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The Effect of Carbon Source and Concentration on *in Vitro* Shoot Proliferation of MM.106 Apple Rootstock

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ABSTRACT

The effect of fructose, sucrose, glucose, sorbitol, and maltose at various concentrations on *in vitro* shoot proliferation on MM.106 apple rootstock was studied. Shoot tips were cultured in MS medium containing 0.4 µM indole-3-butyric acid (IBA), 4.43 µM benzylaminopurine (BAP), 0.6% agar and 0, 30, 60, 90, or 120 mM of the above carbohydrates. Type and concentration of sugars had a significant effect on shoot number and length. Sorbitol at 90 mM was the most effective carbon source for shoot proliferation and it improved plant regeneration. Shoot number was lowest at 30 and 120 mM sucrose, 30 mM glucose and at all concentrations of maltose. Shoot survival in the presence of all carbohydrates was >85% after four weeks. The reduction in shoot length and number was more pronounced in maltose compared with the other sugars.

Keywords: sorbitol, *Malus*, proliferation, sucrose, sugar concentration

INTRODUCTION

Micropropagation of apples for producing self-rooted plants will open up new areas of research and allow changes in traditional fruit tree propagation. The MM.106 apple rootstock has been extensively used in many countries to produce semi-dwarf trees (Aklan *et al*. 1997; Modgil *et al*. 2005). Therefore, *in vitro* micropropagation is very important for commercial practices. Plant cell, tissue or organ culture normally requires the incorporation of a desired carbon source in to the culture medium (George 1993). The type of carbon source and its concentration affects the shoot proliferation (Coffin *et al*. 1976; Welander *et al*. 1989) in apple.

Sucrose at 3% has been used as the only carbon source for micropropagation of MM.106 apple rootstock (Golosin and Radojevic 1987; Sharma *et al.* 2000), but the effect of different sugar concentrations on shoot proliferation of this apple rootstock has not yet been investigated. In this study, we compared the effects of sucrose, sorbitol, glucose, fructose and maltose on proliferation of MM.106 apple rootstock.

MATERIALS AND METHODS

Plant material and culture conditions

The explants employed were shoot tips of the apple (*Malus domestica* Borkh) rootstock, MM.106 of about 25 mm in length preserved from previous *in vitro* cultures and maintained in a growth room by subculturing shoots monthly in 250 ml polypropylene containers containing 40 ml of MS culture medium (Murashige and Skoog 1962) with 0.4 µM indole-3-butyric acid (IBA), 4.43 µM benzylaminopurine (BAP), 0.6% agar, and 87.6 mM sucrose (Sharma *et al*. 2000). The pH of the media was adjusted to 5.8 before autoclaving at 121°C for 15 min. Shoots were illuminated by cool-white florescent light (50 µmol m^{-2} s^{-1}; 16-h photoperiod) at 25 ± 1°C.

Shoot proliferation

Shoot tips (4 to 5 mm long) were cut from larger explants taken from stock plants and transferred to 25 × 100 mm culture tubes containing 40 ml of MS culture medium with 0, 30, 60, 90, or 120 mM of sucrose, sorbitol, fructose, glucose, or maltose. Shoot tips were placed upright in the medium with five shoot tips per vessel. Each treatment included six replications. Each experiment was conducted and repeated twice. After 6 weeks on proliferation media, shoot multiplication was evaluated by counting the number and length of shoots per explant.

The data were statistically analyzed using analysis of variance (ANOVA) as a factorial experiment in a completely randomized design according to Gomez and Gomez (1984). All statistical analyses were performed using SAS software (SAS 2001). Average of the main effects and their interactions were compared using the Duncan's multiple range test at $P< 0.05$.

RESULTS AND DISCUSSION

The concentration of sugars except for maltose significantly affected shoot number. Sucrose at 90 mM produced significantly more shoots than the other sucrose levels (**Fig. 1**). 60 and 90 mM sorbitol, fructose and glucose produced significantly more shoots than the other concentrations (**Fig. 1**). There was no significant difference between the concentration of maltose on shoot number (**Fig. 1**). The formation of shoots is related to the sugar concentration in the medium (Romano *et al.* 1995; Saadat and Hennerty 2002; Kadota and Niimi 2004; Alkhateeb 2008). Therefore, the role of sugars in the present study could be interpreted as both nutritional and osmotic regulatory functions of these carbohydrates.

As can be seen in **Figs. 1** and **2**, the type and concentration of sugar had a significant effect on shoot number and length. Increasing sugar concentration to 90 mM significantly increased shoot number. Sorbitol at 90 mM produced the most and longest shoots and was significantly different from all other sugars at different concentrations. Shoot

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Research Note

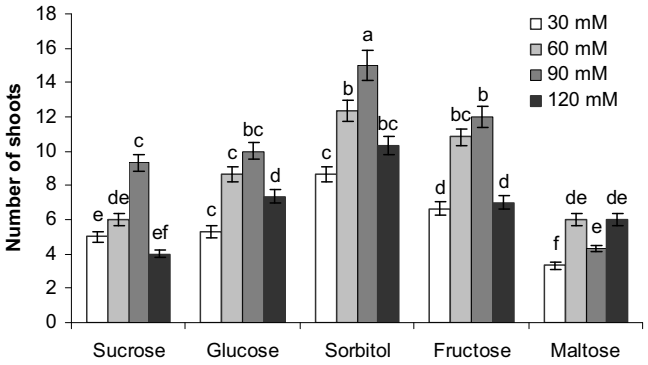

Fig. 1 Influence of carbon sources and their concentrations on shoot number of apple rootstock MM.106. Values with the same superscript letters are not significantly different at $P < 0.05$.

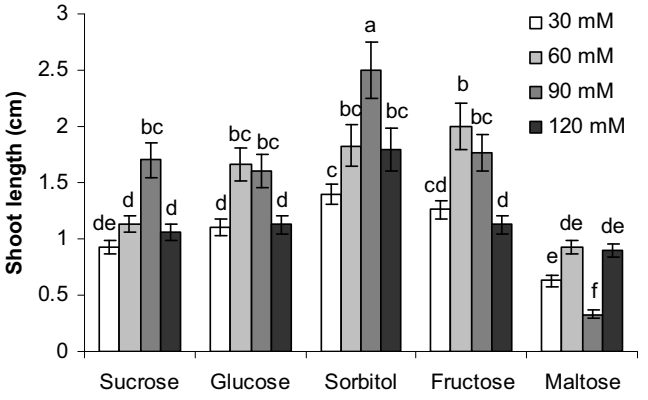

Fig. 2 Influence of carbon sources and their concentrations on shoot length (cm) of apple rootstock MM.106. Values with the same superscript letters are not significantly different at $P < 0.05$.

number and length was lowest at 30 and 120 mM sucrose, 30 mM glucose and at all concentrations of maltose.

Although sucrose at 3% has been used as a carbon source for the proliferation of apple (Golosin and Radojevic 1987; Sharma *et al.* 2000); in this study, however, the highest number of shoots formed on media containing 90 mM sorbitol. Shoot growth of pear and apricot, two other woody Rosaceae species, accelerated when sorbitol was used as a carbon source in proliferation media (Marino *et al.* 1993; Kadota and Niimi 2004). Sorbitol is the main product of photosynthesis and is a transported form of carbohydrate (Bieleski 1982). There are specific enzymes for sorbitol oxidation in apricot microshoots that are responsible for improving shoot production and development when sorbitol is added to a proliferation medium compared with sucrose (Marino *et al*. 1993). We presume that the same reason would explain our results with MM.106 apple rootstock. As 90 mM sorbitol induced the highest shoot number and since shoot length increased compared with all other treatments, we conclude that 90 mM sorbitol was the most effective carbon source for shoot proliferation of MM.106 apple rootstock.

The present study also shows that sorbitol was almost equivalently effective as a carbon source for culture of MM.106 apple rootstock compared with sucrose. Despite the widespread and successful use of sucrose in plant tissue culture, other sugars have also been reported as being suitable carbon sources for *in vitro* culture of many plants (Alkhateeb 2008). However, sucrose may cause hypoxia and ethanol accumulation in cells due to its quick metabolization (Scott *et al*. 1995; Ramarosandratana *et al.* 2001).

Visual observation of apple tissues under higher concentrations showed red and white pigmentation (abnormal growth), compact and solid growth with no sign of shoot formation which could be attributed to osmotic stress due to high sugar concentration. Similar symptoms are quite evi-

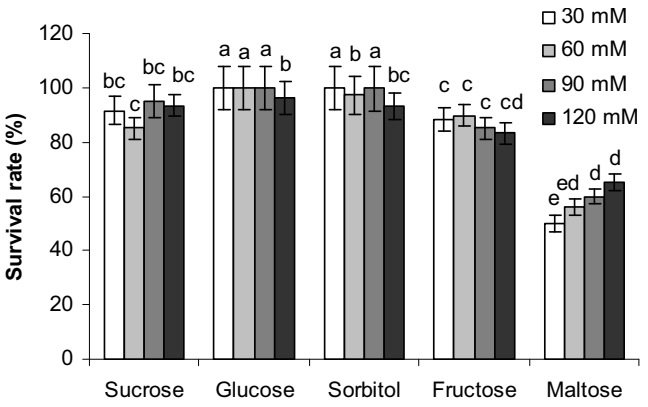

Fig. 3 Influence of carbon sources and their concentrations on survival rate of apple rootstock MM.106. Values with the same superscript letters are not significantly different at $P < 0.05$.

dent in other studies related to carbon sources and concentrations (Hildebrandt and Riker 1949; Millan *et al.* 1992; Romano *et al.* 1995; Cunha and Fernandez- Ferreira 1999; Teixeira da Silva 2004).

Shoot survival in media containing all carbohydrates was over 85%, except for maltose (all concentrations) and 120 mM fructose (**Fig. 3**). These results are in accordance with Kadota and Niimi's (2004) report. They found that shoot survival with all carbohydrates was over 88%; explants treated with sorbitol and glucose tended to survive well followed by explants treated with sucrose and fructose while explants treated with maltose did not survive.

In parallel studies to this study, we also found that carbon source affected the level of hyperhydricity and rooting in AA.106 rootstock (Bahmani *et al*. 2009). Sorbitol at 90 or 120 nM reduced hyperhydricity while 90 mM sucrose improved all rooting components.

In transgenic experiments Zhu *et al*. (2001) obtained transformed apple regenerants only in the presence of sorbitol, while the use of sucrose failed to give any transformants. The results partly can be explained by the fact that, in the Rosaceae, sorbitol and sucrose are the main photosynthetic products and more than 60% of carbon export occurs from source leaves as sorbitol (Loescher and Everard 1996; Noiraud *et al*. 2001).

Media supplemented with no sugar did not produce any new shoots. The importance of sugar for shoot formation was clearly shown in the present study which was indicated by a lack of vegetative growth in the absence of a carbon source. It seems that under these circumstances the quantity of stored carbohydrates within the buds is not enough to support the vegetative growth of tissues. Overall, organ initiation is associated with the utilization of accumulated starch and free sugars of the medium (Thompson and Thorpe 1987). To the best of our knowledge, it seems that *in vitro* shoot formation of MM.106 apple rootstock is a high energy-requiring process and sugars may serve as a source of energy for this process.

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