Water relations and growth of rose plants cultured in vitro under various relative humidities

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Abstract

Stomatal malfunctioning is one of the main reasons why plants desiccate when transferred from in vitro to greenhouse conditions. In order to overcome this problem in Rosa hybrida cv. Madame G. Delbard (R) Deladel, two techniques, bottom cooling and water vapour permeable lid, were used. Both methods aimed to increase the vapour pressure gradient between leaf and atmosphere and consequently to improve plant transpiration.

The results showed that these techniques increased leaf resistance to dehydration and improved stomatal regulation. Water relations of treated plantlets were similar to those generally observed in hardened plants: lower leaf water and osmotic potentials, and lower leaf water content than in the control ones. Osmotic adjustment occurred in treated plantlets maintaining turgor pressure. Each technique also induced some effects on growth during the rooting phase: with bottom cooling, roots were shorter, with permeable lids, apices were necrosed.

These results are discussed in terms of physiological causes and in terms of effect during the following acclimatization.

Abbreviations: AWC – absolute water content, DW – dry weight, FW – fresh weight

Introduction

Upon transfer from in vitro to greenhouse conditions, rooted plantlets have to undergo changes in trophic status (from heterotrophy to autotrophy) as well as in water status (from high to low relative humidity). Desiccation is one of the main reasons why plants cultured in vitro die during this transfer (Brainard & Fuchigami 1982; Maene & Debergh 1987; Short et al. 1987). A poorly developed epicuticular wax layer and malfunctioning stomates lead to an absence in water loss regulation of these plantlets (Sutter & Langahehs 1979; Wardle et al. 1979; Brainard et al. 1981; Ziv et al. 1987). These abnormalities are mostly due to a water saturated atmosphere in the culture vessel and thus to a lack of water potential gradient between gel, plant and air. Therefore, it is important to create conditions in the culture vessels that stimulate transpiration so that in vitro plants, at rooting stage, behave as normal ones (Grout & Aston 1977).

It has been shown previously that promoting water transport through the plants before transplanting can be achieved by lowering relative humidity in the containers (Brainard & Fuchigami 1981; Ziv et al. 1983; Short et al. 1987; Smith et al. 1990; Preece & Sutter 1991).

Several methods have been used to create low relative humidity in the culture vessel including the use of desiccants (Wardle et al. 1983; Short et al. 1987), covering the culture medium with a layer of lanoline (Wardle et al. 1983), the use of culture vessels with porous closures (Short et al.
or walls (Smith et al. 1990) and the use of saturated salt solutions (Ritchie et al. 1990). But most of these methods are not practical and/or dried the culture medium very quickly. Vanderschaeghe & Debergh (1987) used a ‘bottom cooling’ technique and reported that condensation of water vapour on the gel surface by cooling the bottom of the vessels not only reduced relative humidity in the container but also avoided desiccation of the culture medium.

The effects of low relative humidity in the culture vessels on stomatal functioning of plantlets have been largely studied (Brainard & Fuchigami 1982; Short et al. 1987; Ziv et al. 1987; Capellades 1989). However, there are only a few data concerning water relations of these plantlets before transplanting (Ziv et al. 1983). In our study, two techniques were used: ‘bottom cooling’ and ‘water vapour permeable lid’. Treated plants were compared to the control ones for various parameters: leaf water potential components (water and osmotic potentials and turgor pressure), leaf absolute water content, root growth (length and number), apex necrosis and stomatal functioning (water loss of excised leaves).

The aim of this work was to examine whether reduced relative humidity in the culture vessel induces modifications in the water relations of rose plants cultured in vitro, permitting them to regulate water loss.

Materials and methods

Culture medium

The basic medium contained Murashige & Skoog’s (1962) macroelements, microelements and vitamins and 7.5 g l−1 Touzart and Matignon agar. Plant growth regulators (Sigma) were benzyladenine (7.9 μM) and gibberilic acid (0.3 μM) for multiplication medium, and indole-3-acetic acid (2.9 μM) and indole-3-butyric acid (0.5 μM) for rooting medium. 0.2 g l−1 charcoal, 40 g l−1 glucose were added to rooting medium and 30 g l−1 glucose to multiplication medium. The pH was adjusted to 5.6 before autoclaving. All media were autoclaved at 120°C for 20 min.

Culture conditions

Glass jars (height 13 cm, vol. 850 ml) containing 120 ml of multiplication or rooting medium were used. The containers were closed with screw-on polycarbonate lids and placed in a growth chamber where the conditions were 16 h day with 24°C and 70% relative humidity, and 8 h night with 19°C and 90% relative humidity. Light was provided by white fluorescent tubes (Mazda, Aurora). Photosynthetic photon flux density was about 50 μmol m−2 s−1 at plant level.

Plant material

A clone of Rosa hybrida cv. Madame G. Delbard (R) Deladel was used. At the end of the multiplication stage (day 21), usable shoots (0.5 cm < length > 0.3 cm) were decapitated and placed on rooting medium for 12 days. Care was taken to have 20 homogeneous shoots for each jar. During multiplication stage, all jars were maintained under controlled conditions as described above. But, during rooting phase, they were subjected to 3 treatments: control, bottom cooling or water vapour permeable lid. All measurements were made on the youngest fully expanded leaf at the end of the rooting phase (day 33). At this stage, leaf number per plant was not significantly different (p < 0.05) for three treatments. It was 6.1 ± 0.4 in control, 5.7 ± 0.4 in bottom cooling and 6.0 ± 0.5 in permeable lid treatment. These data are the means ± confidence intervals (n = 16).

‘Bottom cooling’

A cooling system with an incorporated pump (3.81 h−1) was used to force cool water through a system of aluminium pipes (diam. 2.6 cm) with exchange wings. In order to ensure good thermal exchange at the bottom of the jars, an aluminium plate (width 10 cm) was placed on top of each tube. Culture vessels were placed on the aluminium plates. Temperature was adjusted on the cooling system to obtain a gradient of about 4°C between bottom and top of the jar. Temperature of the gel was 20.7°C and temperature of the air in the jar was 24.7°C. Condensation then occurred on the gel surface. Relative