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COMPARISON OF SUCROSE AND SORBITOL AS MAIN CARBON ENERGY SOURCES IN MICROPROPAGATION OF PEACH ROOTSTOCK GF-677

TOUQEER AHMAD, NADEEM AKHTAR ABBASI, ISHFAQ AHMAD HAFIZ AND ANSAR ALI

Department of Horticulture,
University of Arid Agriculture, Rawalpindi, Pakistan

Abstract

The influence of two carbon sources, sorbitol and sucrose on *In vitro* shoot proliferation and rooting of peach rootstock GF 677 was compared at 15, 30, 45 and 60 g l^{-1} concentrations. The highest number of shoots per proliferated explant, usable shoots and shoots having maximum fresh weight were obtained on sorbitol at 30 g l^{-1}. GF 677 does not seem to be an efficient utilizer of sucrose for *In vitro* shoot proliferation. Similarly best root development in terms of rooting percentage, number of roots per rooted explant and roots > 1.5 cm in length was also found on sorbitol at 30 g l^{-1}. Sorbitol is therefore better carbon source than sucrose for the development of *In vitro* shoot proliferation and rooting of peach rootstock GF 677.

Introduction

Peach rootstock GF 677, an interspecific hybrid (Peach x Almond) is propagated asexually as clone. It is specially used on alkaline soils because of its resistance to lime induced iron chlorosis (El Gharbi & Jraidi, 1994). It is highly vigorous and performs well on soils with low fertility status (Carrera *et al*., 1998) and also considered suitable for dry areas. However, it is difficult to be multiplied on mass scale through cuttings because of very low rooting ability (Ammer, 1999). A micropropagation system has been successfully developed for peach rootstock GF-677 (Ahmad *et al*., 2003). The growth and multiplication of shoots *In vitro* are affected by many factors (Haque *et al*., 2003), one of which was the concentration and type of exogenous carbon sources added to medium to serve as energy and also to maintain the osmotic potential (De Neto & Otoni, 2003). In cultured plant tissues, the normal function of chloroplast as a source of energy is reduced and a continuous supply of carbohydrates from the medium is therefore necessary. In addition, growth and root initiation are high energy requiring processes that can only occur at the expense of available metabolic substrates, which are mainly carbohydrates (Thorpe, 1982; De Klerk & Calamar, 2002). The establishment of effective shoot proliferation and root development *in vitro* is essential for subsequent success during acclimatization to autotrophic conditions (Premkumar *et al*., 2003).

In general, sucrose is the carbohydrate of choice as carbon source for *In vitro* plant culture, probably because it is the most common carbohydrate in the phloem sap of many plants (Murashige & Skoog, 1962; Thorpe, 1982; Lemos & Baker, 1998; Fuentes *et al*., 2000). However, invertases that are released by the explant into the medium, split sucrose into glucose and fructose (George, 1993; De Klerk & Calamar, 2002). Thus explants are usually exposed to a mixture of sucrose, glucose and fructose. Sorbitol has also been reported as the major photoassimilate in the woody species of *Rosaceae* e.g., *Prunus*, *Pyrus* and *Malus*, where it is also translocated via phloem (Moing *et al*., 1992; Stoop & Pharr, 1993; Sinclair & Byrne, 2003). Therefore, the aim of this study was to compare sorbitol and sucrose on the *in vitro* shoot proliferation and rooting of the economically important peach rootstock GF-677.

*Corresponding author's email: nadeemabbasi65@yahoo.com

Material and Methods

Shoot proliferation of peach rootstock GF 677 was maintained as described previously (Ahmad *et al*., 2003). For better carbon source investigation, shoot tips (2.0 cm long) of stock cultures were transferred to basal culture medium consisted of MS macro and micro elements, MS vitamins, 1 mg l^{-1} BAP and 6.5 g l^{-1} agar with different concentrations of sorbitol and sucrose @ 15, 30, 45 and 60 g l^{-1} Murashige & Skoog (1962). Shoot proliferation in terms of number of shoots per proliferated explant, number of shoots >2.0 cm in length and fresh weight of shoots was recorded after 4 weeks.

Shoot cuttings (>1.5 cm in length) from stock cultures were implanted on MS medium (MS macro and micro elements, MS vitamins, 1.5 mg l^{-1} IBA and 6.5 g l^{-1} agar) with different concentrations of sorbitol and sucrose (15, 30, 45 and 60 g l^{-1}). Root development response was evaluated after 30 days in terms of rooting percentage, number of roots per rooted explant and roots > 1.5 cm in length. In all investigations reported herein, pH of all media was adjusted at 5.7 before autoclaving at 121 ^{o}C for 20 minutes. All cultures were incubated under a 16 h photoperiod illuminated by a cool-white fluorescent light (2500 lx) at 25 ± 1^{o}C.

All experiments were conducted with four replicates and four shoots per treatment in a 125 ml culture jar containing 40 ml of medium and results were evaluated using the method of analysis of variance (ANOVA) and data are presented by ± S.E.

Results

The type and concentration of carbon sources in the culture medium showed the significant effects on the number of shoots per proliferated explant, shoots > 2.0 cm in length and fresh weight of the shoots. Sorbitol was found to be more effective carbon source as compared to sucrose. The highest number of shoots per proliferated explant (10), shoots > 2.0 cm in length (6.0) and fresh weight of shoots (425 mg) were obtained when sorbitol was used at 30 g l^{-1} (Fig. 1). In contrast, cultures grown on sucrose medium showed very poor response as the maximum number of shoots per proliferated explant, shoots > 2.0 cm in length and fresh weight of the shoots were found to be 4.3, 2.4 and 298 mg respectively at the same concentration (Fig. 1). Increasing the concentration of both carbon sources from 30 g l^{-1} to the level of 60 g l^{-1} resulted in inconsistent rather definite inhibitory effect in the number of shoots per proliferated explant, shoots > 2.0 cm in length and fresh weight of the shoots. Similar poor responses were also observed when concentration of sorbitol and sucrose was decreased in the medium from 30 g l^{-1} to 15 g l^{-1} (Fig. 1).

Rooting percentage varied significantly with the type and concentration of two carbon sources (Fig. 2). Maximum rooting percentage (85%) was observed in the medium containing sorbitol at 30 g l^{-1} while the rooting percentage found on the sucrose medium at the same concentration was only 50%. Similarly sorbitol at 30 g l^{-1} resulted in highest number of roots per rooted explant (5.1) and roots >1.5 cm in length (4.0) (Fig. 3) while sucrose resulted in maximum 2.0 number of roots per rooted explant and only 1.0 root >1.5 cm in length (Fig. 4). Moreover, a significant reduction in all the three parameters was noted when explants were exposed to sorbitol and sucrose concentration other than the optimal one.

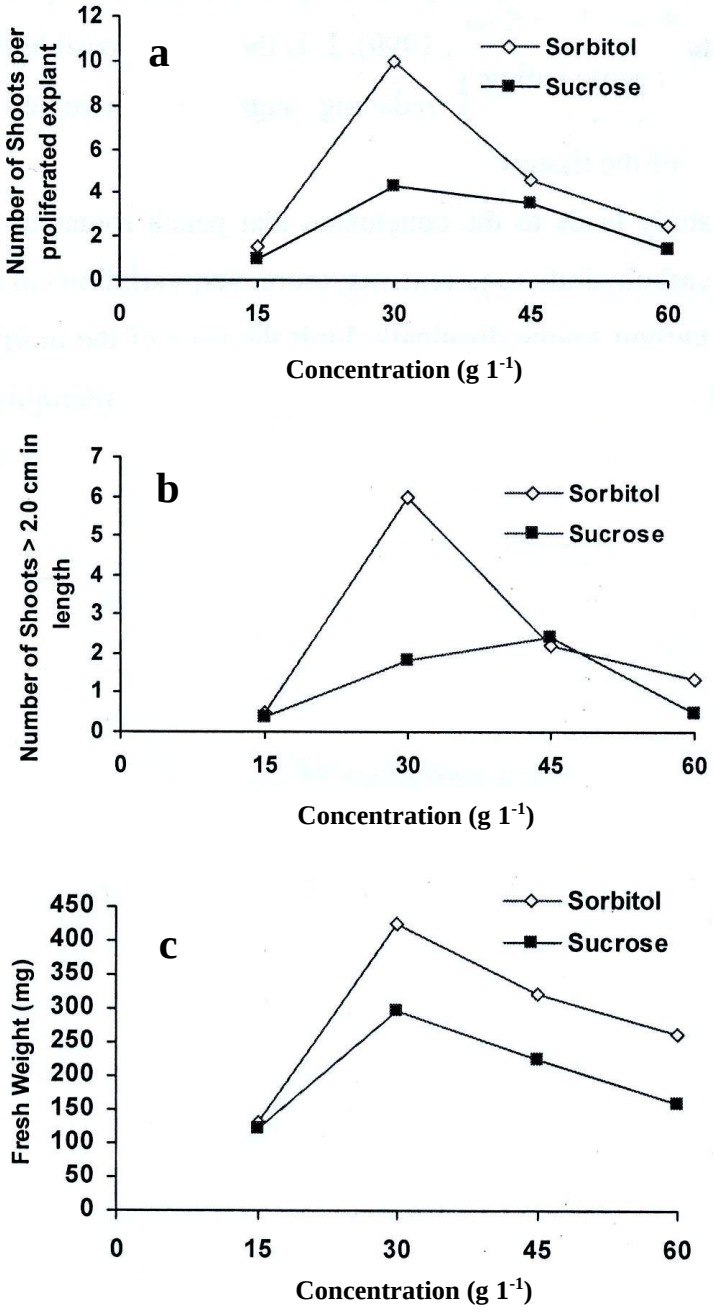

Fig. 1. Mean effect of different concentrations of sorbitol and sucrose on number of shoots per proliferated explant (a), number of shoots >2.0 cm in length (b) and fresh weight of shoots (c) of peach rootstock GF 677.

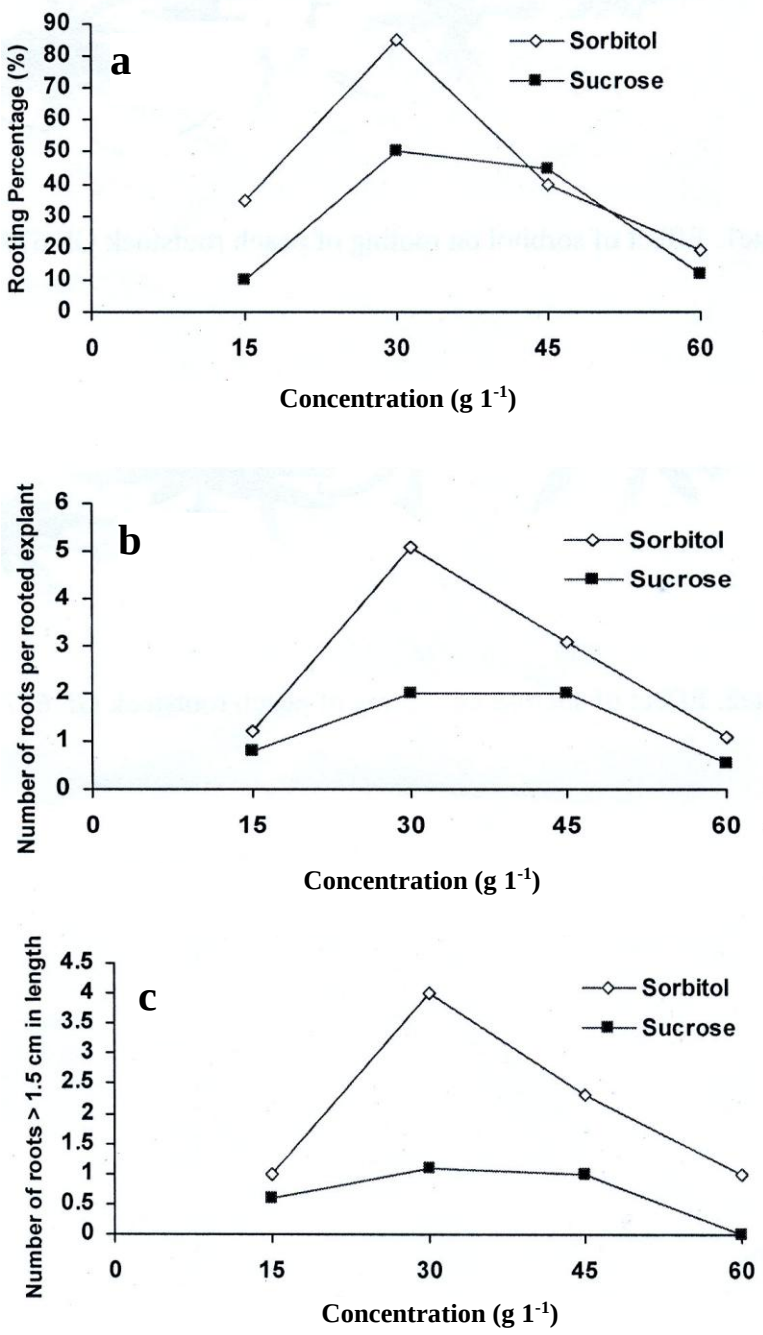

Fig. 2. Mean effect of different concentrations of sorbitol and sucrose on rooting percentage (a), number of roots per rooted explant (b) and number of roots >1.5 cm in length (c).

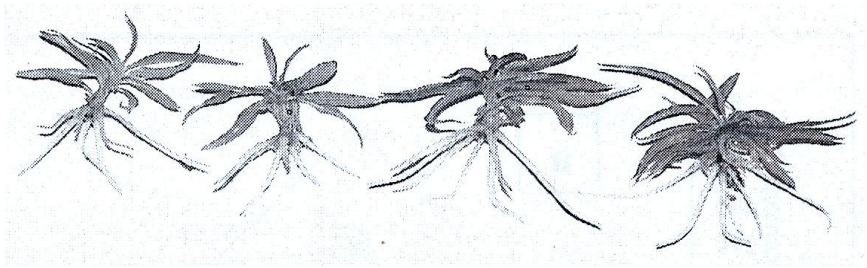

Fig. 3. Effect of sorbitol on rooting of peach rootstock GF 677 at 30 g l^{-1}.

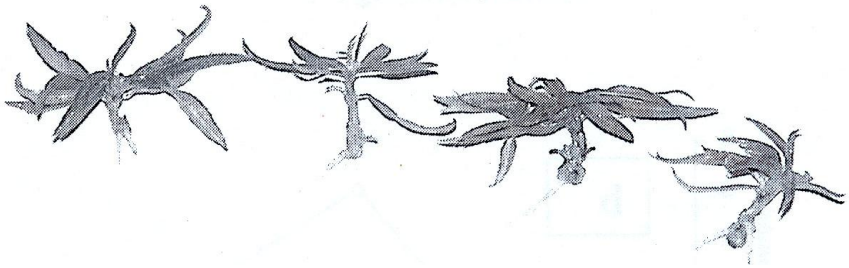

Fig. 4. Effect of sucrose on rooting of peach rootstock GF 677 at 30 g l^{-1}.

Discussion

The internal carbohydrate pool is suggested to have an important role in morphogenesis of several woody species (Kromer & Gamian, 2000; Li & Leung, 2000) and this can be influenced by the exogenous supply of carbon sources (De Neto & Otoni, 2003). Therefore, an attempt was made to find the comparative influence of sorbitol and sucrose on the shoot proliferation and rooting of peach rootstock GF 677. It is evident from the results that peach rootstock GF 677 cultures have quite selective requirements for both the type and concentration of carbohydrates and sorbitol has been found as better carbon source for inducing shoot proliferation as compared to sucrose. In other instances sorbitol has played an effective role in *In vitro* cultures of *Malus* species and other plants of *Rosaceae* family due to being major translocate and metabolite of photosynthesis, respiratory substrate and storage compound *In vivo* in these plants (Pua & Chong, 1985; Moing *et al*., 1992; Kadota *et al*., 2001). More success in shoot proliferation on medium containing sorbitol may be associated with the better utilization of sorbitol by peach rootstock with the sufficient availability of enzymes that help in the hydrolysis of sorbitol. The enzymatic conversion of sorbitol into fructose and glucose can make sorbitol readily available for the tissues and thus used as carbon structure for new growth (Lemos & Baker, 1998). These enzymes are sorbitol dehydrogenase (Negum & Loescher, 1979; Yamaki, 1981) and sorbitol oxidase (Yamaki, 1982). Stoop & Pharr (1993) have confirmed the activity of these enzymes in sinks of sorbitol-translocating plants. More fresh weight of shoots on sorbitol might be due to the fact that high levels of reducing sugars available in the culture medium may speed up cell division thus leading to an increase in the volume and weight of tissues cultured as suggested by other researchers

(Gurel & Gulsen, 1998). Poor response of sucrose in shoot proliferation of peach rootstock might be due to the slow break up of sucrose into glucose and fructose. The availability of invertase enzyme required for the efficient conversion of sucrose into glucose and fructose is less in sorbitol translocating plants (Pua & Chong, 1984). The detrimental effect of concentration higher or lower than the optimal one of both the carbon sources might be due to the accumulation of phenolic compounds in the medium at supra-optimal concentration (Hilae & Te-Chato, 2005) and unavailability of sufficient energy to carry out the metabolic processes at low concentration (De Klerk & Calamar, 2002). Moreover, sugars are perceived by cells as chemical signals, with very high concentrations *In vitro* acting as stressing agents (Steinitz, 1999; Da Silva, 2004).

The results on rooting provide the evidence that the proliferated shoots of peach rootstock GF 677 were able to utilize the sorbitol in a better way as compared to sucrose for root formation. This might be due to the increase in the reducing carbohydrate (fructose and glucose) content in the basal portion of proliferated shoots with sorbitol as compare to sucrose. In *Pinus bansiana*, the accumulation of reducing carbohydrates in the basal region of stem cuttings is related to callusing and rooting (Kumar *et al*., 1999). Furthermore, in rooting of apple, osmotic adjustment regulated by reducing carbohydrates in tissue also influences the initiation of root primordia (Pua & Chong, 1984). These sugars are known to react non-enzymatically with nuclear proteins and cause modifications in proteins (Kumar *et al*., 1999). It is therefore, possible that in the present study these beneficial properties of reducing sugars are involved in shifting the morphogenic pathway of the tissues.

The present study leads to the conclusion that peach rootstock GF 677 cultures have quite selective carbohydrate requirements; moreover, variations in the concentration of the most suitable carbon source drastically limit the pace of the morphogenic process. These results could contribute to the improvement in the micropropagation of this economically important rootstock on commercial scale and warrant its application for other related species.

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