Diversity of Arbuscular Mycorrhizal Fungi on Soybean [*Glycine max* (L.) Merr.] Cultivars KDS 726

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Abstract: The present study deals with the diversity of arbuscular mycorrhizal fungi on Soybean (*Glycine max*) Cultivars KDS 726 from five different localities i.e., Naldurg, Lohara, Tuljapur, Solapur & Omerga Percentage of root colonization varied by ranging (25±1.01) to (88±2.33%). The highest root & root length colonization was found in the Naldurg site while the lowest was in Lohara. Omerga site found Arbsucules, vesicles and hyphal types of colonization as compared to others. Spore density ranged from (326.66±0.11) to (492±2.11/100g) rhizosphere soil. Maximum spore density was found in the Naldurg site (492/100g soil) while minimum in Solapur (326.66±0.11/100g soil). Data of AM fungal spores were analyzed based on count morpho-taxonomy of AM fungi, percentage and root colonization. The data also indicated that *Acaulospora undulata, Acaulospora delicata, Glomus macroaggregatum, Glomus microaggregatum, Glomus funneliformis, Gigaspora sp.* and were found frequently, but *Glomus* genera were dominant. *Glomus macroaggregatum* (80%) was found highest frequency as compared to other species. Both relative abundance (%) and frequency (%) of occurrence were found to maximum *A. delicata* i. e., 42.85% and 80% respectively. *Gi. margarita* shows the lowest RA & F (28.57%) and (40%) respectively. The present study shows that variation controls and correlation between AMF infection and its spore density. In physiochemical parameters, it was observed the soybean varity KDS-726 in pH, EC and Na & slight varying as per the slandered values. But P, Ca, Mn, Zinc, $CaCo_3$, Mn, S, B was found high whereas N and Fe found least in the deposited soil.

Keywords: *Glycine max*, Cultivars KDS 726, Chemical analysis, rhizosphere soil, Myorrhizal fungi, Root colonization, Spore density, localities.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill.) occupies the highest position among crops, being the most important source of good quality concentrated proteins as well as vegetable oil. Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that form a symbiotic relationship with 80–90% of vascular plant species and 90% of agricultural plants Smith and Read (2010). Seeds of soybean have been used in Asia and other parts of the world for many centuries to prepare a variety of fresh, fermented and dry foods (Probst and Judd, 1973). Soy-based beneficial nutritious food products such as tofu, soy milk, soy sauce, miso, etc. have been developed for human feeding while oil-extracted soy meal is used as nutritious animal fodder. As a legume crop, soybean is capable of using atmospheric nitrogen through biological nitrogen fixation and is therefore less dependent on synthetic nitrogen fertilizers. Keeping in view its vast utilities, there is ample justification for its significant contribution to major crop improvement programs throughout the world. Soybean growth is strongly influenced by texture, structure, consistency, porosity, density and soil temperature (Furseth et al., 2012).

AM fungi increase soil binding capacity and act as a biofertilizer. Legumes relatively have a "P" requirement for nodule development and nitrogen fixation. (Bagyaraj et al. 1979; Gupta and Mukerji, 2006; Harley and Smith, 1983; Harikumar, Lakshman et al. 2006; Manimegalai et al. 2011; Suresh and Selvaraj, 2006). Arbuscular mycorrhizae (AM) are symbiotic associations between plants' roots and soil fungi that play an essential role in plant growth, plant protection, and soil quality. The AM fungi grow their filaments in soil and plant roots. These filamentous networks promote bi-directional nutrient movement where soil nutrients and water move to the plant and plant photo-synthases flow in the fungal network. The effectiveness of mycorrhiza in improving plant growth appears to be governed by the interplay between edaphic factors, the host plant, edaphic factors, and the fungi isolate (Bethlenfalvay, Ulrich and Brown, 1985; Hall, 1988).

Soil provides the medium for the production of plant biomass for use as food, feed and fibre. The capacity of soil to supply sufficient quantities and proportions of essential chemical elements (nutrients) and water required for optimum growth of specified plants as

governed by the soil's chemical, physical and biological attributes. Soil is the foundation of an agricultural field and mediates processes essential to the functioning of the system, including the biogeochemical cycling of elements such as carbon and other mineral nutrients; provision of habitat for soil organisms; movement, storage, and decontamination of water; and promotion of plant growth Brady, and Weil (2002).

MATERIALS AND METHODS

Study Site and Rhizosphere Sample Collection

The study was carried out on soybean crop plants during the Kharif or monsoon, season (July - October) from agricultural land of Osmanabad and different localities of the Marathwada region of Maharashtra. annual average rainfall of 679 mm in Marathwada, the region has so far received 840 mm this monsoon. Rhizosphere soil samples of soybeans were usually collected from the 5 different study sites in the Osmanabad district i.e., Naldurg, Lohara, Tuljapur, Solapur and Omerga.

Assessment of arbuscular mycorrhizal status

Root colonization:

Soybean was collected from the 5 different study sites of the Osmanabad district in rainy seasons i.e., monsoon, etc. The secondary root of the Soybean was washed with clean water and removed soil debris and stored in FAA solution (Formalin-Acetic Alcohol). During the study of root assessment, the root was washed 3-4 times to remove the FAA from the root. 20-30 segments of root cut into the 2-3 cm in length, root boiled into the 10% KOH. The root sample wash with sterilized distilled water till to the brown colour of the boiled root changed to colourless. The root was placed into the 5% of HCL for 3-4 min to improve the root staining adeptness. After the HCL treatment root was washed with sterile distilled water 4-5 times and placed into 0.05% of trypan blue stain overnight (12 hours). For making the slide firstly remove the excess stain 4-5 times and wash it with distilled water. The roots were observed in the compound microscope and recorded the results and photographed photograph click with a Sony digital camera (DSC-W310/BC E37) **(**Phillips et. al., 1970). The mycorrhizal infections like arbuscules, hyphae and vesicle colonization were recorded and calculated % root colonization, and % root length colonization by using the formula and photography.

$$\% \text{ Root Colonization} = \frac{\text{Total no of root segments colonized}}{\text{Total no of root segments examined}} \text{x100}$$

Isolation of AM fungal Spores from Soil

Soybean was collected from the 5 different study sites of the Osmanabad district in monsoon seasons. Numerous techniques have been available to recover AM fungi spores from the soil. The basis of this is wet sieving and decanting, which remove the clay, sand and organic matter fractions while retaining spores and other similar-sized soil particles on sieves of numerous with stainless steel mesh (35, 63, 125,150 212 and 355μm). For the isolation, 100g of soil was weighed and added to 1000 ml of water taken in a conical flask. Then the flask was shaken well in a vortex mixture and allowed to sediment for a few seconds and was immediately transferred to a series of sieves. The jar was washed twice with water and added to the sieve's series. This sieving was collected in respected jars by washing them with water. Then the sieving was transferred onto a gridded Petri plate and observed under the binocular microscope 400X (Lawrence and Mayo LM-52-3521). The number of spores was counted and expressed as several spores/100g of soil sample. These isolated spores were picked up using a micropipette and were mounted in Poly Vinyl Lacto Glycerol (PVLG) to make permanent slides and a photography Mobile camera.

Physicochemical parameters of soil

Physico-chemical analysis of infected rhizosphere soil was collected from the study area and used for physicochemical characterization. The soil was spread out on a tray for air drying sieved over 150 mm and used for characterization. Each sample is balanced using a digital balance. The samples were then oven-dried at a temperature of 110˚C for 24 hours and reweighed. The electrical conductivity and pH of compost were measured (Subbiah and Asija, 1956). Nitrogen content was determined by the Kjeldahl method (Sahilemedhin and Bekele, 2000). Organic Carbon was evaluated (Walkley and Black, 1934) technique by oxidizing organic carbon with potassium dichromate and sulphuric acid. Phosphorus in soil was determined by the Olsens method by using a spectrophotometer (Olsen et al., 1954; Bray and Kurtz, 1945). Water soluble and exchangeable Potassium was calculated by the Ammonium acetate method (Hanway and Heidel, 1952) using a Flame photometer. Sodium, Calcium

and Magnesium cations were estimated by EDTA titration (GOI, 2011). Analysis of Ferrous, Manganese, Copper, Boron, Sulphur, Zinc and Molybdenum was done by acid digestion of soil (Jackson, 1967).

Identification of spore

Identification of AM fungal spore was carried out by using INVAM International Collection of Vesicular Arbuscular Mycorrhizal morpho taxonomic criteria and mycorrhizal manuals (Schencket. et al., (1990) and Rodrigues et. al., 2009). Spore density and spore diversity were recorded. According to Schenck and Pérez (1990), Walker (1983); Schußler (2000); Mosse and Bowen 1968; Koske et al. 1986; Muthukumar et al. (2005); Bukhari and Rodrigues (2006) and culture database established by International Collection of Vesicular Arbuscular Mycorrhizal (http://invam.wvu.edu/the-fungi) fungi Glomalean spores were identified.

Relative abundance and frequency of occurrence of AM fungi

AM Fungi spore population was recorded as well and relative abundance and frequency of occurrence were also calculated by using the following formula given by Giovannetti and Mosse (1980).

$$\text{RA (\%)} = \frac{\text{Number of AM fungal spores of a particular species}}{\text{Toal number of AM fungi spore in species}} \text{ x } 100$$

$$\text{F (\%)} = \frac{\text{Number of soil samples possessing spores of a particular AM species}}{\text{Total number of soil samples analysed}} \text{ x } 100$$

Statistical Analyses

All data were statistically analysed and the importance of variances was determined by using a book (Mungikar, 1990).

RESULTS AND DISCUSSION

Physicochemical parameter:

Rhizosphere soil of Soybean cultivars kDS-726 examination of a few sites revealed that it influences water retention, nutrient retention, and soil aeration. Soil fertility varies from site to site and depends on the microbiota. pH, EC, OC, N, P, K, Na, Caco3, Fe, Mn, Zn, Cu, S, and B parameters were studied from the rhizosphere. The pH of the soil is basic2 in nature (7.97). Electric conductivity is moderate (0.47 ds/m^2) and lowest (0.13), respectively. The organic carbon (OC) rhizosphere shows the highest appearance (1.71%) and moderate appearance (0.30%). Nitrogen, phosphorus, and potassium, which are important factors for AMF development, are deficient in renovated fields. The zinc standard is 0.6 to 1 to moderate 1.27. Ferrous and copper were last increased in renovated field soil in the rainy season. Nitrogen has a standard of 125 g/ha (moderate); phosphorus shows 20.60 g/ha; and moderate in the other 15 to 21. Potassium occurs at a maximum very high (768 kg/ha) standard value. The sodium content in the soil during the rainy session (2.14 mmol/L) was higher than the standard value of more than 1. Calcium carbonate ($CaCo_3$) was moderately present in seasons 11.27%, more than 6 to 10, respectively. Iron content is the lowest season. The manganese (12.30 ppm) there is slandered. The zinc (Zn) is 1.27ppm other than the standard highest (0.6 to 1ppm), (Copper (Cu) content was 0.84 highest than 0.2 to 0.4. Sulphur and Boron were absent in all three seasons. Sulphur was the very highest (54.30 ppm) among the standard values (10 to 15 ppm) and boron was 1.08 ppm.

Root colonization and Root length colonization:

The results in percentage (%) of the root colonization and percentage (%) of root length colonization were analyzed from soybeans collected from the different five study sites (Table 1). The highest % root colonization and spore density were recorded in Naldurg as well as the lowest in other study locations. The highest root colonization was found in the Naldurg site while the lowest was in Lohara. The maximum % of root colonization recorded in Naldurg was 88±2.33 and the Lowest % of root colonization found in Lohara was 25±1.01. The highest root length colonization was the maximum % of Naldurg site while the lowest was in Naldurg was 47.2±4.00 and the lowest % of root length colonization was in Lohara is 25±2.10. Root colonization vesicular, arbuscular and hyphal were recorded in this season (Table 2 & 3) In this monsoon season maximum % root length colonization the recorded in Naldurg (47.2±4.00) and the minimum in Lohara (25±2.10) (Fig. 2).

AM fungi spore density

Investigating study of AM fungi Spore density ranged from (326.66±0.11) to (492±2.11/100g) rhizosphere soil. Maximum spore density was found in the Naldurg site (492/100g soil) while minimum in Solapur (326.66±0.11/100g soil). Data of AM fungal spores were analyzed based on count morpho-

taxonomy of AM fungi, percentage and root colonization. The data also indicated that *Acaulospora undulata, Acaulospora delicata, Glomus macroaggregatum, Glomus microaggregatum, Glomus funneliformis, Gigaspora sp.* and were found frequently, but *Glomus* genera were dominant. *Glomus macroaggregatum* (80%) was found highest frequency as compared to other species. Both Relative abundance (%) and Frequency of occurrence (%) maximum observed in *A. delicata* i.e., 42.85 and 80 respectively. *Gi. margarita* show the lowest (%) RA, (%) F (28.57) and (40) respectively. The present study shows that variation controls the % infection and % spore population (fig. 3)

Table-1. Physico-chemical Analysis of rhizosphere soil soybean cultivars KDS-726.

Sr. No.	Parameters	Standard Values ±	Values ±
1	pH	6.1 to 8.5	7.97
2	EC (dS/m)	Less than 1	0.43
3	OC (%)	0.41 to 0.60	1.71
4	N (kg/ha)	281 to 420	125
5	P (kg/ha)	15 to 21	20.60
6	K(kg/ha)	151 to 200	768
7	Na(meq/L)	More than 1	2.14
8	Caco3	6 to 10	11.27
9	Fe(ppm)	4.5 to 10	1.05
10	Mn(ppm)	2 to 5	12.30
11	Zn(ppm)	0.6 to 1	1.27
12	Cu(ppm)	0.2 to 0.4	0.84
13	S(ppm)	10 to 15	54.30
14	Br(ppm)	0.4 to 0.7	1.08

Table 2: Occurrence of Different Soybean (*Glycine max* L. Merr.) KDS-726 Variety.

Sr./No.	Location	Altitude	Latitude
1.	Naldurg	17.838073	76.252052
2.	Lohara	17.902947	76.361791
3.	Tuljapur	18.04651	76.083359
4.	Solapur	17.825449	75.819302
5.	Omerga	17.843552	76.587629

Table 3: Status of percentage (%) of AMF root, root length and type of root colonization.

Sr./No.	Location	AMF Root colonization(n*=25)		
		Rc %	RLc %	Types of Colonization
1.	Naldurg	88±2.33%	47.2±4.00	Vesicles, Arbuscules & Coiled Hyphal
2.	Lohara	25±1.01	25±2.10	Hyphal
3.	Tuljapur	48±2.20	31±2.02	Vesicles, & Coiled Hyphal
4.	Solapur	72±1.12	47.2±1.03	Vesicles & Arbuscules
5.	Omerga	72±0.20	45.6±2.01	Vesicles, Arbuscules & Hyphal

Legends: Values are means of three replications, ±-Standard error, RC- root colonization, RLC- Root length colonization

Table 4: AM fungal spore population rhizosphere of Soybean [*Glycine max* (L.) Merr.] from the different study sites.

Sr./No.	Location (Cultivars Sites)	Spore number /100gm Rhizosphere soil
1.	Naldurg	492±2.11
2.	Lohara	372±4.20
3.	Tuljapur	441.33±3.02
4.	Solapur	326.66±0.11
5.	Omerga	385.66±4.10

Values are means of three replications, ±-Standard error

Table 5: Distribution of species of AM fungal spores in different study sites of Osmanabad District.

Sr./No.	Species of AM Fungi	Study Sites					RA.%	FR. %
		S1	S2	S3	S4	S5		
1.	*Acaulospora gerdemannii*	-	+	-	+	-	28.57	40
2.	*Acaulospora delicata*	-	+	+	-	+	42.85	60
3.	*Glomus macroaggregatum*	+	-	+	+	+	57.14	80
4.	*Glomus microaggregatum*	+	+	-	+	-	42.85	60
5.	*Glomus funneliformis*	+	-	-	-	-	14.28	20
6.	*Gigaspora margarita*	+	-	+	-	-	28.57	40
7.	*Gigaspora candida.*	-	+	-	-	+	28.57	40

Legends:S1-Naldurg,S2-Lohara,S3-Tuljapur,S4-Solapur,S5-Omerga

Fig 1. Showing seasonal variation controls the AMF root colonization (X=100),
A= Arbuscules, **B**=Vesicules, **C**=Coiled hyphae

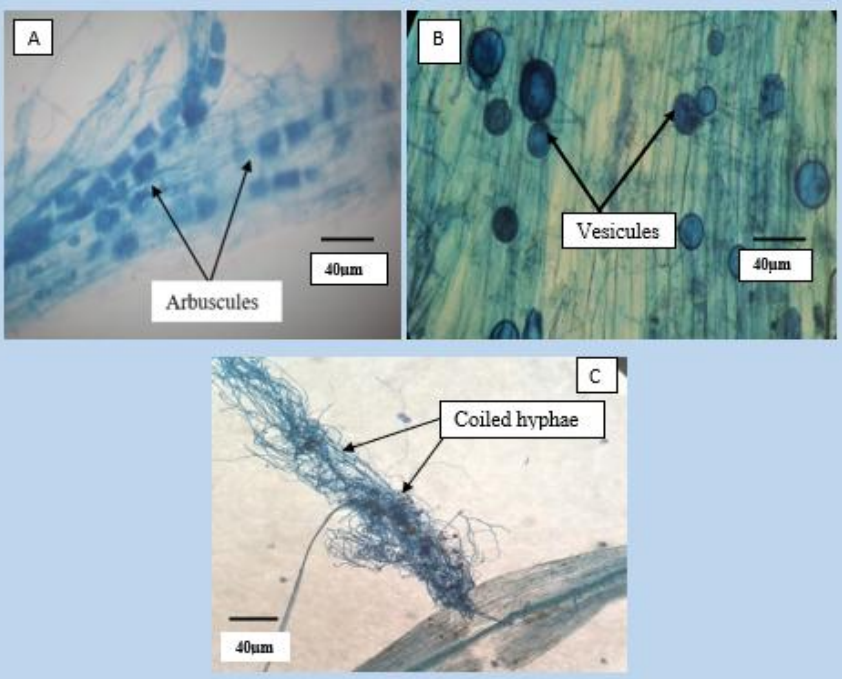
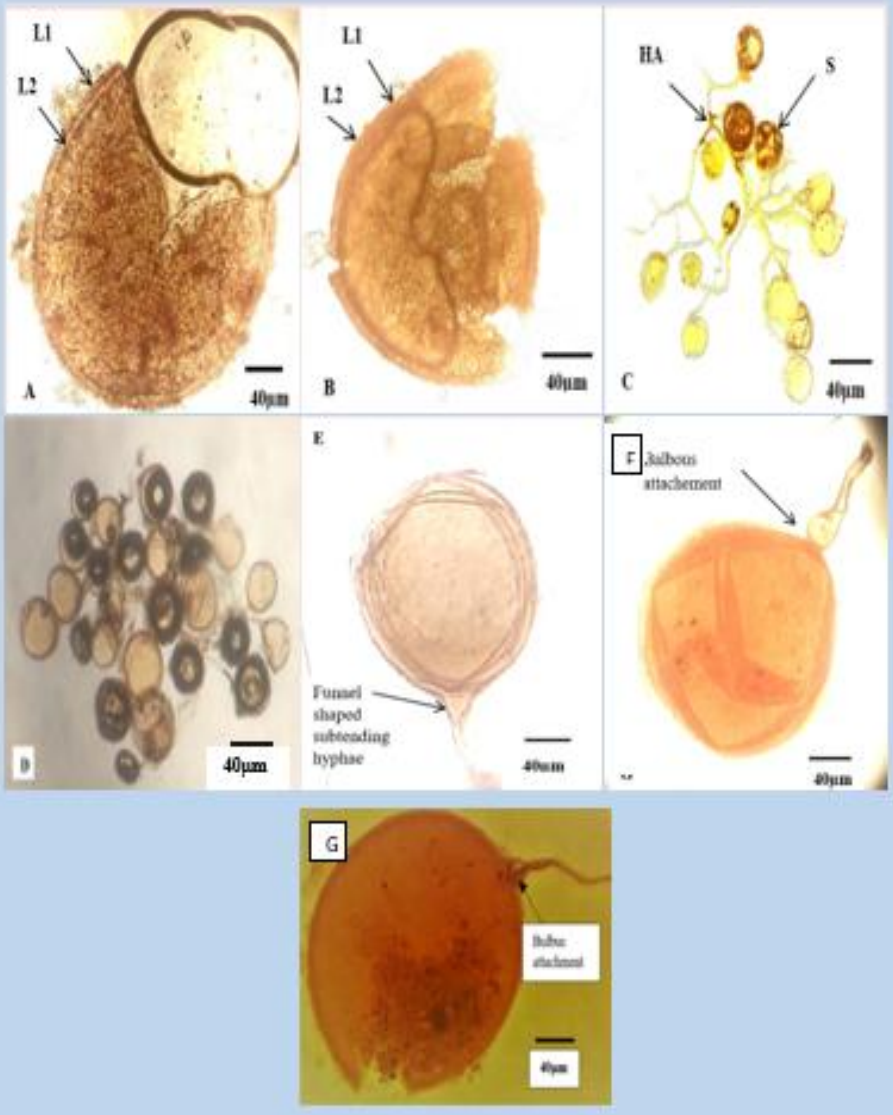


Fig. 2. Diversity of AM fungal spore isolated from the rhizosphere of *Glycine max L.* (X=400),
A= Acaulospora gerdemannii, B= Acaulospora undulata, C= Glomus aggregatum, D= glomus microaggregatum, E= Glomus mossae, F= Gigaspora candida, G= Gigasopra margarita,
S=spore, L1=Layer 1, L2=layer 2, H=Hyphal, Gw1=Germinal wall 1, Gw2=Germinal wall 2



Results observed during the investigation were supported by Vyas and Vyas (2012) where spore density of AMF had a strong positive correlation with soil pH and organic carbon content and a negative correlation with Olsen's P content of the soil. Sreevani and Reddy (2004) studied the relation between soil characters and the occurrence of AMF where a greater number of AM fungal propagates was found in neutral to slightly alkaline (pH 7 to 8) soil whereas alkaline soils (pH higher than 8.0) have not

favoured mycorrhizal fungi. The results concluded that AM fungi play an important role in improving the adaptation to biotic and abiotic plant stresses and alleviating the effects of these stresses on plants. Their role in increasing plant growth and yield, disease resistance, and biotic and abiotic tolerance provides an environmentally friendly solution to reduce the use of hazardous pesticides and industrial fertilizers.

Similarly, Nisha et al. (2010) reported the highest number of AMF spores were found in the rainy season, while moderate numbers were found in the winter and least in summer. Large populations of *Glomus aggregatum* were associated with dense weed populations in a com-soybean sequence (Johnson et al., 1991). Reclamation of River Deposit Soil when amended with renovated land reduced biomass productivity in soybean crop plants compared to cropland soil, but cropland soil showed a significant growth rate, whereas AMF root colonization and spore density were found in both soils (Bhale and Bansode, 2014). Both cropland soil (735 spores per 100 g soil) and renovated + pond soil (730 spores per 100 g soil) had comparable spore densities of arbuscular mycorrhizal fungi (AMF) (Bansode et al., 2014). The density of arbuscular mycorrhizal fungi (AMF) spores in cropland soil was higher (699 spores per 100 g soil) than in combined soil (533/100 g soil). In both soils, Aculospora, Gigaspora, Glomus, Enterphora a nd Scutellospora were found frequently, but and Glomus genera were found dominant, as studied (Bhale et. al. 2014). Johnson et. al. (1991) determined that the distinct fungal communities we observed in soils with different cropping histories probably resulted from differences in the physical, chemical and microbial environments in the rooting zones of corn and soybean.

CONCLUSION

The current study confirms the diversity of AM fungal occurrence in *Glycine max* L. from five different study sites in the district of Osmanabad. Under any environmental condition, mycorrhizal fungi have a significant impact on host plants. Vesicular, arbuscular and hyphal root colonization and four different AMF genera 3 and 7 species were recorded, i.e., *Acaulospora*, *Glomus*, *Gigaspora* from this monsoon season. Glomus species most dominant the highest relative abundance and frequency of occurrence.

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