Micropropagation of Gisela 5 (Cherry Dwarf Rootstock): The Effect of the Type and the Concentration of the Carbohydrates in the Nutrient Medium

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Abbreviations: BAP - 6-benzylaminopurine; IBA - indole-3-butyric acid; NAA - α-naphthaleneacetic acid; IAA - indole-3acetic acid; PPFD - photosynthetic photon flux density; DMRT - Duncan's Multiple Range Test.

Abstract

Gisela 5 Cherry Dwarf Rootstock has a moderate to poor growth and it is very perspective for the development of modern intensive cherry plantations. There are single announcements in scientific literature about micropropagation of Gisela 5.

The aim of the present study was to investigate the effect of the type and the concentration of carbohydrates (sucrose and sorbitol) added to the nutrient medium with auxins (IBA, NAA and IAA) on the multiplication coefficient and the length of the newly formed shootlets in Gisela 5 Cherry Dwarf Rootstock.

The study was carried out on 15 different nutrient media suitable for multiplication, based on MS (1962). The effect of the varying concentrations of auxins (IBA, NAA and IAA) and carbohydrates (sucrose and sorbitol used separately and in a combination) at a constant concentration of the cytokinin BAP (2.5 µM), was followed out.

The results of the investigations showed that the basic effect on both studied characteristics was exerted by the type and the concentration of the carbohydrates. The highest multiplication coefficient (number of shootlets per plant) was achieved when combining sucrose and sorbitol in a 2:1 ratio, followed by the variants of sucrose to sorbitol in a 1:2 ratio. The most distinct differences were reported in the variants on the nutrient medium with IBA and IAA when the coefficient of multiplication was 3-4 times higher compared to the classical variants with sucrose. The best results (multiplication coefficient of 3.72-4.31 at 1.9 cm mean length of the shootlets) were achieved in the variants containing 0.005 µM IBA, 20 g/L of sucrose and 10 g/L of sorbitol.

INTRODUCTION

Before fruit orchards are being established the choice and production of suitable, virus-free vegetative rootstocks for particular types are important. These rootstocks should naturally inherit the biologic and morphologic characteristics of the original plant, they are equalized and could have a slower growth and induce earlier fruit-bearing. The rootstocks of Gisela 5 Cherry Dwarf and Gisela 6 (*Prunus cerasus* × *P. canescens*) have average and even slower growth and have outstanding perspectives for development of modern intensive cherry orchards. For that reason elaboration of methods for their maspropagation in vitro is very important.

There are many factors that have impact on the growth and development of the plants, grown in vitro – light, temperature, composition of the nutrient media (nutrient elements, growth regulators), gas exchange rate of the cultural vessels, etc. All these

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factors have been intensively explored for 30 years. However plants' needs of the source of carbohydrates remain insufficiently explored (Swedlund and Locy, 1993; Leifert et al., 1995). Sucrose is the most widely-used carbohydrate in the plant tissue cultures. It is due to the fact that this disaccharide keeps a relatively good growth of the microplants and is comparatively cheap (Swedlund and Locy, 1993). Regardless of the wide use of sucrose during the last years increasing attention is being paid to other saccarides, suitable for different plant types (Spiegel-Roy and Saad, 1997; Jain et al., 1997).

Despite sucrose, many carbohydrates are synthesized and transported in the plants (Loescher, 1987) and have impact on the processes of differentiation and dedifferentiation of the plant tissues, habits and their physiological state. These carbohydrates vary according to the different botanical families and even species. For that reason it is not a surprise that different carbohydrates in the nutrient media cause specific behaviour of growth in vitro. Many authors note the specific cellular differentiation in some plant types according to their source of carbohydrates (Lemos and Blake, 1996; Karhu, 1997; Bellettre et al., 1999). There is an opinion that the best results are achieved when the types and proportions of the carbohydrates, characteristic for the phloem sap of the respective plant species are used in vitro (Hammat, 1993).

Researches of the species from the genera *Malus* and *Prunus* show the importance of sorbitol in the nutrient media. Sorbitol is a sugar alcohol, that together with sucrose is an initial product from the photosynthesis and is a main transport form of the carbon at the tree types of Rosaceae (Bieleski, 1982; Wallaart, 1980).

The research of the effect of sorbitol on the fruit plants and tissues in in vitro conditions is focused mainly on empirical experiments with different nutrient media. Many experiments specify the positive effect of sorbitol on the initiation of callus, organogenesis of *Malus domestica* (Karhu, 1997), stem multiplication and rooting (Chong and Taper, 1972; Welander et al., 1989; Marino et al., 1993, Pawlicki and Welander, 1994; Kadota et al., 2001).

Ružic et al. (2005) report that sorbitol (115 mM) significantly improves the micropropagation of the cherry 'Lapins' and the pear rootstock 'Pyrodwarf' while fructose advantages the development of the rootstock 'Tabel Edabriz'.

The aim of the present study was to investigate the effect of the type and the concentration of carbohydrates (sucrose and sorbitol) added to the nutrient medium, supplemented with different concentration of auxins (IBA, NAA and IAA) on the multiplication coefficient and the length of the newly formed shootlets in Gisela 5 Cherry Dwarf Rootstock.

MATERIALS AND METHODS

Plant Material

The experiments are made with in vitro propagated plants of Gisela 5 Cherry Dwarf Rootstock.

Establishment of In Vitro Culture

Vegetative shoots of Gisela 5 are taken from virus free source plants, grown in an isolated field of the Fruit-Growing Institute – Plovdiv.

The sterilization of explants is made according to the standard procedure: washing with running water; sterilization with calcium hypochlorite ($Ca(OCl)_2$ 5% solution) for 5, 7 and 9 minutes; three times washing with sterile distilled water for 1, 5 and 10 minutes.

Treated in that way the explants are plated on nutrient media based on MS (Murashige and Skoog, 1962) with and without addition of growth regulators.

Multiplication

The plant material is cultured on MS nutrient media, supplemented with 2,5 μM BAP, 0.005 μM IBA, sucrose 30 g/L, agar 6,5 g/L, pH 5.6 (before autoclaving).

The Effect of Carbohydrates in the Nutrient Medium

Nutrient media for multiplication on the basis of MS (Murashige and Skoog, 1962), enriched with 2,5 μM BAP, agar 6,5 g/L, (pH 5.6) and ranged content of sucrose, sorbitol and of the auxins IBA, NAA, IAA are used in the experiments (Table 1). The shoots (about 5-7 mm) are plated in test-tubes with diameter 22 mm and 5 ml nutrient media. The plants are cultivated in a growth chamber with temperature 22±2°C and photoperiod 16/8 hours (40 μmol m^{-2} s^{-1} PPFD).

In two passages of 3 weeks on the respective nutrient media the following indices are reported:

- number of newly formed shoots up to 5 mm and above 5 mm high;
- average height of newly formed shoots (mm).

Data Analysis

For each variant of nutrient media 20 shoots in three repetitions are set. Data were analyzed by analysis of variance and the means were separated using the Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Establishment of the Explants in In Vitro Conditions

The sterilization procedures that are used at work with fruit species are standard and from all variants 85-100% sterile explants are obtained. The most suitable in regard to the survival of the plants appeared the 5 minutes treatment with calcium hypochlorite for the tips and 7 minutes for the stem segments. With these variants a higher (above 85%) degree of disinfection is achieved, the explants are fresh, green and vital and have a high degree of adaptation to the in vitro conditions.

Multiplication

The results from the testing of different nutrient media for multiplication show that at constant concentration of the cytokinin BAP in the nutrient media main effect on the two studied indices has the type and concentration of the carbohydrates (Figs. 1 and 2). When sucrose and sorbitol are applied together in proportion 2:1 and 1:2 (VM10 - VM15) significantly higher number of shoots with higher length, regardless of the type of the used auxin, is obtained. The highest coefficient of multiplication (number of shoots/plants) is achieved with variants, containing sucrose and sorbitol in proportion 2:1, followed by variants sucrose:sorbitol 1:2 (Fig.1).

The interaction between the source of carbohydrates and the type of auxin is interesting. When only sucrose in nutrient media is used the variant with NAA (VM2) shows three times higher multiplication rate and higher length of the shoots in comparison with the other two tested auxins (Figs. 1 and 2).

When only sorbitol is applied the auxin NAA (VM8) again induces the appearance of more new shoots compared to IBA and IAA (VM7, VM9), but the difference is not so distinctive. In regard to the length of the microplants the three variants with sorbitol (VM7, VM8 and VM9) are almost the same.

The best results (coefficient of multiplication 3.94-4.31 at average height of a shoot 19 mm) are achieved with variants VM13, VM14 and VM15 of nutrient media, consisting of 20 g/L sucrose and 10 g/L sorbitol.

With all tested variants of nutrient media the plants are fresh and in good physiological state. Slight indications of vitrification are observed with variants VM10, VM8, VM11 and VM13.

By analogy with the results received by us, combining sucrose (2.5%) and sorbitol (0.5%) improves the proliferation of *Prunus persica* L. × *Prunus amygdalus* BATSCH. hybrid compared to the independent application of sucrose (3%) (Imani and Abdollahi, 2006).

Marino et al. (1991) also report that sorbitol (116.8 mM) improves the proliferation and decreases the vitrification of the apricot cultivars 'San Castrese' and 'Portici'.

Significant increase in the efficiency of proliferation under the influence of sorbitol (20-30 g/L) is achieved with the Japanese pear (Kadota et al., 2001). The authors report that a system of two phases with solid and liquid nutrient media with 20 g/L sorbitol is even more effective and induces an increase in the number of axillar buds and the fresh mass of the microplants.

CONCLUSIONS

The presence of sorbitol in the nutrient media for multiplication of the Cherry Dwarf Rootstock Gisela 5 stimulates development of side buds and growth of the newly formed shoots. The highest coefficient of multiplication (number of shoots/plant) is achieved with variants, consisting of sucrose and sorbitol in proportion 2:1, followed by variants of sucrose:sorbitol 1:2.

ACKNOWLEDGEMENTS

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Tables

Table 1. Nutrient media for multiplication.

VARIANTS	IBA (μM)	NAA (μM)	IAA (μM)	Sucrose (Su) (g/L)	Sorbitol (Sb) (g/L)
VM 1	0.005			30	
VM 2		0.005		30	
VM 3			0.057	30	
VM 4	0.005			15	15
VM 5		0.005		15	15
VM 6			0.057	15	15
VM 7	0.005				30
VM 8		0.005			30
VM 9			0.057		30
VM 10	0.005			10	20
VM 11		0.005		10	20
VM 12			0.057	10	20
VM 13	0.005			20	10
VM 14		0.005		20	10
VM 15			0.057	20	10

Figures

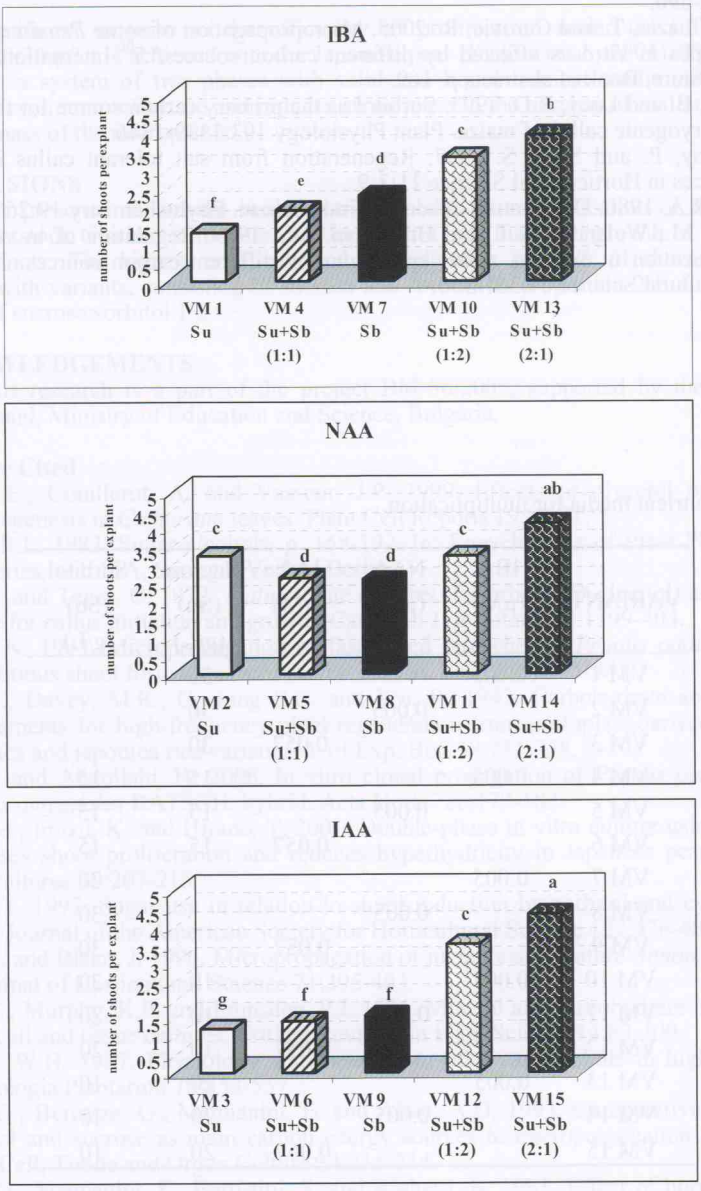

Fig.1. Effect of the Sucrose (Su) and Sorbitol (Sb) in the nutrient media on the multiplication rate of the plants of Gisela 5. Different letters within each column indicates significant difference ($P<0.05$) by DMRT.

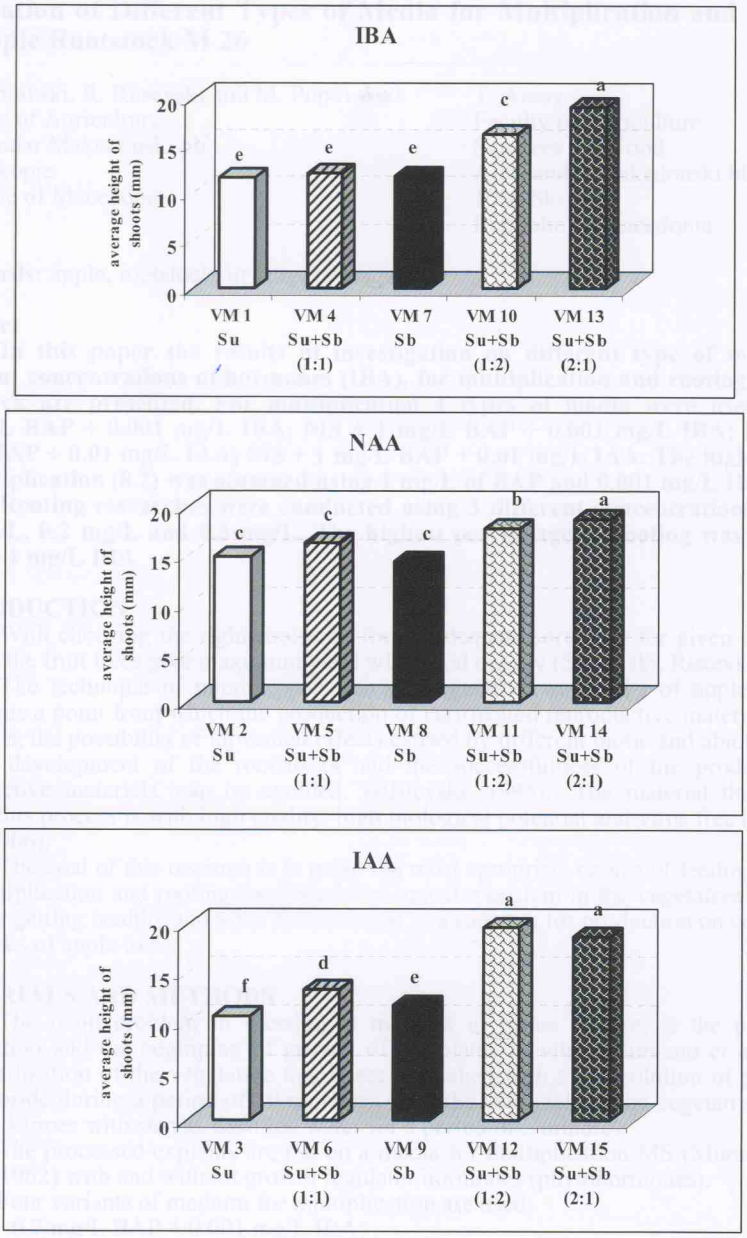

Fig. 2. Effect of the Sucrose (Su) and Sorbitol (Sb) in the nutrient media on the height of the shoots of Gisela 5. Different letters within each column indicates significant difference ($P<0.05$) by DMRT.