

EFFECT OF DIFFERENT CARBON SOURCES ON *IN VITRO* SHOOT PROLIFERATION AND ROOTING OF PEACH ROOTSTOCK GF 677

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Abstract

The effect of different carbon sources on *In vitro* shoot proliferation and rooting of peach rootstock GF 677 was investigated at National Agricultural Research Centre, Islamabad. Over all the results showed that sucrose + glucose (15 gm/l each) was most appropriate carbon source combination in relation to the growth and shoot proliferation of peach rootstock GF 677 while in case of rooting, glucose 20gm/l was more suitable as compared to sucrose 20gm/l.

Introduction

Peach is an important fresh fruit of Pakistan grown on 4,400 ha with a annual production of 43,000 tons. Economic life of peach orchards in Pakistan is 5 to 8 years. The most important factor responsible for short life and yield decline have been identified as the use of an inappropriate rootstock, which produce phytotoxins in the soil and are affected by lime induced iron chlorosis (Samra, 1996). Peach rootstocks have been reported to influence both the performance and the production life of the scion cultivars. Therefore, rootstock choice is an important factor to be considered when establishing a successful orchard (Doud, 1980, Young & Houser, 1980).

Different rootstocks used for peaches in the world are GF 677, GF 557, Nemaguard, St. Julien, GF 655.2, Brompton, GF 43, Damas, GF 1869, GF 305 and Montclar. Each has particular advantages and /or disadvantages for the regions where they are used (Layne, 1987). Under Pakistani soil conditions GF 677 is a potential rootstock as reported by Samra (1996). GF 677 is an interspecific hybrid (Peach x Almond). It is clonally propagated and is specially used on alkaline soils being resistant to lime induced iron chlorosis (El Gharbi & Jraidi, 1994); a major problem in soils here. GF667 is also useful in replant situations being resistant to phytotoxins (Minguzzi, 1989).

GF 677 is highly vigorous, do not produce suckers, resistant to peach rust, crown gall and root knot (Layne, 1987). However, it is difficult to multiply on mass scale through cutting because of very low rooting percentage (Ammer, 1999). Through micropropagation it can be multiplied at a much faster rate as compared with conventional propagation (Kyriakidou & Pontikis, 1983, Ahmad *et al.*, 2003).

Generally, saccharides are known to serve as carbon and energy sources, osmotic agents, stress protectants and signal molecules in plants (Lipavska & Konradova, 2004). Ramage & Williams (2002) have specified that, species that are difficult to propagate, demonstrate that variation in hormone ratios cannot be the sole mechanism controlling *In vitro* developmental processes. Carbohydrate source in media play very basic role in peach shoot proliferation and affects on shoot growth and survival (Priyakumari *et al.*, 2002). Sucrose (2-5 %) is the most popular carbohydrate used with tissue culture (Bridgen, 1994). The cultures in standard media with sucrose (Ahmad *et al.*, 2003) have a very short culture

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life making the working subculture period labor intensive. In the same laboratory data on effect of different carbon sources on sugarcane cultures gave variable culture longevity (Anon., 2004). With literature supporting the same for peach, this study was undertaken to extend subculturing period and to develop well proliferating roots.

The basic purpose of this study was to determine the affect of different carbon sources on *In vitro* proliferation, with particular reference to culture longevity and improved rooting of peach rootstock GF 677.

Materials and Methods

The experiment was conducted at the *In vitro* Preservation Laboratory of Plant Genetic Resources Program (PGRP), National Agricultural Research Center (NARC), Islamabad. The data was taken 35 days after subculturing (DAS) for shoot proliferation and 51 DAS for rooting. Shoot tips (explants) were collected from actively growing plant in the field of Horticultural Research Institute (HRI) at NARC. Culture establishment was carried out following the procedure of Ahmad *et al.*, (2003); using MS (Murashige & Skoog, 1962) media containing 30 gm/l sucrose. Cultures were incubated for 4 weeks at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ under 16 h light (2000 lux).

For shoot proliferation the basic MS media was supplemented with 0.6 mg/l BAP (benzyl amino purine) and used as followed by Ahmad *et al.*, (2003); which was modified for carbon sources. The different forms/levels of carbon sources are given in Table 1. Established cultures were subjected to sub culturing in same media. For rooting, proliferated uniform shoots were transferred to media containing the two carbon sources, either sucrose or glucose at the level of 10, 20 and 30 gm/l.

Data was taken on the following parameters; fresh shoot weight (g), number of shoots, shoot length (cm), number of roots and root length (cm). The experiment was completely randomized design (CRD) consisting of five replications per treatment and 5 explants per replication for shoot proliferation and three replications per treatment and 5 shoots per replication in case of rooting. The data thus obtained were statistically analyzed using Mstate C software-program to detect if significant differences exist for various parameters. LSD was also determined.

Results and Discussion

Peach rootstock GF677 cultures grown on media with different carbon sources and its subsequent rooting is presented in Figs. 1-3. The data on shoot fresh weight are presented in Fig. 1 (LSD 0.16). The analysis of data revealed significant differences for shoot fresh weight among different type and levels of carbon sources at 0.05 level of probability except in suc15+glu15 with sucrose 40 gm/l. The highest mean fresh shoot weight was observed in glucose 40gm/l (0.354gms). Though the lowest mean value of fresh shoot weight was observed in suc15+glu15 and sucrose 40 gm/l (0.101gms); the former (suc15+glu15) had more tender shoots with high proliferation capacity. Although Singha *et al.*, (1987) have reported that sucrose in autoclaved media is partially hydrolyzed to glucose and fructose, such effect was not manifested by partially hydrolyzed sucrose here. The longevity of cultures in glucose 40 gm/l was appreciably extended with 100% cultures survival. However, there was a problem of withering in next sub culturing on the same media. Whereas, sucrose 15 +glucose 15 was found as a suitable carbon source for shoot proliferation because there was no withering problem in plants. The findings from our study are in agreement with those of Priyakumari *et al.*, (2002).

Table 1. MS Shoot proliferation media supplemented with different carbon sources.

Treatments	Carbon sources	Treatments	Carbon sources
T1 (Control)	Sucrose- 30 gm/l	T4	Sucrose- 40 gm/l
T2	Glucose-30 gm/l	T5	Glucose- 40 gm/l
T3	Suc+Glu- 15+15 gm/l	T6	Suc+Glu- 20+20 gm/l

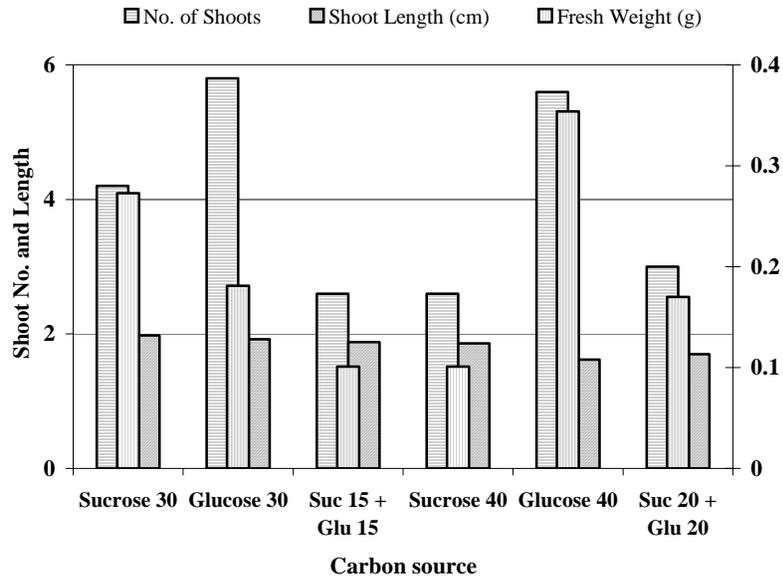


Fig. 1. Shoot growth (number of shoots, shoot length in cm and culture shoot fresh weight) of peach rootstock GF 677 cultures as affected by carbon source and concentration.

Statistical analysis showed significant differences in number of shoot developed in peach cultures, LSD 2.5 (Fig. 1). The treatment, glucose 30gm/l showed highest number of shoots (5.8), whereas lowest value (2.6) was in combination of sucrose and glucose each 15 gm/l and in sucrose 40gm/l. Glucose 30gm/l as source of carbohydrate in GF 677 clearly showed high proliferation rate, but there was chlorosis problem in plants survived at 35 DAS. Muleo *et al.*, (1995) have suggested that apical necrosis might be interfering with apical dominance and development of axillary shoots. However, in our experiment these shoots were also short lived. Therefore, regular sub-culturing after 10-20 days was necessary as in case of control i.e., 30 g/l sucrose. Further, there was also problem of withering in next sub-culturing on the same media. On other hand, sucrose15 + glucose 15 was suitable carbon source for shoot proliferations because cultures remained healthy in this media and there was no withering noticed in next sub culturing on the same media. The shoot length in case of control (Glucose 30gm/l) was significantly highest (1.98 cm) than that recorded in glucose 40gm/l (1.62 cm) and combination of sucrose and glucose each 20gm/l (1.7 cm). Remaining treatment remained at par with control (Fig. 1), LSD 0.27. However, the longevity of cultures were least in control where only 20% culture survived at 35 DAS, therefore shoot length would not be a true criteria for peach culture multiplication. These observations are fully supported by Franc (1998).

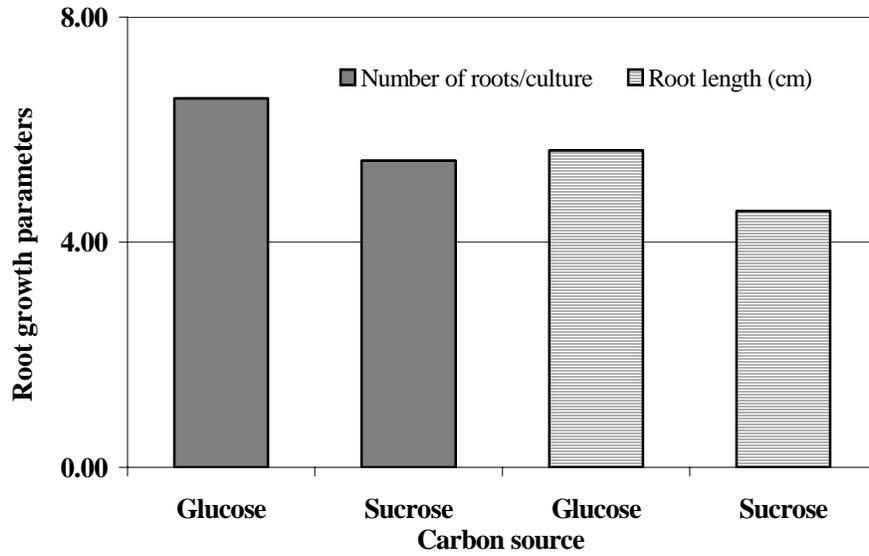


Fig. 2. Root growth (number of roots and root length in cm) of peach rootstock GF 677 cultures as affected by carbon source.



Fig. 3. Root growth in peach rootstock GF 677 cultures as affected by carbon source (left culture supplemented with glucose and cultures in jar at right supplemented with sucrose each 20 g/l). Well-developed roots as well as the luxurious growth of leafy shoots is visible in background from culture vessel base for rooting in cultures supplemented with glucose as compared with sucrose.

In addition to other characteristics rooting of proliferated shoot has great impact on tissue culturing of rootstock GF677. Among the various treatments prolific rooting was observed in media supplemented with either 20 g/l glucose or 20 g/l sucrose. The higher (30 g/l) and lower (10 g/l) concentrations of either glucose or sucrose did not promote proper rooting. The two treatments sucrose 20gm/l and glucose 20gm/l showed differences for rooting by the subcultured shoots (Figs. 2, 3). The highest number of roots (6.55) and root length (5.66 cm) were observed in glucose 20 gm/l at 51 DAS. Number of roots, root length and days required for root initiation (15-20) were also appreciable in case of sucrose 20 gm/l but there was a problem of withering in these cultures. Hence, glucose 20 gm/l was better carbon source for rooting of peach GF677. Similar results have also been reported by Priyakumari *et al.*, (2002). The behavior of shoot proliferation may differ to root proliferation and same sugar type may not prove productive for both. Lipavska & Konradova (2004) state that the recommended concentration vary in different cultures, ranging from 1-6%; however, they are also of the view that in many cases a detailed search for optimum sugar concentration has not been performed.

This study points to the fact that selection of carbon source does play an important role in regulation of growth. The concentrations as well as combinations are also important considerations. It is also realized that greater values of shoot length and biomass in micro-propagation may not be the true indicator. The culture's state of vigor, performance over time and survival also need due consideration.

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