



RESEARCH ARTICLE

STUDIES ON THE EFFECT OF *GLOMUS FASICULATUM* ON THE PHYSIOLOGICAL GROWTH IN *SESAMUM INDICUM* L.

*Ananda Kumar, D. and Siva Kumar, K.

Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India

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ABSTRACT

Plant physiological growth of sesame (*Sesamum indicum* L.) were studied in controlled environment using normal soil and indigenous Arbuscular mycorrhiza (AM) fungi treated soil. The seedlings of sorghum were inoculated with *Gigaspora* species of AM (*Glomus fasciculatum*) and the inoculum was multiplied with help of sorghum seed bed. Sesame seeds were then inoculated into the bed and it was found that the plant height, shoots lengths, roots, biomass of shoot and roots were considerably increased in the mycorrhizal plants. The effect of AM infection was assessed in pot experiment. In this comparative study, specific mycorrhizal fungi had consistent effects on various growth parameters such as the number of leaves, number of roots, shoot length, biomass of shoot and roots and biochemical parameters were observed at various time intervals by statistical analysis, it was confined with mycorrhizal and non-mycorrhizal infected plants. It was found that the ability of isolates to maintain the plant growth effectively in the case of mycorrhizal seedlings shows a maximum absorption of 0.77, shoot length is about 8.34, count of root and leaves are about 8.10, 5.6 respectively under mycorrhizal infection in 30 days of analysis and had a positive effect on the growth at all intervals. Biochemical analysis was carried out to estimate the total chlorophyll, chlorophyll A, chlorophyll B and Carotenoids contents and it was analyzed to be 9.0 mg/g, 8.3 mg/g, 3.6 mg/g, 4.0 mg/g respectively. At the 30th day of analysis for the mycorrhizal plants, it was found to be high in mycorrhizal seedlings which shows the symbiosis had improved the nutrient uptake of cultivated plants. *G. fasciculatum* was found to be the most efficient fungus and exhibited the highest levels of mycorrhizal colonization, as well as the greatest stimulation of physiological parameters.

Key words: AM; Sesamum, Symbiosis

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INTRODUCTION

Arbuscular mycorrhizal fungi are important in sustainable agriculture because they improve plant water relations and have an impact on environment based agrochemicals and high intensity farming, together with examining cost-effective crop production strategies that relegate less financial reliance on expensive artificial inputs, have stimulated the interest in the practical application of mycorrhizae and legumes in agriculture. Mycorrhiza is the symbiotic association between soil born fungi and the roots of higher plants (Rebeca *et al.*, 2013). Arbuscular mycorrhizal fungus improves disease control and they increase mineral uptake, which reduces the use of fertilizers. Improved plant water status and changes in water relations have been attributed to a wide variety of mechanisms, including some mechanisms not directly related to phosphorous nutrition or water uptake (Davies *et al.*, 1996).

*Corresponding author: Ananda Kumar, D.,
Department of Agricultural Microbiology, Faculty of Agriculture,
Annamalai University, Annamalai Nagar –
608 002, Tamil Nadu, India.

In fact, the inconclusive information that has been obtained suggests that more studies will be required to determine the direct or indirect mechanisms which control plant water relations in AM fungus plant symbioses. The abilities of specific fungus plant associations to tolerate drought are of great interest. Mycorrhizal and Non-Mycorrhizal Plants of sesame and Arbuscular Mycorrhiza is the most abundant kind of mycorrhiza described as a universal plant symbioses. Lack of host specificity is even more characteristic of this symbiosis than other types known. Arbuscular Mycorrhiza is a potential biofertilizer (Sullia, 1991). Arbuscular Mycorrhiza Fungi is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant. Mycorrhizal fungi that grow into the root cortex of the host plant and penetrate root cells to form two kinds of specialized structures, arbuscular and vesicles. Mycorrhizal Fungi is specifically designed to reduce transplant stress while improving soil hydration and fertility. Mycorrhizal association can also enable the plant host to access nutrients in an organic form which would be unavailable otherwise. Compare to normal plant roots Mycorrhizal structures can take up Phosphorus from lower concentration effectively (Howeler *et al.*, 1981).

Mycorrhizal association is responsible for up to 80% of the total Phosphorus uptake by plants (Marschner and Dell, 1994). One of the strongest effects of Arbuscular Mycorrhizal Fungi inoculation is an increase in the development of the host plant, which is attributed to an increase in nutrient uptake, particularly those that have low soil mobility and low concentration in the soil solution. A number of interacting factors affecting the successful combination of AM fungi are pH, soil nutrients, Organic matter, moisture and temperature (Malakooti, 2000). Mycorrhiza fungi increase growth, photosynthetic pigments and photosynthesis of host plants by better mineral nutrition. They cause chlorophyll organs of plant to grow by absorbing required carbon, giving nutrients to plant and increasing efficiency of photosynthesis, showed that mycorrhiza. inoculated maize plants have more dry matter than non-inoculated plants due to salinity. Also inoculation of salt stressed tomatoes with mycorrhiza meaningfully increased their dry weight of root and shoots compared to non-Mycorrhiza inoculated plants (Al-Karaki, 2000). Many studies have been reported on the use of growth regulators or mycorrhizal fungi in decreasing harmful effects of environmental stress. Mycorrhizal fungi are unique microorganisms residing in rhizosphere. These fungi form symbiotic colonies with most plants and in addition to increasing inorganic nutrients in plant, they can increase the resistance of plants to environmental stresses by stimulating growth regulators, increasing photosynthesis, and improving regulation of osmotic adjustment (Rabie and Almadani, 2005). The AM fungus, *Glomus fasciculatum* (*Gigaspora* Species) is known to symbiotically associate with Plants and enhance the nutrient content in the plant (Allen, 1991). Studies on AM fungi conducted during last few decades envisaged their occurrence in a wide variety of hosts, different habitats and variability in quality and quantity. Sesame is a flowering plant in the genus *Sesamum* belongs to the family of pedaliaceae. Numerous wild relatives occur in Africa and a smaller number in India. The AM fungi used in this study was *Glomus fasciculatum* species. In this study we compared *Glomus* species with a non mycorrhizal control. And determined the effects of fungal isolates on plant growth, biomass of shoot and root and the biochemical constituents like chlorophyll, carotenoids contents were analyzed.

MATERIALS AND METHODS

In the present investigation sesame CO2 variety were used. The experiment was conducted in at Department of Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram Tamil Nadu, India.

Preparation of inoculum

The pure culture of *Glomus fasciculatum* was inoculated in the mixture of sterile sand and soil in the ratio 1:1. Sorghum was chosen as host plant for this study and to transfer *Glomus fasciculatum* into the root of sesame plant, sorghum was grown in the mixture sand for of three months, after that period the aerial part of the plant was cut off from the soil and discarded. The root portion of sorghum which was colonized with *Glomus fasciculatum*, was cut down into small pieces and mixed into the soil and shade dried it for growing of sesame plant. Plant samples randomly from a total of 10 patches, which were chosen to cover a range of shoot densities from 100-200 shoots m⁻². Plants were dug up with a spade carefully washed free from sediment using a sieve. Plants with undamaged roots

were transferred to plastic bags and transported to the laboratory, where they were kept in cold storage until the next morning when the roots were examined for AM. Ten plants were sampled randomly from each of the 10 patches.

Staining for colonization

For staining the roots were cleared in 10 % KOH at 90 °C for 15 min. (Philips and Haymen, 1970). After the cleaning, the root samples were stained in 0.05 % typan blue in lactoglycerol at 90 °C for 5 min. from each of sesame 1 cm segments was taken for the staining. All the segments were examined for the presence of fungal structures (eg. Vesicles, arbuscles and hyphae) at 200-400 x magnification using Phase contrast microscope (Carl Zeiss, Germany).

Measuring length of plant root: The root lengths were measured by a ruler and recorded in centimeter.

Measuring dry weight of root and of shoot : After separating roots from shoots, each of them was separately placed in aluminum sheet and then put in oven at 80°C for 10 days until their weight was fixed and their dry weight was measured in terms of grams.

Measuring fresh weight of root and of shoot: After separating roots from shoots, their fresh weights were measured in terms of gram.

Measuring the number of roots and leaves:

Number of roots and leaves were counted and recorded in numbers for all time periods.

Biochemical Analysis

Measuring chlorophyll and carotenoid content

Chlorophyll and carotenoid contents of the plants were measured according to the method suggested by Lichtenthaler (1987). In this method, 0.2 g fresh texture of leaf was weighed and then ground in Chinese mortar containing 80 % acetone. Then 5 ml Acetone was added to it and solution volume was reached to 15 ml. Three ml of this solution was poured in a cuvette and its absorption intensity was read in 470, 663, 647 nm by Spectrophotometer. For regulating spectrophotometer, 80 % Acetone was used as witness. Pigment density was determined in terms of mg/g fresh weight of the plant essence.

Estimation of growth rate

Growth rate was estimated through the length of the plant as it grows with varying time intervals and percentage had been calculated from it.

Data analysis and statistical studies

This experiment was performed with three replications based on a completely randomized design. Data analysis was performed by two way ANOVA.

RESULTS

The content of root mycorrhizal colonization

Root colonization in inoculated plants with *Glomus fasciculatum* was determined. This indicates that *Glomus*

Table 1. Studies on the effect of *Glomus fasciculatum* on the Physiological growth in *Sesamum indicum* L

| PARAMETERS | TIME INTERVALS IN DAYS | | | | | |
|--------------------------|------------------------|---------|------|---------|------|---------|
| | 10 | | 20 | | 30 | |
| | AM | Control | AM | Control | AM | control |
| Length of Plant | 2.78 | 2.1 | 4.88 | 3.31 | 8.34 | 5.55 |
| Number of Leaves | 2.60 | 2.02 | 6.08 | 4.08 | 8.10 | 5.94 |
| Length of roots | 1.47 | 1.68 | 3.60 | 3.30 | 5.60 | 4.40 |
| Dry weight of root | 0.2 | 0.007 | 1.20 | 0.10 | 1.98 | 1.15 |
| Fresh weight of root | 0.67 | 0.25 | 1.31 | 0.65 | 1.95 | 1.01 |
| Dry weight of stem | 0.29 | 0.07 | 1.34 | 0.14 | 2.21 | 1.34 |
| Fresh weight of stem | 1.02 | 0.45 | 1.57 | 0.65 | 2.17 | 1.01 |
| Chlorophyll A (mg/g) | 13.0 | 9.0 | 15.0 | 8.0 | 15.6 | 8.3 |
| Chlorophyll B (mg/g) | 6.0 | 3.6 | 6.6 | 3.3 | 7.0 | 3.6 |
| Total Chlorophyll (mg/g) | 17.3 | 9.0 | 17.3 | 8.6 | 18.5 | 9.4 |
| Carotenoids (mg/g) | 5.03 | 3.5 | 6.5 | 4.2 | 6.3 | 4.2 |
| Growth Rate | 0.77 | 0.32 | 0.75 | 0.58 | 0.72 | 0.67 |
| Growth Percentage | 77 | 32 | 75 | 58 | 72 | 67 |

fasciculatum can significantly colonized in root the penetration of fungi into root cells.

Number of roots and leaves

The results of this study showed that number of root and leaves was found to be high in number at treatment with AM than the control. Number of root and leaves were increased was meaningful at and have significant difference was seen between time intervals (10- 30day) of analysis. In mycorrhiza fungus treatment there was an increase in number of root and leaves growth, was found to high as 5.6 and 8.10 at 30th day of analysis respectively

Shoot length

The results of this study showed that length of shoot was found to be high at treatment with AM than the control. Length of shoot was increased was meaningful at $p \leq 0.0001$ and have significant difference was seen between time intervals (10-30 day) of analysis. In mycorrhiza fungus treatment there was an increase in length of shoot growth, was found to high as 8.34 ± 0.2 and for control was about 5.54 at 30th day of analysis respectively

Root dry and fresh weights

The results of this study showed that root dry and fresh weights were found to be high at treatment with VAM than the control. Root dry and fresh weights were increased was meaningful and have significant difference was seen between time intervals (10 -30day) of analysis. In mycorrhiza fungus treatment there was an increase in Root dry and fresh weights were found to high as 1.98 gms, 1.95gms and for control was about 1.15 gms, 1.01gms at 30th day of analysis respectively. The amount of this parameter was meaningfully increased in treating plants with *Glomus fasciculatum* increase in roots dry and fresh weights were observed relative to control plant at 30th day of analysis.

Shoot dry and fresh weights

The results of this study showed that root dry and fresh weights were found to be high at treatment with VAM than the control. Root dry and fresh weights were increased was meaningful and have significant difference was seen between time intervals (10 -30day) of analysis. In mycorrhiza fungus treatment there was an increase in Root dry and fresh weights were found to high as 2.21gms, 2.17gms and for control was about 1.34 gms, 1.01 gms at 30th day of analysis respectively. The amount of this parameter was meaningfully increased in

treating plants with *Glomus fasciculatum* increase in roots dry and fresh weights were observed relative to control plant at 30th day of analysis.

Chlorophyll A

Chlorophyll A content in AM treatments meaningfully increased. However, treating plants with mycorrhiza fungi, meaningfully increased chlorophyll A content than the control. Chlorophyll A content significantly varies with the time intervals between the VAM and control. Chlorophyll A content was found to be high at VAM treatment as 15.6 mg/g and for control as 8.3 mg/g.

Chlorophyll B

Chlorophyll B content in AM treatments meaningfully increased. However, treating plants with mycorrhiza fungi, meaningfully increased chlorophyll B content than the control. Chlorophyll B content significantly varies with the time intervals between the VAM and control. Chlorophyll B content was found to be high at VAM treatment as 7 mg/g and for control as 3.6 mg/g.

Total chlorophyll

Increasing in total chlorophyll content, which is meaningful in AM treatment. In inoculated plants with mycorrhiza fungi, chlorophyll content had meaningfully increased relative to control plant at various time intervals. In this experiment, increasing effects of *Glomus fasciculatum* were more prominent on total chlorophyll content than the control. Total chlorophyll content was found to be high at AM treatment as 18 mg/g and for control as 9.0 mg/g.

Carotenoids

The results of this study showed that increase in carotenoids content in VAM treatment. Furthermore, the increase in carotenoids content was meaningful at all time intervals to treat with *Glomus fasciculatum* than the control plants (Fig. XII). Carotenoids content was found to be high at VAM treatment as 6.3 mg/g and for control as 4.0 mg/g.

Growth rate and growth percentage

Growth rate and growth percentage were showed to be high at VAM treatment at all time interval analysis. Growth rate was meaningfully increased in treatment than the control as 0.72, 0.67 respectively at 30th day of analysis. At all time analysis

of analysis growth percentage significantly increases in treatment than the control as 72 % and 67 % respectively.

DISCUSSION AND CONCLUSION

Inoculation of plants with arbuscular mycorrhizal [AM] fungi has the potential to increase or maintain yields and allow for reduced fertilizer and pesticide application (David et al., 2008). In these systems, the hosts studied are often the dominant species. Their endo mycorrhizal fungi produce a larger amount of extra radical mycelium that behaves as an ecosystem stabilizer, improving the nutrient flux among community components (Al-Agely and Reeves, 1995). The use of AM fungi in forestry appears to be more important than in agriculture because in countries like India no large scale provisions exist to irrigate, fertilize and protect the plantation. The practical use of AM fungi seems to be more appropriate as they are effective in overcoming the stress conditions like draught, disease incidences and deficiency of nutrients (Peter and Rhodes, 1987). Although a few studies have been conducted on AM interaction with the tropical trees (Marx et al., 1971), the results are encouraging as the growth of seedlings and productivity was found to be enhanced in AM treated. The effect of growth parameters in AM inoculated plants showed prompt response in terms of growth and flowering character when compared with Non-Mycorrhizal plants.

Low resistance to water movement through roots and conducting system to the shoots by an increase in vessels or vessel diameter also aids in maintaining water uptake under the stress (Hale and Oracett, 1987). The present investigation revealed that, the treatment AM significantly increased the root length and shoots length at all time of investigation. In cycocel treated plants, there was increase in root length. Similar results were reported by Turner and Begg, (1978). In the present study AM colonization was found in the root samples. The length and leaf surface area are considerably increased in the AM infected plants. From the literature on the interactions between AM- fungi and terrestrial plants, it is clear that AM is mainly involved in facilitating nutrient uptake (Khan et al., 1975) although it has been shown that AM is mainly involved in uptake of phosphorous, nitrogen and other nutrients and exchange for photosynthesis (Smith and Read, 2008). Microscopic pipelines hyphal structure of Mycorrhizal fungi that can transport carbon and minerals to and away from the plants (Barrow, 2004). The cultivators of Maize and *Sesemum* require more amount nutrients in the early stage of development of plant system completely based on the development and performance of roots (Cheunget al., 1987). In this study, AM treated plants had root dry and fresh weights were increased due to osmotic potential of soil and disturbance in water absorption by plant.

Existence of fungus hypha network increases nutrient and water absorption. Fibers of mycorrhizal fungus are divided into two groups; some of them enter the plant system and decrease density of abscisic acid and increase cytokinin content. This action increases water absorption and develops root system of the plant. Second group of fibers are out of root system and secrete organic acids solving phosphorus such as malic acid that increases phosphorus absorption by plant and its dry matter. Phosphorus as one of the elements required for plant increases dry matter because it has an important role in cellular division by regulating plant hormones. Moreover, it has an important role in producing photosynthetic matters and

produces energy in plant and has an important role in cellular division by regulating plant hormones. Moreover, it has an important role in producing photosynthetic matters and produces energy in plant (Khalvatiet al., 2005). The results of shoot dry and fresh weights were increased in this study under AM treatment. In the plants inoculated with mycorrhiza fungus, increased fresh and dry weight of shoot was observed.

This increase in weight can be resulted from the effects of mycorrhiza fungus on absorbing various nutriment such as nitrogen, calcium, potassium, copper, zinc and sulphur. Using mycorrhiza fungus increases plant growth and affects devoting and transferring nutriment between stem and root so that dry weight of shoot is increased by increasing absorption of nutriment and their transfer. Fresh weight of shoot was also increased in *Glomus mosseae*- treated plants in low salinity. Similar results were also obtained about mycorrhizal barley plant in salt stress conditions (Nouriniaet al., 2007). Chlorophyll is known to influence the photosynthetic rate and in turn influence growth and development of cotton. However, under AM treated conditions there will be increase in pigment composition, which induce to increase chlorophyll content. Chlorophyll A, chlorophyll B, total chlorophyll and carotenoids content of leaves were increase in AM treated conditions. Higher persistence of chlorophyll content under stress due to growth regulators and AM may be attributed to decreased chlorophyll degradation and increased chlorophyll synthesis. These results are in accordance with Jayakumar and Thangaraj (1998). AM had significantly higher chlorophyll content at all time analysis of crop growth. The increase in total chlorophyll concentration of drought plants in response to mycorrhizal effects was positively correlated with respective levels of mycorrhizal infection in broad bean plants (Abdel et al., 2002). Such increases were related to the degree of mycorrhizal infection and Huixing (2005) also showed the effect of AM on host plant in drought condition that enhanced resistance to drought stress by increase in chlorophyll content than non AM plants. Thus, our results of enhanced chlorophyll content due to plant growth regulators application and VAM are in agreement with the above discussion. Growth rate in AM treatment were found to be high as VAM act as growth regulators and it influence even at stress conditions (Shekoofeh and Sepideh, 2011). The VAM fungal symbioses were proved to play a vital role in such stress conditions by supplying the nutrients to the host plant. The present study also established that the association of AM fungi with Sesame as sorghum enhanced the growth when compared with control.

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