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Responses of *in vitro* raised bitter gourd plantlets to arbuscular mycorrhiza fungal species

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ABSTRACT

Acclimatization of micropropagated plants corresponds to a transition period when roots become adapted to a substrate with less available nutrients, and to an autotrophic condition. To ameliorate this problem in bitter gourd, 30-day-old, *in vitro* rooted plantlets of bitter gourd cultivars Pusa Do Mausami, Pusa Vishesh and the DBGy 201 were subjected to root inoculation with different arbuscular mycorrhiza fungal (AMF) strains. All the mycorrhizal treatments showed almost two times higher *ex vitro* survival than the control plantlets. Mycorrhization plantlet showed increase in vine length in Pusa Vishesh (194.02 cm) in mixed strain, leaf area in Pusa Vishesh (107.91 cm^2) in *Acaulospora scorbiculata*, chlorophyll in Pusa Do Mausami (3.29 mg/g FW) in *A. scorbiculata*) and total phenols content in Pusa Do Mausami (7.84 µg/g FW) in *E. columbiana*). Photosynthetic rates were enhanced in arbuscular mycorrhizal fungi (AMF) treated plant in Pusa Do Mausami (10.75 µmol $CO_2/m^2/s^1$) in mixed strain in comparison to an uninoculated control. Among the AMF species, mixed strain (Nutrilink®) showed good as high as 38% root colonization for all the cultivars. In this experiment the mixed AMF strain has contributed significantly in survival of the plantlets and plant establishment in the field.

Key words: Bio-hardening, bitter gourd plantlets, physiological parameters, biochemical changes.

INTRODUCTION

Mycorrhizal inoculation of *in vitro* propagated transplants has proven to be effective in respect of tolerance to different stresses, improvement in vegetative growth and mineral nutrient status. It has an advantage to the transplanted propagules in terms of nutrient availability, soil pH, aeration and protects the juvenile axenic plants from infestation of the harmful saprophytes. The beneficial effects brought about due to an array of physiological and biochemical changes imparted in the tissue culture raised plants was found in Chile ancho pepper plants (Estrada-Luna and Davies, 2).

The ultimate success of micropropagation on a commercial scale depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rates. Protocols for biohardening of *in vitro* regenerated plantlets of chilli using *Glomus mosseae, Gigaspora margarita* and mixed arbuscular mycorrhizal fungi (AMF) strains were standardized (Ranjan *et al.*, 8). The *in vitro* raised plantlets were treated with AMF and the maximum survival (97.08%) was recorded with mixed strain of *Glomus mosseae* and *Gigaspora margarita.* The root and shoot length was also maximum when plantlets were treated with mixed AMF strains. In the cucurbitaceous crops like bitter gourd, no such trials have been conducted worldwide. Therefore, the present investigations were carried out to assess the efficiency of different AMF species in improving growth and productivity of *in vitro* raised bitter gourd.

MATERIALS AND METHODS

Micro-propagated plantlets of bitter gourd (*Momordica charantia* L.) cultivars Pusa Do Mausami and Pusa Vishesh and DBGy 201 regenerated at the Central Tissue Culture Laboratory, IARI, New Delhi were used as experimental material for the present study. Thirty-day-old micropropagated plantlets were used for hardening with mycorrhiza after Stage-IV. Six treatments comprised of AMF species like *Acaulospora scorbiculata* (T_1), *Glomus manihotis* (T_2), *Scutellospora heterogama* (T_3), *Gigaspora gigantea* (T_4), *Entrophospora colombiana* (T_5), mixed AMF strains (Nurtilink®, T_6) and control (T_0) were used for the present study. Soil-based AMF cultures were multiplied on Rhode's grass (*Chloris guyana*) as a host plant. In order to ensure sufficient root colonization, rhizosphere soil of Rhode's grass containing mycelia, spores, arbuscules, vesicles and root segments were used as inoculum. The rooted bitter gourd plantlets (30-day-old) were washed with sterile tap water to dislodge the adhering agar-agar and then transferred to plastic pots containing sterilized potting mixture (soil, sand and FYM; 2:2:1) followed by application of 20 g AMF inoculum in the rhizosphere soil. The control treatment had

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only sterile potting mixture. The potted plants were kept in the net house for their further growth and development.

Ex vitro (field) survival percentage and per cent root colonization were measured 45 days after inoculation. Fresh root segments were stained with 0.01% trypan blue in lactic acid (Phillips and Hayman, 7). Growth parameters, *viz*., plant height, root length, leaf number and area were recorded 45 days after inoculation. Leaf area was measured by leaf area meter. Photosynthetic rate and transpiration rate of the intact mature leaves were determined by a portable infrared gas analyzer (LiCor-6400, USA). Relative water content was determined in leaves by the method suggested by Weatherley (10). After 45 days of acclimatization, leaf chlorophyll was estimated as per the method suggested by Barnes *et al.* (1). Total phenols in leaf samples were assayed by the method proposed by Malik and Singh (6). For estimation of enzyme polyphenol oxidase foliar samples were prepared according to the method suggested by Lerner *et al.* (5) with slight modifications. Both catecholase and cresolase activities were measured employing the method suggested by Sanchez-Ferrer *et al.* (9). The experiments were laid out in complete randomized design with three replications. The percentage data were subjected to Arc Sin (√%) transformation before subjecting to ANOVA.

RESULTS AND DISCUSSION

The AMF strains had differential response to the different varieties of bitter gourd. With respect to *ex vitro* survival T_1 and T_4 gave maximum survival of 90 and 90.05% in Pusa Do Mausami, whereas in Pusa Vishesh performed well in T_2, T_4 and T_5 with 90.05% and in DBGy-201 in T_4 (76.41%) and T_6 (65.94%). All the mycorrhizal treatments showed almost two times higher *ex vitro* survival than the control plantlets (Fig. 1). The higher survival rates of mycorrhizal *in vitro* raised plantlets might be due to the development of strong root system and improved uptake of plant immobile nutrients and water. It was clearly evident from this study that the mixed strain inoculated plants had maximum plant height and number of leaves per plant in all the three genotypes (Table 1). The three genotypes responded differently to the different AMF strains. Pusa Vishesh had the maximum plant height in the T_6 treatment (206.72 cm) followed by T5 (192.32 cm). Number of leaves/ plant was recorded the maximum in T_6 (91.80) followed by T_5 (89.09) in the same cultivar whereas the leaf area was highest in T_1 (108.22 cm^2) in Pusa Vishesh followed by T_6 in Pusa Do Mausami (100.69 cm^2). It was revealed from Table 2, that T_6 had the maximum primary root length (85.91 cm) in Pusa Do Mausami followed by T_6 in Pusa Vishesh (78.90 cm, Fig. 2.), treatment T_6 in Pusa Do Mausami (10.31) and Pusa Vishesh (10.06) recorded the maximum plant fresh weight : dry weight ratio. The enhanced growth of micropropagated plantlets due to AMF inoculation and was attributed to significant increase in height, leaf area and fresh weight of roots and shoots (Yano-Melo *et al.*, 11). The positive effect of AMF on growth and nutrient, uptake depends on the plant species as well as strain used. This effect is mainly caused due to profuse branching of lateral roots or their elongation.

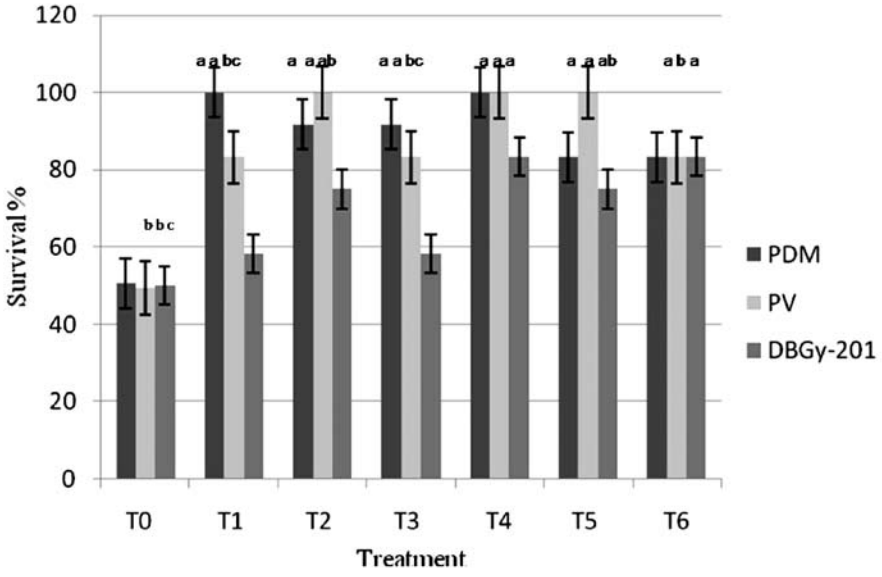

Fig. 1. Effect of arbuscular-mycorrhizal fungi inoculation on *ex vitro* survival (%) of micropropagated bitter gourd plants.

Table 1. Influence of AMF inoculation on plant height, leaf number and area of micropropagated bitter gourd plantlets.

Treatment	Plant height (cm)			No. of leaves/ plant			Leaf area (cm^2)		
	PDM	PV	DBGy-201	PDM	PV	DBGy-201	PDM	PV	DBGy-201
T_0	117.57 ± 6.62^{c}	119.84 ± 7.24^{c}	107.96 ± 2.38^{d}	30.29 ± 2.87^{c}	30.4 ± 3.02^{b}	29.15 ± 0.94^{d}	75.30 ± 1.67^{cd}	79.32 ± 2.23^{b}	74.25 ± 1.46^{c}
T_1	152.15 ± 14.59^{b}	186.85 ± 7.71^{a}	113.37 ± 1.19^{c}	50.94 ± 10.77^{b}	81.73 ± 7.42^{a}	33.64 ± 1.53^{bc}	84.51 ± 6.67^{bc}	108.22 ± 7.77^{a}	67.51 ± 2.69^{de}
T_2	135.27 ± 3.65^{bc}	164.22 ± 2.94^{b}	115.11 ± 1.49^{bc}	34.74 ± 1.74^{bc}	51.72 ± 8.90^{b}	29.42 ± 0.56^{cd}	58.63 ± 4.27^{e}	92.81 ± 16.01^{ab}	65.45 ± 3.32^{e}
T_3	153.06 ± 3.33^{b}	163.62 ± 13.02^{b}	111.21± 1.85^{cd}	41.44 ±1.51^{bc}	54.05 ± 13.55^{b}	35.16 ± 1.35^{b}	68.74 ± 7.22^{de}	84.82 ± 5.75^{b}	72.77 ± 1.45^{cd}
T_4	142.83 ± 6.24^{b}	146.82 ± 4.20^{b}	119.12 ± 1.64^{b}	36.83 ± 2.45^{bc}	36.81 ± 1.37^{b}	33.28 ± 1.42^{bcd}	71.59 ±3.49^{cde}	75.4 ± 6.17	70.8 ± 1.50^{cde}
T_5	189.96 ± 8.11^{a}	192.32 ± 4.69^{a}	119.03 ± 1.47^{b}	88.84 ± 11.12^{a}	89.09 ± 7.57^{a}	37.07 ± 1.37^{ab}	94.20 ± 3.34^{ab}	87.99 ± 0.68^{ab}	84.34 ± 2.10^{b}
T_6	186.36 ± 6.68^{a}	206.72 ± 3.29^{a}	130.16 ± 1.28^{a}	82.68 ± 7.05^{a}	91.80 ± 12.37^{a}	41.07 ± 2.44^{a}	100.69 ± 4.19^{a}	95.15 ± 2.82^{ab}	90.56 ± 1.14^{a}

Mean values followed by the different letter under different treatments within a column are significantly different from each other at P≤0.05; PDM = Pusa Do Mausami, PV = Pusa Vishesh

The data on physiological parameters with respect to plantlets were presented in the Table 3. The photosynthetic rate was highest in Pusa Do Mausami treated with *Glomus manihotis* (T_2, 12.11), whereas *Scutellospora heterograma* (T_3) contributed maximum transpiration in the same variety (6.37). The increased photosynthetic rate could be was due to increased leaf area and increased synthesis of chlorophyll. The increased transpiration rate could be better attributed to better uptake of water as mycorrhiza explores the soil better than roots and increased metabolic activity associated with AMF colonization.

The mycorrhizal inoculation significantly enhanced the root colonization and relative water content. Among the different treatments tried, maximum colonization was found in mixed strain in all the three genotypes which was estimated to 39%. The colonization frequency is due to rapid hyphal recognition of host species and forming a mycelial shield causing better establishment, growth and nutrient uptake of such plants. The differential response to colonise the host species by AMF species varied considerably and depended on the host endophyte combination. Such phenomenon probably also depends upon variations in host preference and soil adaptation. Superiority of mixed cultures can be attributed to that of AMF communities in the present investigation. When plants are colonized by more than one AMF isolates, preference of host for specific isolates of the community is pertinent (Johnson *et al*., 3). Similarly, the relative water content of leaves was highest in Pusa Do Mausami (92.29%; Table 4) treated with mixed strain (T_6). Increased root biomass through branching could be one of the reasons for improved water uptake *via* increased root surface area due to microbial inoculation.

The mycorrhizal inoculation drastically increased the endogenous biochemical constituents like chlorophyll and total phenols contents compared to control (Table 5). Among the different AMF treatments, mixed strain was proved to be best by increasing the tissue chlorophyll a content in all three genotypes being the maximum in Pusa Do Mausami (3.46 mg/g). The chlorophyll b content was the maximum in T_3 in DBGy-201 (0.67 mg/g). The total chlorophyll content was highest in *Acaulospora scorbiculata* (T_1) fungi in genotype Pusa Do Mausami (3.46 m/g) followed by DBGy-201 in the same strain (3.28 mg/g; Table 5). Plantlets inoculated with T_1 strain recorded maximium phenol content in Pusa Do Mausami (8.08 µg/g; Table 5). AMF inoculated plantlets exhibited higher level of catecholase and cresolase activities. These enzyme activities were highest in the mixed strain in all the genotypes and among them, Pusa Vishesh

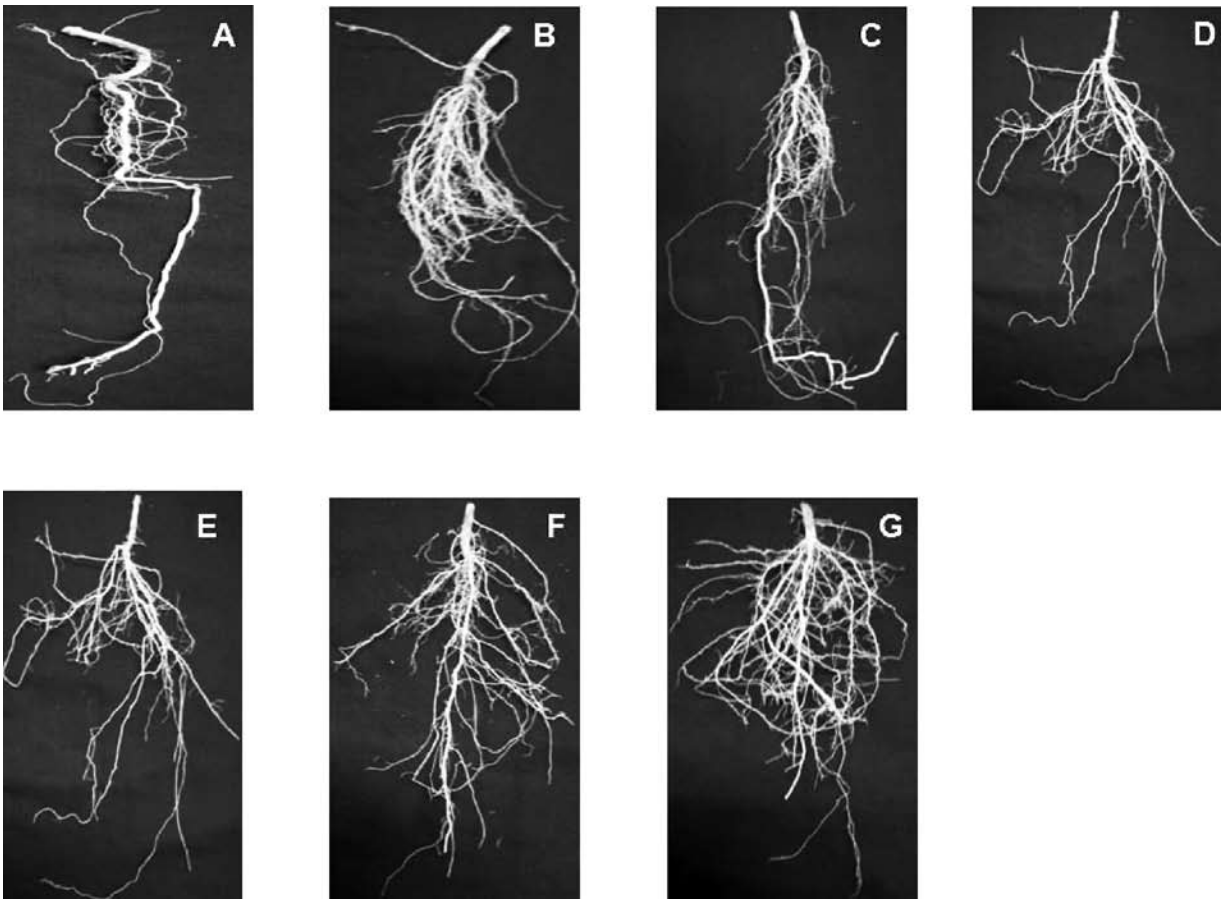

Fig. 2. Effect of AMF inoculation on enzymatic activity in bitter gourd plantlets.

Table 2. Influence of AMF inoculation on length of primary root and plant fresh and dry weight ratio of micropropagated bitter gourd plantlets.

Treatment	Length of primary root (cm)			Plant fresh weight: dry weight		
	PDM	PV	DBGy-201	PDM	PV	DBGy-201
T_0	47.49 ± 1.39^{d}	43.05 ± 2.73^{d}	44.29 ± 1.12^{e}	7.28 ± 0.48^{a}	7.35 ± 0.52^{b}	7.39 ± 0.25^{bc}
T_1	50.07 ± 0.22^{d}	54.94 ± 1.88^{c}	51.21 ± 0.49^{d}	8.06 ± 0.50^{a}	8.12 ± 0.48^{ab}	7.96 ± 0.28^{bc}
T_2	51.86 ± 1.84^{d}	51.61 ± 1.13^{c}	51.91 ± 1.27^{d}	7.36 ± 0.33^{a}	7.31 ± 1.03^{b}	7.23 ± 0.46^{c}
T_3	58.00 ± 2.66^{c}	66.56 ± 2.88^{b}	67.11 ± 1.27^{c}	7.95 ± 1.02^{a}	8.97 ± 0.25^{ab}	8.21 ± 0.11^{b}
T_4	52.10 ± 2.51^{d}	78.85 ± 0.65^{a}	70.15 ± 0.67^{b}	8.49 ± 1.30^{a}	7.88 ± 0.23^{ab}	8.14 ± 0.37^{b}
T_5	66.61 ± 1.25^{b}	72.87 ± 2.34^{ab}	71.46 ± 0.17^{b}	9.99 ± 2.20^{a}	7.74 ± 1.09^{ab}	8.18 ± 0.21^{b}
T_6	85.91 ± 2.06^{a}	78.90 ± 4.36^{a}	77.84 ± 0.67^{a}	10.31 ± 0.22^{a}	10.06 ±1.11^{a}	9.99 ± 0.23^{a}

Mean values followed by the different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$; PDM = Pusa Do Mausami, PV = Pusa Vishesh

recorded the maximum enzymatic activity in this strain (Fig. 3).

Phenolic compounds occur naturally in plant system and are known for their anti-microbial properties and inhibit fungal spore germination, mycelia fungal enzymes and toxin produced by pathogens. *Entrophospora columbiana* was found to induce maximum *in vivo* phenol production in mycorrhizal plantlets. The increased phenolic content might be attributed to enhanced polyphenol oxidase

Table 3. Comparative physiological parameters of bitter gourd genotypes due to mycorrhization.

Treatment	Photosynthetic rate (µmol CO_2 /m^2 /s^1)			Transpiration rate (µmol CO_2 /m^2 /s^1)		
	PDM	PV	DBGy-201	PDM	PV	DBGy-201
T_0	7.12 ± 0.18^{c}	6.96 ± 0.25^{a}	6.85 ± 0.17^{ab}	4.64 ± 0.52^{a}	4.28 ± 0.39^{bc}	3.48 ± 0.09^{bc}
T_1	7.92 ± 0.61^{bc}	8.07 ± 2.71^{a}	4.93 ± 0.37^{c}	6.08 ± 1.07^{a}	5.40 ± 0.39^{ab}	3.56 ± 0.23^{bc}
T_2	12.11 ± 1.75^{a}	6.48 ± 1.30^{a}	6.78 ± 0.33^{ab}	4.33 ± 0.54^{a}	4.99 ± 0.28^{ab}	3.96 ± 0.06^{abc}
T_3	7.74 ± 0.76^{bc}	4.84 ± 0.28^{a}	6.71 ± 0.29^{ab}	6.37 ± 1.15^{a}	6.06 ± 0.83^{a}	4.9 ± 0.64^{ab}
T_4	11.37 ± 2.05^{ab}	5.74 ± 0.73^{a}	6.79 ± 0.27^{ab}	3.79 ± 0.34^{a}	3.50 ± 0.24^{c}	3.46 ± 0.45^{bc}
T_5	7.62 ± 0.89^{c}	5.03 ± 0.66^{a}	5.63 ± 0.59^{bc}	3.67 ± 0.34^{a}	2.98 ± 0.21^{c}	3.12 ± 0.06^{c}
T_6	10.31 ± 1.20^{abc}	6.40 ± 0.39^{a}	7.41 ± 0.61^{a}	5.13 ± 1.51^{a}	5.69 ± 0.40^{a}	5.16 ± 1.06^{a}

Mean values followed by the different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$. PDM = Pusa Do Mausami, PV = Pusa Vishesh

Table 4. Effect of AMF on bitter gourd genotypes on root colonization and relative water content.

Treatment	Root colonization (%)			Relative water content (%)		
	PDM	PV	DBGy-201	PDM	PV	DBGy-201
T_0	12.64 ± 1.16^{d}	11.75 ± 0.49^{e}	11.05 ± 0.43^{e}	73.37 ± 1.25^{b}	76.54 ± 2.04^{b}	74.36 ± 1.01^{b}
T_1	32.61 ± 0.72^{c}	33.61 ± 0.32^{d}	32.82 ± 0.55^{d}	87.74 ± 4.41^{a}	87.37 ± 2.92^{a}	86.60 ± 3.05^{a}
T_2	34.21 ± 0.33^{c}	37.57 ± 0.56^{b}	34.88 ± 0.91^{c}	88.04 ± 1.46^{a}	89.61 ± 1.40^{a}	87.94 ± 1.33^{a}
T_3	33.47 ± 0.32^{c}	35.76 ± 0.61^{c}	34.98 ± 0.23^{c}	86.89 ± 5.28^{a}	88.99 ± 2.52^{a}	86.92 ± 3.10^{a}
T_4	36.43 ± 0.42^{b}	37.58 ± 0.45^{b}	35.81 ± 0.17^{bc}	91.59 ± 3.36^{a}	86.87 ± 1.40^{a}	88.26 ± 0.33^{a}
T_5	37.11 ± 0.41^{b}	36.08 ± 0.25^{c}	36.73 ± 0.24^{b}	88.40 ± 1.60^{a}	85.88 ± 3.78^{a}	86.06 ± 2.28^{a}
T_6	39.01 ± 0.20^{a}	39.52 ± 0.37^{a}	38.88 ± 0.17^{a}	92.29 ± 2.03^{a}	88.44 ± 1.73^{a}	89.65 ± 0.42^{a}

Mean values followed by the different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$. Pusa Do Mausami, PV = Pusa Vishesh

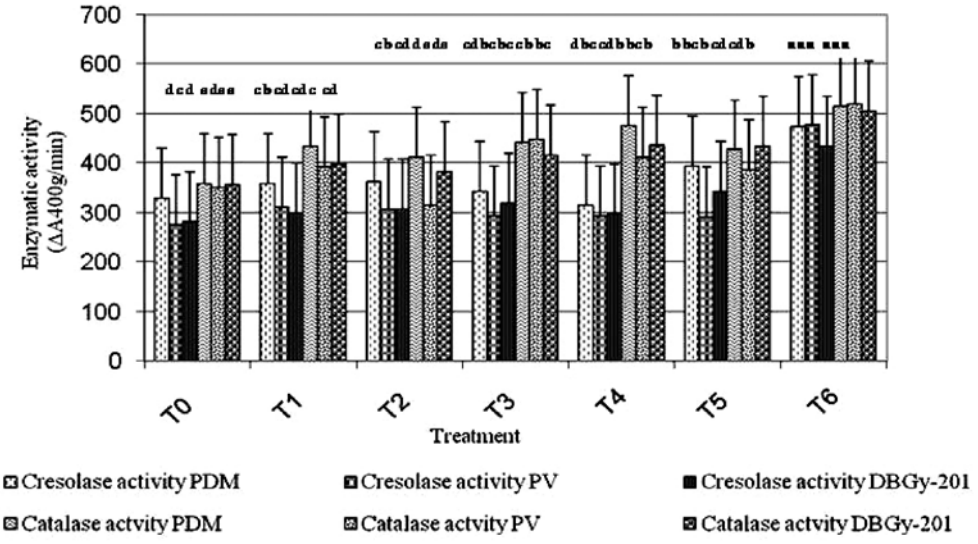

Fig. 3. Effect of different AMF treatments on root growth in bitter gourd plantlets. A = Control (T_0); B = *Acaulospora scorbiculata* (T_1); C = *Glomus manihotis* (T_2); D = *Scutellospora heterograma* (T_3); E = *Gigaspora gigantia* (T_4); F = *Entrophospora columbiana* (T_5); and G = Mixed AMF strains (Nutrilink®, T_6).

Table 5. Biochemical status of bitter gourd plantlets as influenced by AMF inoculation during hardening.

Treatment	Chlorophyll 'a' (mg/g FW)			Chlorophyll 'b' (mg/g FW)			Total chlorophyll (mg/g FW)			Total phenols (µg/g FW)		
	PDM	PV	DBGy-201	PDM	PV	DBGy-201	PDM	PV	DBGy-201	PDM	PV	DBGy-201
T_0	1.52 ± 0.05^{d}	1.47 ± 0.06^{c}	1.48 ± 0.04^{c}	0.61 ± 0.04^{a}	0.61 ± 0.02^{a}	0.66 ± 0.02^{a}	2.74 ± 0.06^{b}	1.88 ± 0.04^{e}	2.21 ± 0.25^{cd}	2.81 ± 0.21^{e}	2.46 ± 0.12^{d}	2.3 ± 0.29^{e}
T_1	2.51 ± 0.31^{b}	2.20 ± 0.03^{b}	2.24 ± 0.24^{b}	0.53 ± 0.06^{ab}	0.51 ± 0.05^{ab}	0.37 ± 0.05^{b}	3.46 ± 0.20^{a}	2.80 ± 0.08^{ab}	3.28 ± 0.17^{a}	4.88 ± 0.11^{cd}	5.24 ± 0.32^{b}	4.54 ± 0.13^{cd}
T_2	2.42 ± 0.08^{bc}	2.38 ± 0.08^{b}	2.39 ± 0.02^{b}	0.38 ± 0.09^{b}	0.30 ± 0.05^{c}	0.21 ± 0.01^{b}	1.59 ± 0.12^{c}	2.30 ± 0.01^{d}	1.94 ± 0.04^{de}	4.95 ± 0.43^{cd}	4.89 ± 0.35^{bc}	4.53 ± 0.32^{cd}
T_3	1.67 ± 0.03^{cd}	1.43 ± 0.06^{c}	1.59 ± 0.02^{c}	0.65 ± 0.06^{a}	0.62 ± 0.02^{a}	0.67 ± 0.05^{a}	1.77 ± 0.05^{c}	1.81 ± 0.05^{e}	1.71 ± 0.04^{e}	4.25 ± 0.05^{d}	4.07 ± 0.05^{c}	4.36 ± 0.04^{d}
T_4	2.16 ± 0.06^{bcd}	2.09 ± 0.04^{b}	2.08 ± 0.03^{b}	0.62 ± 0.06^{a}	0.66 ± 0.04^{a}	0.63 ± 0.05^{a}	2.93 ± 0.06^{b}	2.47 ± 0.01^{cd}	2.31 ± 0.04^{bcd}	6.82 ± 0.23^{b}	5.57 ± 0.34^{b}	6.26 ± 0.14^{b}
T_5	2.19 ± 0.44^{bcd}	2.38 ± 0.09^{b}	2.49 ± 0.05^{b}	0.48 ± 0.06^{ab}	0.36 ± 0.05^{bc}	0.31 ± 0.02^{b}	2.75 ± 0.09^{b}	2.90 ± 0.15^{a}	2.59 ± 0.06^{bc}	8.08 ± 0.08^{a}	7.75 ± 0.32^{a}	7.73 ± 0.30^{a}
T_6	3.46 ± 0.37^{a}	3.24 ± 0.23^{a}	3.23 ± 0.30^{a}	0.52 ± 0.01^{ab}	0.49 ± 0.02^{ab}	0.61 ± 0.03^{a}	2.73 ± 0.05^{b}	2.62 ± 0.07^{bc}	2.69 ± 0.10^{b}	5.15 ± 0.26^{c}	4.70 ± 0.37^{bc}	5.15 ± 0.07^{c}

Mean values followed by the different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$; Pusa Do Mausami, PV = Pusa Vishesh

activity in mycorrhizal plantlets. There was a change in the chlorophyll content (chlorophyll a, b and total) when plantlets were inoculated with different microbial treatments. The increased chlorophyll content of leaves could be attributed to enhance uptake of Mg, Fe and Cu, which are essential for synthesis of chlorophyll (Krishna *et al*., 4). The treatment effects on total phenols was evidenced in foliar parts by recording the high polyphenol oxidase activity with progressing time period. The increase polyphenol oxidase activity may be explained by increased colonization and symbiotic association of host plants with microbes with progressing time period. The activity of cresolase activity was also affected by AMF inoculation; however, its activity was much lower than catecholase activity, which could probably be due to its lag period and great instability.

The results revealed that root colonization of tissue cultured bitter gourd plantlets inoculated with AMF strains have provided conclusive evidences that AMF are potential inoculants for catering transplantation shock during acclimatization under glasshouse condition. The present study suggests that such association brought about different physiological and biochemical parameters of the plants which are necessary to reduce the adverse effect of transplanting shock and enhancing *ex vitro* survival of tissue cultured plants.

REFERENCES

1. Barnes, J.D., Balaguer, L., Maurigue, E., Elvira, S. and Davidson, A.W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophyll a and b in lichens and higher plants. *Env. Exp. Bot*. **32**: 87-99.

2. Estrada-Luna, A.A. and Davies, F.T. Jr. 2003. Arbuscular mycorrhizal fungi influence water relations, gas exchange, abscisic acid and growth of micropropagated chile ancho pepper (*Capsicum annuum*) plantlets during acclimatization and post-acclimatization. *J. Plant Physiol.* **160**: 1073-83.

3. Johnson, C.R. and Hummel, R.L. 1985. Influence of mycorrhizae and drought stress on growth of *Poincirus* x *Citrus* seedlings. *HortSci*. **20**: 754-55.

4. Krishna, Hare, Singh, S.K., Sharma, R.R., Khawale, R.N., Grover, Minakshi and Patel, V.B. 2005. Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization. *Scientia Hort.* **106**: 554-67.

5. Lerner, H.R., Harley, E., Lehman, E. and Mayer, A.M. 1971. Phenylhydrazine- a specific irreversible inhibitor of catecholase oxidase. *Phytochem*. **10**: 2637-40.

6. Malik, C.P. and Singh, M.B. 1980. *Plants Enzymology and Histo-Enzymology*, Kalyani Publishers, New Delhi, 286 p.

7. Phillips, J.M. and Hayman, D.S. 1971. Improved procedure for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment for infection. *Trans*. *British Mycol. Soc*. **55**: 158-61.

8. Ranjan, J.K., Singh, S.K., Chakrabarti, A.K. and Pragya. 2010. *In vitro* shoot regeneration from cotyledonary leaf explant in chili and bio-hardening of plantlets. *Indian J. Hort.* **67**: 43-49.

9. Sanchez-Ferrer, A., Bru, R., Cabanes, J. and Garcia-Carmona, F. 1988. Characterization of catecholase and cresolase activities of Monastrell grape polyphenol oxidase. *Phytochem.* **27**: 319-21.

10. Weatherly, P.E. 1950. Studies in water relation of cotton plants. I. Field measurement of water deficits in leaves. *New Physiol*. **49**: 81-97.

11. Yano-Melo, A.M., Saggin, Jr., O.J., Filho, J.M., Melo, N.F. and Maia, L.C. 1999. Effect of AMF on the acclimatization of micropropagated banana plantlet. *Mycorrhizae*, **9**: 119-23.

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