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Cooler Temperature During Germination Improves the Survival of Embryo Cultured Peach Seed

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Abstract. **Embryo culture techniques are employed to germinate seed of early ripening peach and nectarine [*Prunus persica* (L.) Batsch] cultivars. Generally, the embryos in these genotypes do not mature by the time the fruit matures, thus rendering normal stratification procedures ineffective. In 1998 and 1999, immature embryos from multiple peach genotypes were cultured in an embryo rescue medium (Woody Plant Medium, 3% sucrose, 0.065% agar) at 5 °C for 45 days in the dark. Embryos were then placed under lights at either a cool-temperature (18 °C in 1999 and 20 °C in 1998) or a warm-temperature (30 °C in 1999 and 28 °C in 1998) treatment with a photoperiod of 12 hours for germination and initial growth. After 2–4 weeks, embryos were rated for germination, root number, and top growth. The embryos incubated at the cool-temperature regime not only had better germination, but also had a higher rate of greenhouse survival.**

For many years, embryo culture has been used to rescue embryos that would not survive under normal germination conditions. Many advances have been made in the techniques and the media type used for embryo culture (Bridgen, 1994; Emershad and Ramming, 1994; Ramming 1990). Temperature has been reported to affect embryo enlargement of in ovule cultured peach [*Prunus persica* (L.) Batsch] embryos (Ramming, 1985), the frequency of rosetting of young peach seedlings (Byrne et al., 2000), and the growth of in vitro cultured peach rootstocks (Loreti et al., 1988). No reports were found on the response of embryo cultured peaches to temperature during germination after cold stratification. In fact, many articles do not indicate the temperature used during germination. In recent works, the temperatures indicated were 25 °C (Rizzo et al., 1998) and 15.5 °C (10 d)/25 °C (subsequent growth) (Emershad and Ramming, 1994).

Materials and Methods

In 1998 and 1999, immature embryos from multiple peach genotypes (Tables 1 and 2) were cultured in an embryo rescue medium {Woody Plant Medium (WPM), 3% sucrose, 0.065% agar} at 5 °C for 45 d in the dark (Rizzo et al., 1998). The cultured embryos (13 mm average length) were then placed under florescent lights at either a cool-temperature (20 °C in 1998 or 18 °C in 1999) or a warm-temperature (28 °C in 1998 or 30 °C in 1999) treatment with a photoperiod of 12 h for germination and initial growth. After 2–4 weeks, embryos were evaluated for germination, root number, and in 1999 top growth. Root number was classified as: 1 = less than 5 roots, 2 = 5–10 roots, 3 = 11–20 roots, and 4 = 21 or more roots. Top growth was rated as follows: 1 = dead embryo; 2 = white/green embryo with no shoot formation; 3 = weak growth with shoot dieback; 4 = weak, unhealthy growth; and 5 = healthy shoot formation. If embryos had a root number rating of 2–4 and a top rating from 3–5, they were removed from culture 2–4 weeks after placement under florescent lights, transplanted into 2.5" × 1.5" × 2.5"-pots with Sunshine Mix #4, and acclimated to greenhouse conditions. In 1999, survivability (percentage of live plants) was determined after 6–8 weeks of growth in the greenhouse.

Results and Discussion

In both years, germination and root growth were better or no different for individual genotypes and better on the average at the lower temperature as compared to the higher temperature (Tables 1 and 2). In 1999, this increased germination and root growth is reflected in the greater top growth and survival of embryos germinated at the lower temperature regime. Furthermore, observations showed that higher temperatures caused substantial shoot dieback to occur (Fig. 1).

Although there is a lack of information specifically published on stone fruits, evidence exists that cooler temperatures during germination are beneficial for woody ornamental species (Meyer, 1988). This suggests that the better germination may be correlated to the temperature experienced by the species during seed germination under natural conditions.

This simple procedure increased peach

Table 1. Effects of temperature during germination on the germination and root number of embryo cultured peach seed in 1998.[z]

Cultivar	Total embryos tested	Germination (%)		Root no. rating[y]	
		20 °C	28 °C	20 °C	28 °C
Spring Brite	518	92 a	69 b	3.2 a	2.4 b
Richlady	932	92 a	72 b	3.0 a	2.0 b
Diamond Ray	155	97 a	70 b	3.5 a	2.7 b
Overall	1605	93 a	71 b	3.2 a	2.2 b

[z]Mean separation in rows by Duncan's multiple range test. Means within a row followed by the same letter are not significantly different at the 5% level.
[y]Root number ratings: 1 = less than 5 roots, 2 = 5–10 roots, 3 = 11–20 roots, and 4 = 21 or more roots.

Table 2. Effects of temperature during germination on the germination, root number and top growth of embryo cultured peach seed in 1999.[z]

Cultivar	Total embryos tested	Germination (%)		Root no. rating[y]		Top growth rating[x]		Survival (%)	
		18 °C	30 °C	18 °C	30 °C	18 °C	30 °C	18 °C	30 °C
Sunmist	187	66 a	76 a	2.2 a	2.1 a	4.7 a	4.5 a	62 a	63 a
TX2293-1	138	77 a	51 b	2.3 a	1.9 a	4.5 a	3.7 b	75 a	46 b
TX2293-2	279	67 a	54 a	2.4 a	2.2 a	4.4 a	3.9 b	56 a	49 a
TX2293-3	169	94 a	91 a	2.7 a	2.5 a	4.9 a	4.8 a	81 a	94 a
TX3290-2	112	92 a	58 b	2.7 a	2.3 a	4.7 a	4.1 b	66 a	24 b
TX1B38N	148	68 a	17 b	2.5 a	1.0 b	4.6 a	3.2 b	13 a	6 a
TX2A36	198	81 a	60 a	2.9 a	2.0 b	4.7 a	4.0 b	56 a	39 a
TX2B1	251	79 a	74 b	3.4 a	2.9 b	4.8 a	4.3 b	65 a	33 b
TX2B3	93	56 a	54 a	2.1 a	1.8 a	4.3 a	4.1 a	37 a	48 a
TX2B6	199	89 a	76 a	3.0 a	2.9 a	4.6 a	4.1 a	70 a	20 b
TX2B7N	100	74 a	47 b	2.5 a	1.7 b	4.8 a	3.9 b	33 a	35 a
Overall	1874	75 a	62 b	2.6 a	2.2 b	4.6 a	4.1 b	58 a	42 b

[z]Mean separation in rows by Duncan's multiple range test. Means within a row followed by the same letter are not significantly different at the 5% level.
[y]Root number ratings: 1 = less than 5 roots, 2 = 5–10 roots, 3 = 11–20 roots, and 4 = 21 or more roots.
[x]Top growth rated as follows: 1= dead embryo, 2 = white/green embryo with no shoot formation, 3 = weak growth with top dieback, 4 = weak, unhealthy growth, 5 = healthy shoot formation.

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Fig. 1. 'Richlady' seedlings germinated at cool temperature (**a**) and warm temperature (**b**).

embryo germination an average of 22% in 1998 and 13% in 1999 and increased survival in the greenhouse 16% in 1999. This is a large effect, which emphasizes the importance of controlling temperature when growing out embryo cultured peach embryos and other similar in vitro propagules.

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