Acclimatization of ‘VR043-43’ (*Vitis vinifera* × *Vitis rotundifolia*) grapevine rootstock

Marília Pereira Machado; Luiz Antonio Biasi; Marlice Ritter; Luciana Lopes Fortes Ribas; Flávio Zanette

1 UFPR – Programa de Pós-Graduação em Agronomia.
3 UFPR – Laboratório de Micropropagação de Plantas.
*Corresponding author <biasi@ufpr.br>

ABSTRACT: The pre-acclimatization stage can be used to improve micropropagation protocols and increase the yield of produced plants. The influence of sucrose and photon flux density (PFD) levels on the acclimatization of *in vitro*-grown ‘VR043-43’ (*Vitis vinifera* × *Vitis rotundifolia*) grapevine rootstocks was evaluated. Rooted shoots were obtained from 4-week-old *in vitro* shoots cultivated in QL (Quoirin and Lepoivre, 1977) culture medium supplemented with 15, 30 and 45 g L–1 of sucrose. The experiment was kept in a 25 ± 2ºC growth room, under 16-h photoperiod and PFD of 18 μmol m–2 s–1 or 43 μmol m–2 s–1. Plants were transferred to an intermittent misting system greenhouse for 10 d followed by 20 d of once-a-day watering routine using a handheld hose. Plant height was influenced by sucrose concentration, and shoots produced on media supplemented with 30 g L–1 sucrose were the tallest (5.0 cm). The largest leaf area was obtained with 31.3 g L–1 of sucrose, under the PFD of 43 μmol m–2 s–1 (13.3 cm2). Absence of sucrose in the culture medium led to a significant reduction in leaf area at both PFDs. Shoot (aerial part) dry matter was largest when 30 or 45 g L–1 of sucrose (17.5 and 16.7 mg per plant, respectively) were used. Microcuttings rooted in all sucrose concentrations tested. The highest survival percentage (100%) during *ex vitro* acclimatization was obtained for shoots cultured in media supplemented with 45 g L–1 of sucrose under both PFDs tested.

Key words: tissue culture, sucrose, micropropagation

Introduction

A pre-acclimatization stage, which applies techniques to make the *in vitro* environment more similar to the natural one, is suggested to enhance survival of micropropagated plants under *ex vitro* conditions (George, 1993). The pre-acclimatization stage can be used to improve micropropagation protocols and, consequently, to increase the yield of produced plants (Hoffmann et al., 2001).

The presence of sugar in the culture medium inhibits photoautotrophic capacity of plants, causing reduced growth and death of plants later in the acclimatization process (Kozai, 1991). In addition, presence of sucrose for a prolonged period in the culture medium may also cause loss of chlorophyll synthesizing capacity by the...
cells (George, 1993). However, the presence of a carbohydrate source is essential for in vitro rooting of many species and determinant for transplantation success (Srikandarajah and Mullins, 1981; Grattapaglia and Machado, 1998). Furthermore, in vitro root development usually enhances transplanting success because functioning roots can create a favorable plant water balance (Díaz-Perez et al., 1995).

Gribaudo and Fronda (1993) suggested a reduced sucrose concentration in the culture medium in the phase preceding the transfer of shoots or plants to ex vitro environment. This is done in order to enhance photosynthetic capacity and adaptation of plants to autotrophic conditions. Furthermore, Gribaudo and Fronda (1993) affirmed that an increase in light intensity facilitates the acclimation process since it promotes photosynthesis, improves water relationship between the tissues of the plant and external environment and thickens epicuticular wax in the leaves surface. However, PFD necessary for the in vitro cultivation of organs and tissues may differ depending on the explant type, the plant species and the micropropagation stage (Economou and Read, 1987).

The objective of this paper was to evaluate the influence of sucrose and PFD on the survival of in vitro produced ‘VR043-43’ grapevine rootstock plants during acclimatization.

**Material and Methods**

One-node stem pieces excised from in vitro ‘VR043-43’ grapevine rootstock shoots, which had been previously subcultured five times, were placed in test tubes (25 mm Ø x 150 mm high) containing 10 mL of QL medium (Quoirin and Lepoivre, 1977) with MS vitamins (25 mm Ø x 150 mm high) containing 10 mL of QL medium (Murashige and Skoog, 1962). This medium was supplemented with 100 mg L\(^{-1}\) of myo-inositol, 6 g L\(^{-1}\) of agar, and the following sucrose concentrations: 0, 15, 30 and 45 g L\(^{-1}\). No growth regulator was added. Media was sterilized by autoclaving at 121ºC, for 20 minutes at 1 atm.

The tubes were capped with aluminum foil and sealed with plastic film and kept in an acclimatized room at 25 ± 2ºC with a 16-h photoperiod provided by cool light fluorescent lamps, and PFD of 18 μmol m\(^{-2}\) s\(^{-1}\) for four weeks.

Transplantation of the tissue-cultured plantlets to an intermittent mist system greenhouse was done after four weeks of in vitro culturing. For that, culture tubes were placed inside this environment two days before the transplant. After that, the roots of the plants were washed in order to remove the agar and transferred to 53 cm\(^3\) tubes, containing a commercial substrate.

Plants were kept under the intermittent mist system for ten days subjected to the following watering regime: 15 s watering every 30 min from 8:00 to 17:00, 15 s watering every hour from 17:00 to 23:00; and 15 s watering every three hours from 23:00 to 8:00. After that, plants remained inside the greenhouse for 20 d, under daily irrigation using a hand-held hose.

Two evaluations were performed: one after removing the plantlets from the tubes to the greenhouse and the other after 30 d inside the greenhouse. The variables analyzed were height, number of leaves, percentage of roots, shoot (aerial part) dry matter, root dry matter and the leaf area. The latest was determined by using a Win Rhizo LA 1600 (Regent Instruments Inc.) with 100 W resolution.

A 2 × 4 factorial (two PFDs and four sucrose concentrations) with four replicates of 40 explants per treatment in a randomized complete block design was used. The experiment was repeated once. Results were analyzed using analysis of variance (ANOVA), and means were compared at the 5% level of probability according to Duncan’s multiple range test using MSTATC (Michigan State University) program. For some results, a second-degree polynomial model was fitted. The adjusted equation model was: \(Y = b_0 + b_1X + b_2X^2\) where \(Y\) was the variable, \(X\) the applied treatment, and \(b_0, b_1, b_2\) the coefficients of the model.

**Results and Discussion**

Plant height was positively affected by sucrose concentration up to 30.1 g L\(^{-1}\) (Figure 1). However, greater growth responses were observed for 41B grapevine rootstock (Vitis vinifera ‘Chasselas’ × Vitis berlandieri) aerial parts when 25.0 or 37.5 g L\(^{-1}\) of sucrose were added to the culture medium (Fila et al., 2008). For the du Lot and Chardonnay grape cultivars, as well as to ‘Gravesac’ grapevine rootstocks, plant height was significantly superior when medium was supplemented with sucrose compared to a sucrose-free medium (Galzy and Compan, 1992; Silva and Doazan, 1995).

Sucrose concentration and PFD caused an effect on the number of leaves per plant, and the interaction between both factors was detected by the analysis of variance. The number of leaves increased up to 34.2 g L\(^{-1}\) of sucrose (5.7 leaves), under 18 μmol m\(^{-2}\) s\(^{-1}\); at 43 μmol m\(^{-2}\) s\(^{-1}\), the maximum point on the curve (6.9 leaves) corresponded to the 29 g L\(^{-1}\) sucrose concentration. Higher PFD not only promoted more leaves per plant but also less sucrose was necessary to reach its maximum point.
the two studied factors was detected. The best results in shoot dry matter production, but no interaction between

\[ Y = -0.004194X^2 + 0.242916X + 3.40625 \]

was obtained with 31.3 g L\(^{-1}\) of sucrose, under 43 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) (Figure 3). Borghezan et al. (2003) observed a 12.5 ± 1.3 cm\(^2\) leaf area in ‘VR043-43’ rootstock grapevines when using the culture medium DSD1 (Silva and Doazan, 1995) with 20 g L\(^{-1}\) of sucrose under 40 to 45 \(\mu\)mol m\(^{-2}\) s\(^{-1}\).

Under the lowest PFD tested (18 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)), a higher sucrose concentration (34.2 g L\(^{-1}\)) was necessary to reach the highest point of the regression curve (5.7 cm\(^2\)) (Figure 3). This value was inferior to the one obtained under the highest photon flux density. However, no difference in leaf area was observed under three PFDs (35, 60 and 85 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)) (Viña et al., 2001) in Persea Americana, for instance. Herein, absence of sucrose in the culture medium reduced the leaf area of plants under both PFDs tested (Figure 3). Leaf area of Nicotiana tabacum plants was also lower in the absence of sucrose compared to the medium supplemented with 30 g L\(^{-1}\) of sucrose (Kdlecček et al., 2001).

The analysis of variance confirmed the influence of both factors (sucrose concentration and PFD) on the shoot dry matter production, but no interaction between the two studied factors was detected. The best results in shoot dry matter production were obtained with 30 and 45 g L\(^{-1}\) of sucrose (17.5 and 16.7 mg per plant, respectively) (Figure 4). Such results suggest that sucrose, besides interfering in the root system of in vitro plants, plays an important role in the aerial part formation of the micropropagated plants. For the PFD levels tested (18 and 43 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)), no difference between dry matter averages (11.9 mg per plant and 13.6 mg per plant, respectively) was obtained. However, Tichá et al. (1998) observed a positive effect in biomass and leaf area of Nicotiana tabacum plants with addition of 30 g L\(^{-1}\) sucrose in the culture medium and Galzy and Compan (1992) obtained similar results with Vitis rupestris.

The smallest dry matter of the aerial part (5.0 mg per plant) was obtained when sucrose was absent from the culture medium, probably due the necessity of increasing not only the PFD but in vitro CO\(_2\) enrichment and humidity in the culture tubes environment in order to promote photosynthesis, transpiration and accumulation of dry matter (Kozai and Nguyen, 2003). This is due to the increase CO\(_2\) concentration promotes increase photosynthesis, due to its direct effect on enzyme RUBISCO (ribulose-1.5-biphosphate carboxylase), and the decrease in relative humidity with greater gas exchanges in the tube increases significantly the rate of transpiration, and consequently the absorption of water and nutrients. According to Yoon et al. (2009), in vitro CO\(_2\) enrichment and sugar deprivation promoted most of the growth parameters during in vitro growth and ex vitro acclimatization of Phalaenopsis plantlets.

Rooting occurred regardless the sucrose concentration tested, being the interaction between sucrose concentration and PFD to the rooting percentage, according to the analysis of variance (Figure 5). The highest rooting percentage (95%) was obtained when sucrose was absent from the culture medium, allied to PFD of 18 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) (Figure 5A). The presence

\[ Y = -0.01147X^2 + 0.718133X + 2.097 \]

in the culture medium and Galzy and Compan (1992) obtained similar results with Vitis rupestris.

The smallest dry matter of the aerial part (5.0 mg per plant) was obtained when sucrose was absent from the culture medium, probably due the necessity of increasing not only the PFD but in vitro CO\(_2\) enrichment and humidity in the culture tubes environment in order to promote photosynthesis, transpiration and accumulation of dry matter (Kozai and Nguyen, 2003). This is due to the increase CO\(_2\) concentration promotes increase photosynthesis, due to its direct effect on enzyme RUBISCO (ribulose-1.5-biphosphate carboxylase), and the decrease in relative humidity with greater gas exchanges in the tube increases significantly the rate of transpiration, and consequently the absorption of water and nutrients. According to Yoon et al. (2009), in vitro CO\(_2\) enrichment and sugar deprivation promoted most of the growth parameters during in vitro growth and ex vitro acclimatization of Phalaenopsis plantlets.

Rooting occurred regardless the sucrose concentration tested, being the interaction between sucrose concentration and PFD to the rooting percentage, according to the analysis of variance (Figure 5).

The highest rooting percentage (95%) was obtained when sucrose was absent from the culture medium, allied to PFD of 18 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) (Figure 5A). The presence

\[ Y = -0.00449X^2 + 0.354933X + 3.059 \]

in the culture medium and Galzy and Compan (1992) obtained similar results with Vitis rupestris.

The smallest dry matter of the aerial part (5.0 mg per plant) was obtained when sucrose was absent from the culture medium, probably due the necessity of increasing not only the PFD but in vitro CO\(_2\) enrichment and humidity in the culture tubes environment in order to promote photosynthesis, transpiration and accumulation of dry matter (Kozai and Nguyen, 2003). This is due to the increase CO\(_2\) concentration promotes increase photosynthesis, due to its direct effect on enzyme RUBISCO (ribulose-1.5-biphosphate carboxylase), and the decrease in relative humidity with greater gas exchanges in the tube increases significantly the rate of transpiration, and consequently the absorption of water and nutrients. According to Yoon et al. (2009), in vitro CO\(_2\) enrichment and sugar deprivation promoted most of the growth parameters during in vitro growth and ex vitro acclimatization of Phalaenopsis plantlets.

Rooting occurred regardless the sucrose concentration tested, being the interaction between sucrose concentration and PFD to the rooting percentage, according to the analysis of variance (Figure 5).

The highest rooting percentage (95%) was obtained when sucrose was absent from the culture medium, allied to PFD of 18 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) (Figure 5A). The presence

\[ Y = -0.0015X^2 + 0.1025X + 3.925 \]

in the culture medium and Galzy and Compan (1992) obtained similar results with Vitis rupestris.
of sucrose was fundamental for the development of in vitro roots of strawberry plants, and no rooting response was obtained in its absence (Calvete et al., 2002). However, a 60% increase in rooting of M-26 apple tree rootstocks was obtained when sucrose was reduced from 30 to 10 g L$^{-1}$ (Simmonds, 1983). Herein, there was no negative effect exerted by the higher PFD tested on rooting percentage of the rootstock plantlets. A reduction in rooting percentage was only observed if sucrose was lacking in the culture medium (Figure 5A).

ANOVA confirmed an effect of both factors (sucrose concentration and PFD) and interaction between them on the survival percentage of acclimatized plants. A reduction in rooting percentage was only observed if sucrose was lacking in the culture medium (Figure 5A).

Supplementation of sucrose to the culture medium, under the PFD of 43 μmol m$^{-2}$ s$^{-1}$, promoted better growth and development of in vitro 'VR043-43' grapevine rootstock cuttings. The higher survival in the ex vitro environment corresponded to the higher sucrose concentration in the culture medium.

Conclusions

Supplementation of sucrose to the culture medium, under the PFD of 43 μmol m$^{-2}$ s$^{-1}$, promoted better growth and development of in vitro 'VR043-43' grapevine rootstock cuttings. The higher survival in the ex vitro environment corresponded to the higher sucrose concentration in the culture medium.

Acknowledgements

To the Araucaria Foundation for financial support; to CAPES for a Fulbright scholarship awarded to M.P. Machado and to CNPq for the granting to L.A. Biasi and F. Zanette.

References


Acclimatization of ‘VR043-43’ grapevine rootstock


Received November 13, 2008
Accepted May 14, 2010