Influence of Arbuscular Mycorrhizal Fungus on Growth and Nutrient Status in Chickpea (*Cicer Arietinum L.*) Plant

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Abstract

Background: The greatest challenge of today's agriculture is to feed the growing population and restore the natural resources. World over demand of crops in market is more than production. Indian crop production needs to be doubled and just to maintain the present precipitate consumption. . Excessive use of chemical fertilizers causes environmental pollution both at the manufacturing and application sites. It is therefore most necessary to reduce the dependence on chemical inputs in agriculture. This is possible only through eco friendly approaches of farming system. Besides other biotechnological interventions, the arbuscular mycorrhizal fungi could be used as bio inoculants for promotion of growth, development, quality and yield of vegetables that too under an Integrated Plant Growth substances management system. Mycorrhizal fungi are used in conventional agriculture to improve crop production and productivity.

Methods: A pot culture was performed at Botanical Garden of School of Studies in Botany, Jiwaji University Gwalior to examine the effect of *Glomus hoi* on growth and certain biochemical parameters. Plants were raised in triplicates for different mycorrhizal species through the pot culture, containing sterile soil. Plastic pots of 18" × 12" size were used for this purpose. Pots were placed at a sunny place after the seed sowing. And after the seed germination, plants were irrigated as when required

Results: After germination the inoculated plants along with their controls were be sampled at 30, 60 and 90 days. During sampling it was found that due to AMF symbiosis all growth and biochemical parameters like plant were increased in all AMF treated plants.

Introduction

Arbuscular Mycorrhizal Fungi are obligate symbiotic organisms which are living in association with the roots of most land plants. AMF produce wide network of extracellular mycelium of independent length, Spreading from host roots into the surrounding soils and establishing below ground interconnections among plants belonging to the same or to different taxa. AMF are beneficial soil symbionts establishing mutualistic association with the roots of 80% of Plant species and the large majority of food crops including cereals, Legumes vegetables and fruits (Pepe et al, 2018). Arbuscular mycorrhizal (AM) fungi (AMF), one of soil inhabiting fungi, can form the symbiotic association with roots of higher plants, called AM symbiosis. The symbiosis is characterized by increased water and nutrient uptake diverted from the soil to the fungal partner, and in return receives the Photosynthetic supply from the host plant to the AMF2. Studies showed that AMF strongly enhanced drought tolerance of host plants via varied mechanisms viz., direct absorption of water by mycorrhizal extra radical hyphae at the rate of 375–760 nL water/h, contributing up to 20% of total water absorbed by plant roots³, improvement in osmotic adjustments, enhancement in antioxidant profile coupled with enhanced efflux of hydrogen peroxide into rhizosphere and facilitating the formation of soil water-stable aggregates by both glomalin and mycorrhizal hyphae (Liu C.Y et al, 2018). AMF shows increase in Biomass and Higher Tolerance in Biotic and Abiotic Stress (Lanfranco L. et al, 2018). Arbuscular mycorrhizal fungi (AMF) are considered as a potential biotechnological tool for improving Phytostabilization efficiency and plant tolerance to heavy metal-contaminated soils. However, the mechanisms through which AMF help to alleviate metal toxicity in plants are still poorly understood (Yang Y et al, 2015). During mycorrhiza formation, the arbuscular mycorrhizal fungus undergoes several developmental stages. Spores germinate and arbuscular mycorrhizal fungi shows limited hyphal development in the absence of a host plant during a symbiotic stage and they switch to the pre symbiotic stage which is characterized by extensive hyphal branching in the presence of root exudates (Buee et al, 2000). The plants which forms symbiotic association with mycorrhiza shows increase in growth than non mycorrhizal plants. Arbuscular mycorrhizal fungi increases the growth of some plants like Japanese mint, *Spilanthes calva*, *Withania*

somnifera, *Cartanospermum austral*, *Cymbopogon martini*, *Phyllanthus amarus*, *Gloriosa superba* and *Pueraria tuberosa* (Gianinazzi et al, 2010). AMF helps host plant in reducing stress which is responsible for soil drought. In AMF association, the fungal mycelium helps host plant for water v absorption (Mathur N et al, 2007). In agriculture, the increased uptake of soil minerals by colonized plants means that it is possible to consider reducing substantially the application of fertilizers and pesticides, and at the same time obtain equivalent or even higher crop yields (Jakobsen I et al, 1992). The plants which show mycorrhizal association possess higher concentration of esterase, pectin and endo-polymethyl glacturonase activities than controlled plants (Garcia Romera et al, 1991). The roots of *Arachis hypohaea* forms symbiotic association with *Glomus fasciculatum* shows higher amount of amino acids and proteins (Krishna and Bagyaraj, 1983). Arbuscular mycorrhizal fungi enhance the production of secondary metabolites such as alkaloids and terpenoids (Su Y. Y and Guo L. D, 2007). AMF helps host plant in increasing in nitrogen fixation and increase dry weight of above ground parts of host plant (Nasr and Diem, 1987).

Materials And Methods

A pot culture was performed at the Botanical Garden of School of Studies in Botany, Jiwaji University Gwalior to study the symbiotic association between an arbuscular mycorrhizal fungus (*Glomus hoi*) and Chickpea plant (*Cicer arietinum*). Plants were raised in five replicates for mycorrhizal species (*Glomus hoi*) as well as for non inoculated plants through the pot culture, containing sterile soil. Plastic pots of 18" × 12" size were used for this purpose. A total number of 40 pots were placed, 20 pots were kept as control and 20 pots were inoculated with mycorrhizal spores. In Each pot 3 plants were grown per plant. For procuring AMF free tissues, the seeds were surface sterilized by 4% hypochlorite solution before sowing. Pots were placed at a sunny place after the seed sowing. And after the seed germination, plants were irrigated as when required. After germination the inoculated plants along with their controls was sampled at 30, 60 and 90 days. During sampling plants will be thoroughly watched and were washed several times with the tap water followed by the jet of distilled water. The water adhered to the plant surface was removed by wrapping the harvested plants in blotting papers. Fractionation of the root and the shoot components was carefully done and the fresh weights of the root and the shoot were measured. The detailed methodology for various parameters of growth and biochemical analysis is described as below

Root clearing and staining technique:

Roots were thoroughly washed with the tap water, cleared in 10% KOH by autoclaving for 20–25 minutes at 121°C and 15lbs pressure. Cleared roots were rinsed with water 4–5 times then kept in HCl for 5 min. The HCl solution was decanted and left overnight in 0.05% trypan blue stain as per the method of Philip and Hayman, (1970).

Estimation of root colonization:

Above treated roots were cut in 1 cm long segments, mounted on microscopic slides in Lacto glycerol solution. 10 such root segments were arranged properly on a glass slide and then covered with another glass slide. Slides were observed under compound microscope for mycorrhizal structures viz. hyphae, vesicles and arbuscules as per the method of Bierman and Linderman (1981).

Estimation of growth and development parameters:

Plant height, Plant width, Number of branches, Total number of leaves, Root length, Number of pods, were measured with the scale.

Fresh and dry weights of root and shoot components were measured with the help of laboratory balance.

Estimation of Chlorophyll Content:

For chlorophyll a, chlorophyll b and total chlorophyll the method of Arnon (1949) and Withman et al., (1971) were employed. The fully expanded fresh plant leaves from all the pots trials were collected in the polythene bags and transported to the laboratory. Weighted (0.5gm) fresh leaf material was homogenized and extracted thrice in chilled 80% acetone. The volume of the acetone extract was made up (10 ml) to a known one and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer

Calculation:

The concentration of the chlorophyll pigments was calculated using the following formula

Chlorophyll a = [(12.7 X OD at 663) – (2.69 X OD at 645)] X dilution factor

Chlorophyll b = [(22.9 X OD at 645) – (4.68 X OD at 663)] X dilution factor

Total chlorophyll = [(20.2 X OD at 645) + (8.02 X OD at 663)] X dilution factor.

Estimation of biochemical parameters:

The following methods were employed for certain biochemical analysis of the stem modified tissue.

- Protein estimation by Lowry's *et al.*, (1951) method.
- Estimation of non reducing sugar by Nelson-Somogyi (1952).
- Estimation of reducing sugars by Nelson-Somogyi (1952).
- For ascertaining total carbohydrates by Anthrone method of Hedge and Hofreiter (1962) will be used.
- Estimation of total phenol by the method of Mallick and Singh (1980)

Statistical Analysis Results on growth analysis and chemical components were analyzed by average ± standard error.

Results

Soil

Table 1
Some physical properties of soil which were used for the experiment

Colour	Texture	pH	Electric conductivity (dsm-1)	Organic carbon (percent)	Moisture content (percent)	Water holding capacity (percent)	Density (mg. m -3)
Dark grayish brown	Clay loam	7.32 ± 0.32	0.23 ± 0.02	0.42 ± 0.02	6.45 ± 0.24	1.23 ± 0.10	0.98 ± 0.04

Table 2
Macro and micronutrient analysis of soil which was used for practical.

Nitrogen (kg. ha ⁻¹)	Phosphorous (kgs. ha ⁻¹)	Potassium (kg. ha ⁻¹)	Iron (ppm)	Zinc (ppm)	Manganese (ppm)	Copper (ppm)
168.00 ± 12.06	18.56 ± 1.32	291.24 ± 14.32	3.26 ± 0.20	7.14 ± 0.14	21.04 ± 0.14	4.74 ± 0.16
(The results in the represents Average ± standard error)						

Mycorrhizal plant root colonization

The plants which were inoculated with mycorrhizal spore's shows root colonization ranging from 23.46 to 67.68. The non inoculated plants did not showed any colonization. (Table 3)

Table 3
Root colonization

Days	Percent root colonization
30	23.46 ± 0.18
60	44.32 ± 4.32
90	67.68 ± 6.32
(The results in the represents Average ± standard error)	

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Growth and Biomass

The growth and plant biomass of *Cicer arietinum* were assessed at 30, 60 and 90 days of germination for both inoculated and non inoculated plants. Mycorrhizal treatment showed significant effect on the growth parameters like Plant height, Plant width, root length, Total number of leaves, Total number of flowers and Total number of pods. The plant height at 90days analysis was 61.25 ± 1.76 (average ± standard error) and 52.13 ± 0.54 (average ± standard error) in inoculated and non inoculated plants respectively. Similarly the root length was 19.16 ± 0.59 and 14.8 ± 0.49 in inoculated and non inoculated plants respectively. AMF inoculation shows much increase in number of leaves also, after 90 days of germination in AMF inoculated plants the total number of leaves were 67.15 ± 0.36 and in control plants it was 43 ± 2.22. The flowering starts at 59 days after germination in both inoculated and control plants and the first pod in inoculated plants were seen at 47 days after germination and in non inoculated plants it was seen several days later. (Tables 4 and 5)

Table 4
Various growth and developmental parameters of plants with and without AMF

Days	Plant height (cm)		Plant width(cm)		Root length original to last (cm)		Total number of leaves per plant		Total number of flowers per plant		Total number of pods per plant	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	28.4. $\pm$ 0.01	24.9 $\pm$ 0.01	1.2 $\pm$ 0.03	1 $\pm$ 0.04	14 $\pm$ 0.03	11.17 $\pm$ 0.03	21 $\pm$ 0.02	14 $\pm$ 0.01	-	-	-	-
60	48.36 $\pm$ 1.42	42.96 $\pm$ 1.20	1.8 $\pm$ 0.04	1.19 $\pm$ 0.14	16 $\pm$ 0.04	13.9 $\pm$ 1.5	62.56 $\pm$ 2.22	39.19 $\pm$ 3.55	17.9 $\pm$ 0.47	11 $\pm$ 1.69	-	-
90	61.25 $\pm$ 1.76	52.13 $\pm$ 0.54	2.9 $\pm$ 0.07	2.1 $\pm$ 0.07	19.16 $\pm$ 0.59	14.8 $\pm$ 0.49	67.15 $\pm$ 0.36	43 $\pm$ 2.22	-	-	7.3 $\pm$ 0.002	2.3 $\pm$ 0.001
(The results in the represents Average $\pm$ standard error)												

Table 5
Fresh and dry matter content with and without AMF in the plant (Weight gm. Plant-1)

Days	Root Fresh		Shoot Fresh		Total fresh		Root dry		Shoot dry		Total dry	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	0.38 $\pm$ 0.21	0.12 $\pm$ 0.20	2.3 $\pm$ 0.03	1.19 $\pm$ 0.01	2.68 $\pm$ 0.13	1.31 $\pm$ 0.12	0.09 $\pm$ 0.02	0.07 $\pm$ 0.01	0.45 $\pm$ 0.01	0.32 $\pm$ 0.03	0.54 $\pm$ 0.58	0.39 $\pm$ 0.02
60	0.47 $\pm$ 0.01	0.15 $\pm$ 0.02	2.87 $\pm$ 0.01	1.48 $\pm$ 0.02	3.35 $\pm$ 0.02	1.63 $\pm$ 0.04	0.11 $\pm$ 0.22	0.08 $\pm$ 0.14	0.56 $\pm$ 0.01	0.4 $\pm$ 0.01	0.67 $\pm$ 0.02	0.48 $\pm$ 0.04
90	0.55 $\pm$ 0.03	0.17 $\pm$ 0.01	3.36 $\pm$ 0.03	1.74 $\pm$ 0.01	3.91 $\pm$ 0.03	1.91 $\pm$ 0.01	0.13 $\pm$ 0.13	0.1 $\pm$ 0.02	0.65 $\pm$ 0.02	0.47 $\pm$ 0.12	0.78 $\pm$ 0.03	0.57 $\pm$ 0.02
(The results in the represents Average $\pm$ standard error)												

Chlorophyll content

Under normal conditions (control plants) at 30 days after germination the chl a, chl-b and total chl of *Cicer arietinum* were 0.059 ± 0.002 , 0.120 ± 0.026 and 0.188 ± 0.005 respectively. However there was a significant increase in mycorrhizal treated plants. The chl-a, chl-b and total chl content at 30 days after germination were 0.068 ± 0.001 , 0.120 ± 0.026 and 0.188 ± 0.005 respectively. At every stage of growth i-e at 30, 60 and 90 days after germination the chlorophyll content was slightly increased in mycorrhizal inoculated plants.(Table 6)

Table 6
Chlorophyll fractions and their content in the *Cicer arietinum* with and without the presence of AMF (mg. gm.-1 leaves).

Days	Chl-a		Chl-b		Total chl	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	0.068 ± 0.001	0.059 ± 0.002	0.120 ± 0.026	0.013 ± 0.00	0.188 ± 0.005	0.072 ± 0.00
60	0.129 ± 0.02	0.081 ± 0.04	0.138 ± 0.00	0.162 ± 0.02	0.267 ± 0.003	0.243 ± 0.02
90	0.220 ± 0.01	0.18 ± 0.01	0.249 ± 0.01	0.199 ± 0.02	0.469 ± 0.02	0.379 ± 0.001
(The results in the represents Average ± standard error)						

Nutrient Status/ Biochemical parameters

All the biochemical parameters like reducing sugars, non reducing sugars, total sugars, total protein, and total phenol was gradually increased in those plants which were inoculated with AMF spores at every stage of growth and development. The protein content in AMF treated plants at 90 days after germination was 62.33 ± 0.47 and in non inoculated plants it was 44.0 ± 0.72 . Our results finds that at arbuscular mycorrhizal fungi helps the plant in increasing all the biochemical parameters.(Table 7)

Table 7
Some metabolite fractions as affected by the presence of AMF during growth of *Cicer arietinum* after inoculation along with control plants (mg.gm-1 fresh weight)

Days	Reducing sugar		Non reducing sugar		Total sugar		Total protein		Total phenol	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	11	7.21	43.24	22.66	54.66	29.87	55.41	34.9	36	23.83
	± 0.11	± 0.13	± 0.75	± 0.93	± 0.94	± 0.27	± 0.47	± 0.47	± 0.47	± 0.94
60	68	61.93	44	25	112.66	86.93	59.93	41.4	41	29.8
	± 0.48	± 0.41	± 0.27	± 0.72	± 0.72	± 0.47	± 0.27	± 0.27	± 0.98	± 0.47
90	126 ± 0.93	114	108	92	234.66	206.5	62.33	44.0	47.75	37.00
		± 0.27	± 0.11	± 0.53	± 0.72	± 0.72	± 0.47	± 0.72	± 0.54	± 0.27
(The results in the represents Average ± standard error)										

Discussion

The research field of arbuscular mycorrhiza has been developing interest from past several years. The symbiotic relation between plants and mycorrhiza has seen increased interest for direct recycling of nutrients from organic matter to plants by mycorrhizal fungi. The inoculations of arbuscular mycorrhizal (AM) fungi with plants are effective in enhancing plant development and soil properties (Abdel-Fattah and Asrar, 2012). The present investigation was undertaken to assess the effect of arbuscular mycorrhizal fungi (*Glomus hoii*) on the growth and nutrient status of *Cicer arietinum*. Recently Allah *et al.*, (2015) observed that inoculation of AM fungi significantly increased growth parameters of wheat such as plant height as compared to non inoculated wheat plants. Hafez *et al.* (2013) noted similar observation in mycorrhiza treated olive plants. Lenin *et al* (2010) noted an increase in growth parameters of

four vegetable crops, *Lycopersicum*, *Solanum*, *Capsicum* and *Abelmoschus* with the inoculation of AM fungi. Recently Abdel-Fattah and Asrar, (2012) observed that addition of AM fungi significantly increased growth parameters of wheat such as plant height as compared to non-mycorrhizal wheat plants. Our result shows that AMF being in the symbiotic relationship with crop plant enhances growth and biochemical parameters of the plants. In our experiment inoculation of AMF spores increased growth and developmental characters, chlorophyll content, carbohydrate, protein and several other biochemical parameters in chick pea. Based on our results we suggest that AMF must be explored at all levels to further investigate their role in nature as a bio-fertilizer for sustainable agricultural production.

Conclusion

Mycorrhiza is a beneficial type of fungi that grows in association with most plant roots. Mycorrhizae increase the root's ability to absorb nutrients and water from the soil by increasing the surface absorbing area of roots from 100 to 1,000 times. Mycorrhizae also release powerful enzymes that help dissolve nutrients such as organic nitrogen, phosphorous, and iron. Mycorrhiza plays a vital role in plant growth, disease protection and overall soil quality. There are seven types of mycorrhiza viz: arbuscular, ecto, ectendo, Arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae. Arbuscular Mycorrhizal Fungi (AMF) constitutes a group of root obligate biotrophs that exchange mutual benefits with about 80% of plants. They are considered natural bio fertilizers, since they aid in the acquisition of water and soil nutrients, also help host defense mechanism against pathogens in exchange for photosynthetic products. An increase in crop production is essential to meet the future food demand. Soil fertility and soil structure of agricultural systems is to be managed by effective use of fertilizers with increased profitability and reduced harm to the environment. Microbial inoculants, including arbuscular mycorrhizal (AM) fungi for increasing the efficient use of fertilizers are potential components of such management. The process of re-establishing the natural level of AMF richness can represent a valid alternative to conventional fertilization practices, with a view to sustainable agriculture. The need for AMF as a biofertilizer, with a view to sustainable agriculture, is becoming increasingly urgent since the appropriate management of these symbiotic fungi could potentially decrease the use of agro chemicals. The history of AMF applications in controlled and open-field conditions is now long.. AMF exhibit multifunctional mutualistic symbiosis between plants and members of phylum Glomeromycota. AMF plays a vital role in reduction of plant pathogens. AMF shows the ability to reduce bacterial disease in different crops. Presence of AMF in soil regulates the biogeochemical cycling of organic and inorganic nutrients which maintains soil quality. AMF are also involved in the production of growth hormones such as IAA, Cytokinins, GA3 and vitamins like vitamin B. AMF is very important for sustainable agriculture.

Abbreviations

AMF: arbuscular mycorrhizal fungi; AM: arbuscular mycorrhiza; IAA: Indole acetic acid; Ga3: Gibberellic acid

Declarations

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Author Contribution

SAT Designed the study, Carried out experiment and analyzed data; SP supervised the whole study.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest

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