How Carbon Source and Seedcoat Influence the In Vitro Culture of Peach (*Prunus persica* L. Batsch) Immature Seeds

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Abstract. The effects of carbon source and concentration and of seedcoat were tested on the in vitro germination of peach seeds derived from crosses performed in the field. Seeds were extracted from the fruit and cultured in Woody Plant Medium (WPM) supplemented with sucrose, glucose, or sorbitol at concentrations of 15, 30, and 45 g·L⁻¹. The percentage of germination as well as the root and hypocotyl lengths were measured after the stratification process and before acclimatization. Seedcoat did not have any influence on seed germination in any tested media and genotype. Glucose at a concentration of 15 g·L⁻¹ and sucrose at 15, 30, and 45 g·L⁻¹ resulted in greater stem seedling growth. The root developed the most when seeds were cultured in media with 15 or 30 g·L⁻¹ of sucrose.

Peach breeding programs seek to obtain cultivars with high organoleptic quality while solving a variety of problems related to disease, production, or postharvest storage. As a general rule, the earlier a peach is produced, the greater its market value. However, in early-ripening peach genotypes, fruit maturation precedes embryo maturation (Emershad and Ramming, 1994), which produces small immature seeds and nonviable embryo due to inherent nutritional deficiency (Sharma et al., 1996).

Thus, the use of tissue culture techniques arose in plant breeding in an attempt to rescue certain genotypes that, in natural conditions, would not germinate. In the case of stone fruit, the seedcoat is traditionally removed because the seed isolation from the seedcoat reduces the potential for infection (Dulić et al., 2016). Nonetheless, some authors argue that the presence of the seedcoat favors germination because many enzymes that metabolize carbohydrates are present in the seedcoat (Sinclair and Byrne, 2003). The chilling treatment (stratification) is necessary to meet the cold requirements for seed

to germinate, and other factors, such as plant growth regulators (PGR), culture media, and carbon source, are relevant in seed germination by tissue culture. Although a wide range of culture media and PGR have been used, the culture media composition has little influence on germination (Mansvelt et al., 2015); therefore, other parameters as the carbon source or the culture methodology are given a central role. Sucrose, fructose, glucose, and sorbitol are the most commonly used sugars used as carbon source and osmotic stabilizer (Scozzoli and Pasini, 1991), although their concentration is more important for the osmolarity of the medium than for providing nutrition (Raghavan and Torrey, 1964).

The objective of this study was to determine the role played by the seedcoat and the most suitable carbon source and concentration for peach seeds derived from crosses to germinate and grow.

Materials and Methods

Plant material. A total of 1560 seeds (5–7.5 mm) from well-developed fruits of early-ripening peach trees were used as females (12 crosses). Seeds were removed from the endocarp and surface sterilized in a solution of 2% (v/v) sodium hypochlorite and 0.1% (v/v) Tween 20 for 1.5 h in a laminar flow hood.

Carbon source treatments. After disinfection, seeds coats were removed in half of the seeds and were then distributed randomly in nine media (86 or 87 seeds each) composed of WPM supplemented with 15, 30, or 45 g·L⁻¹ of sucrose, sorbitol, or glucose and 0.6% (w/v) of Plant Propagation Agar (Pronadisa, Madrid, Spain) in sterilized tubes (10 mL/tube). The pH was adjusted to 5.7 with KOH (0.1 N) before

autoclaving for 16 min at 1.1 kg·cm⁻² (122 °C). The tubes were incubated in a climatic chamber at 4 ± 1 °C in constant darkness for 90 d. When stratification was complete, root and stem lengths were measured. The plantlets were moved to a climatic chamber at 25 ± 1 °C with a 16-h light (45 μmol·m⁻²·s⁻¹; GRO-LUX, Sylvania, Surrey, UK) photoperiod, and after 10 d, data for the roots and stems were recorded before acclimatization. The number of germinated seeds was also recorded.

Statistical analysis. Data were first tested for homogeneity of variance and normality of distribution. Significance was determined with an analysis of variance, and Duncan's means separation test at *P* ≤ 0.05 was performed using Statgraphics software.

Results

No differences in germination or growth were found between seeds cultured with or without seedcoat. Thus, data were statistically analyzed together. As a result, high percentages of germination (>80%) were recorded for most of the treatments (Fig. 1). The best results were obtained with sucrose and sorbitol at all concentrations and for glucose at the concentration of 15 g·L⁻¹. Only the media with 30 and 45 g·L⁻¹ of glucose obtained a medium-low percentage of germination (65.08% and 58.10%, respectively).

The length of stems and roots after stratification was higher in the seeds grown in the media with 15 or 30 g·L⁻¹ of glucose, all tested concentrations of sucrose and 15 g·L⁻¹ of sorbitol (Fig. 2). Seeds cultured at 45 g·L⁻¹ of glucose had the shortest roots after stratification. In contrast, after 10 d in the climate chamber, results changed for some treatments (Fig. 2). Plantlets grown in sucrose or at 15 g·L⁻¹ of glucose were the most elongated. Plantlets grown at 15 and 30 g·L⁻¹ sorbitol showed medium elongation of their stems; the culture of plantlets at the concentration of 30 g·L⁻¹ of glucose and 45 g·L⁻¹ of glucose and sorbitol obtained the lowest stem growth. On the other hand, roots grew more in media containing 15 or 30 g·L⁻¹ of sucrose. In the remaining media, roots elongated less.

Discussion

Seed culture media used in peach breeding programs must produce acceptable survival on a wide range of early-ripening accessions to be of practical use because it is not feasible to develop a different medium for each accession derived from every hybridization (Sinclair and Byrne, 2003). Therefore, the importance of finding a general protocol, for both germination and initial plant elongation, is key for an early-ripening peach breeding programs.

The role played by the seedcoat has been controversial. On one hand, the seedcoat contains bioactive compounds, enzymes, and antioxidants (Dong et al., 2010) that promote seed survival and germination under favorable conditions. On the other hand, seedcoats are related to seed dormancy; therefore, seedcoat removal

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Fig. 1. Peach seed germination (%) with different sugar (Glu = glucose; Suc = sucrose; Sorb = sorbitol) concentrations (15, 30, and 45 g·L⁻¹). Lowercase letters indicate differences ($P < 0.05$) between treatments, bars represents $\pm$ SE.

Fig. 2. Plant development after peach seed stratification and after a period in the climatic chamber with the different sugar (Glu = glucose; Suc = sucrose; Sorb = sorbitol) concentrations (15, 30, and 45 g·L⁻¹). Lowercase letters indicate differences ($P < 0.05$) between treatments after stratification; uppercase letters indicate differences between treatments after climate chamber period. Bars represents $\pm$ SE.

has been advised after chilling treatments in *Prunus* (Pawasut et al., 2010). In seeds derived from hybridization in this study, the germination was unaffected by the presence of the seedcoat. In fact, the unique advantage presented by seedcoat removal has been the prevention from contamination (data not shown), as indicated by Dulić et al. (2016).

The differences between germination percentages and growth are thus related only to carbon source and concentration. At equal concentrations, sucrose produces a lower osmotic potential (ψ_s) than glucose and, in turn, a lower ψ_s than sorbitol (Cárdenas and Villegas, 2002); thus, sucrose would add a beneficial ψ_s to the media for peach seeds. However, cells recognize sugars as chemical signals, and a high concentration may act as a stressing agent in vitro (Da Silva, 2004). During elongation, plants grown in vitro require an external source of carbohydrate, a factor that may compensate for the lower availability of light for the plant, restricting the photosynthesis process. During sterilization of media in the autoclave, sucrose is hydrolyzed into fructose and glucose (Yoshida et al., 1973), and the combination of these two monosaccharides may result in better development of in vitro plants, inducing better elongation of shoot and root. Thus, this study has confirmed that only the addition of glucose to culture media decreases germination and induces a delay in stem and root growth.

Although sorbitol produced a high percentage of germination, its effect on the development of roots and stems was low. According to previous reports (Scozzoli and Pasini, 1991; Sinclair and Byrne, 2003), sorbitol is not suitable for inducing embryo enlargement or obtaining a high percentage of growing seedlings. This fact has been associated with poor utilization of sorbitol by peach seeds and seedlings due to the content of enzymes involved in hydrolysis of sorbitol into fructose and glucose, which, as has been reported, are present in the seedcoat. However, in the current study, no differences were found between seeds grown with or without seedcoat. Also, sorbitol performed well in seed germination, compared with glucose, at medium and high concentrations. Thus, the enzymes responsible for sorbitol degradation are probably not located in the seedcoat. Other authors have reported that sorbitol may act mainly as an osmotic regulator in tissue culture and not as a carbon source because it neither supports in vitro shoot growth nor is metabolized in most higher plants (Yaseen et al., 2013).

Literature Cited

Cárdenas, M.A. and A. Villegas. 2002. Osmotic potential of culture medium with different

compounds for the in vitro propagation. *Rev. Fitotec. Mex.* 25(2):213–217.

Da Silva, J.A.T. 2004. The effect of carbon source on *in vitro* organogenesis of *Chrysanthemum* thin cell layers. *Bragantia* 63:165–177, doi: 10.1590/S0006-87052004000200002.

Dong, Q., M.S. Banaich, and P.J. O'Brien. 2010. Cytoprotection by almond skin extracts or catechins of hepatocyte cytotoxicity induced by hydrogen peroxide (oxidative stress model) versus glyoxal or methylglyoxal (carbonylation model). *Chem. Biol. Interact.* 185(2):101–109, doi: 10.1016/j.cbi.2010.03.003.

Dulić, J., V. Ognjanov, S. Ercisli, M. Miodragović, G. Barać, M. Ljubojević, and D. Dorić. 2016. *In vitro* germination of early ripening sweet cherry varieties (*Prunus avium* L.) at different fruit ripening stages. *Erwerbs-Obstbau* 58(2):113–118, doi: 10.1007/s10341-016-0265-y.

Emershad, R.L. and D.V. Rammings. 1994. Effect of media on embryo enlargement, germination and plant development in early-ripening genotypes of *Prunus* growth *in vitro*. *Plant Cell Tissue Organ Cult.* 37:55–59, doi: 10.1007/BF00048117.

Mansvelt, E.L., W.M. Pieterse, S.B.D. Shange, T.C. Mabiya, C. Cronjé, I. Balla, H. Ham, and M.J. Rubio-Cabatas. 2015. Embryo rescue of *Prunus persica*: Medium composition has little influence on germination. In *VIII International Peach Symposium 1084* (pp. 207–210). <http://dx.doi.org/10.17660/ActaHortic.2015.1084.28>.

Pawasut, A., K. Yamane, N. Fujishige, K. Yoneyama, and Y.T. Tamaki. 2010. Influence of seed coat removal and chilling on abscisic acid content and germination in ornamental peach (*Prunus persica* Batsch) seeds. *J. Hort. Sci. Biotechnol.* 85(3):248–258.

Raghavan, V. and J.G. Torrey. 1964. Effects of certain growth substances on the growth and morphogenesis of immature embryos of *Cap-sella* in culture. *Plant Physiol.* 39:691–699, doi: 10.1104/pp.39.4.691.

Scozzoli, A. and D. Pasini. 1991. Effects of different media constituents on the development of peach embryos cultured *in vitro*. *Acta Hort.* 300:265–268.

Sharma, D.R., R. Kaur, and K. Kumar. 1996. Embryo rescue in plants—a review. *Euphytica* 89:325–337.

Sinclair, J.W. and D.H. Byrne. 2003. Improvement of peach embryo culture through manipulation of carbohydrate source and pH. *HortScience* 38:582–585.

Yaseen, M., T. Ahmad, G. Sablok, A. Standardi, and I.A. Hafiz. 2013. Review: Role of carbon sources for *in vitro* plant growth and development. *Mol. Biol. Rep.* 40:2837–2849, doi: 10.1007/s11033-012-2299-z.

Yoshida, F., T. Kobayashi, and T. Yoshida. 1973. The mineral nutrition of cultured chlorophyllous cells of tobacco I. Effects of π_{salts} , π_{sucrose} , Ca, Cl and B in the medium on the yield, friability, chlorophyll contents and mineral absorption of cells. *Plant Cell Physiol.* 14:329–339.