

Mycorrhizal fungi associated with mungbean

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Abstract

Objective of the present study was to identify the mycorrhizal flora associated with mungbean [*Vigna radiata* (L.) wilczek] var. NM-98. Seeds were sown in pot soil collected from a well cultivated field of Punjab University Lahore. The plants were allowed to grow till maturity and decomposition of roots in the pots thereafter. Mycorrhizal spores were separated from the soil by wet sieving and decanting techniques and identified with the help of various keys. Four species of *Glomus* namely *G. fasciculatum*, 70 of *G. mosseae*, 6 of *G. macrocarpum* and 4 of *G. reticulatum* were identified which were present in 48%, 45%, 4% and 3%, respectively, of the total. This study concludes that *G. fasciculatum* and *G. fasciculatum* are the most commonly occurring fungi associated with mungbean in Pakistan.

Keywords: Arbuscular mycorrhizae, AM fungi, *Glomus*, Mungbean.

Introduction

Mycorrhizal fungi belong to phylum Glomeromycota, are ancient symbionts that constitute a group of roots obligate biotrophs. They develop hyphal branching with host plants to trigger the morphogenesis process for the successful establishment of symbiotic associations between the plant roots and mycorrhizal fungi (Montesinos-Navarro *et al.*, 2019). This relationship is widespread in nature and provides a broad range of benefits to the host plants including enhanced nutrient uptake, crop growth and production (Javaid, 2008; Delavaux *et al.*, 2017). In leguminous plants, mycorrhizal associations have important roles in directly influencing the increased rate of photosynthesis, heavy metal remediation, enzymatic production, nutrient uptake, enhanced nitrogen fixation by N₂ fixing bacteria, suppression of pests and soil-borne fungal diseases, secondary metabolite production, improved soil aggregation as well as osmotic adjustments under stress conditions (Javaid, 2010; Berruti *et al.*, 2016; Chen *et al.*, 2018). Different mycorrhizal associations have shown a tremendous effect on the ecology and physiology of host plants. Recently, Neuenkamp *et al.* (2019) observed that mycorrhizal inoculations under field conditions facilitated the N₂ fixation and vegetative cover of diverse plants. Martínez-Medina *et al.* (2011) also reported that plants inoculated with mycorrhizal fungi caused a significant decrease in Fusarium wilt disease of melons in comparison of non-inoculated plants. Likewise, Wang *et al.* (2018) attributed an increase in the nutrient uptake and growth of a medicinal plant *Chrysanthemum morifolium* under saline stress conditions by the colonization of *Diversispora versiformis* and *Funneliformis mosseae* alone or in combinations. Hashem *et al.* (2016) have reported an increase in the nodulation, plant growth, stress tolerance and nutrient acquisition of a leguminous shrub *Acacia gerrardii* under abiotic stress in the presence of mycorrhizal fungi. The

present study was carried out to find out the mycorrhizal species associated with mungbean.

Materials and Methods

Isolation of mycorrhizal fungi

Seeds of *V. radiata* var. NM-98 were surface sterilized with 3% H₂O₂ for 10 minutes followed by four washings with sterilized water. These seeds were grown in field soil. After 20 days of sowing when seedlings became colonized with mycorrhizal fungi, plants were uprooted carefully and soil was removed from the roots by thorough washing under tap water. The washed roots were surface sterilized with 1% sodium hypochlorite for three minutes and the plants were transferred to pots containing washed and heat-sterilized sand-soil mixture (1:2). After 100 days of transplantation, rhizospheric soil samples were collected from the pots. Mycorrhizal fungal spores were isolated from the soil by wet sieving and decanting technique (Gerdeman and Nicolson, 1963). One hundred grams of each soil sample were dispersed in 500 mL water and the suspension left undisturbed for 30 minutes to allow soil particles to settle. The suspension was then decanted through sieves and the residues on the sieves were washed into beakers. After settlement of the heavy particles, the supernatant was filtered through gridded filter papers.

Identification of mycorrhizal fungi

Each gridded filter paper containing mycorrhizal spores was spread into a glass plate and examined under a binocular dissecting microscope. Intact mycorrhizal fungal spores were transferred using a wet needle to polyvinyl alcohol- lactophenol on a glass slide for identification. The mycorrhizal spores on the slide were examined under compound microscope. Different spores were identified on the basis of shape, size, colour and surface configuration

of spores, and form of subtending hyphae following Trappe (1982) and Schenck and Perez (1990).

Results and Discussion

Abundance of mycorrhizal species

Four different types of mycorrhizal spore were identified. They all belonged to the genus *Glomus* and varied in their intensity of occurrence. There were 75 spores of *G. fasciculatum* (48%), 70 of *G. mosseae* (45%), 6 of *G. macrocarpum* (4%) and 4 of *G. reticulatum* (3%) per 100 g of the soil (Fig. 1). In the present study all the four mycorrhizal species isolated from the rhizospheric soil of *Vigna radiata* were belonged to the genus *Glomus*. It is the most widespread and largest genus within the phylum Glomeromycota (Rodrigues and Rodrigues, 2020). It is known to have maximum number of species (Trappe, 1982, Schenck and Perez, 1990) and is the most widely and commonly occurring mycorrhizal genus in the soil all over the world (Morton, 1985). In the present study *Glomus fasciculatum* and *G. mosseae* were found more abundant in the rhizosphere of the test host plant than the others species. These results are in line with the findings of earlier workers from Pakistan who have reported greater abundance of these two species than others in agricultural fields (Jaluddin and Anwar, 1991), in the rhizosphere of grasses (Irm, 1997), and in saline areas of the country (Naz, 1999).

Glomus fasciculatum (Thaxter sensu Gerd.) Gerd. & Trappe

It was the most abundant mycorrhizal species found in the rhizosphere of mungbean. Chlamydo spores were globose to subglobose, longest dimension at maturity was 50–125 μm . Surface ornamentation - smooth to dull roughened; number of walls two, outer thinner than inner; outer layer hyaline, inner yellow to brown. Spores with cylindrical persistent hyphae with closed pore, hyphal attachments 4–15 μm in diameter. This mycorrhizal species is known to enhance crop productivity in a number of plant species. Its inoculation increased fresh biomass and oil yield in *Mentha arvensis* under salt stress (Bharti *et al.*, 216). It alleviated oxidative damage caused by biotic stress of *Fusarium oxysporum* f. sp. *lycopersici* in tomato (Math *et al.*, 2019). It has the ability to act as a bioconverter of nutrients in the soil, particularly solubilization of P into well consumable form by the plants i.e. the anionic form H_2PO_4^- (Azmat *et al.*, 2016). Synergistic interaction of *G. fasciculatum* and *Pseudomonas monteilii* resulted in increased plant growth and tuber production, and decreased incidence of root rot and wilt diseases in *Coleus*

forskohlii (Singh *et al.*, 2013).

Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe

It was the second most abundant mycorrhizal species found in the rhizosphere of of mungbean. Chlamydo spores were yellow to brown in color, globose to ovoid in shape, and with a typical funnel shaped base of 20–30 μm . Spore size ranged from 120–300 μm . Number of spore walls one, yellow brown. Contents of mature spores white to hyaline globules. Hyphae often thick from 1–4 μm near the spore. Diverse beneficial affects of inoculation of *G. mosseae* have been report in agriculture. It induced resistance in *Catharanthus roseus* against *Spiroplasma citri* infection (Tahat *et al.*, 2014). It stabilized more cadmium in the roots and restricted its translocation to the stems of *Pistacia vera*. In addition, it also alleviated the adverse affects of cadmium on phosphorus absorption, chlorophyll content and total protein (Rohani *et al.*, 2019). It also has the ability to improve phytoextraction of Pb by vetiver grass, *Chrysopogon zizanioides* (Punamiya, 2010). Its inoculation increased yield in papaya by 105.2% (Vázquez-Hernández *et al.*, 2011).

Glomus macrocarpum Tul. & Tul.

It was the less abundant mycorrhizal species in the present study. Its chlamydo spores were globose to subglobose, ranging in size from 100–250 μm . Spore wall was composed of two distinct layers, outer layer was thin (1–2 μm) and hyaline when mounted in water glycerol; inner wall was yellow, 6–12 μm thick. Spore tapers to the point of attachment of a single persistent hypha. Diameter of hypha at the point of attachment ranges from 9–20 μm .

Glomus reticulatum Bhatt. & Muk.

It was the least abundant mycorrhizal species found in vicinity of mungbean roots. The spores were ovate, with reticulate surface at maturity, longest dimension at maturity was 125–175 μm . Spore wall was two layered, 5–8 μm in thickness, outer wall thinner than inner, all the layers were brownish black to black. Spores were with one cylindrical subtending hypha, 5–12 μm in diameter at the point of attachment. Color of subtending hyphae was hyaline to yellow or brown.

Conclusion

This study concludes that *G. fasciculatum* and *G. mosseae* are the most abundant mycorrhizal species associated with mungbean.

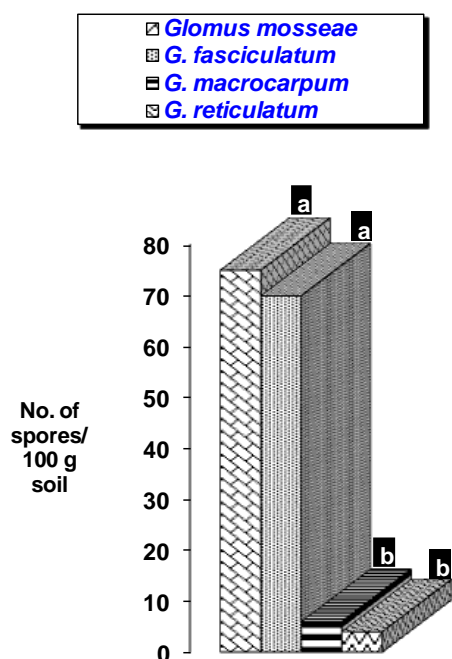


Fig. 1: Mycorrhizal flora isolated from the rhizosphere of *Vigna radiata*. Bars with different letters show significant difference ($P \leq 0.05$) as determined by DMR Test.

References

- Azmat R, Hamid H, Moin S, Saleem A, 2016. *Glomus fasciculatum* fungi as a bio-convertor and bio-activator of inorganic and organic P in dual symbiosis. *Recent Pat Biotechnol.*, **9**: 130-138.
- Berruti A, Lumini E, Balestrini R, Bianciotto V, 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Front. Microbiol.*, **6**: 1559.
- Bharti N, Barnawal D, Shukla S, Tewari SK, Katiyar RS, Kalra A, 2016. Integrated application of *Exiguobacterium oxidotolerans*, *Glomus fasciculatum*, and vermicompost improves growth, yield and quality of *Mentha arvensis* in salt-stressed soils. *Ind. Crop Prod.*, **83**: 717-728.
- Chen M, Arato M, Borghi L, Nouri E, Reinhardt D, 2018. Beneficial services of arbuscular mycorrhizal fungi—from ecology to application. *Front. Plant Sci.*, **9**: 1270.
- Delavaux CS, Smith-Ramesh LM, Kuebbing SE, 2017. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology*, **98**: 2111-2119.
- Gerdemann JW, Nicolson TH, 1963. Spore of mycorrhizal endogone species extracted from soil by wet sieving and decantation. *Trans. Brit. Mycol. Soc.*, **46**: 235-244.
- Hashem A, Abd-Allah EF, Alqarawi AA, Al-Huqail AA, Wirth S, Egamberdieva D, 2016. The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress. *Front. Microbiol.*, **7**: 1089.
- Irm, 1997. Seasonal variation in VA mycorrhiza in the rhizosphere of four allelopathic grasses. M. Sc. Thesis, University of the Punjab Lahore.
- Jalaluddin M, Anwar QMR, 1991. VAM fungi in wheat rice fields. *Pak. J. Bot.*, **23**: 115-122.
- Javaid A, 2008. Role of mycorrhizae in plant nutrition. In: *Microbes in Sustainable Agriculture*. Khan MS, Zaidi A, Mussarrat J (Eds.). Nova Science Publishers, Inc. New York.
- Javaid A, 2010. Role of arbuscular mycorrhizal fungi in nitrogen fixation in legumes. In: *Microbes for Legumes Improvement*. Khan MS (ed.), Springer Wein New York, 2010. pp. 409-426.
- Martínez-Medina A, Roldán A, Pascual JA, 2011. Interaction between arbuscular mycorrhizal fungi and *Trichoderma harzianum* under conventional and low input fertilization field condition in melon crops: growth response and Fusarium wilt biocontrol. *Appl. Soil Ecol.*, **47**: 98-105.
- Math S, Arya S, Sonawane H, Patil V, Chaskar M, 2019. Arbuscular mycorrhizal (*Glomus fasciculatum*) fungi as a plant immunity booster against fungal pathogen. *Curr. Agric. Res.*, **7**: 99-107.
- Montesinos-Navarro A, Valiente-Banuet A, Verdú M, 2019. Mycorrhizal symbiosis increases the

- benefits of plant facilitative interactions. *Ecography*, **42**: 447-455.
- Morton JB, 1990. Evolutionary relationships among arbuscular mycorrhizal fungi in the Endogonaceae. *Mycologia*, **82**: 192-207.
- Naz JZ, 1999. Mycorrhiza induced salt tolerance in *Helianthus annuus* L. var. suncross. Ph. D. Thesis, University of the Punjab Lahore, Pakistan.
- Neuenkamp L, Prober SM, Price JN, Zobel M, Standish RJ, 2019. Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. *Fungal Ecol.*, **40**: 140-149.
- Punamiya P, Datta R, Sarkar D, 2010. Symbiotic role of *Glomus mosseae* in phytoextraction of lead in vetiver grass [*Chrysopogon zizanioides* (L.)]. *J. Hazard. Mater.*, **177**: 465-474.
- Raya-Hernández AI, Jaramillo-López, PF, López-Carmona DA, Díaz T, Carrera-Valtierra JA, John J, 2020. Field evidence for maize-mycorrhiza interactions in agroecosystems with low and high P soils under mineral and organic fertilization. *Appl. Soil. Ecol.*, **149**: 103511.
- Rodrigues KM, Rodrigues BF, 2020. *Glomus*. In: *Beneficial Microbes in Agro-Ecology*. Academic Press. pp. 561-569.
- Rohani, N, Daneshmand F, Vaziri A, Mahmoudi M, Saber-Mahani F, 2019. Growth and some physiological characteristics of *Pistacia vera* L. cv. Ahmad Aghaei in response to cadmium stress and *Glomus mosseae* symbiosis. *S. Afr. J. Bot.*, **124**: 499-507.
- Schenck NC, Perez Y, 1990. Manual for identification of VA mycorrhizal fungi. 3rd ed., INVAM. Univ. of Florida, Gainesville.
- Singh R, Soni, SK, Kalra A, 2013. Synergy between *Glomus fasciculatum* and a beneficial *Pseudomonas* in reducing root diseases and improving yield and forskolin content in *Coleus forskohlii* Briq. under organic field conditions. *Mycorrhiza*, **23**: 35-44.
- Tahat MM, Nejat N, Sijam K, 2014. *Glomus mosseae* bioprotection against aster yellows phytoplasma (16srI-B) and *Spiroplasma citri* infection in Madagascar periwinkle. *Physiol. Mol. Plant Pathol.*, **88**: 1-9.
- Trappe JM, 1982. Synaptic keys to the genera and species of Zygomycetous mycorrhizal fungi. *Phytopathology*, **72**: 1102-1108.
- Vázquez-Hernández MV, Arévalo-Galarza L, Jaen-Contreras D, Escamilla-García JL, Mora-Aguilera A, Hernández-Castroc E, 2011. Effect of *Glomus mosseae* and *Entrophospora colombiana* on plant growth, production, and fruit quality of 'Maradol' papaya (*Carica papaya* L.). *Sci. Hort.*, **128**: 255-260.
- Wang Y, Wang M, Li Y, Wu A, Huang J, 2018. Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress. *PLoS One*, **13**: e0196408.