DOI : 10.5958/0974-0112.2019.00007.0

Indian J. Hort. 76(1), March 2019: 44-49

Mycorrhization alters root morphology, leaf starch and nutrient content of micropropagated banana under water stress

Vartika Srivastava* **and A.K. Singh**

Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh

ABSTRACT

The influence of mycorrhizal inoculation under water stress was studied to observe the effect of different mycorrhizal strains on the root morphology in banana and its consequence on starch and nutrient content of *in vitro* derived banana leaves. We assessed per cent root colonization, number of roots per plant, root length, root fresh weight, root dry weight and its effect on leaf starch content of micropropagated banana plantlets during hardening at 20, 40 and 60 days after mycorrhizal inoculation. The effect of mycorrhization on leaf nutrient (N, P, and K) was recorded at the end of the hardening period. Four treatments including control were replicated four times and 10 plants per replication were maintained in completely randomized design (CRD). Significant increase in number of roots, root fresh and dry weight of mycorrhized banana plantlets was recorded at 20, 40 and 60 days after inoculation over control. Though the numbers of roots remain significantly less in banana plantlets without mycorrhization, the root length was highest throughout the experimental period. Changes in root morphology of mycorrhized plantlets under water stress plays an important role in improved growth as number of lateral roots increase surface area for nutrient and water absorption. Significantly less leaf starch content after 20 days of inoculation in mycorrhizal plantlets were observed which increased significantly after 40 days of inoculation and continued up to 60 days. Foliar N, P, K content was also found to be significantly high in the mycorrhizal plantlets as compared to non-mycorrhized plantlets at 60 days after inoculation.

Key words: *Musa* sp., Arbuscular mycorrhizal fungi, micropropagation, root morphology.

INTRODUCTION

In vitro propagation of banana via shoot-tip culture provides excellent advantages of exponential rate of multiplication, physiological uniformity, disease-free planting material, easy exchange, genetic stability, and rapid growth in the early growing stages compared to conventional materials. Tissue culture plantlets generally devoid of food reserves are functionally delicate and have poorly developed leaves and root system. Root architecture and morphology is not only important to take up nutrients from the soil, but also helps the plant to survive especially under water stress. Despite its greater influence on plant growth, root architecture has not been studied as much as it should be. Among the environmental factors involved in root development (for example, soil structure, temperature, water, nutrient availability), communities of soil microbiota is of immense importance. Arbuscular myorrhizal fungi (AMF) are endophytic and biotrophic mutualistic symbionts that colonizes most crops roots and increases the capacity to absorb certain mineral nutrients especially phosphorous. Banana shows a great ability to establish mycorrhizal symbiosis (Jaizme-Vega *et al*., 6). The mycorrhizal infection causes physiological changes in leaf of the colonized plantlets that lead to improvements in the general condition of the plant and help to alleviate abiotic stresses (Srivastava *et al*., 9). However, despite the great number of studies on banana, there is paucity of information on the effect of rhizosphere colonization on tissue cultured banana root architecture subjected to water stress over the period of time and their consequences on leaf starch and nutrient content which the present study seeks to fill.

*Corresponding author's Email: vartika.srivastava@icar.gov.in

MATERIALS AND METHODS

Dwarf Cavendish banana plantlets were multiplied through *in vitro* shoot-tip culture. Cultures were initiated with isolation of aseptic shoot-tips from the field collected suckers after initial cleaning and surface sterilization with mercuric chloride (0.1%) for 5 min. under laminar air flow cabinet. Explants were kept in MS medium supplemented with 6 mgl^{-1} BAP and 1 mgL^{-1} IAA for multiplication. After sixth sub-culture cycle, plantlets were transferred to rooting media containing half strength MS media supplemented with 1 mgl^{-1} IAA and finally transferred to hardening pots.

Pure cultures of arbuscular-mycorrhizal fungi (AMF) were procured from TERI, New Delhi and

multiplied on Rhodes grass (*Chloris guyana*) as host plant. Uniformly rooted micropropagated plants of cv. Dwarf Cavendish of about 5 cm tall with at least four leaves were selected from the tissue culture bottles for AMF inoculation and transferred to a growth chamber. Inside the growth chamber, each plantlet was transplanted into individual commercial plastic bags (25×15×10 cm^3) containing steam-sterilized mixture of soil, sand and FYM (1:1:1) along with 20 g of AMF inoculums placed immediately below the roots. The inoculated and non-inoculated (control) plantlets after transplanting were irrigated immediately with sterile water and covered with perforated transparent polybags. After transplanting, plantlets were transferred in growth chamber with day-night temperatures ranging from 27±1ºC and relative humidity was maintained up to 70-75%. Day length was maintained to 16 hr with cool white fluorescent lights at 630 µmol $m^{-2}sec^{-1}$. Plantlets were transferred to net-house at 15 days after inoculation.

Four treatments comprised of non-inoculated control (T_1) and three mycorrhizal fungi *viz*., *Acaulospora scrobiculata* (T_2), *Glomus intraradices* (T_3) and mixed AMF strain (T_4) were arranged in a completely randomized design (CRD). Each treatment was replicated four times and ten plantlets were maintained per replication. The observations were recorded at three growth phases 20, 40 and 60 days after inoculation in both mycorrhized and non-mycorrhized plants. Data was subjected to analysis of variance (ANOVA). There was no fertilizer application during the experimental period and soil moisture was maintained close to 60% field capacity throughout to create water stress conditions to study root architectural changes in mycorrhized and non-mycorrhized plantlets.

Plantlets were sampled and assessed for root colonization by staining method as suggested by Phillips and Hayman (8). Extent of root colonization was assessed in twenty segments, averaged and expressed as a percentage of root length. Total number of roots and root length per plant was calculated for control and mycorrhized treatments in five randomly selected plants, averaged and recorded in each replication. Roots were washed with tap water to remove the adhering potting mixture. Thereafter, the excess water on the root surface was removed by gentle swabbing with blotting paper and fresh weights were noted immediately. For dry matter determination of roots, samples were put in tissue paper bags and kept in a hot air oven at 70°C till they showed no change in weights. Starch content was determined in the first fully expanded leaf from top at 20, 40 and 60 days after mycorrhizal inoculation by anthrone method (Dubois *et al*., 5). The leaves of control and mycorrhized banana plantlets were collected after 60 days of acclimatization for N, P and K estimation. Third leaf from top was selected for sampling in four randomly selected plants in a treatment and dried in hot air oven at 70°C for 24 hr. until constant weight was recorded. The dried leaves were then chopped and grinded before being subjected to nitrogen, phosphorus and potassium determination. Total nitrogen content in leaf samples was determined by nitrogen analyzer (Pelican, Model KEL 20L) adopting Kjeldahl method. Phosphorus content in dried leaf samples of control and mycorrhized banana plantlets was determined by method as described by Olsen *et al*. (7). Potassium content in leaf was estimated by atomic absorption spectrophotometer (Elico, Model SL-194) after digestion of the samples in tri-acid mixture [HNO_3: H_2SO_4: 60% $HClO_4$ in a ratio of (75:30:15)]. Dried plant material (50 mg) was taken in the digestion tube and 10 mL tri acid mixture was added to it. Tubes were put on digestion heater (Pelican, Model KES-20L) and heated at 200°C till the solution became colourless. Solution was brought to room temperature and final volume was made to 100 ml with double-distilled water. The concentration of potassium in the solution was determined by atomic absorption spectrophotometer using specific hollow cathode lamp of the elements and standards.

RESULTS AND DISCUSSION

Root colonization percentage increased steadily in all the mycorrhizal treatments during the experimental period and reached maximum at 60 days after inoculation (Fig.2). At the first assessment (20 days after inoculation) mycorrhizal colonization in mixed AMF strain was 51.25 per cent, at 40 days it was 83.75 per cent, and increased to 96.25 per cent at 60 days after inoculation (Fig. 1a). Maximum root colonization was recorded in mixed AMF which was at par with other mycorrhizal treatments after 60 days of inoculation. Yano-Melo and Lima-Filho (10) reported that differences in AMF colonization frequency could be attributed to the differences in mycorrhizal dependency among the host plants and the abiotic factors. In our experiment, superiority of mixed AMF culture may be attributed to that of existing compatible AMF communities. Furthermore, when plants are colonized by more than one AMF isolates, preference of host for specific isolates of the community is noted.

The root system of micropropagated *Musa* plants consists of a series of adventitious roots that develop directly from the corm (subterranean structure). These roots branched in first order laterals, from which secondary roots developed. Mycorrhizal banana plants showed a denser root system than the control

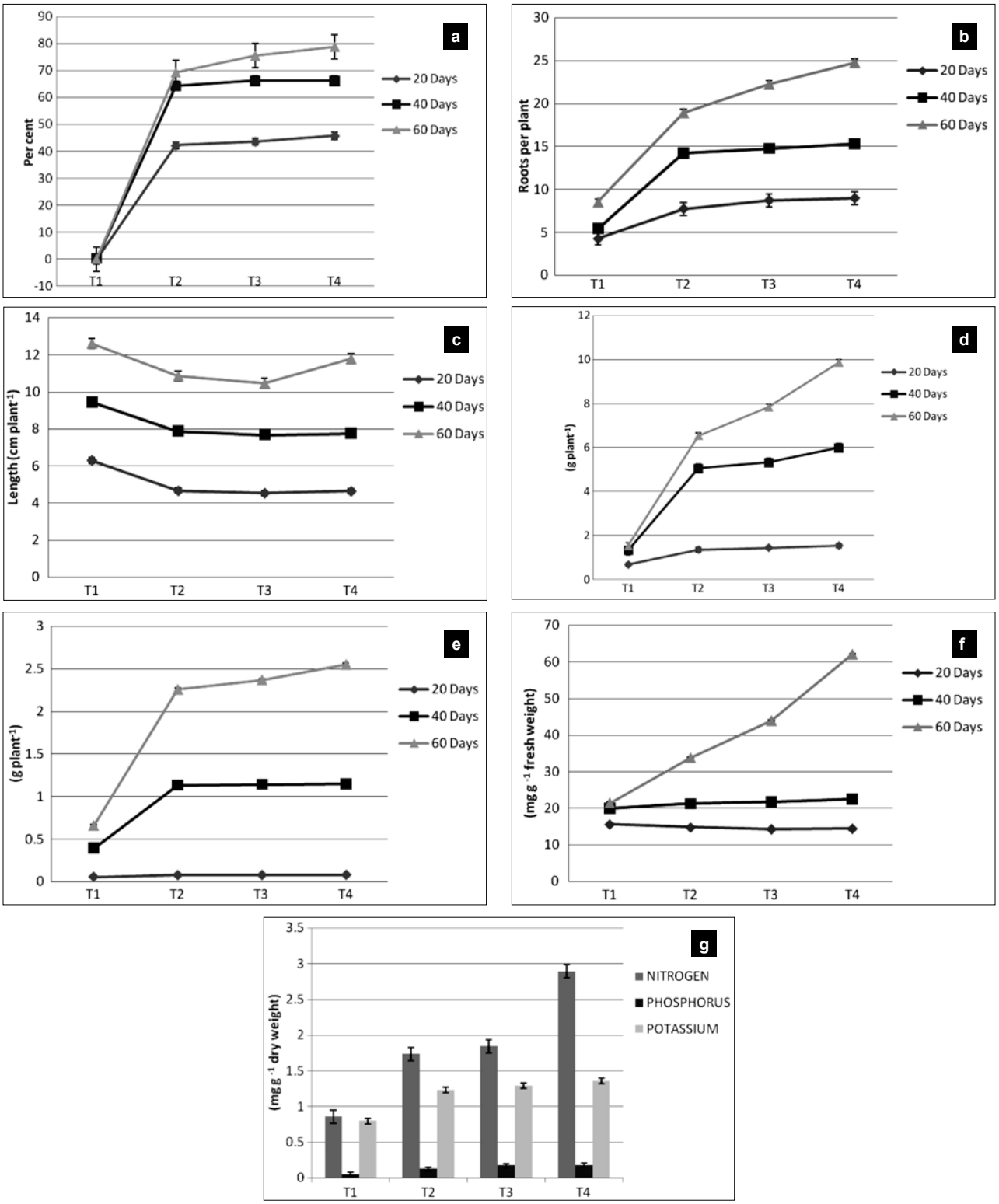

Fig. 1. **(a)** Per cent root colonization, **(b)** Number of roots per plant, **(c)** Average root length per plant, **(d)** Root fresh weight per plant, **(e)** Root dry weight per plant, **(f)** Starch content in leaves of control and mycorrhized plantlets during hardening at 20, 40 and 60 days after inoculation, **(g)** Leaf N, P and K content in control and mycorrhized micropropagated banana plantlets under water stress at 60 days of hardening period (T1: Control; T2: *Acaulospora scrobiculata*; T3: *Glomus intraradices*; T4: Mixed AMF strain).

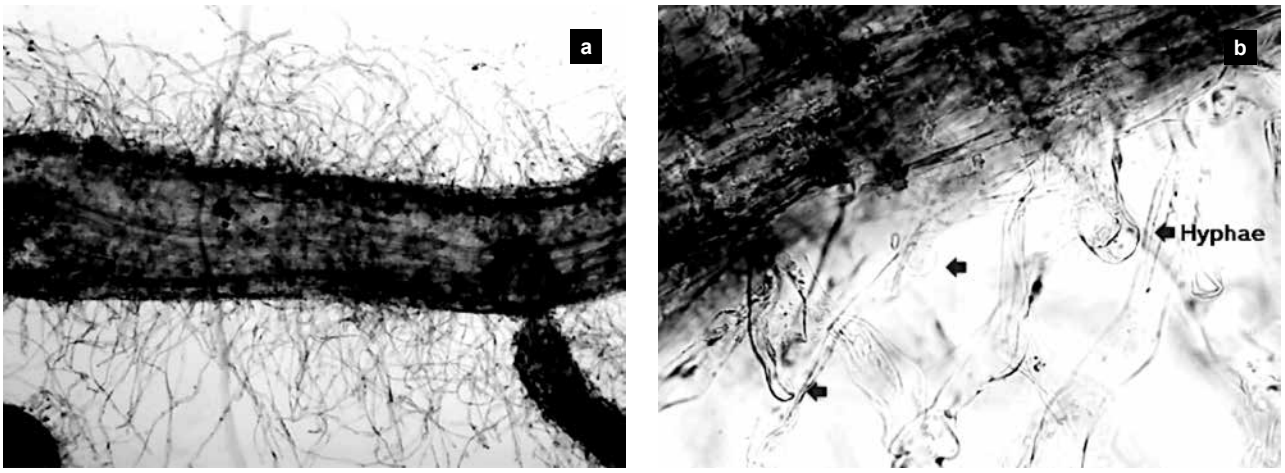

Fig. 2. (a) Banana root section covered with mycorrhizal network, (b) Mycorrhizal hyphae protruding from the root cells.

plants. Adventitious root number increased over time in both mycorrhizal and non-treated plants, although values of mycorrhizal plants were always significantly higher (Fig. 1b).

The most important effect of AMF colonization on the root system of banana plants was an increase of adventitious root branching (Fig. 3). This increase, together with the increase in number of adventitious roots, led to a denser root system. It is generally assumed that a denser root system has a greater absorbing power than an elongated one. Root architecture and morphology provide a lot of useful information about plant species and their ability to take up nutrients from the soil. Mycorrhizal hyphae are more efficient than roots alone in nutrient uptake since they increase the root absorption area. It has been reported that AMF are even able to change root architecture (Jaizme-Vega *et al*., 6). The extensive network of external mycelium, with its absorbing power and explorative functions, in addition to the denser root system, could improve the "growth effect" characteristic of mycorrhizal plants. Moreover, a much branched root system is particularly useful for banana plants, as they are easily uprooted by winds, which are a frequent problem in many cropping areas of the world. In the case of adventitious root length, mycorrhized plantlets showed significantly lower root length than non-treated plantlets, throughout the experimental period (Fig. 1c). The reduction in length of adventitious roots was accompanied by an increase in their number, as also observed in another endomycorrhizal system in which a monocotyledon was involved i.e., *Allium porrum* + *Glomus E3, Ornithogalum umbellatum* + *G. fasciculatum* (Berta *et al*., 2). The increase in the root length under water stress is the first line of defense in all plant species

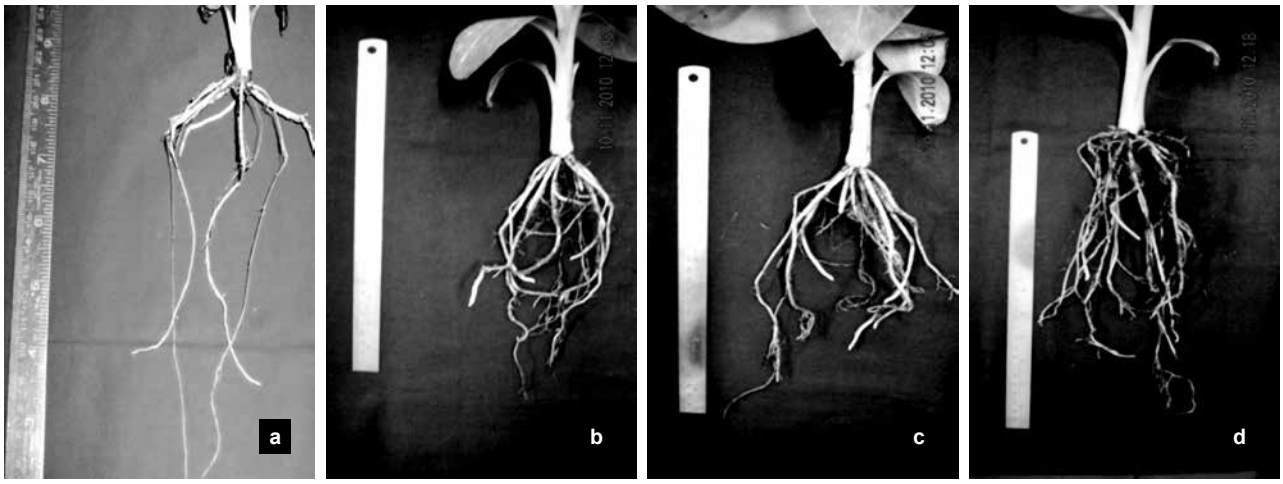

Fig. 3. Root morphology of control and mycorrhizal banana plantlets at 60 days after planting. (a) Control, (b) *Acaulospora scrobiculata*, (c) *Glomus intraradices*, (d) Mixed AMF strains treated plantlets.

to extract water from the deeper zones of soil, this may be the reason of increased root length in non-mycorrhized water stressed banana plants.

Significantly higher root fresh weight was recorded throughout the experimental period in mycorrhized plants as compared to control. The increased fresh weight may be due to enhanced number of lateral roots as well as larger diameter of mycorrhized roots. All mycorrhizal treatments showed at par readings at 20 days after inoculation but later on significant increase in root fresh weight was recorded in mixed AMF strain (Fig. 1d). Maximum root fresh weight at 60 days after inoculation was recorded in mixed AMF strain indicating its beneficial effect on shoot growth and development. It has been observed that with increase in the colonization intensity, dry weight of mycorrhized roots increased gradually. Increased root dry weight in mycorrhized plants was recorded at 20, 40 and 60 days after inoculation but significant difference was observed at 60 days after inoculation where maximum root dry weight was recorded in mixed AMF strain (Fig. 1e). This signifies increased root growth rate and dry matter accumulation due to enhanced photosynthetic efficiency in plants inoculated with mixed AMF strains. The starch content in the leaves of mycorrhized plants was significantly less than that of control at 20 days after inoculation. It might be due to probable hydrolysis of starch in to sugar and its translocation to the root zone to support the symbiotic colonization with mycorrhizal fungi. The starch levels were found to increase at 40 and 60 days post inoculation in mycorrhized plantlets over control (Fig. 1f) which indicates increased photosynthetic rate of mycorrhized plantlets (data not presented) resulting in improved assimilation of photosynthates in the leaves to support greater carbon flow to the root system needed to support the symbiosis. Maintenance of greater photosynthetic capacity during water stress in mycorrhizal plants has also been indicated by higher starch levels in mycorrhized than in non-mycorrhized plants (Davies *et al*., 4).

It has often been reported that nutrient uptake by mycorrhizal plants is faster than non-mycorrhized roots (Bolan, 3). In the present study though plantlets were hardened without fertilizer application, N, P and K levels in the leaves of mycorrhized plantlets were significantly higher as compared to the non-inoculated (control) plantlets after 60 days of acclimatization (Fig. 1g). The higher P and K levels in banana plants grown in low fertility soil condition were observed by Jaizme-Vega *et al*. (6). AMF inoculation was found to increase the fitness of the host plant by increasing uptake of minerals such as P that is relatively immobile in soils. AMF develop intensively inside roots and within the soil by forming an extensive extraradical mycelium which helps the plant in extracting mineral nutrients and water from the soil. In plants, particularly those with restricted or weak root system, hyphal connections act as a bridge between roots and nutrient sites in soil and facilitate efficient uptake of immobile nutrients by host plants (Azcon-Aguilar and Barea, 1). The higher levels for N, P and K observed in mixed AMF treated plantlets may be correlated to the maximum colonization percentage at 60 days after inoculation. Depending on the host plant, colonization by AMF can increase P nutrition. Mycorrhizal structures effectively take up P from lower concentrations in the soil at which normal plant roots fail. AMF helps in increasing nutrient uptake by increasing the surface area of absorptive system (roots) of plants and exploring soil by extraradical hyphae beyond the root hair and P-depletion zone. The absorbed P is then converted to polyphosphate granules in external hyphae and passed to the arbuscule for transfer to the host plant (Azcon-Aguilar and Barea, 1).

The results of this experiment demonstrates that mycorrhizal inoculation has positive effects on root architecture development under water stress conditions which has synergistic effect on plant development by improving water and nutrient uptake. The use of mycorrhizal inoculants could help to simplify and optimize the hardening of tissue culture plantlets, with favorable effects on the quality of the planting material. However, an initial assessment of the mycorrhizal strain suitable for a particular crop should be made in order to adjust the doses and types of inoculants to the specific conditions of each situation. In this experiment, banana plantlets responded to all the mycorrhizal strains used but the response was more prominent in case of mixed AMF treated plantlets.

REFERENCES

1. Azcon-Aguilar, C. and Barea, J.M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens: an overview of the mechanisms involved. *Mycorrhiza*, **6**: 457-64.

2. Berta, G., Fusconi, A. Trotta, T. and Scannerini, S. 1990. Morphogenet ic modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum* L. *New Phytol.* **114**: 207-15.

3. Bolan, N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil*, **134**: 189-207.

4. Davies, F.T., Potter, J.R. and Linderman, R.G. 1993. Drought resistance of mycorrhizal pepper plants independent of leaf P concentration-response in gas exchange and water relations. *Physiol. Plant*. **87**: 45-53.

5. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Calorimetric method for determination of sugar and related substances. *Anal. Chem.* **28**: 350-56.

6. Jaizme-Vega, M.C., Esquivel Delamo, M., Tenoury Dominguezy, P. and Rodriguez-Romero, A.S. 2002. Efectos de la micorrización sobre el desarrollo de dos cultivares de platanera micropropagada. *InfoMusa.* **11**: 25-28.

7. Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *U.S. Dep. Agr. Circ*. 939.

8. Phillips, J.M. and Hayman, D.S. 1970. Improved procedure for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment for infection. *Trans. Brit. Mycol. Soc.* **55**: 158-161.

9. Srivastava V., Singh A. K. and Singh, S.P. 2014. Effect of mycorrhizal inoculation on water stress tolerance of tissue cultured banana (Musa × paradisiaca) plants. *Indian J. Agric. Sci.* **84**: 124-26.

10. Yano-Melo, A.M. and Lima-Filho, J.M. 1999. Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets. *Springer-Verlag*. **9**: 119-23.

Received : June, 2017; Revised : October, 2017;
Accepted : October, 2017