The Rate of Shoot Regeneration from Apple (*Malus domestica*) Leaves Depending on the In Vitro Culture Conditions of the Source Plants

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Abstract

Efficiency of somatic organogenesis depends to a great degree on the physiological status of the in vitro source plants. Important factors are the cultivation conditions – nutrient media, cultivation plates, gas-exchange possibilities, temperature and light regime. The aim of the present study was to investigate the effect of conditions for cultivating the source plants (cultivation plates and nutrient media) on the regeneration capacity of the apple (*Malus domestica*) leaf explants. Studies were carried out with in vitro propagated plants of the apple cultivar 'Chadel'. The source microplants were cultivated on MS nutrient media with the addition of sucrose 10 g/L and modified MS with added BAP 2.5 µM, IAA 0.0571 µM and sucrose 30 g/L. Two types of cultivation plates were used in the experiment – glass jars and plastic vessels with a gas-permeating cover. Leaf segments of the source plants that had been cultivated in each of the four combinations of nutrient media/cultivation plates were set for regeneration. The nutrient media for regeneration were based on MS with added TDZ (7.5 µM), sucrose 10 g/L, sorbitol 30 g/L and different IAA content – 5, 10, 15 and 20 µM. The explants were cultivated in darkness for 15 days, after which – at a photoperiod of 16/8 hours (40 µmol m^{-2} s^{-1} PAR) at a temperature of 22±2°C for 25 days. The best results (over 80% regeneration) were achieved when using explants of plants grown in plastic vessels with a gas-permeating cover in modified MS nutrient medium.

INTRODUCTION

Physiological status of the in vitro source plants is a key factor for the efficiency of somatic organogenesis. It depends to a great degree on the gas-exchange possibilities of the cultivation plates and plant growth regulators in the nutrient media.

It was established that the accelerated ventilation significantly stimulated the development of the in vitro plants. Such results were obtained about the in vitro growth of *Solanum* (Kubota and Kozai, 1992). Analogous were the data about gerbera and *Ficus lirata* published by Jackson et al. (1991), as well as the results obtained by Solarova (1996) about carnation and potato when the vessels were not firmly closed. Those effects were explained by the authors with the increased influx of CO_2 in the culture vessels during the light period as well as with the avoidance of the accumulation of ethylene and toxic concentrations of CO_2 at the end of the dark period.

Nacheva and Ivanova (2006) announced that the improved gas-exchange between the culture vessels and the ambient air in the in vitro propagated apple rootstock MM 106 had a positive influence on the general structuring of the photosynthetic apparatus. The shootlets grown with a gas-permeating cover were lower, with shorter but thicker stems and their leaf blades were broadly open. Under the effect of the improved ventilation the growth of dry matter of the whole in vitro plants and of the leaves increased. Both the percentage of dry matter to a unit of fresh one and the relative share of leaves (based on dry matter) to the whole plant also increased. Rootlets emerged earlier. The chlorophyll and carotenoid content also increased. More intensive $^{14}CO_2$ photofixation was reported (Nacheva and Ivanova, 1998).

The aim of the present study was to investigate the effect of the cultivating conditions for the source plants (cultivation plates and nutrient media) on the regeneration capacity of the apple (*Malus domestica*) leaf explants.

MATERIAL AND METHODS

Studies were carried out with 13-15-day-old in vitro propagated plants of the apple cultivar 'Chadel'.

The source microplants were cultivated on two nutrient media:

- growth regulators free MS (Murashige and Scoog, 1962) nutrient media for elongation (E) with the addition of sucrose 10 g/L;
- modified MS (Murashige and Scoog, 1962) nutrient media for multiplication (M) with added BAP 2.5 μM, IAA 0.0571 μM and sucrose 30 g/L.

Two types of cultivation vessels were used in the experiment:

- glass jars (600 ml);
- plastic vessels with a gas-permeating cover (Combiness, Belgium, gas exchange – 10 GE/day).

Leaf segments of the source plants that had been cultivated on each of the four combinations of nutrient media/cultivation plates were set for regeneration.

Approximately 1/3 of the first three fully opened leaves was dissected, wounded with a surgical blade and placed abaxial side up on the cultural media.

The nutrient media for regeneration were based on MS (Murashige and Scoog, 1962) with added TDZ 7.5 μM, sucrose 10 g/L, sorbitol 30 g/L and different IAA content – 5, 10, 15 and 20 μM.

The explants were cultivated in darkness for 15 days, then – at a photoperiod of 16/8 hours (40 μmol m^{-2} s^{-1} PAR) at a temperature of 22±2°C for 25 days.

RESULTS AND DISCUSSION

It was established that when leaf segments of in vitro plants having been cultivated on hormone-free nutrient medium (E) in glass jars were used, the regeneration success was greatly influenced by the IAA concentration in the regeneration medium. The highest regeneration percentage (88%) was obtained on media with 5 μM of IAA. With the increase of IAA concentration the regeneration percentage decreased, and, at 20 μM of IAA it was only 38% (Fig. 1).

When leaf segments of in vitro plants having been cultivated on hormone-free nutrient medium (E) in plastic vessels with a gas-permeating cover were used, the regeneration percentage was comparatively low (35-39%) and it did not depend on the concentration of IAA in the regeneration medium.

Cultivation of the source microplants on a nutrient medium for multiplication (M) containing cytokinin led to a significant increase of the regeneration percentage. In explants from plants having been cultivated in glass jars, the regeneration percentage was 55-70%. The leaf segments of plants having been cultivated in plastic vessels with a gas-permeating cover demonstrated a very high regeneration percentage (80-90%) (Fig. 2). In both cases any interdependency between the regeneration percentage and the auxin IAA concentration was not observed. A possible reason for the results obtained was the cytokinin (BAP 2.5 μM) added to the nutrient medium of the source plants that most probably led to increasing the level of endogenous cytokinins and, respectively, to increasing the regeneration capacity of the plant tissues.

In the different variants 10-40% of the explants formed more than 5 regenerants (Fig. 3). The best results in respect to that characteristic were achieved in a regeneration medium with 10 μM of IAA added, for plants cultivated in glass jars in a hormone-free nutrient medium E (40%), and, in a regeneration medium with 5 μM of IAA added, for plants cultivated in glass jars in a nutrient medium for multiplication M (35%).

The results obtained confirmed the importance of the different sealing materials for the development of the in vitro plants.

In a number of studies the significance of the type of the cultivation vessels used and the level of gas-exchange in micropropagation was underlined (Nacheva and Ivanova, 2006).

In experiments with pear, 'GF677' and quince 'BA29' it was demonstrated that for the regeneration processes the gaseous environment inside the cultural vessels was an important factor for the growth and differentiation of plant cultures (Marino et al., 1995, 2008; Marino and Berardi, 2004).

Our previous experiments with plums and apples as well as the present study made us believe that the cultural conditions of micropropagated source plants influenced the behavior of the explants at the time of regeneration. The type of cultural vessels, sealing materials and nutrient media determined the gas environment (ethylene, O_2, CO_2, etc.) of the plants. Thus the endogenous hormone level and the structure of the leaves were changed and the regeneration capacity of the plant tissues was affected.

In the present study, we described a system that made it possible to improve the efficiency of somatic regeneration. A key feature was the control of the in vitro culture conditions of the source plants. The best results were achieved when using explants of plants grown in plastic vessels with a gas-permeating cover on cytokinin enriched nutrient medium. Such an approach could be useful in the work with other species difficult to regenerate.

As a result of the research more than 2,000 somaclones of the apple cultivar 'Chadel' were obtained and propagated. All clones are to be tested for resistance to fire blight (*Erwinia amylovora*).

CONCLUSIONS

- The results of the present study confirmed the importance of the physiological conditions of the source plants for the success of the somatic regeneration.
- The best results (over 80% regeneration) were achieved when using explants of plants grown in plastic vessels with a gas-permeating cover on modified MS nutrient medium with added BAP 2.5 μM, IAA 0.0571 μM and sucrose 30 g/L.
- As a result of the research more than 2,000 somaclones of the apple cultivar 'Chadel' were obtained and propagated. All the clones are to be tested for resistance to fire blight (*Erwinia amylovora*).

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Figures

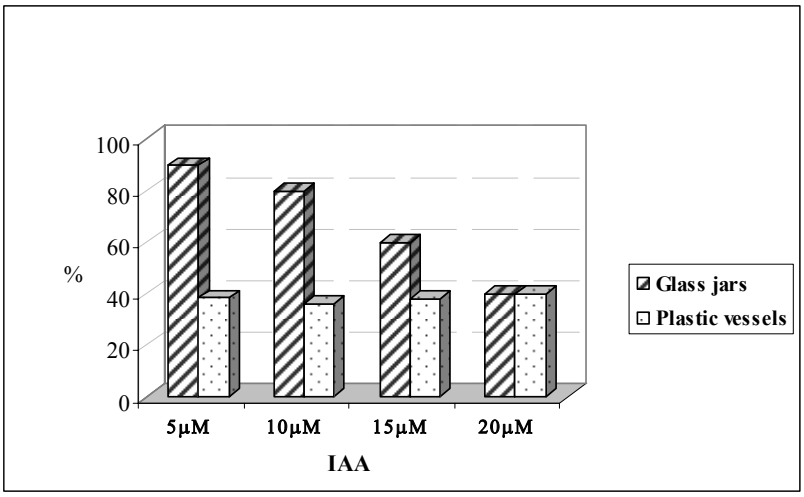

Fig. 1. Regeneration of leaf segments of in vitro plants of the apple cultivar 'Chadel' cultivated in a hormone-free nutrient medium (E) in glass jars and plastic vessels with a gas-permeating cover – the effect if IAA concentration.

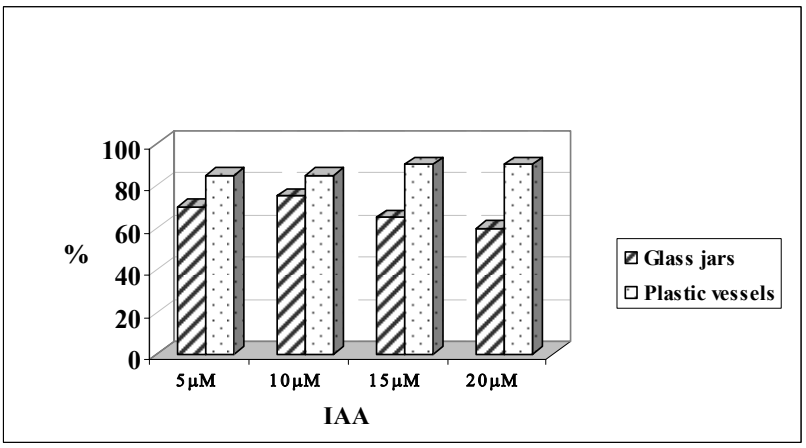

Fig. 2. Regeneration of leaf segments of in vitro plants of the apple cultivar 'Chadel' cultivated in a nutrient medium for multiplication (M) in glass jars and plastic vessels with a gas-permeating cover – the effect if IAA concentration.

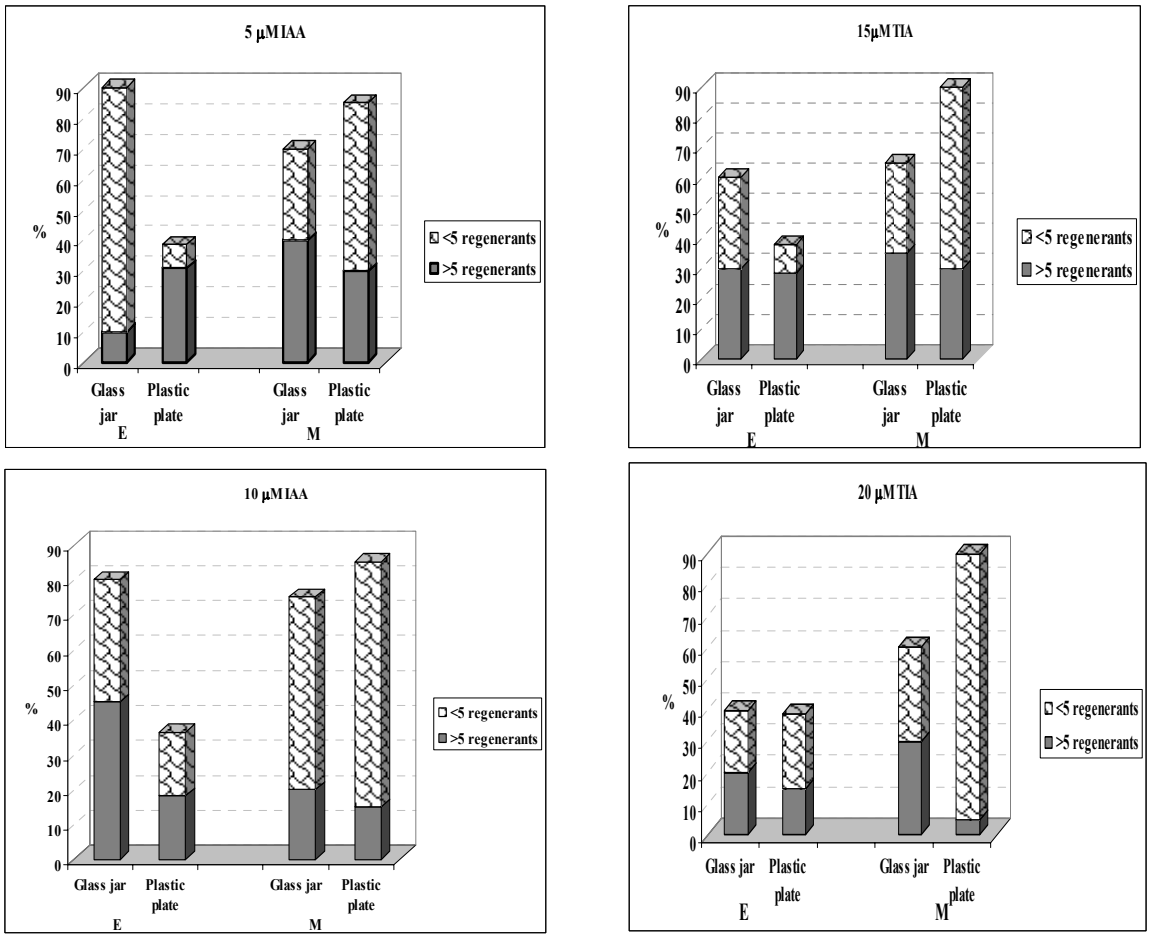

Fig. 3. Effect of the cultivation conditions of the in vitro source plants of the apple cultivar ‘Chadel’ on the percentage of explants forming less than 5 and more than 5 regenerants.