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Full Length Research Paper

Effect of vesicular arbuscular mycorrhizal fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions

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The symbiotic association between certain plants and microorganisms plays an important role in soil fertilization, and improves their growth and mineral nutrition. The symbiotic association between vesicular arbuscular mycorrhizal (VAM) fungi and roots provides a significant contribution to plant nutrition and growth. VAM mycelium in soil results in greater efficiency of nutrient absorption particularly for slowly diffusing mineral ions, especially phosphorous. The present investigation aims at studying the effects of VAM fungi on the physiological changes of the selected tree seedlings viz., *Cassia siamea, Delonix regia, Erythrina variegata, Samanea saman* and *Sterculia foetida.* The chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, protein, nitrate, nitrogen, phosphorus and potassium content increased in VAM fungus treated seedlings compared with non-mycorrhizal tree seedlings. The total soluble sugars and soluble starch contents in the leaves of all the selected tree species in the present study showed a decrease in mycorrhizal seedlings than non-mycorrhizal seedlings.

Key words: Vesicular arbuscular mycorrhizal, *Cassia siamea*, *Delonix regia*, *Erythrina variegata*, *Samanea saman*, *Sterculia foetida.*

INTRODUCTION

The vesicular arbuscular mycorrhizal (VAM) fungi, the obligate biotrops, lead a symbiotic life which is characterized by the transfer of nutrients, especially phosphorus, which have been taken up from the soil by the fungi, and in turn they obtain carbohydrates provided by the host plants (Hampp and Schaeffer, 1995; Smith and Read, 1997). The main benefit to the VAM by the host plant is the provision of an ecological niche because VAM cannot grow independently. VAM fungi which symbiotically colonize plant roots form associations with more than 80% of plant species found in nearly every habitat in the world (Smith and Read, 1997). These VAM fungi show great diversity in their abundance. VAM fungi have occurred for ages and are known to occur in different ecosystems like aquatic environment (Bajwa and Javald, 1997), humid tropics (Janes et al., 1995; Siguenza et al., 1997), tropical rain forest (Guadarrama and Sanchez, 1999), sand dunes (Koske and Halverson, 1981), savannas (Saif, 1986), heavy metal polluted soils (Griffoen et al., 1994) and even in saline soils (Mandeel and Gull, 1999).

The symbiotic association between VAM fungi and roots provides a significant contribution to plant nutrition and growth (Smith and Read, 1997). VAM mycelium in soil results in greater efficiency of nutrient absorption particularly for slowly diffusing mineral ions, especially phosphorous as observed by Smith et al. (2000). In addition to phosphorous, VAM mycelium also enhances the uptake of nitrogen in the form of NO_3 (Frey and Schuepp, 1993; Morte et al., 2000) and also increase the potassium content in plants (Azcon and Barea, 1992; Maksoud et al., 1994). VAM fungi also increase the uptake of Ca, Mg, Cu, Zn and Fe (Marschner and Dell, 1994; Alkaraki and Clark, 1999).

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VAM fungi significantly increase the net photosynthesis by increasing total chlorophyll and carotenoid contents ultimately increasing carbohydrate accumulation. The VAM fungi have also increased stomatal resistance, thereby reducing the rate of transpiration (Mathur and Vyas, 1995). Shrestha et al. (1995) have shown that photosynthesis and transpiration rates of mycorrhizal *Satsuma mandarian* trees are higher than non-mycorrhizal trees. Mycorrhizal turf, creeping bent grass has maintained significantly higher chlorophyll concentration than non-mycorrhizal turf during the drought period. Wright et al. (1998) also showed that mycorrhizal *Trifolium repens* L. exhibited a higher specific leaf area and increased rate of photosynthesis compared with non-mycorrhizal plants.

The chlorophyll content, fresh weight and leaf area are higher in mycorrhizal plants than in non-mycorrhizal plants but differences are significant only under draught stress conditions (Morte et al., 2000). Borges and Chaney (1993) have observed that the concentration of soluble sugars and starch in roots increased with increasing photosynthesis levels for all treatments, although mycorrhizal seedlings have greater concentrations of soluble sugars in roots than non-mycorrhizal seedlings. The accumulation of sugars and a number of other organic solutes is the most important among the factors involving carbohydrate metabolism (Kameli, 1990). The higher rates of glucose accumulation throughout the period of water stress in uninoculated seedling of wheat have been observed by Kameli and Losel (1993).

Proline accumulation correlates with draught resistance in various plant species. The free proline concentration is lower in leaves of VAM plants than the non-mycorrhizal plants at the end of the water stress period, pointing to a different water stress tolerance in corn (Mcumiller and Hofner, 1991). VAM inoculated alfalfa plants were better adapted than non-mycorrhizal plant to compare with the water deficit and increased concentration of proline (Golcoechea et al., 1998). The present work aims at studying the effects of VAM fungi on the physiological changes of the selected tree seedlings; *Cassia siamea, Delonix regia, Erythrina variegata, Samanea saman* and *Sterculia foetida.*

MATERIALS AND METHOD

Extraction and estimation of chlorophyll and carotenoid pigments

Leaf material (100 mg) was ground with a chilled pestle and mortar in diffuse light using 5 ml of 80% cold acetone and the homogenate was centrifuged at 3000 x g for 2 min. Aliquots of 5 ml of 80% cold acetone were added to the pellet and centrifuged till it was non-green. The supernatants were pooled and protected from light prior to the estimation of chlorophyll pigments. The concentration of chlorophyll was calculated using the formula of Arnon (1949).

Total Chl = $0.0202 \times A_{645} + 0.00802 \times A_{663}$ mg/ml
Chl a = $0.0127 \times A_{633} - 0.00269 \times A_{645}$ mg/ml
Chl b = $0.0229 \times A_{645} - 0.00488 \times A_{663}$ mg/ml

The content of total carotenoid in the 80% acetone extract was assessed using an absorbance co-efficient A_{473} 1% = 2500 according to Goodwin (1954) using following formula

Carotenoid = $A_{480} + (0.144 + A_{638}) \times A_{645}$

Biochemical assays

Proteins in crude extract were estimated using the method used by Lowry et al. (1951). Starch concentration was determined following the method of Mc Cready et al. (1950) and total soluble sugars were estimated following the method of Dubois et al. (1956). The amount of nitrate formed was measured by the method of Cataldo et al. (1975). Estimation of praline was done according to Bates (1973). The dried plant materials were ground in porcelain mortar with porcelain pestle and the N content was determined by the modified micro-Kjeldahl method (Umbreit et al., 1972). Acid-soluble total phosphorus was estimated by method of Fiski-Subba Rao as modified by Bartlett (1959). Potassium was estimated by flame photometric method.

RESULTS AND DISCUSSION

A change in a number of biochemical parameters has been observed in VAM fungus infected plants. In the present study, we observed that VAM fungus enhanced the contents of chl a, chl b, total chlorophyll and carotenoid in all the VAM inoculated seedlings. The chl a content increased by 23% in *D. regia,* 30% in *E. variegata*, 24% in *S. saman* and 26% in *S. foetida* in VAM fungus treated plants compared with their respective controls. Similarly, chl b also showed an increase in all mycorrhizal seedlings and the increase was 23% in *C. Siamea*, 25% in *D. regia*, 32% in *E. variegata*, 26% in *S. Saman* and 28% in *S. foetida* (Figures 1 and 2).

The total chlorophyll content increased by 21% in *C. siamea*, 24% in *D. regia*, 31% in *E. Variegata*, 25% in *S. Saman* and 27% in *S. foetida* in mycorrhizal inoculated seedlings compared with uninoculated controls. The carotenoid content also increased in VAM fungus inoculated seedlings and the increase was 20% in *C. siamea*, 22% in *C. siamea,* 25% in *E. Variegata*, 21% in *S. Saman* and 23% in *S. foetida* (Figures 3 and 4).The increase in chl a, chl b, total chlorophyll and carotenoid contents observed in the present study is in concurrence with the reported findings of Gemma et al. (1997) who have observed that mycorrhizal *Agrostis plastids* has significantly higher chlorophyll concentration.

Mycorrhizal plants have higher total chlorophyll and carotenoid contents than the non-mycorrhizal plants (Morte et al., 2000; Mathur and Vyas, 1995). Mycorrhizal seedlings show a greater increase in the rate of photosynthesis than their controls which may be due to increase in the content of total chlorophyll (Shrestha et al., 1995; Wright et al., 1998).

The total soluble sugars and soluble starch contents in the leaves of all the selected tree species in the present study showed a decrease in mycorrhizal seedlings than non- mycorrhizal seedlings. The decrease in soluble su-

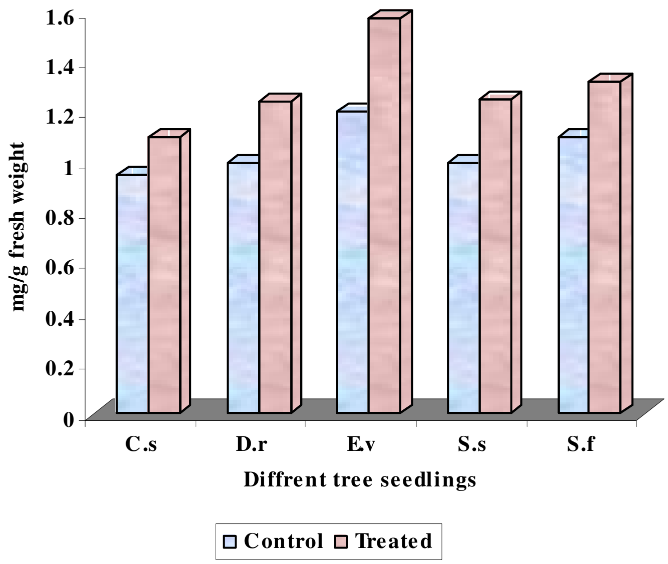

Figure 1. Effect of VAM on chlorophyll a content of different tree seedlings.

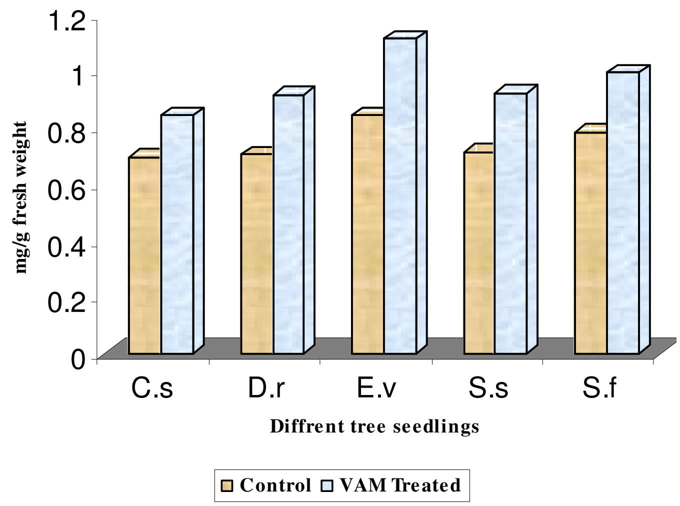

Figure 2. Effect of VAM on chlorophyll b content of different tree seedlings.

gars was 18% in C*. siamea*, 21% in *D. regia* 24% in *E. variegata*, 21% in *S. saman* and 22% in *S. foetida*. The available soluble starch decreased by 18% *C. siamea*, 20% in *D. regia* 23% in E*. variegata*, 21% in *S. saman* and 22% in *S. foetida* (Figures 5 and 6).

The decrease in soluble sugars and soluble starch in the leaves may be due to the fact that the VAM fungi utilize 10 - 20% of net photosynthate in exchange for the transfer of nutrients to the host to lead a symbiotic life (Allen, 1991). The decrease in soluble sugars and soluble starch may be due to the translocation of carbohydrate produced by the host to the fungal partner (Fitter, 1991).

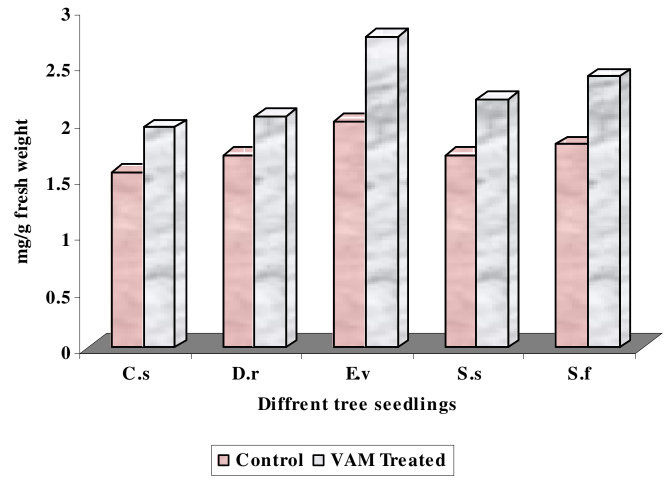

Figure 3. Effect of VAM on total chlorophyll content of different tree seedlings.

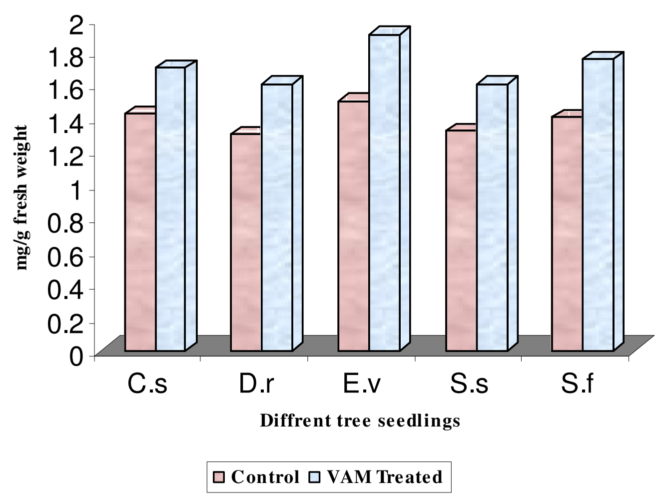

Figure 4. Effect of VAM on carotenoid content of different tree seedlings.

Johnson et al. (1997) have found that since VAM fungi are obligate biotrophs, the host should have a substantial influence on the VAM fungi through the regulation of carbon supply (Kozlowski 1992; Hampp and Schaeffer, 1995; Schaeffer et al., 1997).

In the present study, the leaf protein content of all mycorrhizal inoculated seedlings showed a greater increase than the non-mycorrhizal seedlings. The increase was 23% in *C. siamea*, 25% in *D. regi,* 30% in *E. variegata*, 26% in *S. saman* and 28% in *S. foetida* (Figure 7).

The effect of VAM in the total leaf nitrate content is presented in Figure 8. It was observed that the NO_3 content increased more in VAM inoculated seedlings compared with the non-mycorrhizal seedlings. The increase was 19% in C*. siamea*, 20% in *D. regia* 23% in *E.*

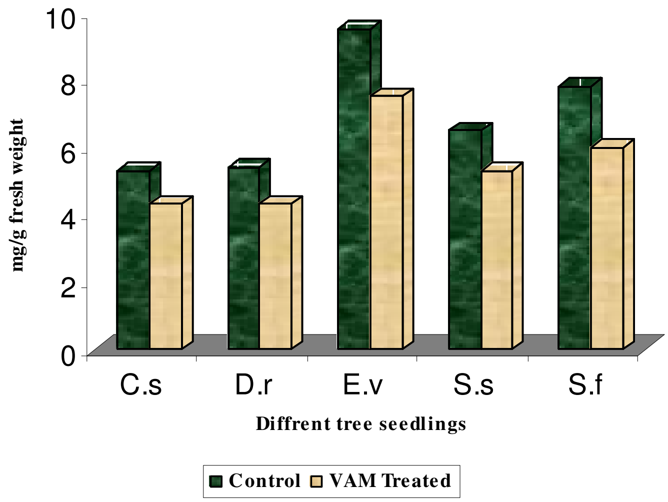

Figure 5. Effect of VAM on soluble sugars of different tree seedlings.

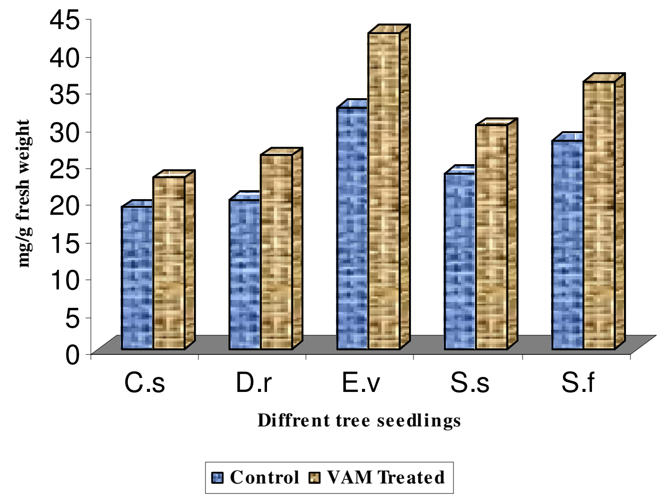

Figure 7. Effect of VAM on protein content of different tree seedlings.

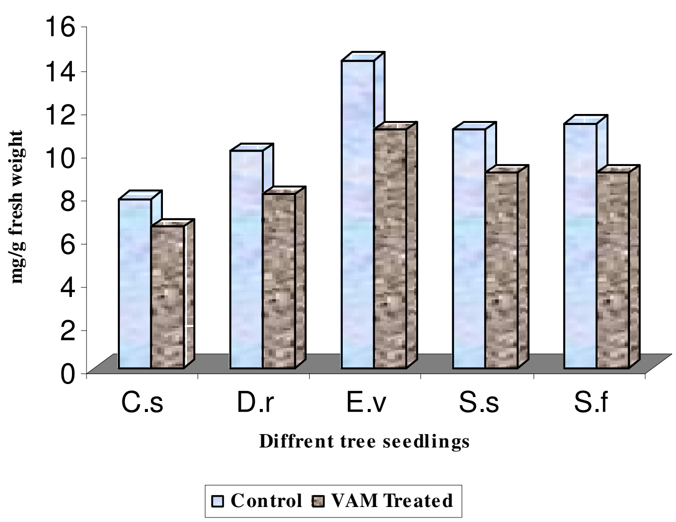

Figure 6. Effect of VAM on soluble starch of different tree seedlings.

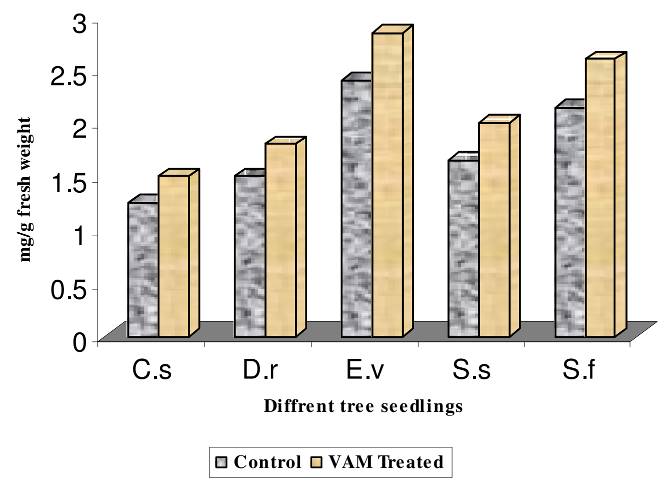

Figure 8. Effect of VAM on nitrate content of different tree seedlings.

variegata, 20% in *S. saman* and 21% in *S. foetida*. Our results are in concurrence with the reported findings of Johansen et al. (1993) which have shown that mycorrhizal hyphae are able to transport inorganic N as NO_3. According to Cuenca and Azcon (1994), the increase in growth and acquisition of nutrients by plants fertilized with NO_3-N and inoculated with VAM showed that VAM mycorrhizae have a capacity for the foliar nutrient analysis was carried out in all the mycorrhizal inoculated seedlings. The present study showed an increase in nutrient content especially nitrogen (N), phosphorous (P) and potassium (K) (Table 1).

Nitrogen content was increased by 15% in *C. siamea*, 17% in *D. regia* 23% in *E. variegata*, 19% in *S. saman* and 21 in *S. foetida* of VAM inoculated seedlings (Table 1). Maximum increase in N content was observed in *E. variegata* followed by *S. foetida.* Our findings are in concurrence with Frey and Schuepp (1993) who reported that VAM hyphae improve the capacity of higher plants to acquire inorganic nitrogen. Uptake and transport of N through VAM external hyphae have been reported by Johansen et al. (1992). Radioisotopic studies have revealed that external mycelium can utilize soil inorganic nitrogen very efficiently (Frey and Schuepp 1992; Johansen et al., 1993, 1994). VAM plants have access to all forms of N, unavailable to non-VAM plants (Tobar et al., 1994 a, b).

VAM fungus inoculated mycorrhizal seedlings showed a greater increase in phosphorus content than the un-

Table 1. Effect of VAM fungus on nutrient uptake (N, P, K in %) in different tree seedlings grown under nursery conditions.

Plant name	Nitrogen (N)		Phosphorus (P)		Potassium (P)	
	Control	VAM	Control	VAM	Control	VAM
Cassia siamea	1.19±0.02	1.37±0.03	0.02±0.00	0.25±0.02	1.04±0.01	1.14±0.02
Delonix regia	1.33±0.03	1.56±0.01	0.18±0.00	0.23±0.03	1.0±0.02	1.11±0.01
Erythrina variegate	1.80±0.02	2.21±0.02	0.27±0.00	0.37±0.02	1.82±0.03	2.10±0.03
Samanea saman	1.39±0.01	1.65±0.01	0.22±0.00	0.29±0.01	1.47±0.01	1.63±0.01
Sterculia foetida	1.50±0.02	1.82±0.01	0.25±0.00	0.33±0.02	1.63±0.02	1.84±0.02

Each value is the mean of 3 replicates ± SD.

inoculated controls. The increase was 25% in *C. siamea*, 28% in *D. regia* 37% in *E. variegata*, 30% in *S. saman* and 33% in S*. foetida*. The increase in phosphorous content was more pronounced in *E. variegata* and *S. foetida* (Table 1). The results of the present study coincide with the reported findings of Jackobsen et al. (1992) who reported that the fungal hyphae growing beyond the rhizospheric soil increase the absorptive surface area of the root, which result in a greater efficiency of nutrient absorption, especially slowly diffusing mineral ions like phosphorus (Kothari et al., 1991; Li et al., 1991). VAM fungus not only increases the plant growth but also enhances phosphorous uptake (Tarafdar and Marschner, 1994; Srivastava et al., 1999; Shrihari et al., 2000; Smith et al., 2000).

Potassium also found to increase in VAM fungus inoculated seedlings more when compared to the un-inoculated controls. The increase was by 10% in *C. siamea*, 11% in *D. regia* 15% in *E. variegata*, 11% in *S. saman* and 13% in *S. foetida* (Table 1). These results are in harmony with Azcon and Barea (1992) who reported an increase in K content in the shoots of VAM inoculated Lucerne. Maksoud et al. (1994) reported that VAM inoculation significantly increases the potassium content in tamarind seedlings. Morte et al. (2000) reported that the mycorrhizal plants accumulate more potassium in shoots and roots than the non-mycorrhizal plants.

Conclusion

In the present study, the inoculation of VAM in all the selected tree seedlings; *C. siamea*, *D. regia*, E*. variegata*, *S. saman* and *S. foetida* enhanced the physiological and biochemical characteristics. The changes may be due to enhance most of water uptake and nutrient absorption, especially P from the soil, which may be due to VAM fungus, since VAM hyphae extend beyond the root hair zone increase the absorptive surface of the host. Although all the selected five tree species showed a good response to VAM fungus inoculation, the growth and nutrient uptake were more pronounced in *E. variegata* and *S. foetida* than in other tree species.

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