Variability in Sorbitol : Sucrose Ratios in Mature Leaves of Different Peach Cultivars

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Abstract. The aim of this study was to investigate variability in the sorbitol : sucrose ratio (SSR) in source leaves of different peach [*Prunus persica* (L.) Batsch] cultivars. Four- and 5-year-old trees of 58 cultivars were examined. Mature leaves were sampled on three dates in middle to late summer and analyzed for neutral soluble sugars using high-performance liquid chromatography. Differences in SSRs were observed. In most cultivars, the sorbitol content was at least twice that of sucrose. The maximal range of SSR occurred on the third date and ranged from 1.5 to 4.3. There was a date $\times$ genotype interaction ($P < 0.01$). When cultivars were grouped by country of origin, the mean ratios of the Japanese group were lower than those of the Italian and American groups for all three sampling dates. The SSRs of nectarines were higher than those of peach and canning clingstone-type cultivars. In general, variations in SSR were due mostly to differences in sucrose content. The SSR was negatively correlated with flowering date. These results indicate variability in SSR in peach germplasm, variability that seems to be related to the geographical origin of the cultivars.

Sorbitol is a sugar alcohol found in higher plants (Lewis, 1984). It is a major final product of photosynthesis and, together with sucrose, represents the main form of C translocated in some species of the Rosaceae family (Bialeski, 1982). Within the Rosaceae, the presence of sorbitol has been used as a taxonomic criterion (Plouvier, 1963). Sorbitol is common in the subfamilies Spiraeoideae, Maloideae (formerly Pomoideae), and Prunoideae (Plouvier, 1963; Wallaart, 1980) and may be related to the basic chromosome number (Wallaart, 1980).

Sorbitol has been implicated in the response of plants to some environmental conditions. Temperature-regulated accumulation of sorbitol has been reported in apple trees (Williams and Raese, 1974). During cold periods, sorbitol accumulates to high concentrations in tissues and tracheal sap and may have a role in cold hardiness of the Rosaceae trees (Whetter and Taper, 1966). In addition, the amount and activity of sorbitol-6-phosphate dehydrogenase (alditol-6-P : NADP 1-oxidoreductase, EC. 1.1.1.200), a key enzyme in sorbitol synthesis, increase in loquat (*Eriobotrya japonica* Lindl.) leaves at low temperatures (Hirai, 1983). Further, sorbitol has been implicated in the osmotic adjustment of apple (Wang and Stutte, 1992) and cherry (*Prunus* spp.) leaves (Ranney et al., 1991) in response to drought. In apple, sorbitol accounts for up to 50% of the total osmotic adjustment. (Wang and Stutte, 1992).

In mature leaves of peach seedlings, sorbitol synthesis may account for more than half of the newly fixed CO₂, depending on photosynthetic rates (Gaudillère et al., 1991). The participation of glucose and fructose in short-term C partitioning is minor (Moing et al., 1992). Glucose-6-phosphate is the common precursor of sorbitol and sucrose (Hirai, 1981), although nothing is known concerning the factors controlling C partitioning between the two sugars.

It is possible that different cultivars have different C partitioning properties depending on genetic and geographic origin. The

aim of the present study was to examine the variability of sorbitol : sucrose ratio (SSR) in source leaves of several *P. persica* cultivars from different geographical origins. Cultivars differing in SSR will be useful in photosynthetic C partitioning studies in sorbitol-synthesizing species and in studies on the role of sorbitol in plant adaptation to stress. To our knowledge, there are no previous reports concerning differences in SSR between cultivars of peach or any other member of the Rosaceae.

Materials and Methods

During 1992 in an orchard at the Fruit Breeding Station of the Institut National de la Recherche Agronomique, Bordeaux, France, 4- and 5-year-old trees of 58 peach cultivars were examined. Trees were grown in a rich sandy loam soil without irrigation, under a typically oceanic climate, 45 km south of Bordeaux (45° N). An open-vase training system in north-south oriented rows was adopted with a 2 $\times$ 5-m spacing. The sampling was designed for genetic variability analysis, in that one leaf from each of two trees of a given genotype was sampled on 20 July, 21 Aug., and 23 Sept., respectively. Thirteen cultivars still bore fruit at the first sampling date. Mature leaves that were fully exposed to sunlight were sampled from the sixth to eighth node from the shoot apex from nonbearing current-season branches (30 to 50 cm long) localized on the eastern side of the crown at >2 m above ground level. A preliminary trial showed that SSR of leaves from the same tree under the same illumination exhibited <10% variation. In contrast, leaves from the same tree under different illumination were more variable (data not shown). Samples were collected between 9:00 and 11:00 HR (solar time). One square centimeter of one leaf per tree was immediately dropped in 80 ethanol : 20 water (v/v) at 35°C. Neutral sugars were purified and analyzed using high-performance liquid chromatography as described by Moing et al. (1992). SSR was expressed on a microgram per microgram basis. Data were analyzed using the F test, and means were compared using Tukey's mean separation test. Confidence intervals (95%) for all pairwise differences between means were used when there were unequal cell sizes (SAS Institute, 1989). Homogeneity of variance (Fernandez, 1992) and normal distribution (SAS Institute, 1989) of data from all the groups compared were previously verified. Pearson correlation coefficients were also calculated.

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Table 1. Fruit type, country of origin, and sorbitol : sucrose ratio (SSR) in mature leaves of *Prunus persica* on three sampling dates. The plant material was orchard-grown near Bordeaux, France.

Cultivar	Fruit type	Country of origin	SSR ^z (w/w)		
			20 July	21 Aug.	23 Sept.
Akatsuki	Peach	Japan	2.34	2.16	2.01
Alexandre	Peach	United States	2.49	2.82	2.40
Appia	Peach	Italy	2.44	2.66	2.27
Armking	Nectarine	United States	2.00	2.19	2.29
Babygold 5	CCTP ^y	United States	2.60	2.74	2.53
Babygold 6	CCTP	United States	2.01	2.35	2.29
Baifeng	Peach	China	1.86	2.27	1.93
Baladin	CCTP	France	2.20	2.23	2.24
BigTop	Nectarine	United States	3.20	3.54	3.48
Caldesi 84	Nectarine	Italy	2.73	3.55	3.94
Caldesi 2000	Nectarine	Italy	2.77	2.81	2.67
Caldesi 2010	Nectarine	Italy	2.68	2.60	2.90
Caldesi 2020	Nectarine	Italy	2.28	3.67	3.83
Carolina Red	Nectarine	United States	2.51	3.18	3.44
Catherina	CCTP	United States	2.40	2.60	2.24
Derby	Peach	United States	2.51	2.82	2.62
EarliGiant	Nectarine	United States	2.76	3.84	3.18
Fidelia	Peach	United States	2.74	3.46	2.76
Flavorcrest	Peach	United States	2.42	2.88	2.27
Fuzalode	Nectarine	France	2.40	2.28	2.29
Gang Shan Bai	Peach	China	2.20	2.87	2.21
INRA G.F. 5441	Nectarine	France	2.73	3.08	3.07
INRA G.F. 5445	Peach	France	2.47	2.81	2.62
INRA G.F. 5495	CCTP	France	2.56	2.63	2.47
Irie Hakuho	Peach	Japan	1.61	2.12	1.53
Jing Hong	Peach	China	2.13	2.76	2.28
Jing Yan	Peach	China	2.98	3.12	2.48
July Lady	Peach	United States	2.85	2.91	3.14
June Star	Nectarine	United States	2.26	3.10	3.23
Kern Sun	Peach	United States	2.40	2.64	2.29
Kodaira-wase	Peach	Japan	2.07	1.85	2.18
Majestic Giant	Nectarine	United States	2.53	3.05	3.83
Maria Carla	Nectarine	Italy	3.00	3.55	3.86
Maria Delizia	Peach	Italy	3.06	3.20	1.97
Maria Grazia	Peach	Italy	3.29	3.64	3.03
Mike Grand	Nectarine	United States	2.82	3.99	4.29
Maysun	Peach	United States	2.51	2.81	2.82
Nerine	Peach	France	2.44	3.12	2.63
New Kurakata	Peach	Japan	1.88	1.79	1.62
Odaha Hakuho	Peach	Japan	1.88	1.83	1.50
Primerose	Peach	United States	2.69	3.48	3.19
Prita	Nectarine	France	1.88	2.11	2.30
Queen Crest	Peach	United States	2.85	2.15	1.79
Queen Giant	Nectarine	United States	2.55	3.59	3.52
Red Top	Peach	United States	2.89	2.90	2.57
Redwing Ros	Peach	France	2.56	3.60	3.24
Robin	Peach	United States	2.12	2.48	1.73
Saturn	Peach	United States	2.55	3.37	2.29
September Sun	Peach	United States	2.92	3.66	3.27
Sha Zhi	Peach	China	2.31	2.67	1.89
Springtime	Peach	United States	2.12	2.85	2.36
Summer Sun	Peach	United States	2.56	3.03	3.04
Sundance	Peach	United States	2.70	3.55	3.22
Tasty Free	Nectarine	United States	2.94	4.17	3.07
White Lady	Peach	United States	2.46	3.22	2.83
Wu Yue Xian	Peach	China	2.66	3.13	2.33
Yu Hua Rou	Peach	China	2.41	2.44	2.31
Yu Lu	Peach	China	1.99	2.21	1.84
Overall mean			2.50	2.90	2.64
MSD ($\alpha = 0.05$)			1.429	1.562	1.848

^zMeans of two replicate trees per cultivar. The F test detected significant differences due to cultivar. Tukey's separation lines are not indicated because there are many groups formed and the presentation is not easy; thus, only the minimum significant differences (MSDs) are given.

^yCCTP = canning clingstone-type peaches.

Results

There was a wide range of variability in leaf SSR (Table 1), which ranged from 1.61 to 3.29 on 20 July, 1.79 to 4.17 on 21 Aug., and 1.5 to 4.29 on 23 Sept. Sorbitol levels were up to twice that of sucrose in most genotypes. However, the Japanese cultivars New Kurakata and Odaha Hakuho always had a SSR lower than 2:1 and could be discriminated clearly from some Italian ('Maria Carla' and 'Maria Grazia'), American ('Big Top', 'Tasty Free', 'Mike Grand', and 'September Sun'), and other cultivars with ratios higher than 3:1.

There were also significant differences between cultivars for sucrose, sorbitol, glucose, fructose, and total soluble sugar contents (data not shown). However, variations in SSR were due mostly to changes in sucrose content, as indicated by correlation analysis between SSR and sugar contents (Table 2).

The overall mean SSR increased between 20 July and 21 Aug. and then decreased on 23 Sept. (Table 3). However, there was a date $\times$ genotype interaction ($P < 0.01$), which meant that changes in the ratios with time were not similar for all cultivars. Most cultivars showed a maximum value on 21 Aug., although some (e.g., 'Fuzalode', 'Caldesi 2000', 'Yu Hua Rou', and 'Baladin') showed little or no change (Table 1).

Overall mean sorbitol contents peaked on 21 Aug., when sucrose content was lowest. Glucose and fructose contents decreased from 20 July to 23 Sept., while total sugar content was lowest on 23 Sept. (Table 3). The total sugar content did not vary >5% of the mean.

When grouped according to country of origin, the mean of the Japanese group had SSR lower than those of the Italian and American groups for each date (Table 4) due to a high sucrose content of the Japanese group (data not shown). There were no differences in sorbitol content among groups. However, the Japanese cultivar Akatsuki did not have a SSR different from some Italian (e.g., 'Appia' and 'Caldesi 2010') or American (e.g., 'Armking', 'Babygold 6', and 'Springtime') cultivars.

SSR variability was also statistically significant in relation to other factors. For example, when cultivars were grouped according to fruit type, differences were found on the second and third dates (Table 5), with the mean SSR of nectarines being higher than those of peaches and canning clingstone-types. In addition, the correlation between SSR and flowering date was also significant ($P \leq 0.001$). The Pearson correlation coefficients were -0.336 on 20 July, -0.381 on 21 Aug., and -0.412 on 23 Sept. In general, far eastern cultivars flowered 5 to 7 days later than the others.

Discussion

SSR was chosen as the comparative parameter because it is independent of leaf weight for a given area sampled and it allows for comparing data from different cultivars, species, or growing conditions. Since sorbitol and sucrose are the major translocatable photosynthetic products, we can assume that SSR primarily reflects the partitioning of exportable C in leaves.

In apple leaves, SSR has been 3:1 or higher, depending on leaf age (Loescher et al., 1982) and water availability during growth (Wang and Stutte, 1992). SSR for mature leaves of cherry trees under a normal watering regime have been shown to be $\approx 5:1$ (Ranney et al., 1991). In our work, most of the cultivars examined show at least twice as much sorbitol than sucrose and, in some cases, ratios higher than 3:1.

As the pedigree charts of most evaluated cultivars are not available, it is not possible to relate SSR to their genetic origin.

Table 2. Pearson correlation coefficients between sorbitol : sucrose ratio and neutral sugars in mature leaves in each sampling date.

Sugar ($\mu\text{g}\cdot\text{cm}^{-2}$)	Correlation coefficients		
	20 July	21 Aug.	23 Sept.
Sucrose	-0.725^{**}	-0.791^{**}	-0.736^{**}
Sorbitol	0.133	0.134	0.213*
Glucose	-0.113	-0.141	-0.261^{**}
Fructose	-0.101	0.017	0.101
Total sugars	-0.200^*	-0.277^{**}	-0.205^*

***Significant at $P \leq 0.05$ or 0.01, respectively; $n = 116$.

Table 3. Overall means for sorbitol : sucrose ratios (SSRs) and neutral sugars in mature leaves according to sampling dates.

Sugar ($\mu\text{g}\cdot\text{cm}^{-2}$)	Sampling date ^z		
	20 July	21 Aug.	23 Sept.
SSR	2.50 c	2.90 a	2.64 b
Sucrose	195.49 b	188.34 b	208.62 a
Sorbitol	475.61 b	522.79 a	517.26 a
Glucose	157.91 a	148.14 b	100.82 c
Fructose	93.47 a	74.39 b	55.76 c
Total sugars	922.48 a	933.66 a	882.45 b

^zMean separation within lines by Tukey's test ($\alpha = 0.05$), $n = 116$.

Table 4. Sorbitol : sucrose ratios (SSRs) of cultivars pooled according to their country of origin on three sampling dates.

Country of origin	No. of cultivars	SSR ^z (w/w)		
		20 July	21 Aug.	23 Sept.
Italy	8	2.78 a	3.21 a	2.96 a
United States	29	2.59 ab	3.10 ab	2.84 a
France	8	2.41 ab	2.73 ab	2.61 ab
China	8	2.32 bc	2.68 bc	2.16 bc
Japan	5	1.95 c	1.95 c	1.72 c

^zIn each column, values with the same letter are not significantly different. Confidence intervals (95%) of Tukey's mean separation test were used for all pairwise comparisons.

Table 5. Sorbitol : sucrose ratios (SSRs) in mature leaves of cultivars pooled according to fruit type on three sampling dates.

Type of fruit	No. of cultivars	SSR ^z (w/w)		
		20 July	21 Aug.	23 Sept.
Nectarine	17	2.58 a	3.19 a	3.21 a
Peach	36	2.48 a	2.82 b	2.41 b
CCTP ^y	5	2.35 a	2.51 b	2.35 b

^zIn each column values with the same letter are not significantly different. Confidence intervals (95%) of Tukey's mean separation test were used for all pairwise comparisons.

^yCanning clingstone-type peaches.

However, it is clear that, in general, those Japanese cultivars evaluated differ from European and American cultivars. This difference is explained mainly by a higher sucrose content in the Japanese group. The *P. persica* species is highly variable and capable of broad adaptation to environments and cultural practices (Rom, 1988). Pomologists have divided the species into two ecological groups adapted to either subtropical or temperate cli-

mates. However, modern cultivars may carry genes from both these groups (Monet, 1992). Japanese peaches are derived mainly from genotypes introduced from the humid regions of Shanghai, China (Yoshida, 1988), and are considered to be in the subtropically adapted group (Monet, 1992). This fact may explain the lack of any difference in SSR between Japanese and Chinese cultivars in our results. The results from all three sampling dates confirm the variability due to origin and allow us to conclude that ability to synthesize or export either sucrose or sorbitol may depend on ecological origin.

We observed differences in SSR among nectarines and cultivars of other fruit types. The nectarine is a fuzzless peach and is classified as *P. persica* var. *nucipersica* (Schneid.) (Frecon, 1988). To date, nothing is known concerning the inheritance of either the sorbitol-synthesizing capacity or the enzymes of the sorbitol or sucrose pathways and whether such inheritance may be related to glabrous fruit character. Isozymic variability in a number of enzymes has been observed and associated with either freestone or clingstone-type cultivars (Moore, 1988). However, the existence of isozymic variability in sorbitol-6-phosphate dehydrogenase remains unknown.

Loescher et al. (1982) have shown that, in apple leaves, the relative contents of sorbitol and sucrose change with photosynthetic rate, which will vary seasonally due to differences in light level and temperature. Such seasonal variation in sugar content has also been reported by Chong (1971). We also observed significant differences in the amounts of individual sugars and in SSR between dates.

SSR seems to be negatively correlated with flowering date. The early flowering cultivars have ratios higher than those that flower later. In addition, no relation was found between leaf SSR and time of fruit harvest (Saunier, 1988). At the first sampling date, leaves from trees bearing fruit were no different in SSR than leaves of those already harvested. In addition, nonharvested cultivars exhibited no trends in SSR between dates that were not common to all cultivars.

This study has indicated variability in SSR in peach germplasm. This variability is due mainly to differences in sucrose content rather than to sorbitol content, and appears to be related to the geographical origin of cultivars. Apparently, in the Shanghai region and Japan (Yoshida, 1988), natural or artificial selection favored sucrose accumulation and not a lower sorbitol content, as might be expected for trees in a nonstressing environment. Therefore, we can only speculate that sorbitol accumulation may be a general feature of short-term drought stress (Bieleski, 1982; Ranney et al., 1991; Wang and Stutte, 1992). Cultivars differing in SSR would be of value in studying the metabolism mechanisms involved in adaptation of sorbitol-producing plants to stressful environments, and possibly as a selectable trait in breeding programs to improve drought resistance. Finally, genotypes differing in SSR will be useful in studying the regulation of C partitioning and export of sorbitol and sucrose from the source leaves.

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