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ORIGINAL PAPER

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Mycorrhizal effects on the acclimatization, survival, growth and chlorophyll of micropropagated *Syngonium* and *Draceana* inoculated at weaning and hardening stages

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Abstract Micropropagated plantlets of *Syngonium podophyllum* and *Draceana* sp. were inoculated during an early weaning stage of acclimatization with a mixed indigenous consortium of arbuscular mycorrhizal (AM) fungi. Both species were colonized but a significantly higher colonization was observed (38%) in *Draceana* than *S. podophyllum* when it was harvested after 20 weeks. *Draceana* plants showed little difference in the extent to which they were colonized, when examined either at the weaning stage or hardening stage; however, *S. podophyllum* plants at the weaning stage were better colonized than at 20 weeks. Survival was high in inoculated plants at lower fertility in both hosts. Moreover, *S. podophyllum* showed better stolon production than uninoculated controls at both fertility levels, though the increase was higher at lower fertility. *Draceana* showed no response in shoot height to any treatment. Chlorophyll accumulation in both hosts was significantly influenced by inoculation, fertility and stage (weaning and hardening). A significant increment in shoot P uptake was also observed in both hosts related to inoculation, stage and fertility. Inoculation with the AM consortium had a significant and favourable effect on acclimatization of micropropagated *S. podophyllum* and *Draceana* at the weaning stage, saving almost 15 days in the total hardening process.

Key words Mixed indigenous consortium · Micropropagated plants · Acclimatization period · Substrate fertility · Chlorophyll content

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Introduction

Micropropagation of plants is vital to the biotechnology industry. Over the last decade, micropropagation has been a powerful tool for cloning and propagation of horticultural plants (Chu and Kurtz 1990), especially ornamentals (Ammirato et al. 1989). Micropropagation techniques increase the scale and speed of production and yield a healthier product. However, several problems limit the widespread use of micropropagation, of which the transfer of in vitro plantlets to ex vitro conditions is the most critical. High mortality is often observed upon transfer to ex vitro conditions because the cultured plants have functionally impaired stomata and a poorly developed cuticle and root system (Wang et al. 1993). Although some plant species acclimatize quickly due to faster root and shoot growth, others do not and this leads to an extended weaning stage, often accompanied by high losses and a large increase in the use of fertilizer, pesticides and other chemicals.

Several studies conducted to increase the growth rate and reduce mortality of plantlets at the stage of acclimatization have focused on controlling environmental conditions, for example, by increasing light intensity and altering CO_2 concentration (Kozai and Iwanami 1988; Laforge et al. 1990). Another approach to reduce the stress of acclimatization and enhance growth of micropropagated plantlets is to inoculate with arbuscular mycorrhizal (AM) fungi (Lovato et al. 1996). AM fungal colonization of horticultural plants can improve growth by increasing uptake of P, Zn and other minerals (Gianinazzi and Gianinazzi-Pearson 1986; Thomas et al. 1989; Sieverding 1991; Pearson and Jakobsen 1993), reducing disease (Caron 1989), increasing transplanting uniformity, reducing both transplant mortality (Biermann and Linderman 1983) and injury (Menge et al. 1978b), improving water relations of the host plant (Gianinazzi et al. 1990) and increasing drought tolerance (Davies et al. 1992). As micropropagation technology eliminates all microorganisms, including AM

fungi, from growth substrates, the absence of mycorrhiza requires the use of a nutrient-rich environment to guarantee satisfactory growth at outplanting. It has been demonstrated that AM colonization of micropropagated plantlets can improve rooting and enhance root production, survival rate (Strullu 1985) and overall growth (Wang et al. 1993).

The present study was undertaken to study (1) the effect of endomycorrhization on the length of the production cycle of two species of micropropagated plants (*Syngonium podophyllum* and *Draceana sp.*), (2) their survival rates as influenced by inoculation and (3) the optimization of substrate fertility using endomycorrhizal inoculation.

Materials and methods

Planting material

Micropropagated plantlets of *S. podophyllum* Schott. and *Dracaena* sp. were obtained from the Micropropagation Technology Park at Tata Energy Research Institute (TERI). These plant species were obtained either at the weaning stage on agar rooting media or as 7-day hardened plants transferred to a substrate containing peat-perlite mixture.

Growth substrates

The growth substrate was a sandy loam soil (pH 7.5, P 0.53 ppm, K 124 ppm, N124 ppm) obtained from the TERI field station at Gual Pahari, Haryana which is situated at 28°35′N and 77°12′E, 255 m above sea level, and receives about 500 mm of rain annually. The potting medium was either untreated (low fertility, Lf), or amended with composted farmyard manure (pH 7.5, P 38 ppm,K 142 ppm, N 186 ppm, higher fertility, Hf). Unsterile substrates were used throughout the study.

AM inoculation

The crude AM inoculum consisted of a mixed indigenous culture (containing *Glomus*, *Gigaspora* and *Scutellospora* spp.) which was multiplied and assessed for the number of viable infective propagules as described by Gaur et al (1998). Mycorrhizal treatments were at the level of 500 infectious propagules per plant. The inoculum was mixed uniformly with the substrate in each pot. Control plants received equal quantities of autoclaved inoculum.

Experimental set-up and design

The experiment was initiated in October 1996 as a completely randomized factorial design with a $2\times2\times2$ structure of two species from each of two growth stages [weaning stage (NR) or hardened plants (HD)], inoculated and uninoculated, and two fertility levels [unamended soil (Lf) or compost amended soil (Hf)] in the potting substrate, making a total of 8 treatments per host. Each treatment was replicated 15 times. The plants, taken from either the agar medium (weaning-stage plants) or the peat-perlite potting mix (hardening stage), were washed with sterile deionized water and transplanted into polybags (1.25 kg of substrate). The plants were kept in a mist chamber (RH 75%; temperature 24 °C) for 4 weeks and observed for survival. After 4 weeks, the plants were taken out and transplanted into large earthen pots (3.5 kg substrate per pot) and kept in a greenhouse (30 ± 2 °C, RH 60%). The plants were watered on alternate days.

AM colonization

Root samples were taken at 4 weeks and 20 weeks by destructive sampling and were homogenized thoroughly. The colonization percentage was determined by the method described by Biermann and Linderman (1981) after clearing and staining (Philips and Hayman 1970).

Biomass assessment and nutrient analysis

In *S. podophyllum*, the number of stolons was recorded at 15-day intervals. Shoots were severed just above the crown, weighed while fresh, rinsed in distilled water, dried at 70 °C for 48 h, weighed again for dry biomass, ground to pass through a screen (0.5-mm pore size) and digested in H_2SO_4. The P content in the digest was determined using the method of Jackson (1973). Chlorophyll content was determined colorimetrically by grinding the freshly harvested shoots in xylene (Stumpf and Conn 1981).

Statistical analysis

Recorded data on all the parameters for each treatment were analysed using the three-way ANOVA using least significant difference (LSD) with Duncans multiple range test at the 5% significance level. Standard deviation within the treatments was calculated using Costat software (Cohort, Berkeley, Calif.).

Results

At 4-weeks, the mortality was higher in NR plants than in the HD plants. Survival of *S. podophyllum* was lower at high fertility in both NR (68% in inoculated and 60% in uninoculated plants) and HD (78% in inoculated and 75% in uninoculated plants) plants than at lower fertility (83% in inoculated and 70% in uninoculated for NR, and 86% survival in inoculated, 78% in uninoculated for HD). AM fungal inoculation at either the NR or HD stage resulted in increased survival, though the effect of AM inoculation was higher in NR than in HD. AM inoculation in all the treatments resulted in significantly ($P<0.05$) higher shoot P and cholorophyll content than the uninoculated controls in *S. podophyllum*. The values were highest in inoculated plants at Hf and lowest in uninoculated plants at Lf. A significant effect on dry matter was recorded due to AM inoculation of either NR or HD plants, and plants cultivated at Hf had a higher dry matter than those at Lf (Table 1). In contrast, AM inoculation of *Dracaena* resulted in significant ($P<0.05$) increases in shoot P and leaf chlorophyll content but not in dry matter (Table 2).

At the 20-week harvest of *S. podophyllum*, the P status of the shoots was enhanced significantly by AM inoculation in all the treatments (Table 1). Plants cultivated from the NR stage showed higher shoot P than those cultivated at the HD stage. Also, shoot P was higher at Hf than at Lf. Shoot P (2.25 μg/g) was highest in plants raised from the NR stage at Hf and lowest in plants cultivated from the HD stage at Lf. Leaf chlorophyll content was higher in plants raised from the NR

Table 1 Influence of AM inoculation at non-rooted (NR) or hardened stage of *Syngonium podophyllum* plants on various plant and fungal parameters at 4- and 20-week harvests. *Means followed by the same letters are not significantly different* (*Hf* higher fertility, *Lf* lower fertility, *Myc* mycorrhiza)

Stage of inoculation	Fertility level	Inoculation	Shoot P ($\mu g\ g^{-1}$)		Chlorophyll ($\mu g\ g^{-1}$)		Dry matter (g)		Mycorrhizal colonization (%)	
			4 weeks	20 weeks	4 weeks	20 weeks	4 weeks	20 weeks	4 weeks	20 weeks
NR	Hf	Myc+	0.63e	2.25a	2.21e	10.13a	3.21b	21.63a	8.0	20.0
	Hf	Myc−	0.56f	1.90b	1.61g	10.03a	2.99d	18.28b	0.2	4.0
	Lf	Myc+	0.42g	1.85bc	1.80f	9.92a	2.68e	18.58b	12.0	32.0
	Lf	Myc−	0.32h	1.40de	1.30h	9.64a	2.72e	16.25c	0.0	6.5
HD	Hf	Myc+	0.92a	1.63bcd	4.62a	8.19b	3.15c	15.33d	6.0	17.0
	Hf	Myc−	0.81b	1.51cde	3.52c	7.96b	3.21b	15.02d	1.2	2.5
	Lf	Myc+	0.78c	1.65bcd	3.83b	8.31b	3.32a	15.40d	8.0	30.0
	Lf	Myc−	0.7d	1.22e	2.86d	8.07b	3.11c	14.11e	1.5	4.0
	LSD ($P<0.05$)		0.023	0.332	0.112	0.872	0.04	0.715		
Interaction	Inoculation		***	***	***	ns	***	***		
	Fertility		***	***	***	ns	*	***		
	Stage		***	***	***	***	***	***		
	Inoculation × fertility		ns	ns	ns	ns	ns	ns		
	Inoculation × stage		ns	ns	*	ns	ns	***		
	Fertility × stage		***	ns	***	ns	***	***		
	Inoculation × fertility × stage		*	ns	ns	ns	***	**		

than the HD stage. However, neither AM inoculation nor fertility level had an effect on leaf chlorophyll content.

A significant ($P<0.05$) difference between plants raised from the two stages was noted for dry matter; plants raised from NR stage accumulated significantly higher dry matter than those from the HD stage at both fertility levels. Total dry matter yield increased significantly after inoculation with AM fungi in all treatments except hardened plants at Hf. Interaction between inoculation, stage and fertility, and stage was significant only for dry matter in *S. podophyllum* (Table 1). The number of stolons at the 20-week stage was increased significantly by AM inoculation in all treatments. The effect of inoculation was greater at Lf than at Hf. In NR-stage plants, an increase of 46% in stolon number was observed at Hf and an increase of 68% at Lf, whereas, in HD plants an increase of 69% in Hf and an increase of 50% in Lf was observed due to inoculation.

In *Dracaena*, AM inoculation resulted in a significant increase in shoot P in all treatments except hardened plants at Lf. An effect of AM inoculation on leaf chlorophyll content was noted only at Lf in plants inoculated at either the NR or HD stage. Total dry matter was not significantly ($P<0.05$) affected by AM inoculation, but plants at Hf showed a higher dry matter than those cultivated at Lf. There was no significant difference in shoot height among the treatments (Table 2).

In *S. podophyllum*, percent colonization by AM fungi was higher in plants raised from the NR stage than those from HD stage at both the 4-week and 20-week harvests. Also, plants cultivated at Hf showed a lower colonization percentage than those cultivated at Lf (Table 1). In contrast, colonization percentage was not effected by the fertility level in *Dracaena*. At the 20-week stage, colonization percentage was not significantly different in all inoculated treatments (Table 2).

Discussion

In our study, AM fungal infection increased the number of surviving plants after outplanting and this effect was more evident at the weaning than at the hardened stage. Normally, acclimatization requires 6 weeks after transplanting the plantlets from test tubes to the greenhouse. During this period, AM infection was initiated and fungi colonized the hosts. In contrast, in a study by Wang et al. (1993), AM infection was not recorded in the first 4 weeks following inoculation of *S. podophyllum, Gerbera* or *Nephrolepis* spp. The authors concluded that AM fungi do not effect survival of micropropagated plants.

Several other studies on the early stage of post-*in vitro* acclimatization have reported this phase to be a critical step in the micropropagation cycle, at which the lack of beneficial microorganisms can adversely effect survival and growth of *in vitro* produced plantlets (Pons et al. 1983). In our study, mycorrhizal inoculation at the weaning stage increased the survival percentage markedly, a result that is in line with previous studies showing that inoculation with AM fungi at the time of transplantation from axenic to *in vivo* conditions significantly improves survival and growth of micropropagated plantlets (Ravolanirina et al. 1989; Branzanti et al. 1992; Sbrana et al. 1994). Different explanations for this phenomenon have been proposed. AM fungi were suggested to promote renewed shoot apical growth of micropropagated plants (Fortuna et al. 1998). Inocula-

Table 2 Influence of AM inoculation at non-rooted (NR) or hardened stage of *Draceana sp.* plants on various plant and fungal parameters at 4- and 20-week harvests. *Means followed by the same letters are not significantly different* (*Hf* higher fertility, *Lf* lower fertility, *Myc* mycorrhiza)

Stage of inoculation	Fertility level	Inoculation	Shoot P ($\mu g\ g^{-1}$)		Chlorophyll ($\mu g\ g^{-1}$)		Dry matter (g)		Mycorrhizal colonization (%)	
			4 weeks	20 weeks	4 weeks	20 weeks	4 weeks	20 weeks	4 weeks	20 weeks
NR	Hf	Myc+	0.88b	2.12a	2.98d	13.02a	3.86b	22.88a	14.0	35.0
	Hf	Myc−	0.72d	1.98ab	2.02e	12.86a	3.42d	21.97a	1.5	12.0
	Lf	Myc+	0.70d	1.92ab	2.04e	12.72a	3.01f	19.75b	14.0	38.0
	Lf	Myc−	0.58e	1.41cde	1.52f	11.72b	2.62 g	19.12bc	2.3	16.5
HD	Hf	Myc+	0.99a	1.76abc	5.34a	9.94c	4.18a	22.88a	12.0	36.0
	Hf	Myc−	0.84c	1.30de	4.62b	9.92c	3.82c	23.01a	1.8	13.0
	Lf	Myc+	0.83c	1.31de	4.58b	8.93d	3.10e	19.01bc	12.0	36.0
	Lf	Myc−	0.72d	1.26e	3.91c	7.82e	3.04f	18.03c	2.0	15.0
	LSD ($P<0.05$)		0.020	0.365	0.058	0.568	0.35	1.436		
Interaction	Inoculation		***	***	***	***	***	ns		
	Fertility		***	*	***	***	***	***		
	Stage		***	***	***	***	***	ns		
	Inoculation × fertility		***	ns	***	**	***	ns		
	Inoculation × stage		***	ns	ns	ns	***	*		
	Fertility × stage		***	ns	ns	**	***	ns		
	Inoculation × fertility × stage		***	ns	***	**	***	ns		

tion at the weaning stage produces plants with a more effective root system for uptake of P and other nutrients (Berta et al. 1990) as well as making them more resistant to transplant stress and root pathogens (Powell and Bagyaraj 1984; Cordier et al. 1996). Development of the external hyphal network may also have been more rapid in plants inoculated at the weaning stage (Graham et al. 1982).

In our experiment, mycorrhizal inoculation and P fertilization significantly affected shoot P content, suggesting that the growth stimulation of mycorrhizal plants may be explained by higher mineral concentrations in foliar tissues. Effectiveness of AM in increasing growth and P uptake has been related to the speed and extent of root colonization by AM fungi (Sanders 1977; Biermann and Linderman 1983). In *S. podophyllum,* AM inoculation produced higher number of stolons per plant than the controls, which may also be attributed to improved nutrition status (Frey and Shuepp 1993) or alteration in hormonal balance and physiological stress, or uneven photosynthate distribution, in mycorrhizal plants (Allen 1992).

AM colonization in the two hosts showed a varying response in the present study. AM colonization was reduced by increased fertility level with *S. podophyllum* but not with *Draceana*. Thus, both P application (Menge et al. 1978a; Sharma et al. 1996) and the host-fungus combination (Lambert et al. 1979; Ross 1971) influence endophyte development in host plants. Inoculated plants at lower fertility were comparable to uninoculated plants at higher fertility. This supports the previous observations that AM inoculation can partly replace higher fertilization and that the effect of AM inoculation is diminished at higher fertility (Branzanti et al. 1992).

In conclusion, this study demonstrates that AM fungi can establish a symbiotic association with the two host plant species tested and that an early endomycorrhizal inoculation during acclimatization ensures maximal survival and growth of micropropagated plants after outplanting. However, the involvement of other physiological processes in mycorrhizal and P-treated plants, such as hormonal relationships (Radin 1984) and the effect of potting mixes (Branzanti et al. 1991) cannot be excluded. Thus, a better insight into the physiological events (e.g. root growth and photosynthesis) in plants during acclimatization will considerably increase the potential of AM fungal inoculation of micropropagated plantlets.

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