possibly due to competition between the two divalent cations during the absorption process. A similar competition has been reported by Kannan (5) and Maas (6).

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# Effects of Growing Media and Aerial Environments on Acclimatization of *In Vitro*-Grown Miniature Rose Plantlets[1]


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## Abstract

Initial acclimatization of *in vitro*-rooted plantlets of miniature rose (*Rosa chinensis* var. *minima* 'Red Ace') in high humidity and continuous light (most similar to the *in vitro* environment) increased plant growth (33% greater root area and 34% greater shoot area) relative to plants acclimatized under intermittent mist during early *ex vitro* growth stages, but later transfer to standard greenhouse conditions caused a temporary lag in continued growth. Direct transfer from *in vitro* culture conditions to a greenhouse mist bench inhibited growth during acclimatization, but permitted more rapid growth rate (28 and 30% more shoot and root area, respectively) during transition to the greenhouse growing environment. Direct transplant of *in vitro* rooted microcuttings to a growing medium containing soil resulted in high survival rate and circumvented the need for an interim potting medium, whereas transplant to Jiffy-9 pellets resulted in the highest plant losses.



**Index words:** greenhouse production, image analysis, micropropagation, *Rosa chinensis* var. *minima* 'Red Ace', 'Red Ace' miniature rose, tissue culture


## Significance to the Nursery Industry

Procedures used by individual growers to acclimatize miniature roses produced *in vitro* to greenhouse conditions vary from use of high humidity tents or fog chambers to intermittent mist. During the transition from *in vitro* culture conditions to the *ex vitro* growing environment, any abrupt changes in the production environment will exert significant influence on the growth and quality of the final product. While miniature rose plantlets acclimatized in a low light, high humidity chamber exhibited superior overall survival and growth during the initial acclimatization process, plantlets transferred directly from *in vitro* culture to a greenhouse


[1]Received for publication January 24, 1991; in revised form June 3, 1991. This work was partially supported through a Eugene S. Boerner graduate fellowship.


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mist regime were more adapted to transfer to a non-misted greenhouse bench during subsequent production stages. These studies suggest that an initial acclimatization environment parallel to the *in vitro* environment, with very gradual adjustment to lower humidity and higher light levels may allow the benefits of both methods to be preserved. Growing medium had little influence on acclimatization efficiency, suggesting that a standard growing medium during acclimatization may be used to avoid costly interim media and labor intensive transplanting procedures.

## Introduction

Miniature roses (*Rosa chinensis* var. *minima*) are an increasingly important crop (1, 13). Since they are clonally propagated on their own roots (5, 8), miniature roses are more suited to micropropagation than roses that require another rootstock for production. Pot roses produced *in vitro* reportedly have a more commercially-desirable growth habit (denser, more uniform branching) than those propagated by stem cuttings (4, 7). These advantages suggest that miniature roses are an ideal candidate for rapid, disease-free, commercial production via micropropagation.

Although successful *in vitro* propagation techniques for miniature roses have been reported (4, 12), causes of inadequate survival and performance during acclimatization have been reported by researchers (3, 9, 12) and commercial producers (J. Walters, Kimbrow Walter Roses, Grand Saline, Tex., personal communication). Altering the acclimatization environment could offer a means to improve the response of *in vitro*-produced plantlets.

In this experiment, micropropagated, *in vitro*-rooted miniature rose plantlets were evaluated in a range of growing media and aerial acclimatization environments to determine the effects on survival and quality of the finished product. Implications for standardizing efficient commercial production techniques are discussed.

## Materials and Methods

Shoots of 'Red Ace' miniature rose were proliferated on a basal Murashige and Skoog (MS) salts medium (11) containing, in mg/liter (ppm): ascorbic acid, 50; myo-inositol, 100; polyvinylpyrrolidone (PVP-10), 150; thiamine-HCL, 0.5; pyridoxine-HCL, 0.5; nicotinic acid, 0.5; glycine, 2; disodium ethylenediaminetetraacetic acid dihydrate, 37.25 and ferrous sulfate heptahydrate, 27.8 [Fe-EDTA]; sucrose, 30,000; BBL agar (Becton Dickinson, Cockeysville, Md.), 6,500; 6-benzyladenine (BA), 2; and naphthaleneacetic acid (NAA), 0.1. Rooting medium was similar except for reduced salts (0.25 strength), no PVP-10, Fe-EDTA or BA, and reduced agar concentration (5,500 mg/liter). After adjusting the pH to 5.7, 50 ml (1.7 oz) of the proliferation medium was dispensed into GA7 vessels (Magenta Corp., Chicago, Ill.) or 15 ml (0.5 oz) of rooting medium into 25 × 150 mm (1.0 × 5.9 in) test tubes, and autoclaved for 17 minutes at 121°C (250°F).

Established shoot cultures were maintained by subculturing 1.5–2.0 cm (0.6–0.8 in) tip segments at 6-week intervals. After 4–5 weeks on shoot proliferation medium, shoots were excised and inserted vertically to a depth of approximately 0.5 cm (0.2 in) into rooting medium. Both proliferation and rooting phases were incubated under a photosynthetic photon flux (PPF) of 15 $\mu$mol $m^{-2}$ $s^{-1}$ measured with a Li-Cor model LI-190 meter (approximately 809 lux) of continuous cool-white fluorescent lighting at 25 ± 1°C (77 ± 2°F). After 3 weeks, rooted plantlets were transplanted into 6.4 × 6.4 × 8.9 cm deep (2.5 × 2.5 × 3.5 in) plastic pots containing: 1) a medium of 1 soil:1 peat:1 perlite (by vol.), 2) expanded Jiffy-9 pellets partially slit lengthwise to minimize root damage during transplanting and implanted in the aforementioned mix, or 3) peat:sand (1:1 by vol). The pH of the soil-containing peat:sand medium was 5.6 and 5.3, respectively. Fifteen plantlets in each of the growing treatments were acclimatized for a 2-week period in one of the following environments: 1) a 5 sec per min mist regime in the greenhouse, 2) a 5 sec per 5 min mist regime in the greenhouse, 3) a continuous, 2-week high humidity (nearly 100%) regime in an illuminated chamber, or 4) a regime similar to #3, with supplemental humidification discontinued after 1 week of acclimatization.

The mist treatments were located on adjacent benches in the same greenhouse with mist and a day temperature regime of 28 ± 2°C (82 ± 4°F) applied between 0700 and 1900 hr and a night temperature or 24 ± 2°C (75 ± 4°F). Midday PPF ranged from 150–240 $\mu$mol $m^{-2}$ $s^{-1}$ (approximately 8090-12,900 lux) during the course of the experiment. In the illuminated growth chambers, continuous light was supplied by cool-white fluorescent lamps with PPF of 40–50 $\mu$mol $m^{-2}$ $s^{-1}$ (approximately 2100-2690 lux) at the top of the canopy. The temperature in the chambers was 28 ± 1°C (82 ± 2°F). Relative humidity (RH) was maintained at 95–100% with the aid of an ultrasonic humidifier. When supplemental humidification in the illuminated chamber was discontinued (treatment #4), ambient RH ranged between 60–70%.

Half of the plants were harvested and analyzed for shoot and root area, length and dry weight immediately following the acclimatization period, and the remainder were transplanted into 10 cm (4 in) clay azalea pots containing a 1 soil:1 peat:1 perlite growing mixture (by vol), at a pH of 5.6 and grown in a greenhouse for 6 additional weeks. Irrigation was performed as necessary and fertilization occurred on a weekly basis with the application of 250 ppm nitrogen of 20N-8.6P-16.6K (20-20-20) fertilizer. Day/night temperatures were maintained at 22/18 ± 5°C (72/64 ± 10°F). Again, natural daylight was not supplemented. At the end of the 6-week greenhouse growing period, the remaining plants were harvested and analyzed for shoot and root area, length, and dry weight.

Survival rates were determined immediately after acclimatization and 2 and 6 weeks after transfer to the greenhouse. Percentages of plants reaching the flowering stage and the number of days to flowering for these plants were recorded. The flowering stage was considered to be the point where sepals had separated or reflexed such that approximately one-half of the bud area revealed the petal color.

At each of the harvests, after rinsing the roots of samples, plants were placed atop a light box to determine the length and area of shoot and root systems with the aid of a microcomputerized video image analysis measurement system (14, 15). After image capture and analysis, root and shoot systems were dried for 72 hours at 75°C (135°F) and dry weight determined.

The statistical procedure included a completely randomized block design with three replications of each treatment in each of the five blocks. One factor consisted of the three potting media, and the second consisted of the four environmental regimes. Each experiment was repeated once over

time with the same number of replications per treatment. Statistical significance of the data was determined using a two-way ANOVA and mean separation using Tukey's Multiple Comparison method.

## Results and Discussion

Plant response during the early stages of acclimatization was generally enhanced in humidified chambers as compared to mist treatments. After 2 weeks of humidification in the chambers, both root and shoot growth (area and length) were significantly greater (Table 1). Similar trends were observed when data from the two humidified chamber treatments were pooled and compared to data from combined mist bench treatments. For example, shoot and root area from chamber treatments (637 and 186 $mm^2$ [0.99 and 0.29 $in^2$], respectively) were significantly greater than that from mist treatments (479 and 134 $mm^2$ [0.74 and 0.21 $in^2$], respectively). Variables were not different between plants exposed to 1 or 2 weeks of chamber humidification with the exception of a 19% increase in shoot area in the 2-week treatment. Increased shoot area without a corresponding increase in shoot dry weight suggests that the continuous high humidity conditions promoted larger but thinner leaves. Within the two mist treatments, plants responded similarly except that longer roots occurred when the interval between mist applications was extended. Decreased shoot growth in the greenhouse may have resulted from 25 to 30% lower relative humidity and variable, higher light levels on the mist bench.

Later in the growth cycle, after 6 weeks of production in the greenhouse, these same trends in shoot and root growth parameters were no longer evident. Root area was over 30% greater for plants acclimatized in the short interval mist treatment (5 sec/min) as compared to plants acclimatized in the humidity chambers (Table 1). This indicated that the small initial root system measured after 2 weeks under mist acclimatization did not impede later vigorous root development. Although the rose plants grew vigorously in the "ideal" conditions of the chambers during acclimatization, they were less hardened for transfer to the greenhouse environment. Plants from the mist acclimatization treatments more readily adapted to the stringent greenhouse environment than plants from the humidity chambers.

The high humidity chambers promoted superior plant performance during early acclimatization, probably since the environment was more similar to the *in vitro* environment. The highest survival rate was also achieved by plants which had received the 2-week humidified chamber treatment. The second highest survival was for the frequent mist treatment (Table 2). This suggests that elevated humidity in the early stages of acclimatization is critical to maximize the efficiency of acclimatization. The mist environments were limiting to both root and shoot growth (compared to the chamber) during the initial weeks *ex vitro*. Once transferred to the nonhumidified greenhouse environment, however, the growth rate of plants from mist acclimatization treatments exceeded the humidified chamber treatments, although survival was slightly depressed for plants under the longer mist interval (Tables 1 and 2). Since both humidified chamber treatments had similar results in nearly all growth parameters measured at 2 and 8 weeks, the 1 week reduction in humidity level was either too abrupt or did not impose enough stress upon the plants to promote adequate transition to the harsher greenhouse environment without a reduction in growth. Although some plant losses were incurred during the humidified chamber acclimatization stage, no additional losses occurred following transplant from the high humidity chambers to the greenhouse. In contrast, losses of up to 13% in some treatments were noted for plants from mist treatments after transfer to the greenhouse. Losses after this evaluation stage were negligible, and unrelated to treatments.

Results herein reflect the significance of the transition stages on overall plant response, and underscore that growth during and after acclimatization can be best promoted by maintaining a very gradual transition from the high humidity *in vitro* growth environment to the ultimate greenhouse growing conditions. Foliar water loss during acclimatization of *in vitro* produced plants is more serious for woody than for herbaceous plants (7). Growers have experimented with a variety of systems including gradual hardening of rooted plantlets while still *in vitro*, mist systems, humidity tents, and fogging systems to facilitate the gradual transition from tender *in vitro* plant to acclimatized greenhouse specimen.

**Table 1. Effect of acclimatization in four different environments on development of tissue culture-derived miniature roses. Data were recorded 2 weeks after transfer to acclimatization environments and an additional 6 weeks after transfer to the greenhouse. Values are means across all three rooting medium treatments.**

| Parameter | | Mist regime | | Humidified chamber | |
|---|---|---|---|---|---|
| | | 5 sec/ min | 5 sec/ 5 min | 1 week | 2 weeks |
| | | 2 weeks | | | |
| Shoot | Area ($mm^2$) | 524 bc[z] | 436 c | 569 b | 704 a |
| | Dry wt (mg) | 17.7 ab | 16.6 b | 20.3 a | 20.2 a |
| | Length (mm) | 35 bc | 32 c | 40 ab | 40 a |
| Root | Area ($mm^2$) | 119 c | 148 bc | 177 ab | 197 a |
| | Dry wt (mg) | 5.7 a | 6.0 a | 5.7 a | 6.0 a |
| | Length (mm) | 29 c | 43 b | 60 a | 62 a |
| | | 8 weeks | | | |
| Shoot | Area ($mm^2$) | 5980 a | 5272 a | 4376 a | 4690 a |
| | Dry wt (mg) | 324 a | 299 ab | 251 b | 257 b |
| | Length (mm) | 102 a | 115 a | 94 a | 98 a |
| Root | Area ($mm^2$) | 1941 a | 1173 b | 1125 b | 1491 ab |
| | Dry wt (mg) | 518 a | 451 ab | 421 b | 425 b |
| | Length (mm) | 113 a | 131 a | 128 a | 120 a |

[z]Means separation within rows by Tukey's test, 5% level; n = 30.

**Table 2. Cumulative loss percentages 2 weeks after transfer to the greenhouse growing environment. Percentage values were calculated from 30 replicates per treatment.**

| Growing medium | Mist regime | | Humidified chamber | | Average percent loss |
|---|---|---|---|---|---|
| | 5 sec/ min | 5 sec/ 5 min | 1 week | 2 weeks | |
| Jiffy-9 | 13 | 13 | 7 | 7 | 10 |
| Peat/sand | 0 | 13 | 13 | 0 | 6 |
| Soil mix | 0 | 7 | 0 | 0 | 2 |
| Average percent loss | 4 | 11 | 7 | 2 | |

Space and labor considerations may dictate that growers rely on only one transition facility and level of environmental control, yet the transitions between acclimatization and growing environments are a primary source of losses (7, 9). The results of these tests suggest that superior post-acclimatization growth might be facilitated by a more gradual transition from high to low humidity (e.g. gradual lengthening of the intermittent mist interval) and/or maintenance of light levels closer to those of the eventual growing environment.

Time to flower was not affected by acclimatization environment (data not shown). These results were similar to those of Mor and Kofranek (10) who found no difference in development of rose cuttings grown continuously in a growth chamber and those transferred from mist to the greenhouse.

Interestingly, trends in shoot and root growth as a function of humidity regime were consistent over all three growing media treatments (Table 3), despite the influence of growing medium on overall survival rate (Table 2). No significant differences existed immediately after acclimatization in shoot and root area and root length as a function of soil treatments in any of the aerial environments (Table 3). Similar results were obtained at the 8 week evaluation. Furthermore, there were no differences in growth parameters as affected by rooting media alone (data not shown). However, use of Jiffy-9 pellets resulted in a 10% cumulative loss during acclimatization and early greenhouse (Table 2). The increased losses after using the pellets may be a consequence of differential drying rates between the pellet and surrounding soil mix. Also, many of the brittle, fragile *in vitro*-generated roots were unavoidably damaged during transplant to the split pellets. This effect would likely not occur for the less fragile *ex vitro*-rooted microplants. Although most of the past research published on *in vitro* production of miniature rose has emphasized *in vitro* rooting (2, 3, 5), *ex vitro* rooting is currently used in some commercial systems to increase production efficiency. Current research is in progress to evaluate the response of *ex vitro*-rooted miniature roses under alternative acclimatization regimes and to determine if similar trends are produced.

Our results affirm that abrupt environmental changes (even *minor* changes) at any stage during acclimatization must be avoided in order to maximize the productivity of *in vitro*-produced miniature roses. Use of standard growing mix during acclimatization may streamline production methods and avoid costly interim media and labor intensive transplanting procedures. Furthermore, effective evaluation of acclimatization protocols should not be confined to immediately post-acclimatization, but should be extended to assess plant development from transplant through flowering.

**Table 3. Interaction of acclimatization environment and root-zone medium on production of tissue culture-derived miniature roses. Data were recorded 2 weeks after transfer to acclimatization environments.**

| Humidity regime | Growing medium | Parameter | | |
|---|---|---|---|---|
| | | Shoot area ($mm^2$) | Root area ($mm^2$) | Root length (mm) |
| Mist | | | | |
| | Jiffy-9 | 477 ab* | 1171 b | 32 b |
| 5 sec/min | Peat/Sand | 524 ab | 115 b | 26 b |
| | Soil mix | 570 ab | 126 b | 27 b |
| | Jiffy-9 | 457 b | 165 ab | 44 ab |
| 5 sec/5 min | Peat/Sand | 430 b | 153 ab | 45 ab |
| | Soil mix | 420 b | 128 ab | 39 ab |
| Humidified Chambers | | | | |
| | Jiffy-9 | 550 ab | 165 ab | 55 ab |
| 1 week | Peat/Sand | 611 ab | 181 ab | 64 ab |
| | Soil mix | 551 ab | 184 ab | 60 ab |
| | Jiffy-9 | 649 a | 185 a | 64 a |
| 2 weeks | Peat/Sand | 749 a | 176 a | 59 a |
| | Soil mix | 669 a | 229 a | 63 a |

*Means separation within columns (mist and chambers) by Tukey's test, 5% level; n = 10.

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