vesicular arbuscular mycorrhizae (VAM) can improve plant acquisition of soil minerals by soil exploration and can better enable a plant to withstand environmental stresses. Soil micro fauna can be important vectors of spore dispersal, and so may be linked to the spatial and temporal dynamics of VAM activity in soils (Rabatin and Stinner 1989). Crop rotation affects VAM species composition and dominance in the field. Even weed dynamics in agricultural fields have been associated with changes in VAM spore populations. A crop monoculture can favour some VAM species over others, which will vary in accordance with the host crop (Johnson et al., 1991). Continuous monoculture can have a negative effect on VAM colonization compared with fields under crop rotation. VAMF are now going to be an integral part of sustainable agricultural practice, reclamation of the land and restoration of denuded habitats for both routine conventional crops as well as newly introduced or to be introduced other crops (Wang and Qui, 2006).

REFERENCES

Bethlenfalvay GJ, Schreine RP, Mihara KL and McDaniel H (1996). Mycorrhizae, biocides and bio control: 2. Mycorrhizal fungi enhance weed control and crop growth in a soybean cocklebur association treated with the herbicide bentazon. *Applied Soil Ecology* **93**(1) 205-214.

Bremner JM and Keeney DR (1966). Determination of isotope-ratio analysis of different forms of nitrogen in soils: 3 exchangeable "ammonium, nitrate and nitrite" by extraction distillation methods. *Proceedings - Soil Science Society of America* **30**(1) 577-582.

Dela Cruz RE (1987). Status of Bio-reforestation in the Philippines. Paper presented during the pre *Workshop on Bio-Reforestation* sponsored by IUFRO Japan, Bogor, Indonesia March, **40**(5) 25-29.

Duponnois R, Plenchette C, Thioulouse J and Cadet P (2001). The mycorrhizal soil infectivity and arbuscular mycorrhizal fungal spore communities in soils of different aged fallows in Senegal. *Applied Soil Ecology* **17**(1) 239-251.

Gerdemann and Nicolson (1963). Vesicular-arbuscular mycorrhizae formed on maize and tulip tree by Endogone fasciculata. *Mycologia* 57(1) 562-575.

Hayman DS (1970). Methods for evaluating and manipulating of vesicular-arbuscular mycorrhiza. In: *Microbiologycal Methods for Environmental Microbiology*, editors-JM Lynch and JM Grainger, (Academic Press, London, United Kingdom), **2**(2) 95-117.

Research Article

Johnson NC, Copeland PJ, Crookston RK and Pfleger FL (1991). Mycorrhizae: possible explanation for yield decline with continuous corn and soybean. *Agronomy Journal*, **84**(1) 387-390.

Kawaguchi M and Minamisawa K (2010). Plant microbe communications for symbiosis. *Plant Cell Physiology*, **51**(9) 1377-1380.

Kendrik B and Berch S (1985). Mycorrhizae application in Agriculture and forestry. In: *Comprehensive Biotechnology*, (Pergamen Press Oxford) 109-150.

Kumar T and Ghose M (2008). Status of arbuscular mycorrhizal fungi (AMF) in the Sundarbans of India in relation to tidal inundation and chemical properties of soil. *Wetlands Ecology Manage* **16**(1) 471-483.

McGee PA (1989). Variation in propagule numbers of vesicular arbuscular mycorrhizal fungi in a semi-arid soil. *Mycologia Research* 92(1) 28-33.

Mohammad MJ, Hamad SR and Malkawi HI (2003). Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. *Journal of Arid Environment* 53(1) 409-417.

Muthukumar T and Udaiyan K (2002). Arbuscular mycorrhizal fungal composition in semi-arid soils of Western Ghats. Southern India *Current Science* **82**(6) 624-628.

Olsen SR, Cole CV, Watanabe FS and Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture, Washington, D.C.

Puppi G and Bras A (1990). Nutrient and water relations of mycorrhizal white clover. Agriculture, *Ecosystems and Environment* **29**(5) 317-322.

Rabatin SC and Stinner BR (1989). The significance of vesicular arbuscular mycorrhizal fungal-soil macroinver-tebrate interactions in agro ecosystems. *Agriculture, Ecosystems and Environment* **27**(1) 195-204.

Schenck NC and Perez Y (1990). Isolation and culture of VA mycorrhizal fungi. In: *Isolation of Biotechnological Organisms from Nature*, editors: Labeda DP, (McGraw-Hill Book Co, New Yorks, New York) 2(2) 237-258.

Wang B and Qui YL (2006). Phylogenetically distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16(5) 299-363.