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Variability in Sorbitol : Sucrose Ratio in Mature Leaves of Different *Prunus* Species

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Abstract. **Sorbitol is a sugar alcohol, present with sucrose in Rosaceae trees, which seems to have a role in plant response to environmental stress. The aim of this study was to investigate variability in sorbitol : sucrose ratio in source leaves of 53 species or hybrids of *Prunus*. The studied taxa, representing three subgenera and 11 sections of the *Prunus* genus, were chosen from the *Prunus* collection at the Institut National de la Recherche Agronomique, Bordeaux, France. Young mature leaves were sampled on three dates in spring and summer and were analyzed for neutral soluble sugars using high-performance liquid chromatography. There were differences in sorbitol : sucrose ratio according to sampling date and according to taxon. Sorbitol content increased and sucrose content decreased from May to July, leading to an increase in sorbitol : sucrose ratio. For each date, there was a high variability within botanical sections for sorbitol : sucrose ratio. The highest variability between species for sorbitol : sucrose ratio was in July, with *P. cocomilia* having the lowest ratio (1.15, w/w) and *P. fremontii* having the highest ratio (5.59, w/w). When species were pooled according to their geographical zone of origin, species originating from Japan showed the lowest sorbitol : sucrose ratio for all sampling dates. In July, species originating from Japan, Europe, and central to western North America had sorbitol : sucrose ratio significantly lower than that of species originating from Europe to western Asia, China to eastern Asia, and central to eastern North America. These results indicate that variability in sorbitol : sucrose ratio exists in the *Prunus* germplasm and seems to be related to the geographical origin of the species. Moreover, variability in sorbitol to sucrose ratio is high in the germplasm of different *Prunus* taxa.**

Sorbitol is a sugar alcohol present in higher plants (Lewis, 1984). Sorbitol, sucrose, and starch are major final products of photosynthesis in some subfamilies of the Rosaceae, including Prunoideae (Wallaart, 1980), with sorbitol and sucrose translocated in the phloem (Bieleski, 1982) of these subfamilies. Plouvier and Bertrand (1955) first discussed the taxonomic significance of sorbitol in Rosaceae. Later, Wallaart (1980) screened 68 taxa of Rosaceae, including 19 species of Prunoideae, for sorbitol content in leaves. He found sorbitol in the Spiraeoideae, Maloideae, and Prunoideae. Escobar-Gutiérrez and Gaudillère (1994) showed that there was a variability in the sorbitol : sucrose ratio (SSR) in peach germplasm, which could be related to the geographical origin of the cultivars.

Sugar alcohols may play a role in the response of plants to environmental stress. Sorbitol has been implicated in the osmotic adjustment of cherry (Ranney et al., 1991) and apple (Wang and Stutte, 1992) leaves in response to drought stress. It also may be involved in cold hardiness (Raese et al., 1978; Whetter and Taper, 1966). Another sugar alcohol, mannitol, has been implicated in adaptation to salinity in celery (Everard et al., 1994; Pharr et al., 1995; Stoop and Pharr, 1994).

In mature leaves of peach seedlings, sorbitol synthesis may account for up to half of the newly fixed CO_2 (Moing et al., 1992); however, factors controlling carbon partitioning between sorbitol and sucrose are unknown. Although glucose-6-phosphate is the common precursor of sorbitol and sucrose synthesis (Hirai, 1981), the regulation of their biosynthesis differs. Nicotinamide-adenine-dinucleotide-*P*-dependent sorbitol-6-phosphate dehydrogenase (EC. 1.1.1.200] is the key enzyme for sorbitol synthesis (Bieleski and Redgwell, 1977; Tao et al., 1995), whereas sucrose-phosphate synthase (EC. 2.3.1.14) is the key enzyme for sucrose synthesis (Stitt et al., 1987). Increased photosynthesis via elevated CO_2 (Pan and Quebedeaux, 1995), favors photosynthate partitioning into sorbitol rather than sucrose in apple leaves. Decreased photosynthesis via low light favors photosynthate partitioning into sorbitol rather than sucrose in peach leaves (Escobar-Gutiérrez et al., 1995). These apparently contradictory results suggest that sorbitol is preferentially synthesized under various environmental conditions.

The genus *Prunus* includes numerous species of economical significance in horticulture today (i.e., almond, apricot, peach, cherry, plum, and prune). Their common ancestor, the first diploid

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Table 1. Botanical classification, name, origin, and tree characteristics for the 53 species or hybrids of *Prunus* spp. studied for sorbitol : sucrose ratio.

Subgenus	Section	Species	Authority	Geographic origin	Genetic origin	Common name	Habit^z
Amygdalus	Euamygdalus	*persica*	L. Batsch.	China	Selfing of 'Fair Haven'	Peach	Tree
	Euamygdalus	*pollardii*	Hort.	Australia	*P. amygdalus* x *persica*		Tree
Cerasus	Eucerasus	*avium*	L.	Europe, western Asia	'Lapins'	Cherry	Big tree
	Eucerasus	*caproniana*	L.	Western Asia, southeastern Europe		Amarelles	Tree
	Eucerasus	*cerasus*	L.	Southeastern Europe, Western Asia		Sour cherry	Tree
	Eucerasus	*fruticosa*	Pall.	Eastern central Europe Siberia		Ground cherry	Shrub
	Eucerasus	*schmittii*	Rehd.	unknown	*P. canescens* x *avium*		Small tree
	Eucerasus	*semperflorens*	(Ehrh.) W. Koch.	Western Asia, southeastern Europe	*P. cerasus semperflorens*		Small tree
	Lobopetalum	*pseudocerasus*	Lindl.	Northern China			Small tree
	Mahaleb	*mahaleb*	L.	Europe, western Asia	'Sainte Lucie' INRA64	Mahaleb cherry	Tree
	Microcerasus	*besseyi*	Bailey	America, Manitoba to Wyoming, Kansas, and Colorado		Western sand cherry	Shrub
	Microcerasus	*japonica*	Thunb.	China, eastern Asia		Japanese bush cherry	Shrub
	Microcerasus	*microcarpa*	C.A. Mey	Asia Minor	Related to *P. incana*		Shrub
	Phyllomahaleb	*maximowiczii*	Rupr.	Manchuria, Korea, Japan, eastern Asia			Big tree
	Pseudocerasus	*apetala*	Sieb. & Zucc.	Japan	Related to *P. incisa*		Small tree
	Pseudocerasus	*conradinae*	Koehne	Central China, eastern Asia			Tree
	Pseudocerasus	*dawyckensis*	Sealy	Western China	Related to *P. canescens*		Small tree
	Pseudocerasus	*hillieri*	Hillier	unknown	*P. incisa* x *sargentii*		Tree
	Pseudocerasus	*incisa*	Thunb.	Japan, eastern Asia			Small tree
	Pseudocerasus	*juddii*	E. Anders.	unknown	*P. sargentii* x *yeodensis*		Tree
	Pseudocerasus	*nipponica*	Matsum.	Japan, eastern Asia			Small tree
	Pseudocerasus	*pandora*		Japan, eastern Asia	*P. subhirtella* x *yedoensis*		Tree
	Pseudocerasus	*sargentii*	Rehd.	Japan, eastern Asia			Big tree
	Pseudocerasus	*serrula*	Franch.	Western China, eastern Asia			Tree
	Pseudocerasus	*serrulata*	Lindl	Japan, China, Korea, eastern Asia		Japanese cherry	Big tree
	Pseudocerasus	*subhirtella*	Miq.	Japan, eastern. Asia		Higan Cherry	Small tree
	Pseudocerasus	*yedoensis*	Matsum.	Japan, eastern Asia	*P. serrulata* x *subhirtella*	Yoshino-Zakura	Big tree
	Pseudocerasus	*vere*		unknown	Related to *P. serrulata*		Big tree
Prunophora	Armeniaca	*mandshurica*	(Maxim.) Koehne	Manchuria, Korea		Manchurian apricot	Small tree
	Euprunus	*blireiana*	André	unknown	*P. cerasifera* x *mume*		Tree
	Euprunus	*bokhariensis*	Schneid.	Kashmir	Related to *P. salicina*	Bokar plum	Tree
	Euprunus	*caspica*	(Koval. & Ekim.) Erem.	Europe western Asia	Related to *P. cerasifera*		Tree
	Euprunus	*cerasifera*	Ehrh.	Western Asia, Caucasus	Cherry plum, Myrobalan		Tree
	Euprunus	*cocomilia*	Ten.	Italy	Related to *P. cerasifera*	Italian plum	Small tree
	Euprunus	*domestica*	L.	Western Asia, Europe	'Quetsche d'Alsace'	Garden plum	Tree

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Table 1. Continued.

Subgenus	Section	Species	Authority	Geographic origin	Genetic origin	Common name	Habit^z
Prunophora	Euprunus	*fruticans*	Weihe	Europe	*P. spinosa* x *insititia*		Small tree
	Euprunus	*insititia*	L.	Western Asia, Europe		Bullace, Green gage	Small tree
	Euprunus	*longipedicellata*		Unknown	Related to *P. spinosa*		Small tree
	Euprunus	*salicina*	Lindl.	China, eastern Asia		Japanese plum	Tree
	Euprunus	*simonii*	Carr.	Northern China		Apricot plum	Tree
	Euprunus	*spinosa*	L.	Europe, northern Africa, western Asia		Blackthorn, sloe	Shrub
	Euprunus	*ussuriensis*	Kov. & Kost.	Manchuria, western Asia		Manchurian plum	Tree
	Prunocerasus	*alleghaniensis*	Porter	America, Connecticut to Pennsylvania		Alleghany plum	Small tree
	Prunocerasus	*americana*	Marsh.	America, Massachusetts, Georgia, northern Mexico, Utah		Goose plum	Tree
	Prunocerasus	*hortulana*	Marsh.	America, New York, Kentucky, and Tennessee to Iowa and Oklahoma		Wild goose plum	Tree
	Prunocerasus	*maritima*	Marsh.	America, Maine to Virginia		Beach plum	Shrub
	Prunocerasus	*mexicana*	S. Wats.	America, southwest Kentucky and Tennessee		Big-tree plum	Big tree
	Prunocerasus	*orthosepala*	Koehne	Kansas	*P. angustifolia* x *americana*		Shrub
	Prunocerasus	*subcordata*	Benth.	America, Oregon to California		Pacific plum	Small tree
	Prunocerasus	*umbellata*	Ell.	America, South Carolina to Florida		Black sloe	Small tree
	Marianna		unknown	*P. cerasifera* x *munsoniana*		Marianna plum	Tree
Prunophora x Amygdalus		*fremontii*	Watson	North America	*P. armeniaca* x *amygdalus*		Tree
Prunophora x Amygdalus		x *amygdalus*		unknown	Related to *P. fremontii*		Small tree

^{z}Growth habit: big tree: >12 m; tree: between 6 and 12 m; small tree: < 6 m tall.

Prunus species, arose in central Asia. The phylogenetic relationships between *Prunus* species has been described by Watkins (1976). According to Watkins, species from the Microcerasus section may be closer to the ancestral *Prunus* species than commercial Eucerasus cherries. Species from the Eucerasus section were early derivatives of the ancestral *Prunus* species. Watkins further suggests that the plums have an intermediate position in the *Prunus* phylogenetic tree and have at least five geographic centers of origin, depending on the group of species (see Table 1), with *P. domestica* being the most recent crop species to have arisen within the Prunophora subgenus. The separation of plums into Prunocerasus and Euprunus seems to have a geographical rather than a genetic basis. *Prunus persica* seems to have originated from China and appears to be closer to the genetic center of *Prunus* than *P. amygdalus*. Numerous data on the genetic resources of *Prunus* species have been gathered by Moore and Ballington (1990).

Our aim was to examine the variability of the SSR in source leaves of several species or hybrids of *Prunus* from different taxa and geographical origins. Species differing in SSR will be useful in studies on the role of sorbitol in plant adaptation to stress. This survey of sorbitol : sucrose variability among species or hybrids may indicate high or low ability to tolerate drought or salinity and could be used as a basis for future experiments in which stress is applied to some selected species or hybrids.

Materials and Methods

Nearly all the species or hybrids represented in the *Prunus* collection at the Unité de Recherches sur les Espèces Fruitières et la Vigne, Institut National de la Recherche Agronomique, Bordeaux, France, were studied for their leaf carbohydrate content. The complete name, section, geographical and genetic origins, common name, and tree growth habit of all the genotypes studied are presented in Table 1. The botanical classification, geographical and genetic origins are from Rehder (1947). For some species absent from Rehder (1947), a description was found in Krüssmann (1986). These two classifications are the most commonly used for *Prunus* (Dosba et al., 1994); they differ for subgenus Cerasus. We chose Rehder's classification, which comprises 77 species, preferable to Krüssmann's classification, which comprises 140 species, because the former classification still is used widely. The common name of cherries is from Rehder (1947) and that of plums is from

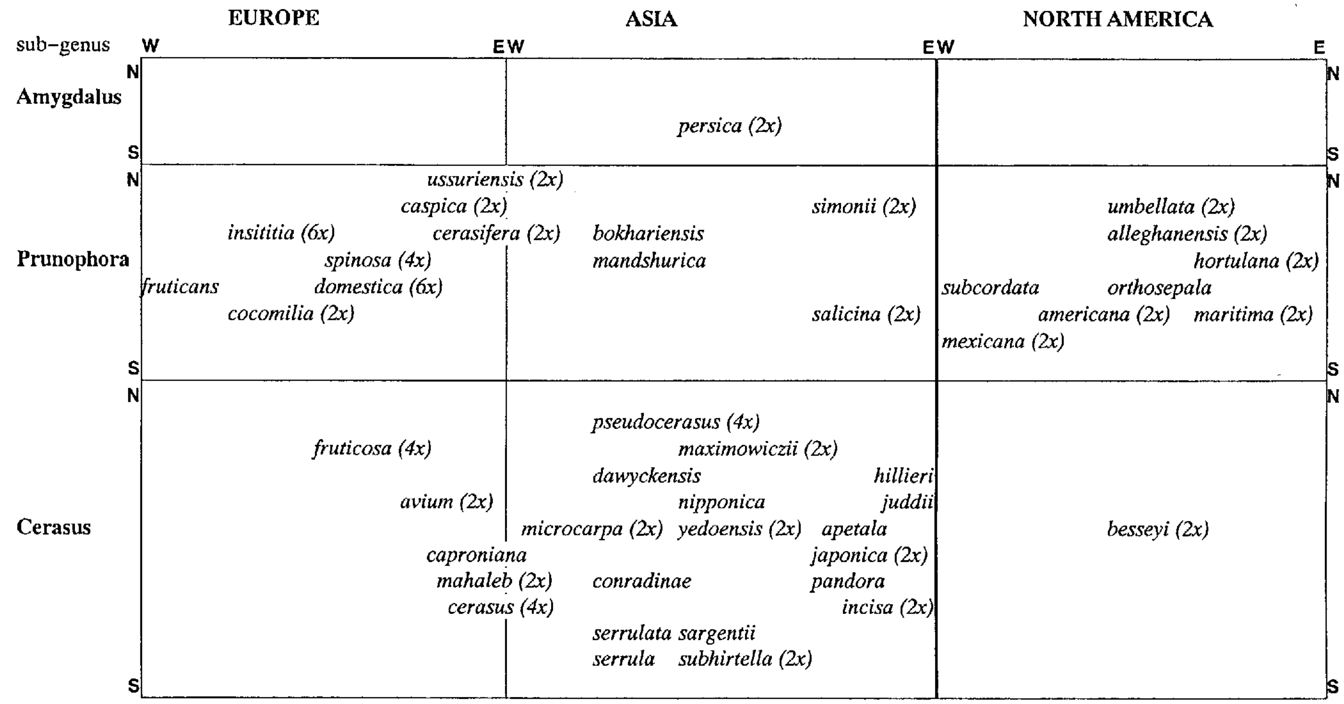

Fig. 1. Simplified scheme showing the geographical origin and chromosome number of 44 of the *Prunus* species studied. The geographical origin is according to Rehder (1947) and Krüssmann (1986). For each continent, the east–west (E–W) distribution is indicated. The north–south (N–S) distribution is approximate. When known from the literature (Darlington and Wylie, 1955; Ramming and Cociu, 1990; Watkins, 1976), the chromosome number of the species is indicated by the ploidy level in parentheses. The ploidy level of the interspecific hybrids *Marianna* and *pollardii*, determined on our plant material (Salesses and Bonnet, personal communication), was 2x. The basic chromosome number in *Prunus* is equal to 8.

Rehder (1947) or Ramming and Cociu (1990). Tree growth habit was based on our observations in the orchard and the description of Rehder (1947) and characterizes the potential size of the tree without pruning. The geographical origins of the species and their chromosome numbers, found in the literature (Darlington and Wylie, 1955; Ramming and Cociu, 1990; Watkins, 1976) or determined on our plant material (Salesses and Bonnet, personal communication), are summarized in Fig. 1.

During 1995, in the orchard of the *Prunus* collection of the Institut National de la Recherche Agronomique, one clone for each of the 53 species or hybrids of *Prunus* was chosen for the soluble carbohydrate study. The trees were 5 to 7 years old, and each species or hybrid was represented by only one clone that had been grafted on a rootstock from genus *Prunus* 1 year before planting in the orchard. Species of the subgenus Amygdalus were grafted on seedlings of GF 305 (*P. persica*), most species of subgenus Cerasus were grafted on Colt (*P. avium* x *pseudocerasus*) rootstock, and most species of subgenus Prunophora were grafted on Marianna GF8.1 [supposed to be (*P. cerasifera* x *munsoniana*)x *cerasifera*; Salesses 1977). Trees were grown in a sandy loam soil, with a 3 × 4.6-m spacing, under a typically oceanic climate near Bordeaux (45° N), with average annual minimal and maximal temperatures of 2.6 and 26.4 °C, respectively. Standard fertilization and pest control practices were as needed. Localized irrigation was applied in spring and summer to compensate for potential evapotranspiration, calculated according to Penman and cumulated for 4 d minus rain.

Table 2. Overall means for dry weight per unit area, soluble sugar content, and sorbitol : sucrose ratio (SSR) in mature leaves of 53 *Prunus* species or hybrids according to sampling date.

Variable[z]	Sampling date 19 May	21 June	19 July
Dry wt (mg·cm^{-2})	7.0 c[y]	8.3 b	8.8 a
Glucose (mg·g^{-1})	9.8 a	10.0 a	10.2 a
Fructose (mg·g^{-1})	8.1 a	6.3 b	5.7 c
Sorbitol (mg·g^{-1})	67.6 c	80.3 b	87.4 a
Sucrose (mg·g^{-1})	50.2 a	40.2 b	33.2 c
SSR (w/w)	1.38 c	2.10 b	2.88 a

[z] Mean of n replicates with n = 53 for dry weight, 212 for soluble sugar content, and SSR, except for sorbitol and sucrose in May where n = 204.
[y] Mean separation within lines by Tukey's test at $P < 0.05$.

Four leaves (one leaf per shoot on four shoots) were sampled for soluble carbohydrate determination on 19 May, 21 June, and 19 July 1995, respectively. On each sampling date, the presence of fruit on the trees was noted visually with four classes: no fruit, low fruit, normal fruit, high fruit load. Mature leaves fully exposed from the sixth to eighth node from the shoot apex on current season branches localized on the eastern side of the crown were sampled. All samples were collected between 11:15 and 12:30 (solar time). For each leaf, two 0.4-cm^2 disks were immediately dropped in 80 ethanol : 20 water (v/v), and two other disks were collected for dry mass determination. All samples were kept on ice in an ice-box during collection in the orchard. For each genotype, the eight disks for mass determination were pooled before lyophilization and dry mass measurement. The samples were immediately extracted and re-extracted in 50 ethanol : 50 water (v/v) for 20 min at 80 °C. Extracts were pooled, and their neutral sugars were purified and analyzed using high-performance liquid chromatography as described by Moing et al. (1992), except that ion exchange resins were mixed together. Sugar quantification was done with Millenium software (Waters, Milford, Calif.), using external standards from Sigma Chemical Co. (St Louis).

Sugar contents were expressed on a dry mass basis using the mean dry mass per unit area determined for each genotype. SSR were expressed on a microgram per microgram basis. Total soluble sugar content is the sum of sorbitol, sucrose, fructose, and glucose contents, which were the four major soluble sugars in leaves. Data were analyzed using the F test, and means were compared using Tukey's mean separation test, using general linear model procedure of SAS software (SAS Institute, 1989). Pearson's correlation coefficients were calculated using CORR procedure of SAS software.

Results

Effect of sampling date and fruit on leaf soluble sugar contents. Dry mass per unit area of mature leaves increased from May to July (Table 2). Sorbitol, sucrose, glucose, and fructose were the four major soluble sugars in mature leaves of all species. The mean glucose content on a dry mass basis of all species remained constant, while the mean fructose content significantly decreased from May to July. However, the sum of glucose and fructose always represented <14% of the mature leaf soluble sugars. The leaf sorbitol content of the studied species or hybrids was always >30 $mg \cdot g^{-1}$. The mean sorbitol content on a dry mass basis significantly increased, while the mean sucrose content decreased from May to July (Table 2). These changes resulted in an increase in the mean SSR from 1.38 in May, to 2.88 in July.

Less than half of the studied clones produced fruit in 1995, probably due to unfavorable environmental conditions during flowering for early flowering species. Only *P. americana, insititia, longipedicellata,* and *domestica* had high fruit production. On the third sampling date, only 30% of the studied trees were still bearing fruit. The presence of fruit on each tree, at the time of leaf sampling, had a negligible effect on leaf SSR (data not shown).

Variability between subgenus and between sections for leaf ssr. When species were grouped by subgenus (data not shown), no significant difference appeared between the mean for SSR in subgenus Cerasus, represented by 26 species, and that of subgenus Prunophora, represented by 23 species, for the three sampling dates. When more than two species per section were studied (i.e., for Eucerasus, Microcerasus, Pseudocerasus, Euprunus, and Prunocerasus), there appeared to be a high variability within each section for the SSR measured in June and July. In July, for example, SSR ranged from 2.26 to 5.35 for Eucerasus, 1.41 to 4.00 for Pseudocerasus, 1.15 to 3.87 for Euprunus, and 1.86 to 4.28 for Prunocerasus.

Variability among species for leaf ssr. For SSR, the F test detected a significant effect ($P < 0.01$) of date, species, and their interaction. Therefore, SSR for the 53 species or hybrids at the three sampling dates are presented (Table 3).

Variability among species increased from May to July. SSR ranged from 0.81 to 2.25 in May, 1.09 to 3.88 in June, and 1.15 to 5.59 in July. Variations in SSR were due to changes in sucrose and sorbitol content, as indicated by correlation analysis between SSR and soluble sugar contents (Table 4). No significant correlation between leaf SSR and leaf dry mass per unit area appeared in May and June (data not shown), while a significant negative correlation (–0.298) appeared in July.

Significant differences in SSRs related to taxonomic differences were detected. SSRs of genetically related species (see Table 1) were not significantly different ($P < 0.05$, Table 3). There were no significant difference in the high SSR value between either *P. persica* and *P. pollardii*, or *P. pollardii* and *P. fremontii*. *Prunus schmittii* (*P. canescens* x *avium*) had an SSR that was not significantly different from that of *P. avium* in May and June, although it was significantly different from that of *P. avium* in July. *Prunus*

Table 3. Sorbitol : sucrose ratio (SSR) in mature leaves of species or hybrids of *Prunus* spp. on three sampling dates. The plant material was grown in orchard near Bordeaux, France.

		Sorbitol : sucrose ratio ($g \cdot g^{-1}$)		
Section	Species[z]	19 May	21 June	19 July
Euamygdalus	*persica*	1.32	2.35	4.21
	pollardii	1.12	2.53	4.31
Eucerasus	*avium*	1.24	2.04	4.66
	caproniana	1.73	2.91	5.35
	cerasus	1.12	2.10	2.75
	fruticosa	1.54	2.62	2.26
	schmittii	1.17	1.89	2.51
	semperflorens	1.90	2.51	2.90
Lobopetalum	*pseudocerasus*	1.34	2.08	2.04
Mahaleb	*mahaleb*	1.49	2.40	3.41
Microcerasus	*besseyi*	0.87	1.09	2.20
	japonica	0.95	1.30	2.19
	microcarpa	1.96	2.45	3.40
Phyllomahaleb	*maximowiczii*	1.80	2.38	2.85
Pseudocerasus	*apetala*	1.03	1.24	1.82
	conradinae	0.97	3.04	4.00
	dawyckensis	1.01	1.60	3.33
	hillieri	1.03	1.38	1.87
	incisa	0.81	1.24	1.67
	juddii	1.03	1.82	1.73
	nipponica	0.95	2.05	2.27
	pandora	0.84	1.13	1.41
	sargentii	1.06	1.33	1.58
	serrula	1.69	3.20	4.04
	serrulata	0.93	1.99	1.92
	subhirtella	1.46	2.24	2.20
	yedoensis	0.98	1.17	1.74
	vere	1.00	1.53	2.38
Armeniaca	*mandshurica*	1.59	3.88	2.84
Euprunus	*blireiana*	2.12	2.31	3.18
	bokhariensis	1.96	1.68	2.67
	caspica	1.92	2.34	2.92
	cerasifera	1.39	2.93	3.87
	cocomilia	1.33	1.37	1.15
	domestica	1.34	1.99	2.19
	fruticans	1.63	2.17	2.64
	insititia	1.26	1.93	2.87
	longipedicellata	2.25	1.88	2.73
	salicina	1.48	2.04	3.87
	simonii	1.30	1.67	2.66
	spinosa	1.23	1.91	2.89
	ussuriensis	1.98	2.43	2.91
Prunocerasus	*alleghaniensis*	1.32	2.01	3.98
	americana	1.51	2.11	2.48
	hortulana	1.54	2.51	4.15
	maritima	1.61	3.46	4.28
	mexicana	1.21	1.83	1.86
	orthosepala	1.31	2.08	2.71
	subcordata	1.58	3.32	2.76
	umbellata	1.29	1.85	3.93
	marianna	1.16	1.44	1.76
	fremontii	1.44	2.23	5.59
	x *amygdalus*	1.96	2.46	2.62
	Overall mean	1.38	2.10	2.88
MSD (0.05)[y]		0.54	0.89	1.82

[z] Means of four leaves per species. The F test detected significant differences due to species.

[y] Tukey's separation lines form too many groups; therefore, only the minimum differences (MSD) are given.

hillieri, *P. juddii*, *P. pandora*, and *P. yedoensis* had a low SSR, which was neither significantly different between these four species nor significantly different from that of their parent species (*incisa* and *sargentii*, *sargentii* and *yedoensis*, *yedoensis* and *subhirtella*, *subhirtella* and *serrulata*, respectively). *Prunus fruticans* (*P. spinosa* x *insititia*) had SSR not significantly different from that of its parent species. *Prunus orthosepala* had SSR not significantly different from that of one of its parent species, *P. americana*.

Prunus apetala, said to be related to *P. incisa*, had SSR not significantly different from that of the former species. However, there were two exceptions since the SSRs of *P. cerasus* and *P. caproniana*, related species, were significantly different in July. *Prunus cerasifera* or *P. caspica* and *P. cocomilia*, supposedly related species, also had significantly different SSR in June and July.

Prunus maritima (beach plum), with a native range of coastal areas with mesic sandy soils (Rieger and Duemmel, 1992), had one of the highest SSR measured in Prunophora in June and July, which could be related to some adaptation to salinity. *Prunus besseyi* (western sand cherry), had an intermediate SSR, relative to other species, although this species, originating from semi-arid grasslands (Rieger and Duemmel, 1992) must have developed some adaptation to drought stress. *Prunus subcordata* (Pacific plum), adapted to mesic to xeric soils, had an intermediate SSR relative to other species in July.

When the chromosome number of the species was known (Fig. 1) and when ploidy presented variability inside a section (i.e., Eucerasus, Euprunus), no relation appeared between leaf SSR (Table 3) and ploidy level.

Relations between leaf SSR and geographical origin of the species. For most of the species, the geographical zone of origin is not precisely known. It was described by Rehder (1947) or Krüssmann (1986) in terms of portion of continents rather than precise climatic or ecological habitat. However, when species were pooled according to their geographical zone of origin, significant differences between groups appeared (Table 5, $P = 0.05$). In May, the highest SSR was observed in species from Europe to western Asia and was significantly different from that of species originating from western North America, China to eastern Asia, and from Japan to eastern Asia. In June, the SSR of species originating from China to eastern Asia was the highest SSR in our study but was not significantly different from that of species originating from Europe, Europe to western Asia, or North America. In July, the highest SSR was in species from eastern North America. It was significantly higher than that of species from western North America, Europe, and Japan to eastern Asia. The SSRs of species from Europe and western North America were not significantly different from that of species originating from Japan to eastern Asia, which had the lowest ratio at that time.

Species originating from Japan to Eastern Asia had the lowest SSR for all three sampling dates (Table 5). This resulted from lower sorbitol contents (data not shown).

Table 4. Pearson correlation coefficients between sorbitol : sucrose ratio and soluble sugars in mature leaves of 53 species or hybrids of *Prunus*, in each sampling date.[z]

Sugar	Correlation coefficients		
(mg·g^{-1})	19 May	21 June	19 July
Sorbitol	0.597**	0.577**	0.477**
Sucrose	–0.434**	–0.588**	–0.692**
Fructose	0.046	–0.035	–0.019
Glucose	0.230**	0.134	0.036
Total soluble sugars	0.235**	0.214*	0.113

[z] n = 204 in May and 212 in June and July.

*, **Significant at $P < 0.05$ or 0.01, respectively.

Table 5. Sorbitol : sucrose ratio (SSR) in mature leaves of 53 *Prunus* species or hybrids pooled according to their geographical zone of origin in northern hemisphere, as described by Rehder (1947) or Krüssmann (1986). The geographical origins of the species presented in Table 1 were gathered into six zones.

	No. species	SSR (w/w)		
Zone of origin	or hybrids	19 May	21 June	19 July
North America				
Center to west	3	1.22 cd[z]	2.08 ab	2.27 b
Center to east	6	1.43 a–c	2.34 a	3.59 a
Europe	3	1.50 ab	2.05 ab	2.02 b
Europe to western Asia	13	1.58 a	2.28 a	3.29 a
China to eastern Asia	12	1.28 bc	2.25 a	3.03 a
Japan to eastern Asia	8	1.02 d	1.53 b	1.80 b

[z] Mean separation within columns by Tukey's test at $P < 0.05$.

Discussion

Physiological significance of SSR. As sorbitol is not metabolized (Loescher et al., 1982) and sucrose is little metabolized in mature leaves of Rosaceae, SSR is the direct result of the balance between sorbitol and sucrose synthesis and their export through phloem. SSR was chosen as a comparative parameter because it is independent of leaf thickness, which is highly variable between species [specific leaf area (on a dry mass basis) from ≈5 to 13 mg·cm^{-2}, in our study] and of leaf starch storage, which is highly variable within species and between dates (A.M., unpublished data) and modifies leaf mass for a given area. We verified that fruiting had a negligible effect on leaf SSR in our conditions.

SSR is known to vary with leaf age (Loescher et al., 1982) and water availability (Wang and Stutte, 1992). However, the SSRs observed in the Bordeaux collection of *Prunus* species are in agreement with previously published data in *Prunus* species (Escobar-Guttiérrez and Gaudillère, 1994; Ranney et al., 1991). Sorbitol was detected in contents always >30 mg·g^{-1} (on a dry mass basis) in all the species or hybrids tested.

Although the orchard was irrigated, there were significant changes in leaf sorbitol and sucrose contents and SSR during the growth season. The general tendency was an increase in sorbitol content from May to July, but some species did not show this pattern. Seasonal variations in the leaf sucrose and sorbitol content already have been reported in apple (Chong, 1971; Loescher et al., 1982) and peach tree (Escobar-Guttiérrez and Gaudillère, 1994). Changes in photosynthetic rates, induced by changes in light or water availability, affected carbon partitioning between sorbitol and sucrose in young peach trees (Escobar-Guttiérrez et al., 1995). Seasonal changes in photosynthetic rate in the orchard in this study could proceed from similar factors and result in changes in SSR through changes in partitioning of photosynthetic carbon. Variability between species was greatest in July, probably due to these seasonal changes.

SSR and taxonomy. Our study completes the inventory of sorbitol in *Prunus* species. Forty-one species and three sections (Lobopetalum, Phyllomahaleb, and Prunocerasus), of the 53 species and 10 sections analyzed in this study, had not been cited by Wallaart (1980), and to our knowledge, most of them had not been characterized previously for sorbitol content. The range of variability detected in our study for SSR was higher than that for clones or hybrids of the species *P. persica* (Escobar-Guttiérrez and Gaudillère, 1994). In our study, we did not estimate variability within each of the species studied.

Genetically related species had similar SSR. However, a high variability was observed within botanical sections. Therefore, SSR, even if measured in the same location at the same time, is not a good taxonomic criterion. The genomic composition of the different species of one section may explain part of this variability. Chemotaxonomic studies using secondary products (Challice, 1981), isozyme studies (Mowrey and Werner, 1990), and more recently, DNA fingerprints (Yoshida et al., 1995) provide more valuable information than SSR for phylogenetic studies of *Prunus* species.

SSR AND ADAPTATION TO ENVIRONMENT. The geographical zones of origin of the *Prunus* species are usually described in terms of parts of continents rather than more precise climatic zones. However, in July, two groups of zones of origin could be distinguished: western North America, Europe, and Japan to eastern Asia, where species had the lowest SSRs, and eastern North America, Europe to western Asia, and China to eastern Asia, where species had the highest SSRs. In general, the former group is mainly characterized by subtropical moist or temperate climates and the latter group by zones with a continental climate. There might be some exceptions since one species originating from western North America comes from a dry continental region. The species originating from Japan and eastern Asia showed the lowest SSR from May to July, resulting from low sorbitol contents. Most of these species are genetically related. This Japanese group may be more adapted to a subtropical humid climate than to a temperate climate. This is in agreement with what Escobar-Guttiérrez and Gaudillère (1994) observed within the peach germplasm, where peach cultivars originating from Japan also had low leaf SSR. Our study should be completed by an estimation of intraspecific variability for leaf SSR in some species other than peach. However, there appears to be a relationship between the environmental conditions of the zone of origin of the species and the genetic capacity for sorbitol synthesis in its leaves.

When the native habitat of the species was known, the situation for some species originating from North America (Rieger and Duemmel, 1992), no clear relation could be observed between SSR measured in Bordeaux and supposed resistance to drought, which may result because our study was performed on irrigated trees and, although a "basal" value of SSR seems to be related to some adaptation to the climate of the zone of origin of the species, additional changes in SSR are inducible only by stress (e.g., drought) conditions (Wang and Stutte, 1992).

Controlled drought or salinity stress might enhance the seasonal changes for SSR in our experiment. This study may be the basis for future experiments in which stress would be applied to selected species or hybrids.

This study indicated high variability in SSR in the germplasm of different taxa of *Prunus*. This variability is due to differences in sucrose and sorbitol content and can be related to the geographical origin of the species. Recent studies, using genetic transformation, seem to indicate that mannitol synthesis, induced in tobacco transformants, improves their resistance to salinity (Tarczynski et al., 1993). Tobacco transformants producing sorbitol are currently being used to evaluate their response to environmental stress (Tao et al., 1995) to verify the protective role of sorbitol. Related *Prunus* species differing in SSR will be useful in studying the potential relationship between sorbitol concentrations and stress tolerance mechanisms for plants that naturally produce sorbitol. If the protective role of sorbitol is confirmed by further experiments in which stress is applied to selected *Prunus* species, sorbitol content, SSR, or both may be considered as a selection trait in breeding programs aimed at improving resistance to salinity or drought.

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