Impact of mycorrhizal fungus *Glomus mosseae* on plant growth and photosynthetic pigments in black gram

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Abstract

The aim of this study was to evaluate the impact of arbuscular mycorrhizal (AM) fungus *Glomus mosseae* Gerd. and Trappe on plant growth and development, including root proliferation, chlorophyll, and carotenoid content in three black gram (*Vigna mungo* L.) accession NULL-7, MLT-9, and MLT-10. The study revealed that the *G. mosseae* inoculated mycorrhizal plants had the comparatively higher percentage of mean plant height 9% to 31.25% root length 15.64% to 28.83% plant fresh biomass 18.20% to 29.70%, root biomass 18.44 to 25.53, total chlorophyll 8.23% to 10.43%, and carotenoid levels 24.05% to 33.33%, chlorophyll A 7.74% to 22.88 % and Chlorophyll B 8.16% to 19.01% as compared to non-mycorrhizal control plants. The present findings suggest that symbiotic association between *G. mosseae*, and *V. mungo* have substantially affected various plant growth parameters and photosynthetic pigments, which might be important and promising for long-term black gram production.

Keywords: Arbuscular mycorrhizal fungi, Black gram, *Glomus mosseae*, Mycorrhizal plants, Photosynthetic pigments.

Introduction

Pulses are an integrated part of many diets across the globe, and they have great potential to improve human health, conserve our soil, protect the environment and contribute to global food security (Banaras *et al.,* 2020; FAO, 2020). Black gram (*Vigna mungo* L.), also known as uradbean, is a dicotyledonous, annual major pulse crop in India, Pakistan, and other south Asian countries (Bisht *et al*., 2005; Javaid, 2009). It is a rich source of vitamin B, protein, calcium, iron, potassium, thiamine, niacin, riboflavin, and essential minerals such as Na, K, Mg, and P (Tresina *et al*., 2005).

In several crops, plant-mycorrhizal symbiotic association positively stimulated the plant growth and metabolites related to plant development (Javaid *et al.,* 1993; Bajwa *et al.,* 2000). Soil microorganisms such as AM fungi represent a key link between plants and soil mineral nutrient exchanges. Thus, they are gaining importance as natural fertilizers. AM fungi are obligate symbionts belonging to the phylum Glomeromycota (Schußler *et al*., 2001), which form mutuality symbioses with about 80% of land plant species, including several crops (Brundrett, 2009). AM fungal hyphae exclusively colonize the root cortex and form highly branched structures inside the cells, i.e., arbuscules, which are considered the functional site of nutrient exchange (Balestrini *et al*., 2015). When the plant and the fungus come into contact, arbuscules and vesicles are formed at the proximal end of the hyphae by endomycorrhizal fungi, then inside the roots across the interface mineral nutrients, and carbohydrates are exchanged between the symbiotic partners (Smith and Read, 2008; Allen, 2011).

It has recently been suggested that in natural environments, a non-mycorrhizal condition should be viewed as abnormal for most species (Berruti *et al.,* 2016). However, there is a marked diversity among AM fungal communities below ground, depending on plant species diversity, soil type, season, or a combination of these factors (Smith and Smith, 2012; Javaid and Khan, 2019). In addition to an improved nutritional supply, AM interactions provide other benefits to plants, such as enhanced growth and development (Kumar *et al.,* 2020), drought and salinity tolerance (Auge *et al*., 2015), and disease resistance (Pozo and Azcon- Aguilar, 2007). Therefore this study aims to evaluate the impact of *G. mosseae* on plant growth parameters and photosynthetic pigments in black gram.

Materials and Methods

Mycorrhizal culture and inoculums preparation

The fungal spores of *G. mosseae* were procured from CMCC (Centre for Mycorrhizal Culture Collection), The Energy and Resources Institute (TERI), New Delhi, India. The pure culture of *G. mosseae* was inoculated in the mixture of sterile sand and soil in the ratio of 1:3. Wheat was chosen as a host plant for inoculums preparation,

wheat was grown in the mixture for two months. After that, these preparations were used as source inoculums for field trials during Rabi season in farmers' agriculture field in Ambikapur, Chhattisgarh, India.

Plant material and growth conditions

The impact of AMF was investigated on three black gram accessions *viz.* NULL-7, MLT-9, and MLT-10, acquired from Rajmohini Devi College of Agriculture and Research Station, Ambikapur, Chhattisgarh, India. According to conventional agricultural methods, healthy seeds were collected and surface sterilized with the fungicide thiram before planting in the field. The experiment was conducted using *G. mosseae* inoculated plants and non-mycorrhizal controls. For the mycorrhizal plants, 100 g of prepared *G. mosseae* inoculum was mixed with the soil. Then black gram seeds were shown in the field, a 5 cm distance between plant to plant and 25 cm between row to row, were maintained, and 25 seeds were shown in a row of each accession. Mycorrhizal and non-mycorrhizal plants were grown in three replications to evaluate plant growth and photosynthetic pigments in the field for each accession.

Assessment of *G. mosseae* root colonization

Some experimental black gram plants were collected after seven days of growth to assess *G. mosseae* root colonization. Leaves, shoots, and roots were sampled separately. Sub-samples of healthy and fresh roots were collected to evaluate mycorrhizal colonization. Root samples were washed and cleaned with 10% KOH at 90 °C for 60 minutes before dyeing with lactophenol cotton blue after soaking in 1% HCl for 5 minutes (Phillips and Hayman, 1970). Mycorrhization was evaluated by the fraction of each root segment's length that included the endophytic components hyphae, coils, vesicles, and arbuscules.

Measurement of plant growth

Plant height and root length of all the three black gram accessions NULL-7, MLT-9, and MLT-10 were evaluated with a tapeline and noted in centimeters after 60 days of sowing. Roots and other portions of the seedlings were thoroughly rinsed with de-ionized water, fresh and dry weight of root biomass, and the plant was taken and then dried in an oven at 70 ^{o}C for 48 hours to a stable weight. Three different plants were chosen for each treatment group in the study.

Chlorophyll and carotenoid content

The amount of chlorophyll and carotenoids in all the black gram accessions was measured using Lichtenthaler's technique (1987). For this, we weighed 0.2 g of fresh leaf material and then crushed in acetone-soaked mortar pestles. Then 5 mL acetone was added, bringing the total volume of the solution to 15 mL. For this investigation, 3 mL of the solution were put into a cuvette, and the absorption intensity was examined using a spectrophotometer at 470, 647, and 663 nm. For this experiment, 80% acetone was used as a reference. The pigment concentration of the plant extract was determined in mg g^{-1} of leaf fresh weight.

Statistical analysis

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

Results and Discussion

Plant growth and development

AM fungal inoculation increased plant growth and development; in three black gram accessions NULL-7, MLT-9, and MLT-10 increased percentage of mean plant length obtained were 9% to 31.25% and root length were 15.64% to 28.83% in mycorrhizal plants as compared to non-mycorrhizal plants (Table 1, Fig. 1.). These findings were in line with those of Ramakrishnan and Selvakumar (2012). According to their results, the shoot and root length of plants inoculated with *G. mosseae* was much longer than control. AM fungi treatment enhanced the overall plant growth (Bass and Kuiper, 1989), encouraging cell proliferation, development, and playing an important role in shoot developmental morphology. The findings of this study demonstrate that AM fungus had a substantial impact on growth parameters. This study supports the results of Moradi *et al.* (2013), who revealed that the application of *G. mosseae* significantly enhanced root length. The increased plant growth has been attributed to the improved Cu and P uptake (Al-Karaki and Clark, 1998; Javaid *et al.,* 2009b). The increased growth of the mycorrhizal plant in terms of plant heights and roots compared to the non-mycorrhizal control plant could also be due to an increase in anabolic processes, especially photosynthesis, due to enhanced nutrient uptake and mobilization of various fundamental nutrients and water (Begum *et al*., 2019).

The root biomass of *G. mosseae* mycorrhizal black gram plants was increased in both fresh and dried roots. In all three black gram accessions, the increased percentage of mean fresh plant biomass was 18.20% to 29.70%); root biomass was 18.44 to 25.53 in mycorrhizal plants compared to non-mycorrhizal plants (Table 2). Similarly, Gupta and Janarthanan (1991) observed that *G. aggregatum* treatment enhanced biomass in *Palmarosa*. Likewise, Gogoi and Singh, 2011 reported that *Piper longum* plant inoculated with sps, *G. fasciculatum*, and *G. clarum* had increased fresh and dry root weight. The

root biomass differed between three different black gram accessions NULL-7, MLT-9, and MLT-10, probably due to AM fungi host choice (Sailo and Bagyaraj, 2005). *Scutellaria integrifolia* shows the same increase in plant biomass as the mycorrhizal plant (Joshee *et al*., 2007). In addition, (Karthikeyan *et al*., 2008) have observed that inoculating *Catharanthus roseus* with *G. mosseae* enhanced the plants' roots, fresh, and dry weight. AM fungi *G. fasciculatum* also boosted the dry root biomass in numerous medicinal plants, according to Karthikeyan *et al.* (2009). This shows that an increase in AM inoculated plants' fresh and dry weight was attributable to the establishment of external mycelium. A similar finding was reported by Gupta and Janarthanan (1991), who observed enhanced biomass in *Palmarosa* plants infected with *Glomus* species. Mycorrhizal plants enhanced plant height, shoot and root dry masses in Citrus tangerine and *Poncirus trifoliate* (Wu and Xia, 2006). Gogoi and Singh (2011) validated this finding, claiming that inoculating *Piper longum* with *Glomus* species greatly enhanced fresh and dried root biomass.

Chlorophyll and carotenoid content

Before flowering, the absolute chlorophyll, chlorophyll A, chlorophyll B, and carotenoid content of three black gram accessions were measured in mycorrhizal and non-mycorrhizal control black gram plants. The increased percentage of mean absolute chlorophyll content in mycorrhizal plants was 8.23% to 10.43%, and carotenoid levels 24.05% to 33.33 %, in all three mycorrhizal accessions as compared to control non -mycorrhizal plants (Table 3). Similarly, the mean increased percentage of chlorophyll A was recorded 7.74% to 22.88 %, and chlorophyll B was recorded 8.16 % to 19.01%, in mycorrhizal plants compared to control non-mycorrhizal plants (Table 4). The relationship between chlorophyll content and photosynthetic rate and fluorescence of chlorophyll has long been known. Thus, higher chlorophyll levels in mycorrhizal plants have been linked to faster photosynthetic rates or higher Mg and N levels, both of which are important components of chlorophyll (Mathur and Vyas, 1995). Sanchez-Blanco *et al*. (2004) found a similar pattern, with increased total carotenoid and chlorophyll content in mycorrhizal plants compared to non-mycorrhizal plants. Many researchers have documented high chlorophyll concentrations in mycorrhizal plants. They have suggested that colonization of arbuscular mycorrhizal roots improved chlorophyll biosynthesis, contributing to higher photosynthesis rates and plant growth (Davies *et al*., 1993). These findings were consistent with Sharma *et al*. (2008) findings which showed that *G. mosseae* infected plants had higher total chlorophyll content. According to a previous study, mycorrhizal plants showed increased chlorophyll concentration than non-AM plants (Mathur and Vyas, 1995). Davies *et al.* (1993) and Abdelmoneim *et al*. (2014) both demonstrated that mycorrhizae cause an increase in photosynthetic pigments, which is associated with faster photosynthesis in plants. A better rate of photosynthesis or higher Mg and N, which are the major ingredients of chlorophyll, are connected with higher chlorophyll concentrations in mycorrhizal plants (Mathur and Vyas, 1995). Similarly, the chlorophyll content of mycorrhizal plants was shown to be higher than that of non-AM plants (Mathur and Vyas, 1995). The findings revealed that black gram plants with a symbiotic relationship with the AM fungi *G. mosseae* mycorrhiza had higher chlorophyll and carotenoid content. Enhanced chlorophyll production has also been linked to increased photosynthesis or higher N and Mg, which is essential chlorophyll elements, in AM incubated, plants (Mathur and Vyas, 1995).

Conclusion

The current findings revealed a symbiotic relationship between black gram and *G. mosseae* promoted the growth parameters and photosynthetic pigments in the host plant. The use of AM fungus *G. mosseae* as biofertilizers to promote soil fertility and plant nutrient uptake might be economical and environment-friendly. Thus, it may be concluded that a symbiotic relationship between *G. mosseae* and black gram could be significant and promising for sustainable black gram cultivation.

Table 1: Plants length and root length of mycorrhizal and non-mycorrhizal black gram plants.

S. No.	Plant Accession	Non-mycorrhizal		Mycorrhizal	
		Plant height (cm)	Root length (cm)	Plant height (cm)	Root length (cm)
1	NULL-7	33.66±5.50	15.53±1.36	39±1	17.96±0.251
2	MLT -9	32±3	14.5±2.5	42±7.36	18.33±6.11
3	MLT-10	33+5.1	12±1	36±3.6	15.46±3.93

Table 2: Total fresh plants and root biomass of mycorrhizal and non-mycorrhizal black gram plants.

S. No.	Plant Accession	Non-mycorrhizal		Mycorrhizal	
		Total plant biomass (g)	Root biomass (g)	Total plant biomass (g)	Root biomass (g)
1	NULL-7	3.03±0.45	0.49±0.32	3.93±0.57	0.60±0.09
2	MLT -9	3.57±0.54	0.45±0.16	4.22±0.61	0.81±0.13
3	MLT-10	3.78±0.08	0.47±0.12	4.56±0.39	0.59±0.06

Table 3: Total chlorophyll and carotenoids (mg g^{-1} FW) content of mycorrhizal and non-mycorrhizal black gram plant.

S. No.	Plant Accession	Non-mycorrhizal		Mycorrhizal	
		Total chlorophyll	Carotenoids	Total chlorophyll	Carotenoids
1	NULL-7	11.90±1.12	0.79±0.20	12.88±1.19	0.98±0.25
2	MLT -9	12.07±0.44	0.72±0.18	13.33±0.66	0.96±0.13
3	MLT-10	10.75±0.74	0.82±0.44	11.78±0.87	1.07±0.17

Table 4: Chlorophyll A and Chlorophyll B (mg g^{-1} FW) of mycorrhizal and non-mycorrhizal fresh black gram plant.

S. No.	Plant Accession	Non-mycorrhizal		Mycorrhizal	
		Chlorophyll A	Chlorophyll B	Chlorophyll A	Chlorophyll B
1	NULL-7	7±0.98	4.90±0.20	7.76±1.04	5.30±0.25
2	MLT -9	7.36±0.58	4.71±0.16	7.93±0.61	5.10±0.85
3	MLT-10	6.51±0.45	4.05±0.97	8.00±0.97	4.82±0.93

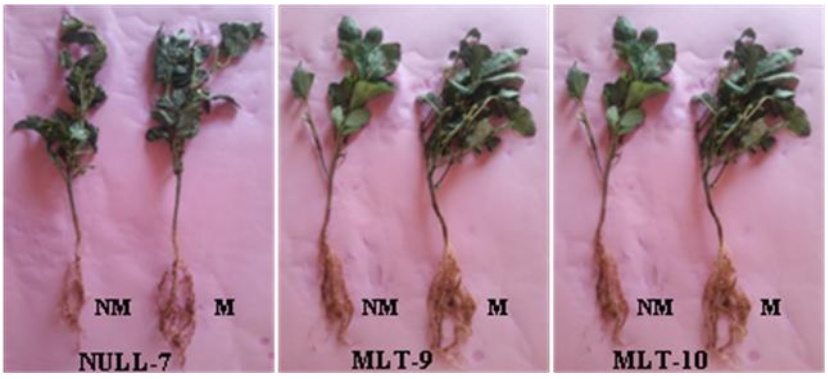

Fig. 1: Non-mycorrhizal (NM) and mycorrhizal (M) plant and root growth pattern of different black gram accessions a) NULL-7, b) MLT-9, c) MLT-10.

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