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Original Research Article

Effect of Carbohydrates on *in vitro* Shoot Growth of Various *Prunus* Species

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Abstract - Carbohydrate sources are one of important factors associated with macro- and micro nutrients and phytohormones *in vitro* culture medium for shoot growth. The optimal carbohydrates for eight species of the genus *Prunus* which are economically important fruit crop was evaluated at the initiation and elongation stages. All carbohydrate seemed utilized for the bud break and leaf growth at the early stage of culture. However, shoot elongation and fresh weight of species tested were superior in the medium containing 90 mM of fructose or glucose rather than sucrose. There was no difference between glucose and fructose. Adventitious shoots from the axillary buds were induced in most species but no significant differences were observed except for two species (*P. salicina* 'Shiro' and *P. tomentosa*). These result demonstrated that glucose and fructose were suitable carbohydrate sources for diverse *Prunus* species than sucrose, although the response to the carbohydrates in the medium were slightly different in the species.

Key words - Carbohydrates, *Prunus* species, Shoot elongation

Introduction

Prunus is a genus of more than 200 species of trees and shrubs. Some species such as peaches, plums, cherries, apricot and almond have been cultivated for centuries as fruit crops and ornamental plants in many countries including the United States. Germplasm of these crops have been exchanged among countries to improve production. *Prunus* species are propagated by vegetative cuttings that may be infected with obligate parasite pathogens and are therefore regulated as a 'prohibited genus'. Imported *Prunus* germplasm must enter the U.S. through the USDA quarantine program to prevent the introduction of pathogens, especially exotic ones (Waterworth, 1993). Infected germplasm was often discarded in the past, potentially resulting in the loss of unique or difficult-to-obtain species/varieties. More stringent quarantine regulations by many countries along with an increased awareness of the value of germplasm have made germplasm acquisition and exchange more difficult and expensive. Therefore, efforts to eliminate pathogens from infected accessions are becoming an important part of germplasm quarantine and exchange programs.

In vitro tissue culture with or without therapeutic (thermo or chemo) treatments is an efficient method to obtain pathogen-free plants. Several protocols have been reported to eliminate viruses infecting *Prunus* species by tissue culture, but the protocols were tested only for some pathogens in limited species and cultivars (Navarro *et al*., 1982; Stein *et al*., 1991; Howell *et al*., 2001; Rizqi *et al*., 2001). Application of *in vitro* pathogen elimination technique depends on establishing plant materials in tissue culture. General environmental conditions such as light intensity, photoperiods and temperatures for *in vitro* culture are often acceptable to abroad range of species; however, different species and cultivars may respond quite differently to a culture medium. This creates challenges for quarantine programs that deal with many different species and cultivars of *Prunus*. Therefore, development of a universal medium that supports the *in vitro* growth of diverse *Prunus* germplasm accessions is a worthwhile objective.

Carbohydrates are very important energy sources for explants that are not autotrophic *in vitro* (Kozai, 1991), especially at initiation stage (Cheong, 2000). They affect development

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and proliferation of leaves and shoots or differentiation of leaf, shoot and root tissues. While sucrose is commonly used for *in vitro* cultivation of many crops, monosaccharide hexoses (glucose or fructose) have resulted in better growth in some species (Harada and Murai, 1996; Kalimina and Brown, 2007). In this study, we investigated the effect of three carbohydrate sources on growth of eight different *Prunus* species as part of the culture medium optimization.

Materials and Methods

Plant materials

Stems from actively growing plants can be used for *in vitro* culture establishment. Mother plants were grown in 12" pot under insect-proof screenhouse which is covered by screen to prevent insect but maintain natural temperature with irrigation system for 4-5 years. In this study, stem cuttings from eight different *Prunus* species (*P. avium*, *P. cerasifera*, *P. dulcis* 'Peerless', *P. mahaleb*, *P. mandshurica*, *P. persica* 'GF305', *P. salicina* 'Shiro' and *P. tomentosa*) were collected from plants in the screenhouse during August and September.

In vitro culture Establishment

Stems were harvested from plants in the screenhouse and brought to laboratory for *in vitro* culture establishment. Spray 70% ethanol and wipe with paper towel then wash thoroughly with tap water containing a few drops of tween 20 (tween 20 water) and dry with paper towel. Stems were cut into single-node samples approximately 2.5-3.0 ㎝ in length, with more stem remaining below the bud than above. Samples were surface sterilized with water containing tween 20 then shake on the orbital shaker at 160 rpm for 20 min with 1% NaOCl (active ingredient). Samples washed with distilled sterile water and were shaken for 30-40 min at 130 rpm and rinsed with the sterile water for at least two times. Stem nodes were dried on the sterilized paper towel on the petri-dish in the laminar-hood, were trimmed both ends of the explant and placed onto initiation medium in a G7 Magenta box.

Culture media and Environmental conditions

Culture media were based on the McCown Woody Plant Medium (Lloydand McCown, 1981) with increased nitrogen (400 ㎎/L NH_4NO_3) and potassium (990 ㎎/LKNO_3). For leaf and shoot regeneration, 1 μM 6-benzylaminopurine (BA) and 1 μM gibberellic acid (GA_3) were supplemented in the medium before autoclave at 121℃ for 15 min. All media was adjusted to pH 5.8 and solidified with 7.5 g/L of agar (Phytotech A111). To investigate the effect of carbohydrate sources on *in vitro* growth of the explants, three different carbohydrate sources-fructose (16 g/L = 90 mM), D-glucose (16 g/L = 90 mM) or sucrose (30 g/L = 90 mM) were added into the medium. Fifty milliliters of the medium were dispensed into a G7 Magenta box (Sigma-Aldrich, V8505) and autoclaved for 15 min. Eight to ten explants were cultivated in each culture vessel and replicated 5 (45-50 explants for total) of each carbohydrate. Cultures were kept at 20℃ (8 hrs) and 22℃ (16 hrs) with a 16/8hr photoperiod (16 hr light and 8 hrs dark) under cool white fluorescent lights (40-50 μmol m^{-2} s^{-1}).

Growth measurement

Observation and measurement were conducted during four weeks of culture. Fresh weight (FW) of induced shoot, number of explants with new shoots and leaves from the axillary bud and adventitious shoots were measured.

Data analysis

Explants contaminated by fungi or bacteria were discarded and not included in the data collection. Ten surviving explants per replicate, three replicates per experiment were measured. All data were analyzed by two way ANOVA test at the level of p = 0.05 using SPSS 15.0 (SPSS Inc.).

Results

Bud opening (Culture initiation)

Explant buds started to expand, and scales opened within 2 weeks of culture before new leaves emerged. The eight species had different responses to the carbohydrate sources. *P. cerasifera*, *P. salicina* 'Shiro' and *P. tomentosa* responded well on all three carbohydrate sources, resulting in high bud opening rates (Table 1). The bud opening rates were reduced slightly for *P. avium* and *P. mahaleb* on the medium with fructose and *P. persica* 'GF305' on the medium with sucrose. However, there were no significant differences among the

Table 1. Bud opening rates of eight Prunus species on media with different carbohydrates

Species	Fructose	Glucose	Sucrose
P. avium	55.0	88.8	72.5
P. cerasifera	97.5	98.7	95.0
P. dulcis	73.3	82.5	64.2
P. mahaleb	56.3	81.3	68.8
P. mandshurica	86.3	76.3	63.8
P. persica 'GF305'	66.3	75.0	52.5
P. salicina 'Shiro'	87.5	87.5	85.0
P. tomentosa	85.0	86.3	91.3

Not significant among carbohydrates at level of p = 0.05.

Table 2. Adventitious shoot induction rate (%) of eight *Prunus* species on media with different carbohydrates

Species	Fructose	Glucose	Sucrose
P. avium	16.3	27.5	27.5
P. cerasifera	47.5	53.8	66.3
P. dulcis	0.0	8.3	8.3
P. mahaleb	42.5	57.5	71.3
P. mandshurica	5.0	12.5	25.0
P. persica 'GF305'	10.0	17.5	30.0
P. salicina 'Shiro'	41.3 ab[z]	57.5 a	13.8 b
P. tomentosa	50.0 ab	71.3 a	15.0 b

[z]Same letter means no significance within a species by carbohydrates at level of p = 0.05. Not significant among carbohydrate sources at level of p = 0.05 for six species.

species, and most opened buds produced leaves. Fifty percent of explants of 'GF 305'on the medium with sucrose opened but did not initiate leaf and shoot growth and eventually died.

Shoot elongation and adventitious shoot formation

Two weeks after culture initiation, growth patterns of the explants started to show differences (Fig. 1). Leaves continued to grow for all eight species, but shoot elongation varied among the species. By four weeks, *P. cerasifera* and *P. mandshurica* elongated well on the media with fructose and glucose, and *P. tomentosa* elongated at a slightly slower rate. Leaves of *P. avium*, *P. mahaleb*, *P. persica* 'GF305' and *P. salicina* 'Shiro' expanded well but shoot elongation was delayed. *P. dulcis* had the slowest growth rate. The results showed that carbohydrate sources affected the shoot growth.

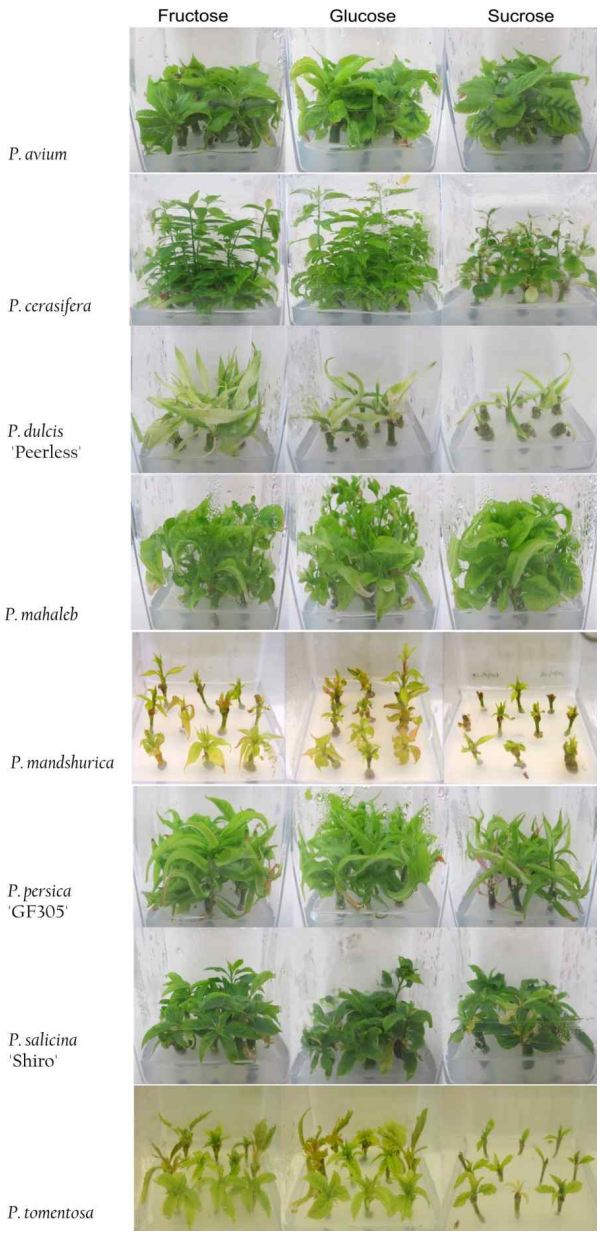

Fig. 1. Growth of explants of eight *Prunus* spp. on media with three different carbohydrates at 4 weeks (2 weeks for *P. mandishurica*) after culture initiation.

Most species did not grow well in the medium containing sucrose, but there was no difference between those on the media with fructose or glucose.

Rates of adventitious shoot formation were also different among the species (Table 2). With the exception of *P. dulcis*, all other species adjusted well to *in vitro* conditions as shoots elongated and adventitious shoots formed. *P. cerasifera* and *P. mahaleb* formed more adventitious shoots regardless of the

carbohydrate sources, and *P. salicina* had more adventitious shoots in the medium with glucose or fructose than with sucrose. *P. dulcis*, *P. mandshurica* and *P. persica* 'GF305' only formed a few adventitious shoots, especially on the medium with fructose. Although *P. dulcis* had a few leaves on the fructose and glucose media, shoots did not develop from most explants. There were also significant differences in growth traits such as leaf size and shoot thickness among the species.

Fresh weight of elongated shoot

The carbohydrates affected the fresh weight of the explants (Table 3). The fresh weights of *P. mahaleb* ranged from 187.8-283.9 g, the highest among all the species, followed by *P. cerasifera* and *P. avium*.*P. dulcis* produced the least fresh weight. Most species produced higher fresh weights on the medium with glucose or fructose. There was strong correlation between the fresh weights and shoot elongation/adventitious shoot formation. Overall growth of eight species on the media with different carbohydrates is summarized in Table 3.

When the shoots derived from bud were excised and transferred to the medium which is supplemented with 1μM of BA and GA_3, they continued to elongate and developed more leaves. However, the shoots induced on sucrose containing medium did not grow well after transfers to the same medium and became yellowed and wilted. Most shoots of *P. dulcis* developed leaf chlorosis, excessive callus formation on the petiole, apical bud and basal end son the initial medium and became wilted. This was especially evident on the medium containing sucrose.

Discussion

The effect of carbohydrates in culture medium was evaluated for *in vitro* culture of various *Prunus* species, especially for shoot growth at the initiation stage. A bud on the node is a general material *for in vitro* culture establishment. The explants are not fully autotrophic and need energy sources such as carbohydrates (Yaseen *et al*., 2013). Thus carbohydrates are important sources in culture medium because it plays important role in morphogenesis of several woody species controlling the expression of many plant genes (Koch, 1996; Kromer and Gamian, 2000; Li and Leung, 2000; De Neto and Otoni, 2003). Although sucrose has been used for energy source in many plant tissue cultures, a number of carbon sources besides sucrose also plays positive role for the *in vitro* shoot and root development in *Prunus* species and other species too (Ahmad *et al*., 2007; Yaseen *et al*., 2009a; 2009b; Bahmani *et al*., 2009). Rates of leaf and shoot development of all tested species were similar on all media. However, growth of leaves and shoots after the bud opening was significantly different among *Prunus* species on different media.Overall, almost species had elongated shoots with different size of leave and length and resulted significant difference of fresh weight. Shoots on the medium containing sucrose could not elongated well enough like others, glucose and fructose with few exceptions, *P. avium* and *P. mahaleb*. The differences were obvious for *P. cerasifera* even though it grows better in sucrose containing medium than other species. The result was similar to the previous work by Kalinina and Brown (2007) that peach rootstock GF305 responded better to fructose than

Table 3. Fresh weight (㎎) of eight *Prunus* species on media with different carbohydrates

Species	Fructose	Glucose	Sucrose	Summary
P. avium	157.6 a[z]	119.2 a	42.8 b	G = F > S
P. cerasifera	93.4 a	100.6 a	71.4 b	G = F > S
P. dulcis	30.6 a	29.8 a	30.0 a	G = F = S
P. mahaleb	228.5 ab	283.9 a	187.8 b	G ≥ F ≥ S
P. mandshurica	38.6 a	35.9 a	22.7 b	G = F > S
P. persica 'GF305'	54.6 a	62.3 a	25.5 b	G = F > S
P. salicina 'Shiro'	42.3 b	62.7 a	20.3 c	G > F > S
P. tomentosa	62.7 a	69.9 a	27.9 b	G = F > S

[z]Same letter means no significance within a species by carbohydrates at level of p = 0.05.

sucrose. Sorbitol is one of promising carbohydrate source *in vitro* culture for Rosaceae family (Ahamad *et al*., 2007; Bahmani *et al*., 2009; Yaseen *et al*., 2009a, b). Ahamad *et al*. (2007) had best result on almond shoot proliferation and fresh weight on 3% of sorbitol and better root formation on sorbitol than sucrose. There was no significant difference among the carbohydrate sources in *P. dulcis* which was worst growth among the species in this study. Compared to other species, shoot and leaf growth of *P. dulcis* was very slow on the media with different carbohydrates. It is considered that other factors associated with the type of carbohydrates such as low concentrations of phytohormones influenced the growth of almond or depends on cultivar because some cultivar of almond could grow well in the medium of sucrose (Channuntapipat *et al*., 2003). The concentration of carbohydrates might not be adequate for this species as seen in the case of grape culture (Lee *et al*., 2013). More experiments needed to identify a better media formulation for *P. dulcis.*

The results show that seven of the eight species tested grow better in medium supplemented with glucose or fructose than with sucrose. Glucose or fructose did not have adverse effects on growth of the eight *Prunus* species and could be used as carbohydrate sources instead of sucrose.

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