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Effect of mycorrhizal inoculation on water stress tolerance of tissue cultured banana (*Musa* × *paradisiaca*) plants

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The drought is considered one of the major abiotic stresses which adversely affect crop productivity throughout the world. It may result due to less rainfall, insufficient irrigation, use of irrigation water consisting of heavy salt concentration, percolation or sandy soil texture. The drought and salinity cause osmotic stress which affects growth and development of plant (Zhu *et al*. 1997). As the tissue cultured plantlets are very delicate and prone to biotic and abiotic stresses during *ex vitro* acclimatization, use of bioprotectants like AMF (arbuscular-mycorrhizal fungi) has been suggested by many workers for improving the growth and survival of tissue cultured plantlets (Gaur and Adholeya 1999, Marin *et al*. 2003, Krishna *et al*. 2005). Banana, being a monocot with shallow root system and a big leaf area index, needs large amount of water during hardening and water stress during this stage is detrimental. Micropropagated plantlets are usually acclimatized under high humidity and need frequent water application for higher rate of survival (Srikul and Turner 1995). This investigation was carried out to observe the survival of micropropagated banana plantlets during hardening under restricted water and nutrient supply and with aid of mycorrhizal inoculation which have been reported beneficial for encountering stress conditions. Thus, understanding the physiological adjustments behind the adaptive mechanism induced by mycorrhizae to various stresses encountered in tissue cultured plants would be of significant research importance.

The experiment was conducted in tissue culture laboratory of the Department of Horticulture, Institute of Agricultural Sciences, BHU, Varanasi during 2010-11. The micropropagated Dwarf Cavendish banana (*Musa* × *paradisiaca* L.) plants were raised in the laboratory and transferred for hardening. Each plantlet was transplanted into individual commercial plastic bags (25×15×10cm) containing steam-sterilized mixture of soil, sand and FYM (1:1:1) along with 20 g of mycorrhizal inoculums (procured from TERI, New Delhi) placed immediately below the roots. Treatments were comprised non-inoculated control (T_1), *Acaulospora scrobiculata* (T_2), *Glomus intraradices* (T_3) and mixed arbuscular mycorrhizal strain (T_4). The inoculated and non-inoculated (control) plantlets after transplanting were irrigated immediately with sterile tap water and covered with perforated transparent polybags. After 30 days of full acclimatization period, plantlets were allowed to dry until soil water content reached 60% field capacity. Physiological changes in water stressed control and mycorrhized plantlets were recorded after 10 days of stress period. Neither fertilizer nor fungicide or pesticide was applied to the plantlets during the experimental period. Plantlets were sampled for root colonization by staining method as suggested by Phillips and Hayman (1970). Physiological parameters, viz. stomatal conductance, and photosynthetic rates were measured using Infra Red Gas Analyzer (IRGA, LiCOR 6200) and expressed in µmol $CO_2/m^2/s$. The RWC (relative water content) in the recently matured leaf was determined using the method suggested by Weatherley (1950). All the treatments were replicated four times and ten plantlets per replication were maintained. The observations were recorded at 10 days after imposing to water stress in inoculated and non-inoculated plants. Data were subjected to analysis of variance (ANOVA). Percentage values were arcsin transformed before statistical analysis.

At 30 days after acclimatization and 10 days of water stress period, maximum root colonization per cent (83.75) was recorded in mixed AMF which was at par with *Glomus intraradices* (83.75%) and *Acaulospora scrobiculata* (81.25%) treatment. Mycorrhizal colonization under water stress did not differ among the inoculated treatments though mixed AMF treated plantlets showed better responses towards physiological and morphological adjustments of the plantlets

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(Table 1). Superiority of mixed AMF culture may be attributed to existing compatible AMF communities (Johnson *et al*. 1991). The survival rate in non-inoculated banana plantlets was only 65%. Moreover, high mortality in controlled plantlets may be due to fungal attack after transfer to pots, whereas mycorrhizal treatments showed 100 per cent survival throughout the experimental period which indicated their role in disease control. In our study the mycorrhizal inoculation significantly increased the number of surviving plants under water stress, a result which is in line with the earlier findings of Gaur and Adholeya (1999), Estrada-Luna and Davies (2003), Marin *et al*. (2003) and Krishna *et al*. (2005) in micropropagated *Syngonium*, chile ancho pepper, persimmon and grape plantlets, respectively.

Under water stress, visible wilting was observed in both control and mycorrhized plantlets. Relative water content (RWC) in mycorrhized plantlets was found to be significantly higher than that of control plants after 10 days of water stress (Table 1). In mixed AMF strains and *Glomus intraradices* treated plantlets the leaves showed more water content (93%) than non-mycorrhized plant. The increased RWC in the leaves of mycorrhized plantlets under water stress might be due to an improvement of the water uptake by mycorrhizal root system through extra-radical phase (Ruiz-Lozano and Azcon 1995). In a study of micropropagated mycorrhizal strawberry plantlets, Herandez-Sebastia *et al.* (1999) suggested that the higher concentration of water soluble compounds in plant tissue could be a reason for higher RWC of the whole plantlets. In this experiment mycorrhized plants had higher photosynthetic rates and stomatal conductance, which could be associated with the higher CO_2 influx into mesophyll tissues, as indicated by higher stomatal conductance. Mixed strain of AMF was found to be significantly superior in terms of increased photosynthesis and stomatal conductance (Table 1). Around 70% increase in photosynthetic rate and 33% increase in stomatal conductance were observed in mycorrhized plants as compared to the non-mycorrhized plants under water stress. The enhanced photosynthetic rates in inoculated plants suggest that mycorrhizal plants may be able to assimilate more CO_2 and thereby accumulate more biomass. A significant increase in photosynthetic rate of AMF inoculated *Ziziphus nummularia* seedling was observed by Mathur and Vyas (1995). Higher photosynthetic ability in micropropagated mycorrhized grape plantlets at 60 days of acclimatization was also reported by Krishna *et al.* (2005).

The plant biomass production in control (non-mycorrhized) and mycorrhized plantlets was recorded after 10 days of water stress. Significantly more plant height, number of leaves per plant, leaf area, fresh and dry shoot weight were recorded in the mycorrhized banana plants compared to the non-mycorrhized control plants under moisture stress condition (Table 2). The mixed AMF treated

Table 1 Effect of arbuscular-mycorrhizal fungi (AMF) inoculation on root colonization, survival and physiological status of micropropagated banana plantlets after 10 days of water stress

Treatment	Per cent root colonization	Per cent survival	Relative water content (%)	Stomatal conductance (mol/m^2/s)	Photosynthetic rate (µmol/m^2/s)
T_1	0.00 (0.00)	65.0 (53.73)	85.14	0.030	10.20
T_2	81.25 (64.35)	100 (90.01)	88.95	0.047	13.36
T_3	83.75 (66.23)	100 (90.01)	90.67	0.052	13.58
T_4	83.75 (66.23)	100 (90.01)	90.93	0.090	14.54
Mean	62.18 (52.05)	93.75 (75.53)	88.92	0.055	12.54
SEm±	1.78	1.56	0.52	0.011	0.12
CD(P=0.05)	3.89	3.41	1.14	0.025	0.26

Table 2 Effect of arbuscular-mycorrhizal fungi (AMF) inoculation on plant biomass of micropropagated banana plantlets after 10 days of water stress

Treatment	Plant height (cm)	Number of leaves/plant	Leaf area (cm^2/plant)	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)
T_1	7.42	4.81	107.63	5.40	0.557
T_2	11.60	6.10	248.83	8.75	1.495
T_3	12.66	6.32	277.02	9.33	1.720
T_4	13.69	6.76	285.36	9.67	1.870
Mean	11.34	6.00	229.71	8.29	1.411
SEm±	0.20	0.16	4.11	0.23	0.041
CD(P=0.05)	0.45	0.36	8.96	0.50	0.091

plantlets showed 54% increase in plant height, 71% increase in number of leaves per plant and 29% increase in dry shoot weight as compared to non treated control. Mixed AMF and *Glomus intraradices* treated plantlets showed at par values in leaf area and shoot fresh weight and showed 38% and 57% increase respectively, over control under water stress. The results on plant biomass augmentation in mycorrhized plantlets reinforce those obtained by Lin and Chang (1987) who also obtained increased height, diameter of the pseudostem and dry matter weight of banana plantlets inoculated with species of *Glomus*. Duan *et al*. (1996) found higher stomatal conductance in mycorrhizal plants than in controls, similar results were obtained in our experiment. In our experiments, inoculation with AMF increased plant biomass of micropropagated banana plantlets during acclimatization period, which might have increased the rates of photosynthesis. Growth promotion was slightly better with use of mixed AMF species. This corroborates the findings of Yao-Qing *et al*. (2004).

SUMMARY

Study showed that mycorrhizal inoculation during the hardening phase of banana plantlets raised through tissue culture was highly beneficial and a good strategy for sustainable plant production and protection. The mycorrhizal inoculation not only improved the survival rates but also imparted increased biomass as compared to the non-treated plantlets under water stress. The physiological changes in the leaves of mycorrhized plantlets revealed a significant increase in photosynthetic rate, stomatal conductance and relative water content under water stress which contributed to higher survival as compared to non-mycorrhized plantlets. Though, the banana plantlets responded to all mycorrhizal treatments but those treated with mixed AMF strains showed significantly better growth and physiological adjustments under water stress.

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