Chemical Changes in Developing Seeds of 'Independence' Nectarine and 'Fay Elberta' Peach

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Abstract. To learn why embryos of early ripening stone fruits abort or fail to germinate, the growth and nutrition of developing seeds of 'Independence' nectarine and 'Fay Elberta' peach (*Prunus persica*, Batsch.) were compared. Seeds were collected at weekly intervals, beginning 2 months after full bloom until the fruits were ripe. Fruit diameter, seed and embryo lengths, and fresh weights of nucellus and endosperm were recorded. Parts of the seeds were analyzed for soluble carbohydrates, fats, and total N. At the same phenological stages of fruit development, concentrations of these seed fractions were nearly equal for both cultivars. Percentage composition of all fractions varied with time, but increased on a per-seed basis. Sucrose was the major soluble carbohydrate in embryos of both cultivars. Nitrogen content of the embryos, on a percent dry matter basis, gradually decreased from the 12th week after full bloom to harvest.

Embryos of early ripening stone fruits either abort or fail to germinate because the crop is harvested while the embryos are still immature. Internal competition between the developing fruit and vegetative growth, as well as that within the fruit itself, i.e., among the growing embryo, dignifying endocarp, and enlarging mesocarp are contributing factors; but we found no data to support this postulation. The developing fruit is a strong sink and, consequently, redirects the movement of photosynthates (Hansen, 1970) and causes remobilization of food reserves (Ryugo and Davis, 1959).

Developing seeds promote fruit set (Baldini, 1986) and alter fruit size and shape (Molisch, 1921; Nakagawa et al., 1968; Tukey, 1933). They produce auxin- and gibberellin-like substances (Dennis and Nitsch, 1966; Powell and Pratt, 1966; Luckwill et al., 1969; Luckwill, 1970) that may influence flower bud formation. In this study, the nutritional status of developing seeds of an early ripening nectarine was compared to that of a midseason ripening peach to observe if concurrent internal competition for soluble carbohydrates, fats, and total N during fruit growth may contribute to poor embryo development.

Materials and Methods

Developing fruits were sampled at random from mature bearing trees of 'Independence' nectarine and 'Fay Elberta' peach on the campus of the Univ. of California, Davis. Samples were collected at weekly intervals from 8 weeks after full bloom (AFB) until the fruits were ripe. Initially, three 100-fruit samples were harvested; sample size was reduced to 30 as the fruits enlarged. After recording the suture and polar diameters of the fruits, ovules were removed and the seed and embryo lengths measured. Fresh weights of embryo, nucellus, and endosperm were then obtained.

Ethanol-soluble sugars and sugar alcohols. Five grams each of nucellus, endosperm, and embryo were homogenized with 80% ethanol. The homogenate was filtered and the filtrate brought to 100 ml. After adding 1 mg of trehalose as an internal standard

to a 1-ml aliquot of sample, the mixture was evaporated to dryness. Sugars in the residue were silylated (Sweeley et al., 1963) and brought to 500 μ l with dry pyridine. A 1- μ l aliquot, equivalent to 50 mg of fresh tissue, was injected into a gas chromatography (Varian, Model 1400) equipped with a 3.18 $\times$ 1500-mm stainless steel column packed with 3.8% SE-30 coated onto Chromosorb Q. The temperature was increased from 100 to 265°C at 6°C/min. Nitrogen (40 ml·min⁻¹, was the carrier gas. An area integrator (Shimadzu, Chromatopac, Model C-R3A) attached to the chromatography was used to determine the soluble carbohydrate concentrations. Standard solutions (1 mg·ml⁻¹) were prepared using trehalose as the internal standard.

Fat content. An aliquot of dried sample placed in a porous thimble was refluxed 6 hr with diethylether on a fat extractor (Labconco). The sample was transferred to a weighing bottle, the ether was evaporated, and the lipid residue weighed.

Total nitrogen. Kjeldahl N was determined on a 100-mg sample of dried material according to Carlson (1978), using the Westcan Model 360 ammonia analyzer. Means and SES were determined for sugar, sugar alcohols, and N levels.

Results

Fruit development. Although the times of ripening of 'Independence' and 'Fay Elberta' differed by 5 weeks, the double-sigmoid growth curves of their fruit and seed tissues were nearly congruent and similar to those previously published for peach (Blake, 1925; Powell and Pratt, 1966). Embryos of 'Fay Elberta' were longer than those of 'Independence' but the fresh weights were the same (Figs. 1 and 2). The nucellar tissues of both 'Independence' and 'Fay Elberta' attained maximum weight 9 weeks AFB; the endosperms of the respective cultivars reached maximum size 11 and 12 weeks AFB (Fig. 2). Embryos of the two cultivars became macroscopic and began to develop concurrently as the endocarps became lignified.

Ethanol-soluble carbohydrates, Kjeldahl N, and fat. In the nucellus in both cultivars, the concentration of total soluble sugar was nearly constant, ranging between 11 and 12 mg·g⁻¹ fresh weight (FW), whereas N content remained between 1.5 and 2.0 mg·g⁻¹ (Table 1). Glucose and fructose were the two principal identifiable sugars. An unknown compound consistently appeared after the inositol peak on the gas chromatographic tracings of 'Fay Elberta' extracts. Its level, as glucose equivalent, was higher than were levels of other soluble carbohydrates.

Soluble sugars in the 'Independence' endosperm increased

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Fig. 1. Fruit diameters and seed and embryo lengths (mm) of 'Independence' nectarine and 'Fay Elberta' peach.

Fig. 2. Growth curves of 'Independence' nectarine and 'Fay Elberta' peach seeds and their parts.

Table 1. Levels ($\text{mg}\cdot\text{g}^{-1}$ fresh weight) of individual and total soluble carbohydrates and total nitrogen after full bloom (AFB).

Weeks AFB	Sugars and sugar alcohols						Total sugar	Total nitrogen
	Fructose	Glucose	Sorbitol	Inositol	Sucrose	Unknown		
<i>'Independence' nucellus</i>								
8	4.1 ± 0.32	5.5 ± 0.67	1.7 ± 0.09	0.7 ± 0.01	0.6 ± 0.01	---	11.1 ± 1.64	1.7 ± 0.02
9	2.6 ± 0.39	5.5 ± 0.42	1.4 ± 0.46	0.9 ± 0.05	0.4 ± 0.03	---	10.7 ± 1.29	1.5 ± 0.07
10	2.5 ± 0.03	5.7 ± 0.42	1.5 ± 0.02	1.8 ± 0.19	0.5 ± 0.01	---	12.0 ± 0.61	1.7 ± 0.21
<i>'Fay Elberta' nucellus</i>								
8	1.9 ± 0.02	2.4 ± 0.08	0.6 ± 0.01	0.5 ± 0.06	0.2 ± 0.07	6.0 ± 0.79	11.6 ± 1.03	2.1 ± 0.07
9	1.2 ± 0.10	3.0 ± 0.14	0.7 ± 0.06	0.7 ± 0.02	0.2 ± 0.02	5.4 ± 0.46	11.3 ± 0.25	2.0 ± 0.03
10	1.6 ± 0.01	2.0 ± 0.19	0.7 ± 0.15	1.1 ± 0.04	0.3 ± 0.01	6.0 ± 0.39	11.6 ± 0.70	1.9 ± 0.03
11	1.3 ± 0.05	2.0 ± 0.16	0.7 ± 0.06	1.4 ± 0.09	0.4 ± 0.07	6.6 ± 0.22	12.3 ± 0.10	1.0 ± 0.08
<i>'Independence' endosperm</i>								
10	2.4 ± 0.12	2.0 ± 0.28	0.2 ± 0.03	0.4 ± 0.03	1.6 ± 0.29	---	6.7 ± 0.69	2.9 ± 0.03
11	3.0 ± 0.20	2.3 ± 0.56	0.3 ± 0.08	0.9 ± 0.02	2.3 ± 0.09	--	8.8 ± 0.85	3.4 ± 0.05
12	2.8 ± 0.50	2.6 ± 0.57	0.3 ± 0.10	1.4 ± 0.23	2.4 ± 0.43	---	9.5 ± 0.63	3.9 ± 0.24
<i>'Fay Elberta' endosperm</i>								
10	1.5 ± 0.14	2.9 ± 0.15	0.2 ± 0.03	0.3 ± 0.06	0.8 ± 0.30	1.3 ± 0.18	7.0 ± 0.79	2.7 ± 0.04
11	1.1 ± 0.09	2.3 ± 0.09	0.5 ± 0.09	0.5 ± 0.04	1.3 ± 0.07	1.5 ± 0.23	7.1 ± 0.12	3.4 ± 0.13
12	1.3 ± 0.08	3.8 ± 0.25	1.1 ± 0.08	0.8 ± 0.02	1.6 ± 0.18	2.0 ± 0.39	10.7 ± 0.07	4.0 ± 0.16

from 6.7 to 9.5 mg, whereas N content rose from 3 to 4 $\text{mg}\cdot\text{g}^{-1}$ FW during the same period (Table 1). In 'Fay Elberta', sugars increased from 7 to nearly 11 mg, whereas N increased from 3 to 4 $\text{mg}\cdot\text{g}^{-1}$ FW. Glucose, fructose, and sucrose were the principal sugars. The concentrations of the unknown substance, as glucose equivalent, were within the range of the other sugars (Table 1).

Fresh weights of embryos from both cultivars were 617 mg at harvest (Fig. 2), but the dry weights of 'Independence' and 'Fay Elberta' embryos were 148 and 389 mg, respectively. Total sugar content of 'Independence' embryos decreased steadily from the 10th to the 15th week AFB to 6.5% dry matter (Fig. 3) or 1.6% FW (Fig. 4). Total sugar of 'Fay Elberta' increased to 30% of dry matter 12 weeks AFB, but it subsequently decreased to 6% dry matter (Fig. 3) or 3.8% FW (Fig. 4), which is 2.4 times that of 'Independence' embryos.

Sucrose fluctuated considerably, but it was the predominant sugar in the embryos of both cultivars (Fig. 5). Sorbitol and

glucose levels in 'Fay Elberta' increased from the first to the final sample, with minor fluctuations. Total hexose level in 'Independence' was consistently low, ranging from 2.4 to 11.4 $\text{mg}\cdot\text{g}^{-1}$ FW.

Fat content of developing embryos of 'Independence' and 'Fay Elberta' increased steadily, attaining 41% and 56% fat of dry matter (Fig. 3) or 100 and 351 $\text{mg}\cdot\text{g}^{-1}$ FW at harvest, respectively (Fig. 4).

Nitrogen content of embryos from both cultivars increased from the initial to the last sample on a fresh-weight basis; that of 'Independence' reached nearly 1.2%, whereas that of 'Fay Elberta' attained 2.6% (Fig. 4).

Discussion

Sorbitol and sucrose are the two main translocated sugars in the Rosaceae. The deposition of starch in the integuments during the day indicates that these two carbohydrates are converted to

Fig. 3. Seasonal changes insoluble sugars, N, and fat expressed as percent dry weight of developing 'Independence' nectarine (upper) and 'Fay Elberta' peach (lower) embryos. The accumulation of dry matter by embryos is expressed as a percent of fresh weight.

Fig. 4. Seasonal changes in total soluble sugars, N, and fat contents in developing embryos of 'Independence' nectarine (upper) and 'Fay Elberta' peach (lower).

glucose in the bundle sheath surrounding the vascular tissue or the parenchyma cells of the seed coat. Glucose, other sugars, and organic compounds then diffuse into the nucellus because there are no vascular connections between the seed coat and the nucellus. Sorbitol is oxidized to glucose in the nucellus by the enzyme sorbitol oxidase (Yamaki and Ryugo, 1986).

The embryo sac is thought to derive carbohydrates, nitrogenous compounds, and mineral elements initially from the nucellus in which it is imbedded. The endosperm enlarges through free nuclear division and engulfs the proembryo and suspensor cells. The fate of the suspensor cells, synergids, and the antipodal cells in the *Prunus* spp. is yet unknown. In the *Capsella*, suspensor and basal cells may participate in absorbing and transporting nutrients from tissues adjacent to the embryo (Schulz and Jensen, 1969). In some species, the swollen basal cell of the suspensor is evident in nearly mature seeds (Esau, 1960). The diffusion of nutrients through the endosperm at this free nuclear stage must be symplastic.

Not only do the sizes of the nucellus and endosperm fluctuate, but the changes in sugar ratio found in this study between these tissues also suggest that they are both metabolically active and not merely serving as storage tissues or avenues of transport.

Other evidence of metabolic activity in the nucellus-endosperm tissues is the synthesis of gibberellin-like substances in ovules of the almond (Ryugo, 1976), a species closely related to peach.

The simultaneous reduction in total sugars and an increase in fat (Figs. 3 and 4) in the embryo are attributed to interconversion, albeit via different pathways, because they belong to a dissimilar family of compounds. Duffus and Duffus (1984) have outlined the possible pathways by which these occur.

Whereas carbon-labeled sorbitol injected into immature mesocarp tissue of 'French' prune was metabolized predominantly to sucrose and, to a lesser degree, to glucose and fructose, very little radioactive sucrose administered to the same tissue was converted to sorbitol (Hansen and Ryugo, 1979). Thus, whereas the mesocarp tissue of prune contains enzymes capable of converting sorbitol to other sugars, embryos of peach and nectarine apparently have only one. Of the four sorbitol-related enzymes, only sorbitol oxidase was detectable in the immature embryos of 'Fay Elberta' (Yamaki and Ryugo, 1986). The role of the unidentified substance that is readily silylated in extracts of 'Fay Elberta' is yet unknown.

The percentage N varies between 1.2% and 2.6% of the embryo fresh weight, but because N constitutes only a small fraction of the nitrogenous compounds, such as amino acids, proteins,

Fig. 5. Seasonal changes insoluble carbohydrates in developing embryos of 'Independence' nectarine (upper) and 'Fay Elberta' (lower); the latter contained a presumptive sugar (unknown).

protochlorophyll, and nucleotides present in the cotyledons, the total weights of those substances far exceed those of sugars.

Although the final embryo fresh weights of the two cultivars were the same, the rate of dry matter accumulation was slower in 'Independence' than in 'Fay Elberta'. Therefore, the dry weight and N content of 'Independence' embryos at harvest were 40% and 44%, respectively, of 'Fay Elberta'. The slow rate of importation of photosynthates and nitrogenous compounds by the

'Independence' embryos is attributed to the concurrent increase in sink strengths of the enlarging mesocarp and the dignifying endocarp. The relatively low levels of reserve food would account for the poor viability of embryos in early ripening stone fruit cultivars. These embryos can be rescued if they are dissected out early and grown on artificial medium.

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