High Survival Rates During Acclimatization of Micropropagated Fruit Tree Rootstocks by Increasing Exposures To Low Relative Humidity

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Abstract

Micropropagated plants are usually cultured in in vitro environments, with a high relative humidity and low light intensity, taking nutrients and energy from the culture medium. During acclimatization, physiological and structural changes allow micropropagated plants to adapt to the new environmental conditions, mainly to low relative humidity and high light intensity. As a result, plants become autotrophic and develop as normal plants. However, acclimatization is not easy in many species obtaining low survival percentages. In this work we present a method of acclimatization that yields high survival rates, based on the controlled exposure to low relative humidity. Micropropagated plants, exposed daily to low relative humidity, showed high survival rates during acclimatization under a plastic tunnel in the greenhouse. In contrast, only few plants survived when humidity was kept high continuously, indicating that the exposures to low relative humidity may stimulate the changes to become a functional plant. Care should be taken to keep the substrate well watered, in order to provide enough water to restore water loss from transpiration. In this way, plants soon started to adapt and new leaves were visible during the first week of acclimatization. In addition, water transport through the roots was stimulated since plants were able to recover from a moderate wilt after the exposures to low relative humidity.

INTRODUCTION

Micropropagated plants need to adapt to the new environmental conditions, when transplanted to soil, through a process of acclimatization. Adaptation includes a number of hardening changes related with the control of water loss, and autotrophy. Micropropagated plants are usually cultured in in vitro conditions, with a high relative humidity and low light intensity, taking nutrients and energy from the culture medium. To increase plant survival after transfer to soil, different strategies were utilized. The use of antitranspirants to reduce water loss when exposed to a lower relative humidity, although can be a helpful treatment, did not fully solved the problem of plant growth and survival. The use of the latex polymer Anti-Stress 550 as an antitranspirant for acclimatizing micropropagated plantlets was investigated with in-vitro-rooted microshoots of walnut clone TRS (Voyiatzis and McGranahan, 1994). The survival rate of plants treated with latex and kept uncovered in the greenhouse was higher than that of the controls (66.5 and 55.0%, respectively). Earlier, several film-type antitranspirants were tested on tissue-cultured chrysanthemums and carnations (Sutter and Hutzell, 1984) at the time of transfer to a greenhouse. Although a silicone formulation had the greatest effect in reducing transpiration and water stress in transferred plants, it also stunted plant

growth. All other treatments with antitranspirants were ineffective in improving vigor and survival of plants compared with the non-treated controls. In addition, antitranspirants showed phytotoxicity and most antitranspirants needed to be applied at concentrations lower than those recommended by the manufacturers. However, they found that control plants, acclimatized in a humidity tent, were significantly larger and more vigorous than plants in any other treatment.

Plant growth regulators have also been applied to improve acclimatization. Gibberellic acid (GA3), was applied on black cherry (*Prunus serotina*) plantlets to study the effect on terminal bud break and subsequent stem elongation (Kavanagh et al, 1993). A foliar spray of GA3 was effective in stimulating stem elongation, but the effect was temporary. GA3 treatments did not fully substitute for a chilling period. On the other hand, the use of the growth inhibitor paclobutrazol in the rooting medium of *Rosa* and *Anigozanthos bicolor*, combined with the exposure to reduced relative humidity did not improve acclimatization either; however, paclobutrazol showed a positive effect on growth at low concentration (Henderson et al, 1994).

A different strategy was used by Koga et al. (1999) to acclimatize micropropagated strawberry plants by the combination of long periods in rooting medium (54 days) with water spraying (3 times per day by microsprinklers) of potted plants covered with cheesecloth, obtaining high survival rates (96-100%).

During acclimatization, physiological and structural changes allow micropropagated plants to adapt to the new environmental conditions, mainly to low relative humidity and high light intensity. Morphological and physiological plants changes have been described including functional stomata (Marín et al., 1988), efficient water transport through the roots and acquisition of sun-leaf structure (Marín and Gella, 1988) and the sugar and starch metabolism of *Spathiphyllum* plantlets (Van Huylenbroeck and De Riek, 1995), that were translocated to the emerging roots, and afterwards, starch accumulation began.

As a result, plants become autotrophic and develop as normal plants. Although, differences between micropropagated plants and seedlings were noted, since plantlet growth rates during acclimatization were lower than growth rates of similar *Prunus serotina* seedlings and their leaf area was correspondingly less (Drew et al., 1992). However, acclimatization is not always an easy step, since in many species, low survival percentages were obtained. In this work we present a method of acclimatization that yields high survival rates, based on the controlled exposure to low relative humidity

MATERIALS AND METHODS

Micropropagated *Prunus* plants (*P. cerasus*, *P. × amygdalopersica*, *P. cerasifera*, *P. insititia*, *P. cerasifera × munsoniana*), with a root length of 2 cm or longer, were transferred to trays with Jiffy-pots and a peat-perlite (1:1) or peat-vermiculite (1:1) substrate. Trays were placed on the surface of a layer of foam, previously soaked with water in a shaded greenhouse; then plants were treated with a fungicide (Baycor), and an insecticide (Exagama 90) applied to the substrate. Afterwards, they were covered by a plastic tent to create a high relative humidity (RH) environment. Plastic tent was removed daily to reduce the RH at increasing periods. The first days, plants were exposed to low RH until moderate wilt symptoms appeared, which happened at about 10-15 minutes. After one week, plants could tolerate 30 minutes or more, increasing the exposure time half an hour every week.

Watering was made frequently to provide enough water to replace water loss. Osmocote was used as a slow-release fertilizer added to the substrate.

Two months after transfer to soil, plants were transplanted to pots (1 liter) with the same substrate and grown until they were transplanted outdoors to a frame (about 50 cm tall).

RESULTS AND DISCUSSION

Acclimatization of micropropagated plants with increasing exposures to low RH was successful, with a broad average of 90 % survival, two months after transfer to soil. Figure 1 shows the pattern of the survival rate evolution during the acclimatization of 3 different batches (88, 113 and 76 plants) of the cherry rootstock 'Masto de Montañana' (*Prunus cerasus* L.). This pattern was similar to that found during the acclimatization of different micropropagated fruit tree species. No plant death was found during the first two weeks, suggesting that even non-functional plants could stand exposures to low relative humidity during this period. However, after these first two weeks, death of weak plants could be related to nutrient storage depletion. In general, plants looked healthy and new leaves, with a larger size, developed. Root system was well developed and actively growing root tips easily grew through the jiffy-pots at the time of the transplant to pots.

Plant response to the new environment was very quick and it was possible to observe new developing leaves after one week of transfer. The plastic tent maintained a high RH inside near 90-95% that decreased to that of the greenhouse (about 50-60%) when the plastic tent was removed. After the period of exposure to low RH plants recovered readily under the plastic tent, showing a good replacement of water loss; thus, stimulating water transport through the roots. It was critical to keep the substrate well watered, in order to provide enough water to restore water loss from transpiration. Water transport to the leaves also provides nutrients and other metabolites that can affect positively the production of photoassimilates. Since the ability to switch from a heterotrophic to an autotrophic state can be responsible for survival, an increase of transpiration and water transport that are favored in open environments, as we used here, may be the key of success during acclimatization.

Care should be taken to control pests efficiently, since they greatly affect survival rate. Apart from fungi, control of Sciaridae was very important because their larvae damaged tissues (roots and stems) hidden under the substrate. The use of the described pesticides was quite efficient and the frequency of treatments depended on the incidence of pests. Previous trials indicated that plants maintained at high RH in trays covered by small plastic tents did not succeed and survival percentages were low; however, they looked healthy for the first two weeks, but no new leaves developed. After this initial period plants declined and died, which suggested that desiccation was not the cause of low survival percentages, but a lack of metabolic activity that could not restore the needed reserves.

Micropropagated plants, exposed daily to low relative humidity, showed high survival rates during acclimatization under a plastic tunnel in the greenhouse. In contrast, only few plants survived when humidity was kept high continuously, indicating that the exposures to low relative humidity may stimulate the changes to become functional. The described adaptations that plants suffer during acclimatization matched well with this, since sun-leaf structures are adapted to higher light intensity and autotrophy, while functional stomata are linked with photosynthetic activity and control of water loss in low RH environments.

The acclimatization process described was successful for different fruit tree species, some of them difficult to root by conventional methods; thus, allowing micropropagation to be used as an alternative method of vegetative propagation.

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Fig. 1. Acclimatization of three different batches of micropropagated plants of the cherry rootstock 'Masto de Montañana' (*Prunus cerasus* L.)