Role of arbuscular mycorrhizal fungi on plant growth and photosynthetic pigments in *Vigna radiata*

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Abstract

The aim of the present investigation was to evaluate the impact of arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* on plant growth, including root proliferation as well as chlorophyll and carotenoid contents of mungbean [*Vigna radiata* (L.) Wilczek]. We investigated the role of AMF in the field condition during Rabi season in Ambikapur Chhattisgarh, India and data were analyzed using two-way ANOVA. Plant height (50.33±4.72 cm, 46.33±5.50 cm), root growth (22.83±2.75 cm, 19.66±4.72 cm), plant fresh biomass (17.54±1.08 g, 12.55±8.24 g), chlorophyll (11.93±0.83 mg g^{-1} FW, 9.58±3.69 mg g^{-1} FW) and carotenoid contents (0.67±0.10 mg g^{-1} FW, 0.5±0.14 mg g^{-1} FW) of mungbean plants were higher in the mycorrhiza inoculated as compared to the non-inoculated plants. The study revealed symbiotic association of *G. mosseae*had marked effects on various growth parameters and photosynthetic pigments, which may be potentially significant and promising for sustainable mungbean cultivation.

Keywords: Arbuscular mycorrhizal fungi, *Glomus mosseae,* Mungbean growth, Photosynthetic pigments.

Introduction

Mycorrhizae are symbiotic fungi that form a beneficial relationship with the plant. In terrestrial ecosystems, mycorrhizae are the most significant symbiotic fungi form relationships with 80% terrestrial plants (Brundrett, 2009). The mycorrhizal association may be categorized in seven types (arbuscular, arbutoid, ecto-mycorrhizal, endo-mycorrhizal, ericoid, orchid and monotropoid) based upon the fungus involved and their structures generated in the host root by the fungus-plant interaction. The arbuscular mycorrhiza fungi (AMF) are the most widespread of these various forms of symbiotic relationships. That is the most beneficial obligatory symbiotic soil fungi that are predominantly found in the soil, and usually colonize roots and form a symbiotic relationship with several plant species (Manila and Nelson, 2013). Arbuscules are responsible for transporting absorbed water and nutrients from the soil to the roots of plants (Javaid, 2009; Sandeepa, 2013). Many researchers have reported that widely occurring AMF genera *viz. Glomus, Gigaspora, Scutellospora, Acaulospora* and *Entrophospora* play a significant role in plant growth enhancement by raising the nutrients uptake from soil (Hodge *et al.,* 2001; Kasliwal and Srinivasamurthy, 2016). *Glomus mosseae* is considered an important species of *Glomeraceae (Glomeromycota),* which forms symbiotic relationships with higher plants and more frequent AM symbiotic fungi (81%) with mungbean in south India (Ray *et al.,* 2007; Javaid and Khan, 2019). It has been reported that AMF colonization improves growth, nodulation and nitrogen fixation in mungbean (Javaid *et al*., 1993, 1994). As a consequence of the colonization of the AM fungi, plant-AMF symbiosis increases water uptake, plant growth and photosynthesis with a favorable effect on the relative water content (Chen *et al*., 2017). The development of an extensive hyphal network of AM fungi allows plant hosts to absorb water and minerals from the soil, enabling a higher photosynthetic rate (Yang *et al*., 2016; Chandrasekaran *et al*., 2019). AMF plants have a higher rate of transpiration in the leaves, which is connected to a higher stomatal conductance required for photosynthesis and carbon transfer to the mycorrhiza (Auge, 2001; Maggio *et al*., 2004). Several previous researches have shown that AM fungi increase photosynthesis in plants by reducing stomatal conductance, increasing PSII efficiency, and increasing the expression of some chloroplast genes (Sheng *et al*., 2008; Hajiboland *et al*., 2010; Porcel *et al*., 2015). Mungbean, also well-known as greengram, is one of India's most popular pulse crops and high-protein food. The whole and split grains are used as dal or made into flour. Mungbean is very nutritious as it contains a high level of protein, vitamins and calcium (Hou *et al*., 2019). Mungbean is cultivated all the year on all sides of peninsular India, and during Kharif, spring and summer seasons in north-India. Thus the aim of this study is to assess the impact of *Glomus mosseae* on plant development and photosynthetic pigments in mungbean.

Materials and Methods

Glomus mosseae culture was propagated and maintained in a pot, further it was inoculated in soil. Seeds of mungbean were shown in pre-inoculated and non-inoculated soil to assess the plant development and photosynthetic pigments in mungbean in the field condition.

Mycorrhizal culture and preparation of inoculum

Spores of *G. mosseae* were procured from CMCC (Centre for Mycorrhizal Culture Collection), The Energy and Resources Institute (TERI) New Delhi, India. *G. mosseae* culture was maintained following the trap culture method (Selvakumar *et al.,* 2016) in the pot using autoclaved (120 ºC, 0.2 M Pa, 20 min) sand and commercial peat soil (1:3 w:w) as the culture medium and wheat as the host plant. After the successful trapping process, the mixtures containing AMF spores, mycelium, sandy soil and mycorrhizal corm fragments were used as the inoculums. Every 100 g of the prepared inoculums had spores were inoculated and mixed with soil for a field trial during Rabi season in Ambikapur, Chhattisgarh, India

Plant material and growth conditions used to sterilize the soil

The effect of AMF was studied on three mungbean accessions MLT-11, MLT-12, IVT-29, which were obtained from Rajmohini Devi College of Agriculture and Research Station, Ambikapur, Chhattisgarh, India. Seeds were surface sterilized by fungicide carbendazim, and then it was sown in field following the standard agricultural practices for cultivation. The experiment was performed in mycorrhizal *G. mosseae* inoculated and non-mycorrhizal control, in case of mycorrhizal plant, 100 g of prepared inoculums *G. mosseae* were mixed with the soil.

Evaluation of *G. mosseae* root colonization

After seven days of growth, some experimental mungbean plants were harvested to evaluate *G. mosseae* root colonization. Separate samples of leaves, roots, and shoots were taken. To assess mycorrhizal colonization, sub-samples of fresh and healthy roots were obtained. Rinsed root samples were cleaned with 10% KOH at 90 ºC for 60 minutes, then it was stained in lactophenol cotton blue (LPCB) after soaking in 1% HCL for 5minutes (Phillips and Hayman, 1970). The proportion of each root segment's length, which contained any of the endophytic elements - hyphae, coils, vesicles and arbuscules were taken as evidence of mycorrhization.

Plant growth measurement

After 60 days of sowing, plant root length and height of all three selected mungbean accessions were measured with a tapeline and recorded in centimeter, roots and other sections of the plants were rinsed three times with de-ionized water, fresh weight of root biomass and plant was taken, and then dried in an oven at 70 ºC for 48 hours to a stable weight. In grams total root dry weights were calculated based on the percentage water content of total root fresh weight and remaining roots. For each treatment group, three separate plants were selected for the study.

Chlorophyll and carotenoid content

The amount of chlorophyll and carotenoids in the plants was determined using the method proposed by Lichtenthaler in 1987. In this process, 0.2 g of fresh leaves were weighed and afterwards grounded in pestle and mortar having 80% acetone. Then 5 mL acetone was applied, to bring the total volume of the solvent to 15 mL. Three milliliters of this solution were poured into a cuvette, and the absorption strength was measured at 470, 663, and 647 nm using a UV-visible Spectrophotometer (EI-1371) with 80% acetone as a witness. The pigment density of the plant extract was measured in milligrams per gram of fresh weight.

Statistical analysis

This experiment was performed in three replication based on a completely randomized design, and the data generated in the study was analyzed using two way ANOVA at 5% level of probability.

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After 60 days of sowing, root length and plant height of all three selected mungbean accessions were measured with a tapeline and recorded in centimeter, roots and other sections of the plants were rinsed three times with de-ionized water, fresh weight of root biomass and plant was taken, and then dried in an oven at 70 ºC for 48 hours to a stable weight. In grams, total root dry weights were calculated based on the percentage water content of total fresh root weight and remaining roots. For each treatment group, three separate plants were selected for the study.

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Results

Plant height

In this study, plant height was higher in arbuscular mycorrhizal fungi **(**AMF) *G. mosseae* mycorrhizal plants than in non- mycorrhizal (NM) mungbean plants. Plant heights in mycorrhizal plants were 50.334.72 cm in MLT-11, 46.6610.21 cm in MLT-12, and 39.337.32 cm in IVT-29, respectively, while non-mycorrhizal (NM) plants were 46.335.50 cm in MLT-11, 36.665.77 cm in MLT-12, and 33.53.5 cm in IVT-29 (Table 1). A considerable difference at $p \leq 0.001$ in plant height was recorded between non-mycorrhizal control and mycorrhizal plants (Fig.1). The symbiosis between mungbean plant and *G. mosseae* promoted plant height growth than in control, indicating a favorable and higher impact on mungbean plants.

Root length

Root length of three mungbean accessions was studied just after fruiting in non-mycorrhizal control and mycorrhizal plants. Root length in mycorrhizal (M) plants were 22.83±2.75 cm in MLT-11, 19.66±5.29 cm in IVT-29 and 19.16±1.60 cm in MLT-12 respectively, while in non-mycorrhizal control plants it was 19.66±4.72 cm in MLT-11, 18.16±0.28 in IVT-29 and 13.33±1.52 cm in MLT-12 (Table 1). A considerable difference at $P \leq 0.001$ in highest root length was observed between non mycorrhizal control and mycorrhizal plants. Among three mungbean accessions, the highest root length was recorded in all mycorrhizal (M) plants, which indicate that AMF *G. mosseae* showed the higher impact and positively enhanced the root length growth (Fig. 1).

Fresh plant, root and dry root biomass

In mycorrhizal plant, the maximum fresh plant biomass 17.54±1.08 g was observed in mungbean accession MLT 11, 10.2±4.61 g in MLT 12 and 8.85±4.48 g in IVT 29, which was significantly higher at $P \leq 0.001$ than in non-mycorrhizal control mungbean plant where it was 12.55±8.24 in MLT 11, 4.75±1.05 in MLT-12 and 3.54±1.02 g in IVT-29, respectively. Among the three mungbean accessions, the highest fresh plant biomass was observed in all mycorrhizal (M) plants, indicating that AMF; *G. mosseae* showed the higher impact and positively promoted total plant growth in mungbean plant. Mungbean root biomass, both fresh and dried, was recorded separately in mycorrhizal and non-mycorrhizal control. The maximum fresh root biomass 2.14±1.66 g was observed in n mungbean accession MLT 11, 0.72±0.18 g in MLT 12 and 2.29±1.60 g was observed in mycorrhizal IVT 29, which was significantly higher at $P \leq 0.001$ than in non-mycorrhizal control mungbean plant where it was observed 1.6.55±1.21 in MLT 11, 0.66±0.65 in MLT-12 and 0.91±1.35 g in IVT-29, respectively (Table 2). Similarly, the maximum dry root biomass 0.46±0.24 g was observed in mungbean accession MLT 11, 0.27±0.04 g in MLT 12 and 0.41±0.19 g was observed in mycorrhizal IVT 29, which was significantly higher at $P \leq 0.001$ than in non-mycorrhizal control mungbean plant where it was observed 0.39±0.32 in MLT 11, 0.23±0.14 in MLT-12 and 0.22±0.04 g in IVT-29, respectively. Among all the three mungbean accessions, the highest fresh and dry root biomass were recorded in all mycorrhizal (M) plants, which indicate that AMF; *G. mosseae* showed the higher impact and positively promoted fresh plant, root and dry root biomass in mungbean plant.

Chlorophyll and carotenoid content

Absolute chlorophyll, chlorophyll A, chlorophyll B, and carotenoid content of three mungbean accessions in mycorrhizal and non-mycorrhizal control mungbean plants were investigated before flowering. In mycorrhizal plants, absolute chlorophyll content was observed 11.37±3.21 mg g^{-1} FW in mungbean accessions MLT-11, 11.93±0.83 mg g^{-1} FW in MLT-12 and 9.98±0.00 mg g^{-1} FW in IVT-29 respectively, while in control, non-mycorrhizal plants it was observed 9.48±0.63 mg g^{-1} FW in MLT-11, 9.58±3.69 in MLT-12 and 7.90±0.12 mg g^{-1} FW in IVT-29 (Table 3). Similarly, in mycorrhizal plant chlorophyll A was measured 6.68±1.35 mg g^{-1} FW in mungbean accessions MLT-11, 7.4±0.21 mg g^{-1} FW in MLT-12 and 6.60±0.70 mg g^{-1} FW in IVT-29, respectively, while in control, non-mycorrhizal plants it was recorded 6.13±0.44 mg g^{-1} FW in MLT-11, 6.09±2.11 in MLT-12 and 5.35±0.09 mg g^{-1} FW in IVT-29. In mycorrhizal plant, chlorophyll B was measured 4.69±1.91 mg g^{-1} FW in mungbean accessions MLT-11, 4.53±0.67 mg g^{-1} FW in MLT-12 and 3.37±0.30 mg g^{-1} FW in IVT-29 respectively, while in control, non-mycorrhizal plants it was measured 3.34±0.24 mg g^{-1} FW in MLT-11, 3.49±1.58 in MLT-12 and 2.55±0.10 mg g^{-1} FW in IVT-29 (Table 3). Likewise, in the mycorrhizal plant, maximum carotenoid content 0.66±0.22 mg.g^{-1}FW was measured in mungbean accession MLT 11, 0.41±0.29 mg g^{-1} FW gm in MLT 12 and 0.67±0.10 mg g^{-1} FW was observed in IVT 29, which was significantly higher at $P \leq 0.001$than in non-mycorrhizal control mungbean plant where it was measured 0.36±0.73 in MLT 11, 0.26±0.10 in MLT-12 and 0.5±0.14 g in IVT-29, respectively. This study recorded rise in photosynthetic pigment *viz.* total chlorophyll, chlorophyll A, chlorophyll B, and

carotenoid concentration in mycorrhizal plants. The study revealed that symbiosis association of AMF fungus *G. mosseae* mycorrhiza promoted high chlorophyll and carotenoid content in mungbean plants.

Discussion

The present investigation assessed the effect of *G. mosseae* on root proliferation, biochemical content and plant growth of mungbean plants. During this investigation, all three mungbean accessions were colonized by AMF. In this investigation, the *G. mosseae* mycorrhizal plant indicated preferred plant development over the non-mycorrhizal plant. Mycorrhizal plants essentially expanded root length and plant tallness when contrasted with non-mycorrhizal plants. Comparatively, a similar finding is recorded in *Acorus calamus* (Yadav *et al*., 2011). Improved plant growth has been credited to better takes-up of Cu and P (Al-Karaki and Clark, 1998). The plants grown up with *G. mosseae* had significantly higher biomass at $P \leq 0.001$ than non-mycorrhizal plants (Table 2). The same expansion in plant biomass in the mycorrhizal plant is revealed in *Scutellaria integrifolia* (Joshee *et al*., 2007). The expanded development of mycorrhizal, plant in terms of plant tallness and root than that of non-mycorrhizal control plant may be because of the enhancement in the anabolic procedures, particularly photosynthesis, as a result of improved nutrient uptake and mobilization of different fundamental nutrients and water (Panwar, 1991). Root biomass was higher in both fresh and dried roots in *G. mosseae* mycorrhizal mungbean plant, respectively (Table 2). Furthermore, (Karthikeyan *et al.,* 2008) revealed that the inoculation of *G. mosseae* increased the root, fresh, and dry biomass of *Catharanthus roseus*. Likewise, Karthikeyan *et al*. (2009) reported that AMF *G. Fasciculatum* increased the dry root biomass in many medicinal plants. Gupta and Janarthanan (1991) have reported a similar finding who recorded increased biomass in Palmarosa plant inoculated with *Glomus* species. This finding was further confirmed by Gogoi and Singh (2011); according to them, the inoculation of *Glomus* species significantly increased both fresh and dried root biomass of *Piper longum*. In *Citrus tangerine* and *Poncirus trifoliate,* mycorrhizal plants increased plant height, shoot and root dry weights have also been recorded (Wu and Xia, 2006). This indicates that AMF colonization and the development of external mycelium were responsible for the rise in fresh and dry root biomass of mycorrhizal plants.

The overall chlorophyll, chlorophyll A, chlorophyll B, and carotenoid content of *G. mosseae* mycorrhizal associated mungbean plants increased significantly than in non-mycorrhizal plants (Table 3). Similarly, mycorrhizal plants' chlorophyll content was found to be higher than non-AM plants (Mathur and Vyas, 1995). Moreover, the rise in photosynthetic pigments due to mycorrhizae has been confirmed by Abdelmoneim *et al*. (2014). Our findings indicate that mycorrhizal plants have slightly higher chlorophyll and carotenoid content than non-mycorrhizal plants at $P \leq 0.001$. A related pattern was discovered by (Sanchez-Blanco *et al*., 2004), who observed increased total carotenoid and chlorophyll content in mycorrhizal plants than in control non-mycorrhizal plants.

Similarly, a typical high concentration of chlorophyll in mycorrhizal plants have been reported by many workers, and they have advocated that colonization of arbuscular mycorrhizal roots increased chlorophyll synthesis, which could lead to increased photosynthesis rates and plant development (Davies *et al*., 1993; Mathur and Vyas, 1995). These results are in line with the findings previously reported by Sharma *et al*. (2008) where the enhanced total chlorophyll content was recorded in *G. mosseae* inoculated plants. Previous research has shown that compared to non-AM plants, mycorrhizal plants had higher chlorophyll content (Mathur and Vyas, 1995). Moreover, the rise in photosynthetic pigments linked to a faster photosynthesis process due to mycorrhizae has been confirmed by Davies *et al*. (1993) and Abdelmoneim *et al.* (2014). An elevated concentration of chlorophyll in mycorrhizal plants is associated with a higher rate of photosynthesis or higher Mg and N, which are the main constituents of chlorophyll (Mathur and Vyas, 1995). However, the accumulation of chlorophyll and carotenoids due to this positive plant-fungus relationship needs further investigation.

Conclusion

Mycorrhizal symbiosis association significantly increased the plant's root growth, height, fresh and dry weights, carotenoid, and chlorophyll contents. As the photosynthetic rate is related to chlorophyll content and plant growth, thus it may be concluded that a symbiotic association of *G. mosseae* and mungbean may be potentially significant and promising for sustainable cultivation of mungbean.

Table 1: Total plant height and root length of non-mycorrhizal and mycorrhizal mungbean plant

S. No.	Plant Accession No.	Non-mycorrhizal		Mycorrhizal	
		Plant height (cm)	Root length (cm)	Plant height (cm)	Root length (cm)
1	MLT 11	46.33±5.50	19.66±4.72	50.33±4.72	22.83±2.75
2	MLT 12	36.66±5.77	13.33±1.52	46.66±10.21	19.16±1.60
3	IVT 29	33.5±3.5	18.16±0.28	39.33±7.32	19.66±5.29

Table 2: Fresh plant, root and dry root biomass of non-mycorrhizal and mycorrhizal mungbean plant

S. No.	Plant Accession No.	Non-mycorrhizal			Mycorrhizal		
		Fresh plant biomass (g)	Fresh root (g)	Dry root (g)	Fresh plant biomass (g)	Fresh root (g)	Dry root (g)
1	MLT 11	12.55 ±8.24	1.6 ±1.21	0.39 ±0.32	17.54±1.08	2.14±1.66	0.46±0.24
2	MLT 12	4.75 ±1.05	0.66±0.65	0.23 ±0.14	10.2±4.61	0.72±0.18	0.27±0.04
3	IVT 29	3.54 ±1.02	0.91±0.35	0.22 ±0.04	8.85±4.48	2.29±1.60	0.41±0.19

Data are the mean value of three replicates which was repeated thrice. ± standard deviation

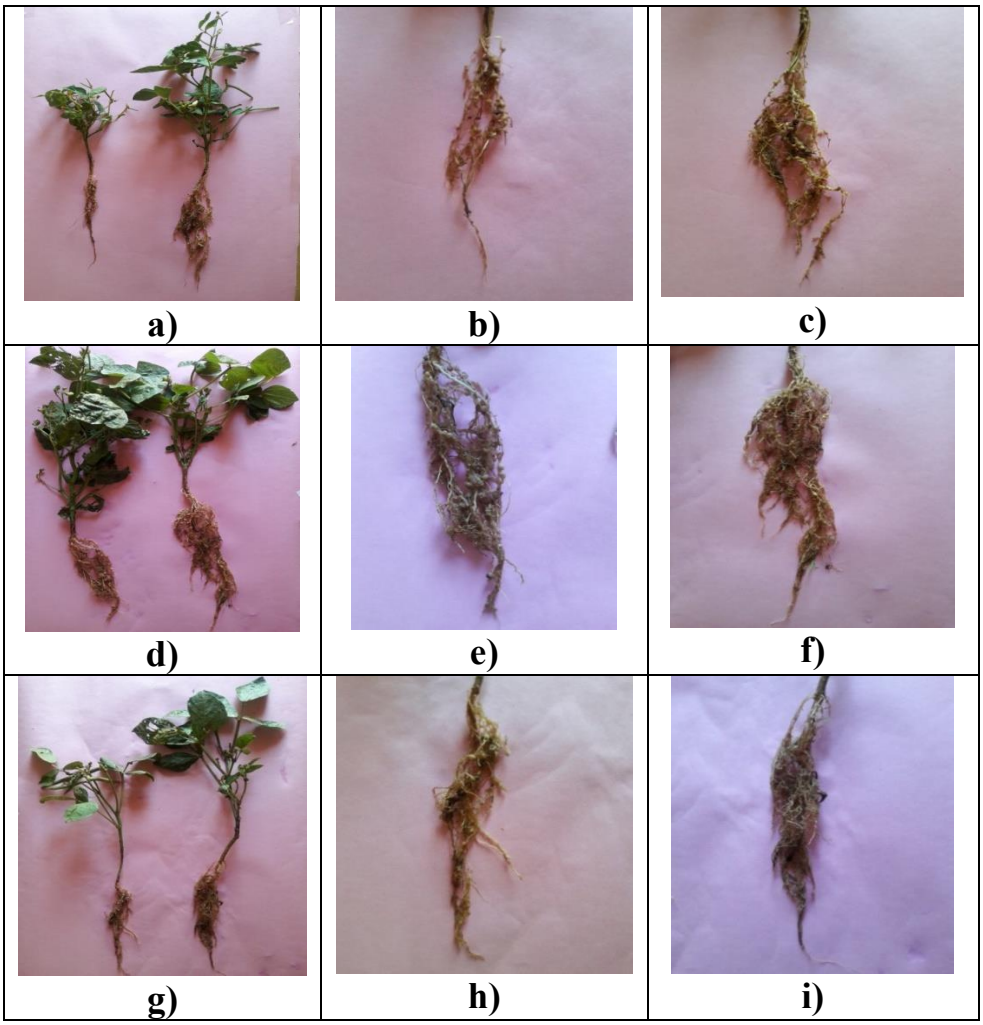

Fig. 1: Total plant and root growth pattern of different mungbean accessions in AMF mycorrhizal (M) and non-mycorrhizal (NM) soil; a) total plant growth of accession IVT-29, b) root profile of non-mycorrhizal (NM) IVT-29, c) root profile of -mycorrhizal (M) IVT-29, d) total plant growth of variety MLT-11, e) root profile of non-mycorrhizal (NM) accession MLT-11, f) root profile of mycorrhizal (M) variety MLT-11, g) total plant growth of accession MLT-12, h) root profile of non-mycorrhizal (NM) accession MLT-12, i) root profile of mycorrhizal (M) variety MLT-1

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