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SHORT NOTE

Rooting and vitality of poinsettia cuttings was increased by arbuscular mycorrhiza in the donor plants

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Abstract In this paper, we provide evidence that the rooting performance of cuttings can be improved by the arbuscular mycorrhizal (AM) symbiosis of donor plants. Poinsettia stock plants were inoculated with the *Glomus intraradices* isolate H510 and grown in three different cultivation systems (two organic and one conventional). AM colonization was not related to P availability in the substrate. Decay of the excised cuttings in response to unfavorable postharvest storage conditions was significantly reduced by AM colonization of the stock plants. In most cases, AM significantly promoted the formation of adventitious roots in the stored cuttings. The strongest effect of AM was found when donor plants were grown in a modified organic substrate; then AM-conditioned cuttings showed higher leaf sugar levels and a changed kinetic of carbohydrates during storage. Analyses of N, P, and K in cuttings did not indicate a nutritional effect. The results support the idea that an altered carbohydrate metabolism and plant hormones can contribute to improved rooting performance of cuttings excised from mycorrhizal donor plants.

Keywords Arbuscular mycorrhiza · Stock plants · Cuttings · Rooting · Sugars

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Introduction

Arbuscular mycorrhiza (AM) having a great influence on overall plant physiology contributes to improved plant health and growth, particularly under suboptimal conditions (Peuss 1958; Hirrel and Gerdemann 1980; Sharma et al. 1992). AM can improve the uptake of water (Auge 2001) and nutrients (George 2000). C assimilation and export from leaves may also be increased in mycorrhizal plants (Douds et al. 2000; Gernns et al. 2001). Changed plant physiology in response to mycorrhizal infection may also be in part related to altered root system morphology such as increased branching or higher numbers of adventitious roots (Berta et al. 1990; Torelli et al. 2000).

The vegetative propagation via cuttings is now a worldwide procedure involving stock plant cultivation at low latitude sites and subsequent storage, transport, and rooting in Central Europe. Such cuttings are stressed by storage and transport, resulting in high senescence and impaired root formation (Kadner and Druege 2004). If AM fungal (AMF) inoculum is added to the cuttings during rooting, development of adventitious roots and subsequent survival can be promoted (Barrows and Roncadori 1977; Verkade and Hamilton 1987; Douds et al. 1995; Lovato et al. 1996; Scagel 2004). However, rooting performance is already predetermined earlier while cuttings are still attached to the stock plant (Haissig 1986), and it is there that it is particularly influenced by the nitrogen and carbohydrate status (Druege et al. 2000, 2004). Considering the physiological influence of AMF on their hosts, we hypothesized that survival and root formation of cuttings can be improved by AM of stock plants.

A greenhouse experiment was conducted with stock plants of poinsettia and focused on organic cultivation with

two fertilizing strategies in comparison with a conventional (nonorganic) system. Because we wanted to explore the practical potential for growers, both cultivation systems were managed under nonlimiting nutrient conditions. The aim of this study was to answer the following questions: (1) How does the symbiosis affect stock plant growth, yield, and the rooting capacity of excised cuttings? (2) How are mineral and carbohydrate concentrations in cuttings affected and related to the rooting response?

Materials and methods

Experimental design and cultivation of donor plants

Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) cv. 'Cortez Red' was used as host plant. The experiment involved a two factorial (cultivation system and inoculation with AMF), randomized block design with four replication plots, each consisting of ten stock plants. Cuttings were rooted in perlite and then planted into 1.5-l plastic pots. For conventional production (C) the commercial peat-based substrate "Einheitserde für Poinsettien" (Patzer Company, Sinntal-Jossa, Germany) was used (mg l^{-1}: N=200–500, P_2O_5=200–400, K_2O=300–600, and micronutrients). During cultivation, after analyzing soil samples, nutrients were supplied (twice a week at maximum) with a nutrient solution containing Flory Basis 1 (14% P_20_5, 38% K_2O, 5% MgO, and micronutrients), NH_4NO_3 (9% NO_3–N + 9% NH_4–N), $Ca(NO_3)_2$ (19% Ca + 14.5% NO_3–N + 1% NH_4–N), and K_2SO_4 (45% K). For organic cultivation (O) we used low peat substrates (Floragard Company, Oldenburg, Germany) consisting of (v/v) 50% peat, 25% plant compost, 25% flax fibers, and 60 kg m^{-3} clay. Organically grown stock plants received a high organic starter fertilization of 1.5 kg m^{-3} horn powder and 3 kg m^{-3} of both horn granules and horn chips (each 13% N) without subsequent fertilization. The organic substrate was in a parallel treatment modified (Om) by reducing the P-rich compost and clay to 5% and 12 kg m^{-3}, respectively, while flax fibers were increased (30%) and coco fibers (15%) were added.

Heating and ventilation in the greenhouse provided an average air temperature of 23.1°C. Depending on plant development, the threshold for automatic shading varied between 630 and 900 µmol m^{-2} s^{-1} (400–700 nm; open air). Three weeks after planting, plants were pinched back to five leaves and then managed as stock plants. Terminal cuttings (4–6 cm, at least three fully developed leaves) were excised from the plants at four harvest dates (Fig. 1), leaving the first two leaves of the axillary shoot on the donor plant.

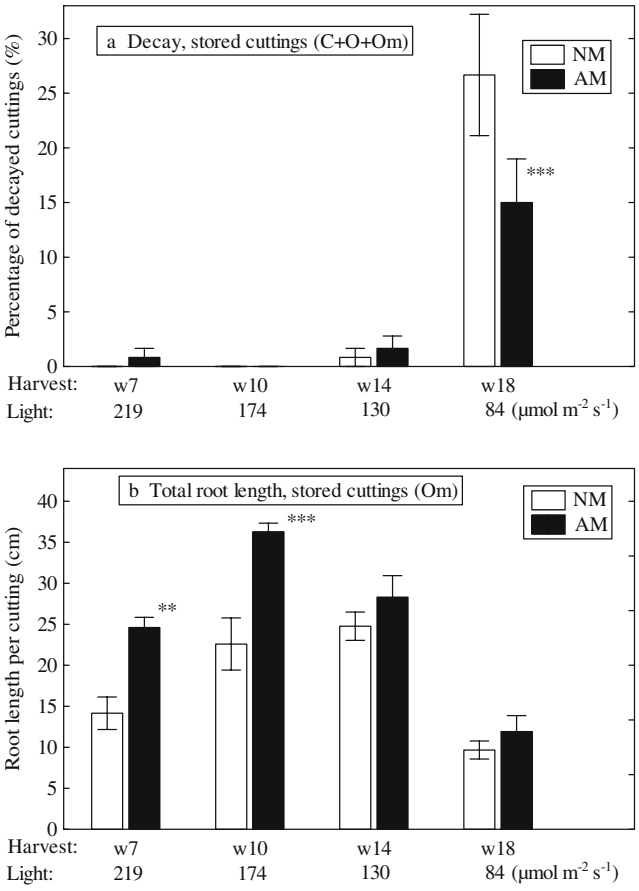

Fig. 1 Effect of AM symbiosis of poinsettia stock plants on **a** decay and **b** total length of adventitious roots formed in excised cuttings after storage. Mean±SE of **a** three cultivation systems (n=12) and **b** Om (n=4). Double asterisks and triple asterisks indicate significant effect of AM at $p \leq 0.01$ and 0.001 (ANOVA and Newman–Keuls). Light: (400–700 nm) 12 h/day during rooting

AM inoculation and quantification of colonization

Stock plants were inoculated (AM) two times: (1) as cuttings in the rooting medium (perlite) and (2) at the time of transplanting into pots, and were compared with non-inoculated control plants (NM). For inoculation, the substrates were mixed with the *Glomus intraradices* (Smith and Schenk) isolate H510 on expanded clay (5%, v/v) as the inoculum carrier (Dehne and Backhaus 1986). Root samples were collected from the stock plants, cleared with 10% KOH, and subsequently stained with 0.05% trypan blue according to Phillips and Hayman (1970). Mycorrhizal infection percentage was estimated by counting the frequency of colonization in 1-cm root pieces (80 pieces per replication plot).

Storage and rooting of cuttings

Cuttings were rooted immediately or previously stored in nonperforated polyethylene bags for 1 week (9–10°C;

dark). The rooting of nine cuttings per replication plot (n=4) was assessed in the greenhouse at temperatures similar to those described for the stock plants. Cuttings were inserted into perlite without application of fertilizers or hormones, and then covered with a perforated polyethylene sheet. After 25 days, the percentage of decayed cuttings (almost all leaves and/or terminal buds destroyed) was counted, and the number and length of roots was determined from the remaining cuttings.

Chemical analyses of substrates and cuttings

Concentrations of NO_3–N, P, and K, pH, and electric conductivity in the substrates were continuously analyzed every second week after extraction with sodium acetate as described by Goehler and Drews (1978). NO_3–N and P were determined colorimetrically (V-550, Jasco Company, Gross-Umstadt, Germany) with chromotropic acid and an ammonium molybdate–vanadate method, respectively. K was measured with a flame photometer (Flapho 4, Carl Zeiss, Jena, Germany). At each harvest, one sample of nine cuttings was collected per treatment for analyses of concentrations of minerals in the dry mass of whole cuttings. The concentration of total N was measured with a CHN-Rapid analyzer (Druege et al. 2000). P and K were measured as described for the substrates after microwave-assisted decomposition of the dried plant samples with 65% HNO_3 (Panholzer 1994). Carbohydrate concentrations were analyzed in the lamina of the oldest and second oldest leaf (pooled) and in the stem base (1 cm) of cuttings immediately after severance and after storage. The sampling of three cuttings per replication (n=4), extraction, and determination of sugars and starch using an enzyme-coupled colorimetric reaction are further described by Druege et al. (2004).

Statistics

The effect of inoculation and interaction with harvest date was analyzed for each cultivation system by ANOVA (Statsoft 2001). Differences between inoculation treatments were tested after Newman–Keuls with a significance level of at least $p \leq 0.05$.

Results

AM colonization and growth response of stock plants

Substantial mycorrhizal infection was observed in week 10 (Table 1) under the high natural light conditions of August (400–700 nm: 183 μmol m^{-2} s^{-1} 12 h/day). The colonization decreased with decreasing light intensity toward the end of the experiment in October (400–700 nm: 80 μmol m^{-2} s^{-1} 12 h/day). A reduction of P in the organic system (treatment Om) was not beneficial for the AM colonization of stock plants. AM frequency data showed a high variation in many treatments because some plants often developed a good colonization, whereas others stayed free of AM. Growth of mycorrhizal and nonmycorrhizal stock plants was not different (Table 1).

Decay and root formation of cuttings

Vitality and root formation of cuttings were improved by the mycorrhiza of donor plants. However, those differences only became apparent when cuttings were exposed to storage before insertion into the rooting medium. After cuttings had been harvested late in October and subsequently stored, a high percentage of decay was observed during the rooting at low natural light intensity as a result of leaf senescence and secondary infection with *Botrytis*

Table 1 Nutrient concentrations, salinity, and pH in the substrate during the growth of poinsettia stock plants (mean±SD; ten sampling dates); mycorrhizal infection frequency (MI; percent of 1-cm root pieces; mean±SD; four replication plots) in week 10 (19 August 2003) and week 18 (10 October 2003) of cultivation; and total shoot fresh weight (FW) per stock plant produced during the whole cultivation period

Parameter	Cultivation system		
	Conventional (C)	Organic (O)	Organic-modified (Om)
Nitrate-N (mg l^{-1})	250.6±119.4	451.2±167.7	122.0±58.9
P (mg l^{-1})	122.2±25.3	143.4±17.2	66.6±32.9
K (mg l^{-1}))	133.9±118.3	656.7±47.8	150.9±97.4
$Salt^{KCl}$ (g l^{-1})	3.0±0.7	4.6±1.0	1.6±0.7
pH	5.5±0.7	5.8±0.9	5.1±0.8
MI week 10 (%)	36±19	46±43	28±27
MI week 18 (%)	5±4	11±14	0
Plant (mycorrhizal) FW (g)	110.2±20.2[a]	60.7±14.0[a]	79.9±7.9[a]
Plant (nonmycorrhizal) FW (g)	92.5±14.2	50.4±13.6	78.9±9.3

[a] No significant effect of mycorrhiza

cinerea (Fig. 1a). The decay was significantly lower in cuttings from mycorrhizal plants. The rooting capacity of stored poinsettia cuttings was significantly increased when donor plants had been inoculated with AMF, even though this depended on the cultivation system. Independent on harvest date, cuttings excised from mycorrhizal stock plants produced a higher number of roots when they were cultivated conventionally (15.1±0.8 for AM compared to 13.1±1.0 for NM; $p \leq 0.05$, $n=16$) or in the modified organic system Om (15.7±1.1 for AM compared to 13.9±1.0 for NM; $p \leq 0.01$, $n=16$). With the Om cuttings, mean root length was also increased by AM (1.6±0.1 cm compared to 1.2±0.1 cm for NM; $p \leq 0.001$, $n=16$). The response of total root length per cutting was strongest for the first two harvests when cuttings rooted under high natural light intensity (Fig. 1b).

Mineral and carbohydrate status of cuttings

Mineral concentrations in whole cuttings remained unaffected by the symbiosis or were slightly lower in the cuttings excised from mycorrhizal plants. Average N, P, and K concentrations in milligrams per gram dry mass ($n=12$) amounted to 46.1, 5.9, and 31.3 for the AM cuttings compared to 50.3, 6.2, and 32.0 for the controls (NM). In the Om system, sugar concentrations in leaves were significantly higher when cuttings were harvested from mycorrhizal plants (Fig. 2a). After storage, which generally decreased leaf sugars, cuttings from mycorrhizal Om plants had by far the highest level of leaf sugars, while the same cuttings also showed the strongest rooting response to AM. Stem carbohydrates were also increased by mycorrhiza; again, the strongest effects were found in the Om system, particularly after storage (Fig. 2b). At harvest, starch in the Om cuttings was not affected by the symbiosis, but the outstanding maintenance of the high sugar pool in the mycorrhiza-conditioned cutting tissues during storage provided an accumulation of starch in the basal stem (Fig. 2c). All these alterations of carbohydrate status were not found when mycorrhizal stock plants were cultivated in the conventional system.

Discussion

In the present study, mycorrhization of *E. pulcherrima* was slow. The overall level of AM colonization often decreases with increasing availability of soluble P (Azcon-Aguilar and Barea 1997), while such responses also depend on the P tolerance of the fungal ecotypes (Nemec 1986). Barrows and Roncadori (1977) found colonization between 71 and 96% when poinsettia plants received low fertilization, whereas colonization with *Gigaspora margarita* was reduced to 30–69% when a four times higher fertilization was applied. The development of AM can be stimulated by organic materials other than peat (Estaun et al. 1999; Linderman and Davis 2001), even though the effects are highly variable among different materials and negative effects have also been reported (Brechelt 1989; Baby and Manibhushanrao 1996). The colonization of the poinsettia with H510 turned out to be less sensitive to P availability and, rather, indicated a stimulating effect of the compost. Irrespective of the higher soluble P in the substrate (Table 1), colonization was highest in the standard organic

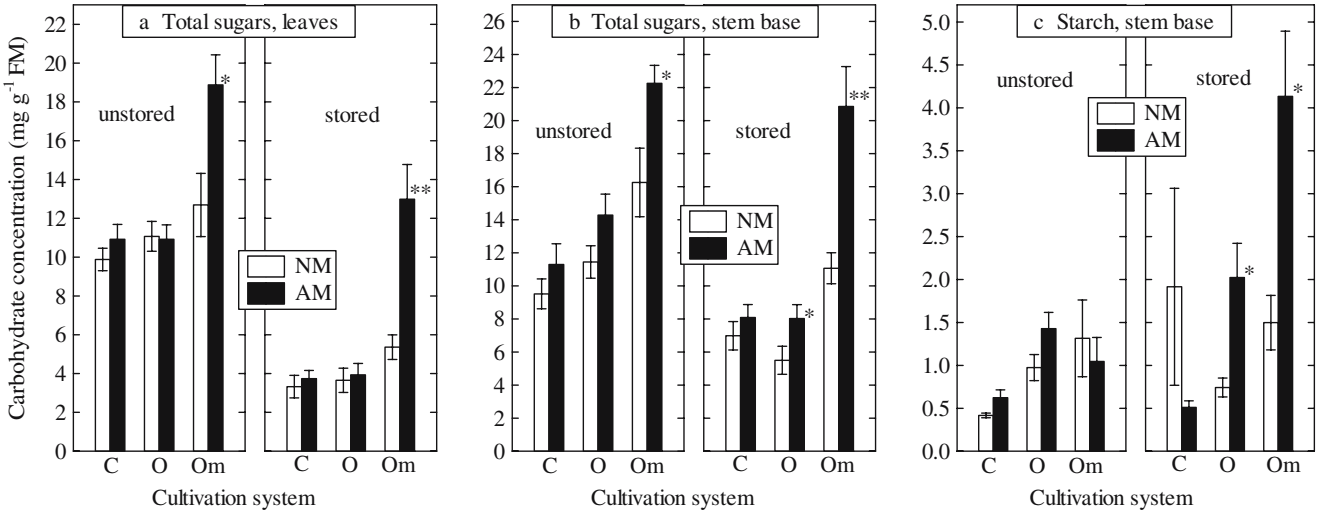

Fig. 2 Concentrations of **a**, **b** total sugars (glucose + fructose + sucrose) and **c** starch in the fresh matter (FM) of **a** the eldest and second eldest leaves and **b**, **c** the stem base (1 cm) of poinsettia cuttings as influenced by cultivation, mycorrhizal symbiosis of stock plants, and storage of cuttings. Means±SE of two harvests (weeks 14 and 18, $n=8$). Single asterisk and double asterisks indicate significant effect of AM at $p \leq 0.05$ and 0.01 (ANOVA and Newman–Keuls)

system with 25% compost when compared with the conventional system (C, no compost) and the Om treatment with the lowest P availability but only 5% compost and extra 15% coco fibers.

To our knowledge, this is the first paper providing evidence that the rooting performance of cuttings can be improved by the AM symbiosis of stock plants, supporting the use of mycorrhiza technology in stock plant management. First, the risk of postharvest decay could be reduced. Also, considering the data of seven other experiments with poinsettia and *Pelargonium hortorum* (Druege and Von Alten, unpublished observation), the maximum percentage of decay in the cuttings remained below 28% when stock plants experienced substantial AM colonization, whereas in cuttings from nonmycorrhizal plants the decay reached up to 50%. Obviously, being independent on cutting decay (compare subpanels a and b in Fig 1), the mycorrhization of donor plants promoted the development of adventitious roots in cuttings when produced conventionally or in the organic system Om and when subsequently exposed to postharvest storage.

No indication is provided for a nutritional effect of AM because N, P, and K concentrations in cuttings were not increased and within the optimum range (Bergmann 1988). Resulting from the particular source to sink balance of the symbiosis, which is also influenced by environmental factors, carbohydrate levels in the shoots or leaves can be increased, decreased, or unaffected by AM (Jongen et al. 1996; Wright et al. 1998; Boucher et al. 1999). The carbohydrate data of the present study indicates that, depending on root zone conditions (i.e., substrate), mycorrhizal stock plants can accumulate higher carbohydrate levels, particularly in the leaves of the young shoots (cuttings), when growing under sufficient light conditions and a higher photosynthetic activity of mycorrhizal plants can be assumed. This could be driven by the higher sink strength of the mycorrhizal root system or result from improved P nutrition (Douds et al. 2000). However, the same P and starch concentrations in the AM and control cuttings at harvest indicate that other mechanisms were involved. Druege and Schoenbeck (1992) showed that inoculation of flax plants with a mixture of AMF containing H510 increased leaf cytokinin levels, transpiration, and photosynthesis in host plants, and that the application of a cytokinin into the vascular system of nonmycorrhizal plants counteracted an abscisic-acid-mediated decrease in assimilation. This supports the idea that a changed ABA to cytokinin balance in the AM plants contributed to improved photosynthesis via increased stomatal opening, which may become particularly relevant under water-deficit conditions (Goicoechea et al. 1997).

The kinetic of carbohydrates in the cuttings during storage (Fig. 2) provides evidence that AM in stock plants can greatly influence postharvest carbohydrate fluxes. Considering that high sugar availability is crucial for both survival and the adventitious root formation in cuttings (Druege et al. 2004), the present results suggest that an altered carbohydrate balance can contribute to improved rooting capacity of AM-conditioned cuttings. The strongest rooting response to AM of the stock plants was observed when cuttings were produced in the Om system and subsequently stored (Fig. 1b). Only the Om cuttings responded to AM with significantly higher leaf sugar levels, which are particularly relevant to adventitious root formation (Druege et al. 2004; Rapaka et al. 2005) and an outstanding maintenance of high carbohydrate availability during storage (Fig. 2). A positive relation between carbohydrate levels and the survival or rooting of AM cuttings may be the consequence of higher reserves, which should involve a strong influence of the source to sink balance of the mycorrhiza in the donor plants. However, it can also result from a carbohydrate-reflected higher photosynthesis. Current photosynthesis during rooting is a crucial factor for adventitious root formation in leafy cuttings, particularly after a storage-induced carbohydrate shortage (Rapaka et al. 2005). These interrelations would explain the less pronounced rooting response of the Om cuttings of the last two harvests to AM, which had to cope with a much lower current light intensity (Fig. 1b). Mycorrhiza in the conventionally (C) grown stock plants increased the rooting of cuttings, too. Here, AM might as well have caused a higher photosynthesis. However, the host plants did not accumulate sugars. Our methodology for quantifying the development of the symbiosis was not fine enough to show whether the AMF biomass in the conventional substrate was significantly higher, consuming the surplus of carbohydrates that could be stored in shoots by the stock plants growing in the Om system. Even more than mycorrhizal biomass within roots, extraradical hyphal biomass might be influenced by substrate composition (Gryndler et al. 2002) and the resulting C costs of the symbiosis might be higher or lower. We have to remember that in our growing systems with high nutrient availability mycorrhizal hyphae were not necessary for plant nutrition. Finally, we have to consider that adventitious rooting is much more complex and is not simply determined by carbohydrate metabolism (Haissig 1986). Obviously, depending on the particular fungus and host plant, AM can increase cytokinin and auxin levels in plant tissues, the latter of which may also be related to assimilate fluxes (Druege and Schoenbeck 1992; Druege et al. 2000; Ludwig-Mueller 2000; Torelli et al. 2000). Regarding the great importance of plant hormones particularly of cytokinins to senescence (Smart 1994) and auxins to adventitious root formation (Kevers et al. 1997), the changed hormonal balance in AM plants should also be involved in the changed survival and rooting of cuttings derived from mycorrhizal stock plants.

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