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Benzyladenine and Shortened Light/ Dark Cycles Improve in Vitro Shoot Proliferation of Peach

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Growth regulators and light can have significant effects on growth and proliferation of shoots in vitro. Benzyladenine (BA) has been generally used for in vitro shoot proliferation of peach *[Prunus persica* (L.) Batsch.] (Almehdi and Parfitt, 1986; Hammerschlag et al., 1987), but BA and thidiazuron (TDZ) have not, to our knowledge, been compared. Cycles of 4 h light/2 h dark have been shown to increase in vitro growth and proliferation of *P. ceracifera* and a *P. persica* × *P. dulcis* hybrid when compared with a 16-h light/8-h dark cycle (Morini et al., 1990). The objective of our study was to determine the influences of light/dark cycles and TDZ vs. BA on peach shoot proliferation.

Actively growing shoot tips, 10 mm long, from greenhouse-grown 'Suncrest', 'Belle of Georgia', and 'Evergreen' peaches were surface-disinfested and placed in 20 × 100-mm petri plates on Almehdi and Parfitt (1986) medium. After 2 months, with monthly transfers to fresh medium, shoots were pruned to three 10-mm-long terminal leaves with stems cut to 6- to 8-mm lengths. These shoot tips were individually placed in 95 × 25-mm vials on gelled (Phytagel at 1 and USB agar at 5 g·liter^{-1}) Murashige and Skoog (1962) medium with 0.02 μM l *H* -indole-3-butync acid; in combination with 1.0 or 10 μM BA; or 0.1, 1.0, or 10 μM TDZ. Cultures were grown on lighted shelves under 55 μmol·m^{-2}·s^{-1} provided by an equal mix, in terms of wattage, of warm-white and Vita-lite (Duro Test, Bergen, N. J.) fluorescent lights in a continuous 16-h light/8-h dark (16/8) or 4-h light/2-h dark (4/2) cycle. After 4 weeks, the basal callus was removed from the shoots and its dry weight recorded. Shoots were then transferred to fresh media. At 8 weeks, shoots (25 mm) were counted, and again basal callus dry weights were recorded. The experiment was designed as a fixed-model 3 **x** 5 **x** 2 factorial with nine replications per treatment.

The 4/2 cycle in combination with 10 μM BA produced the most shoots for all three peach genotypes (Fig. 1). Growth, as indicated by the number of leaves per shoot, was generally greater with the 4/2 than with the 16/8 cycle (data not presented). Fujiwara et al. (1987) found that CO_2 concentrations in culture vessels increase during the dark period, but fall below atmospheric concentration in light, even in vessels designed for gas exchange. The more frequently alternating light/dark conditions of the 4/2 cycle may provide a more uniform and sustained CO_2 level. Morini et al. (1990) suggested that the 4/2 cycle may also affect phytochrome activity, protracting the Pfr form and/or increasing the quantity of pigment. The greatest effect of the 4/2 cycle occurs at the optimum BA level (10 μM) (Fig. 1), suggesting that the benefits of this treatment are related to the shoot growth potential.

The shoot proliferation response to BA and to light/dark cycles observed in this study confirm results obtained with other *Prunus* species (Borkowska and Litwinczuk, 1993; Morini et al., 1990). The combination of the 4/2 cycle and 10 μM BA caused greater shoot multiplication than previously reported for 'Suncrest' (Almehdi and Parfitt, 1986; Hammerschlag et al., 1987). While 'Suncrest' and 'Belle of Georgia' produced few shoots on media containing TDZ, 'Evergreen' responded positively to increased TDZ concentrations, but the effect of TDZ even in 'Evergreen' was not as great as that of 10 μM BA.

Basal callus dry weight increased with concentration. of growth regulators. TDZ caused more callus growth than BA (Fig. 1). This callus may have had a negative effect on shoot proliferation (Preece et al., 1991).

At the concentrations tested, TDZ was less effective than BA for peach shoot proliferation. A 4-h light/2-h dark cycle produced more shoots and greater shoot growth than a 16/8 cycle, particularly with 10 μM BA.

Literature Cited

Almehdi, A.A. andD.E. Parfitt. 1986. In vitro propagation of peach: II. A mediumfor in vitro multiplication of 56 peach cultivars. Fruit Var. J. 40:46-49.

Borkowska, B. and W. Litwinczuk. 1993. Activity of thidiazuron in in vitro shoot cultures of *Prunus* sp. and *Morus alba.* Biologia Plant. 35:63-67.

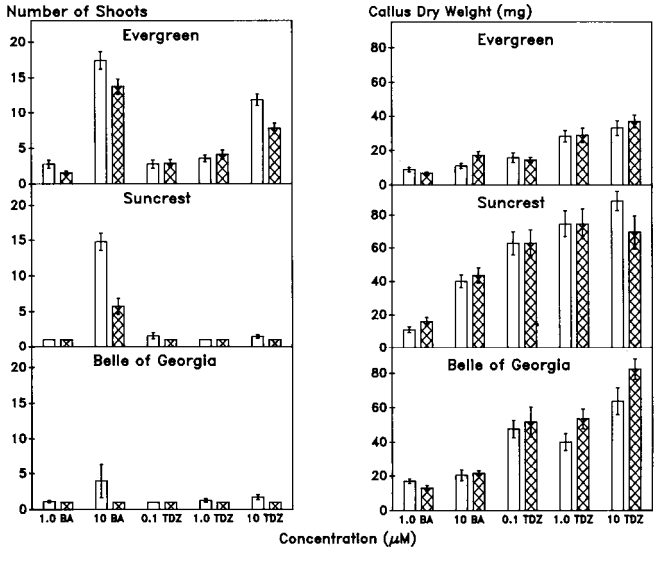
Fujiwara, K.,-T, Kozai, and I. Watanabe. 1987. Measurements of carbon dioxide gas concentration in enclosed vessels containing tissue cultured plantlets and estimates of net photosynthetic rates of the plantlets. J. Agr. Meteorol. 43:21-30.

Hammerschlag,F. A., G.R. Bauchan, and R. Scorza. 1987. Factors influencing in vitro multiplication and rooting of peach cultivars. Plant Cell Tissue Organ Cult. 8:235-242.

Morini, S., P. Fortuna, R. Sciutti, and R. Muleo. 1990. Effect of different light-dark cycles on growth of fruit tree shoots cultured in vitro. Adv. Hort. Sci. 4:163-166,

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Preece, J.E., C.A. Huetteman, W.C. Ashley, and P.L. Roth, 1991. Micro-and cutting propagation of silver maple. I. Results with adult and juvenilepropagules.J.Amer.Soc.Hort.Sci. 116: 142–



. 1. Average number of shoots produced (±standard error) after 8 weeks and total dry weight of basal callus harvested at the end of two 4-week intervals, in response to BA or TDZ concentrations and continuous cycles of 4 h light/2 h darkness (❑) and 16 h light/8 h darkness (⊠).

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