Soluble solid assessment of California *Prunus domestica* germplasm

S.B. Castro and T.M. DeJong

Department of Plant Sciences, University of California, Davis, USA.

Abstract

The California *Prunus domestica* growers and processors promote dried plums as a natural way to achieve digestive regularity without the use of probiotics or synthetic laxatives. The dietary fibre and natural sorbitol content of prune fruit are some of the cornerstones of the prune industry's domestic marketing strategy. Because of the importance of sorbitol, the prune breeding program at the University of California, Davis has begun to evaluate the fruit sugar and sorbitol profiles in its germplasm. Glucose, sucrose, fructose, and sorbitol contents were analysed on fruit from 224 genotypes over two years; 84 were from the program's germplasm collection, 30 were a product of one controlled cross ('D2N-76' × 'Muir Beauty'), and 107 were a product of another separate controlled cross ('D6N-103' × 'MuirBeauty'). Total sugars as determined by high-performance liquid chromatography (HPLC) ranged from 9-26.3 g 100 g^{-1} over two years of analysis; glucose, fructose, sucrose, and sorbitol ranged from 1.6-10.5, 0.3-5.0, 0.4-13.3, and 0.8-9.8 g 100 g^{-1}, respectively. Titratable acidity and pH were also tested on a majority of the fruit each year and ranged between 0.231-2.060 and 3.186-4.790, respectively (*n*=211 per year). The data indicate that most currently used cultivars have high concentrations of sorbitol but there appears to be the potential to increase it by traditional breeding techniques using currently available germplasm.

Keywords: prunes, sorbitol, acidity, sucrose

INTRODUCTION

The California Dried Plum board has been funding the University of California, Davis (UCD) European plum (*Prunus domestica*) breeding program for 31 years. California's main cultivar, 'Improved French' has become a monoculture in the prune farms of California, creating a great need for new cultivars. The goals of the program include the development of new cultivars that can expand the bloom and harvest season and also meet specific criteria such as processability, fruit flesh quality, tree pest resistance, acceptable tree structure, appropriate sugar profiles, and appropriate sorbitol concentrations.

European plums are one of the members of the *Rosaceae* family which have fruit with very high sorbitol concentrations (Richmond et al., 1981; Wrolstad and Shallenberger, 1981). High sorbitol is one of the traits that contribute to prunes being a superior dried product. The desire for high sorbitol concentrations is due to the sugar alcohol's inability to undergo the Maillard reaction (browning) during dehydration (Forni et al., 1992) as well as its positive dietary attributes. Sorbitol also acts as a preservative and humectant, thus allowing the dried product to have high humidity but low water activity (Forni et al., 1992).

Sorbitol is desired by consumers because of its digestive benefits as well as its low status on the glycemic index (Stacewicz-Sapuntzakis et al., 2001). Fruits with a low glycemic index are particularly attractive in the diets of diabetics (Forni et al., 1992). It is also non-cariogenic, meaning it does not promote tooth decay (California Dried Plums, 2013). Fruits with high sorbitol content provide consumers with a natural laxative as well as a sweet flavor with a low glycemic index (Stacewicz-Sapuntzakis et al., 2001).

The purpose of this study was to determine the range of soluble solids, titratable acidity, and pH in the California prune breeding population using historical selections, international cultivars, and genotypes from two controlled crosses that included unselected, self-rooted sibling populations of 30 and 107 individuals. High sorbitol is such an important trait to the

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industry that the UCD breeding program would like to emphasize this trait when planning controlled crosses. More information about the existing germplasm's potential for increasing fruit sorbitol could be very helpful for selecting parents in order to meet program goals with regard to fruit sorbitol content. Most post-harvest fruit research information for *P. domestica* has focused on antioxidant and phenolic content of prunes (Swain and Hillis, 1959; Raynal et al., 1989; Donovan et al., 1998; Sabarez et al., 2000; Fang et al., 2002; Kayano et al., 2003; Kim et al., 2003; Lombardi-Boccia et al., 2004; Slimestad et al., 2009; Rop et al., 2009; Kristi et al., 2011). Research on *P. domestica* cultivar fruit sugar concentrations has been limited. Forni et al. (1992) evaluated 10 international cultivars, Usenik et al. (2008) tested four European cultivars, Turkish adapted European cultivar information was published by Nergiz and Yildiz (1997), and Wrolstad and Shallenberger (1981) reported sugar concentrations of dried 'Improved French', 'Imperial', and 'Robe de Sargent' cultivars. These studies largely reported sugar profiles in relation to a specific experiment being performed, none were meant to show the range of potential concentrations within a set of germplasm. This study was designed to document the sugar compositions of international cultivars grown in California and also to identify the range of variability in sugar composition within sibling populations to get a better idea of the potential for finding new cultivars with desirable sugar profiles through the standard breeding practices that are currently being employed in the breeding program.

METHODS AND MATERIALS

Sugar testing was done in each of two years (2010 and 2011). Sugar and sorbitol concentrations were determined in the fruit of 225 different *P. domestica* genotypes, 137 of which were unselected full sibling seedlings from two different crosses ('D6N-103' × 'Muir Beauty' n=107 and 'D2N-76' × 'Muir Beauty' n=30), the remainder were from named cultivars and selections that are from the breeding program. The bare-root seedlings were planted in 2004 and 2005 in rows with 60 cm spacing at the University California, Davis campus orchards.

The selections and germplasms tested in this study were grafted onto commercial rootstocks and grown in the program's selection orchards at the Wolfskill Experimental Orchards in Solano County, CA or at the Kearney Agricultural Center in Fresno County, CA. The rootstocks used were the conventional rootstocks for prune farming in California including Marianna 2624, Myrobalan seedling, Myro 29c, and M40. These selection orchards were managed using conventional farming methods with irrigation, winter pruning, fertilization, and standard pest control methods.

Trees were harvested in July, August, or September of 2010 and 2011 when the fruit firmness on individual trees decreased to an average flesh pressure of 2.0-2.8 N cm^2 (3-4 PSI). Between 10 and 20 fruits were picked from a tree and weighed to get an average fruit size in g $fruit^{-1}$. Ten fruit slices from ten different fruits were blended in a standard food blender to create an average juice sample. The juice was then strained using a cheesecloth and centrifuged at 4°C at 17,000 × g for 10 min. The clarified supernatant was removed and submitted to the UCD analytical lab for high-performance liquid chromatography (HPLC) sugar analysis as described in Richmond et al. (1981). The remainder of the supernatant was used to determine titratable acidity (TA) and pH testing. TA was expressed in malic acid equivalents using an automatic titrater (Radiometer TitraLab: Tim 850 titration manager and SAC80 sample changer; Radiometer Analytical SAS, Villeurbanne, France).

RESULTS AND DISCUSSION

Ranges of glucose, fructose, sucrose, and sorbitol concentrations, TA, and average fruit weight, are shown in Table 1. The range for sorbitol was 0.20-10.0 g 100 g^{-1}. The highest sorbitol concentration documented was in the California selection 'D8S-50' (product of 'Imp. French' × 'Burton' op) which had 9.7 and 10 g 100 g^{-1} of juice in 2010 and 2011, respectively. This was higher than the California standard 'Improved French' which had 6.9 and 3.1 g 100 g^{-1} in 2010 and 2011, respectively. This high concentration is more than the highest sorbitol value reported by Forni et al. (1992) where 'Sel D8(BO)dark' had 5.3% fresh weight sorbitol.

Table 1. Sugar, sorbitol (via high-performance liquid chromatography; HPLC), titratable acidity, and fruit weight ranges of fruit from 225 different plum genotypes (most data were taken on the same genotypes in two sequential years, 2010 and 2011); 137 of the genotypes were unselected, self-rooted seedlings, the remainder grafted University of California, Davis (UCD) selections or named cultivars.

Sugar trait	*n*	Max	Min	Mean	MSE	SD
Average fruit weight (g fruit^{-1})	216	95	11.2	32.5	0.55	11.4
Glucose (g 100 g^{-1})	222	10.50	1.40	4.67	0.08	1.60
Fructose (g 100 g^{-1})	221	5.70	0.30	1.87	0.05	1.04
Sucrose (g 100 g^{-1})	216	13.30	0.30	5.14	0.13	2.62
Sorbitol (g 100 g^{-1})	222	10.00	0.20	3.37	0.09	1.87
Sum of sugars[a] (g 100 g^{-1})	222	26.30	5.30	14.92	0.19	4.07
Titratable acidity	211	2.06	0.23	0.55	0.01	0.21
pH	210	4.79	3.19	3.96	0.01	0.30

[a]Sum of glucose, fructose, sucrose, and sorbitol

The largest fruit tested was 'Empress' with an average fruit weight of 95 g. The item with the most sucrose was a California breeding selection 'F11N-27' with 13.3 g 100 g^{-1}. Most of the minimum data points for size and soluble solids were from seedlings. This may have been partially due to the fact that the fruit was from seedlings planted at very high densities whereas the selections were grown on rootstocks and spaced wider.

The results from specifically named cultivars are presented in Table 2. Items with the highest sorbitol concentrations were 'D'Ente GF 2733', and selection 'D2N-76' that was used as a parent for one set of seedlings. The fruit with the lowest sorbitol concentrations was from 'Empress' and 'Tragedy'. 'Tragedy' also had one of the highest TAs along with 'Empress' and 'President'. The glucose/fructose ratio has been used in various publications as a taxonomic trait (Wrolstad and Shallenberger, 1981; van Gorsel et al., 1992). In Table 2, the lowest glucose/fructose ratio was 1.87 while 'Tragedy' and 'Moyer' had the highest at 6.75 and 6.38, respectively. These are larger ratios than what has been reported previously. Forni et al. (1992) reported a range from 0.9-5.8 between 14 different cultivars.

Forni et al. (1992) and others have made the observation that cultivars with high sugar content also contain high sorbitol. The correlation between total sugar and sorbitol concentrations in this study (R^2=0.3004) was substantially less than the R^2=0.71 reported by Forni et al. (1992) (Figure 1).

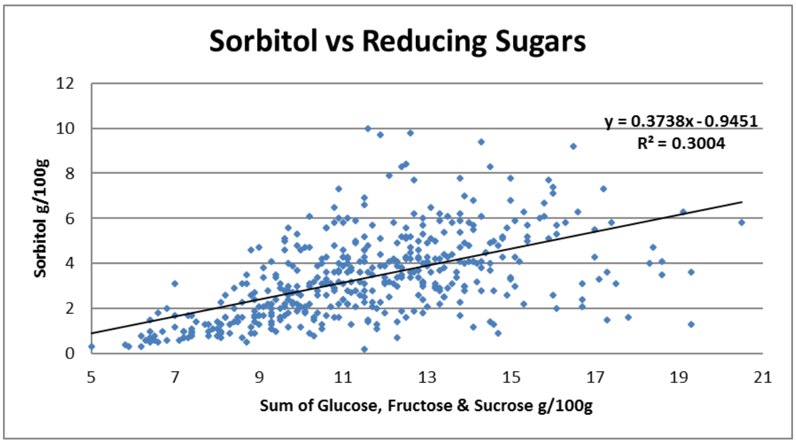

Figure 1. Correlation between sorbitol content and the sum of soluble sugars: glucose, fructose, and sucrose.

Table 2. Sugar and sorbitol concentrations (via high-performance liquid chromatography; HPLC), titratable acidity (TA), and fruit weight of named cultivars and two advanced selections analysed in 2010 and 2011.

Item ID	Glucose ($g\ 100\ g^{-1}$)	Fructose ($g\ 100\ g^{-1}$)	Sucrose ($g\ 100\ g^{-1}$)	Sorbitol ($g\ 100\ g^{-1}$)	Sum of sugars[a]	Glucose/ fructose ratio	TA	Fruit weight[b] ($g\ fruit^{-1}$)
D6N-103[c]	6.80	2.55	1.75	3.65	14.8	2.67	0.44	32.3
D'Ente GF 2733	5.90	1.95	3.65	6.95	18.5	3.08	0.54	26.2
D'Ente GF 303	3.45	1.65	4.25	2.30	11.7	2.13	0.64	23.4
D'Ente GF 652	4.90	2.00	6.50	2.50	15.9	2.45	0.77	27.0
Double Robe	3.10	1.15	5.55	3.95	13.8	2.77	0.52	42.0
Emperor	4.90	2.00	6.40	6.20	19.5	2.45	0.59	30.3
Empress	4.20	1.05	5.80	1.80	12.9	4.36	0.90	76.6
Gerrans E. French	4.25	1.65	6.95	2.15	15.0	2.62	0.60	16.3
Improved French	6.15	2.25	1.65	5.00	15.1	2.74	0.50	26.2
Lorida	4.15	1.40	4.05	2.30	11.9	3.32	0.52	41.4
Moyer	4.30	0.70	6.45	4.80	16.3	6.38	0.43	46.9
Muir Beauty[c]	4.70	1.55	7.50	4.80	18.6	3.19	0.44	43.6
Petite 3x	5.80	1.95	2.60	4.45	14.8	2.97	0.46	26.0
President	3.70	1.50	4.65	3.15	13.0	2.50	1.00	39.6
Stanley	4.40	1.55	5.25	4.05	15.3	2.87	0.60	38.6
Sutter	3.90	1.20	7.50	4.80	17.4	3.38	0.44	26.8
Tragedy	1.95	0.40	5.85	1.15	9.20	6.75	1.73	59.7
Tulare Giant	4.10	2.20	6.30	4.45	17.1	1.87	0.46	65.4
D2N-76[c]	6.30	2.00	5.20	7.30	20.8	3.15	0.43	33.4

[a]Average sum of glucose, fructose, sucrose, and sorbitol.

[b]Fruit weight; an average of at least 10 fruits per year.

[c]Parent of two controlled crosses.

Fruit from the different sets of siblings and half-siblings generated by the breeding program exhibited a range of fruit characteristics (Table 3). All items, with the exception of the two 'Muir Beauty' crosses, were grafted selections and not a representative sample of the range that a particular cross might produce. The cross that generated selections with the highest sorbitol was between 'Improved French' × '6-22-51'. The cross that produced fruit with the lowest concentration of sorbitol on average was 'D6N-103' × 'Muir Beauty'. The TA and the glucose/fructose ratios in Table 3 had less variability and range than was present in the named cultivars that were analysed (Table 2).

The non-selected full sibling seedling sorbitol concentration frequency distributions were further evaluated to examine that trait amongst the progeny (Figures 2 and 3). Fruit of seedlings of both crosses had higher sorbitol concentrations in the fruit juice in 2011 than 2010. The 2010 'D6N-103' data showed more seedlings with increased sorbitol compared to the parents. Despite the fact that a majority of the seedlings had less sorbitol than the parents, there were a few seedlings with an increased amount. This indicates that there is indeed breeding potential within these crosses to further increase sorbitol within the germplasm.

Sucrose concentrations were much more variable than the other sugars and there were many seedlings with much higher concentrations of sucrose than their parents. This also indicates the potential to increase fruit sucrose concentrations by traditional breeding methods. Many anecdotal comments at grower tastings have indicated that higher sucrose items taste more "fruity" and palatable when the fruit is dried. The California breeding program has traditionally been evaluating its seedlings using dried taste evaluations, so the selection of higher sucrose items might have been occurring within our program for the last 20 years without direct testing of individual soluble solid concentrations. In Table 2, the top sucrose concentrations were UCD released cultivars ('Sutter' and 'Muir Beauty' released in 2000 and 2004, respectively). The sucrose concentrations of many of the selections in Table 3 are higher than many of the parental varieties this program started with, most notably 'Improved French'.

Table 3. Average sugar and sorbitol concentrations, fruit weight (g fruit^{-1}), titratable acidity (TA), pH, and glucose/fructose (glu/fru) ratio for a variety of progenies measured in 2010 and 2011. Unless noted, items tested were breeding selections and not seedling trees.

Progeny	*n*	Glucose (g 100 g^{-1})	Fructose (g 100 g^{-1})	Sucrose (g 100 g^{-1})	Sorbitol (g 100 g^{-1})	Sum of Sugars	Glu/fru ratio	TA	Av. fruit weight (g fruit^{-1})
D6N-103 × Muir Beauty[a]	107	4.51	1.84	4.92	2.60	13.7	3.0	0.53	30.8
D2N-76 × Muir Beauty[a]	30	4.07	1.60	6.68	3.45	15.8	3.1	0.54	27.4
2-6E-9[b] half sib	2	5.95	3.70	2.70	3.98	16.3	1.6	0.42	51.9
3-11E-26 × 2-11E-40RR	2	5.13	1.28	6.10	2.93	15.4	4.8	0.59	51.9
5-19-39[c] half sib[d]	12	5.68	2.05	5.11	4.55	17.4	3.4	0.41	33.9
5-19-39[c] × 6-15-62	5	5.82	2.18	5.02	4.79	17.8	3.0	0.40	39.7
5-3-4[c] half sib	3	3.88	1.27	8.65	5.57	19.4	4.0	0.54	34.1
Imp. French half sib[e]	27	5.65	2.27	4.26	4.91	16.7	2.9	0.62	31.0
Imp. French × 6-22-51[f]	4	5.36	2.06	4.21	6.25	17.9	3.2	0.51	23.7
Imperial × 6-17-1	3	4.60	1.48	4.57	5.48	16.1	3.2	0.48	45.0
Primacotes half sib	7	5.16	2.16	4.68	3.56	15.2	3.0	0.68	31.4

[a]Unselected, self-rooted seedlings.

[b]Parentage: standard × 'Primacotes'

[c]Parentage: open-pollinated 'Burton'.

[d]Includes '5-19-39' × '6-15-62' data.

[e]5 includes 'Imp. French' × 6-22-51 data.

The relative concentrations of the individual sugars and sorbitol varied significantly in the two years of analysis (Figure 3). Figure 3 depicts the year to year variations in fruit sugar concentrations among the 'D6N 103' × 'Muir beauty' seedlings as well as how the sugars vary in range according to the different sugar types. These data are consistent with reports for peaches, where sugar concentrations were influenced by the seasonal environment (Brooks et al., 1993; Cantin et al., 2009). In 2010, California had a cool spring and summer, and 2011 had a wet spring and hot summer. The trendline for fructose in Figure 3 was the shortest, whereas sucrose had the longest, indicating that there was a wider spread of variability within the population for sucrose as opposed to fructose. This also potentially indicates that there is a greater potential to breed for increased sucrose than for increased fructose in the germplasm we are working with.

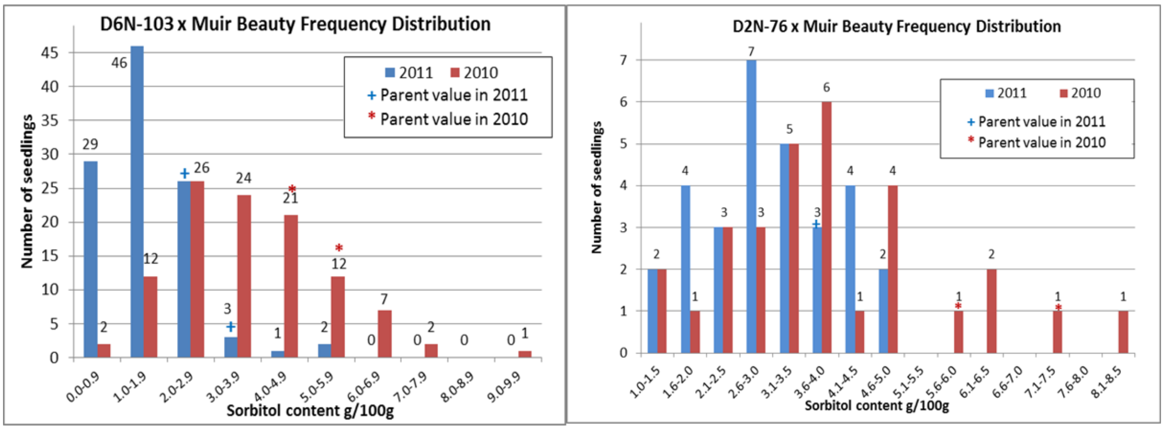

Figure 2. Sorbitol frequency distribution for 'D6N-103' × 'Muir Beauty' seedlings, *n*=107 (left) and for 'D2N-76' × 'Muir Beauty' seedlings, *n*=30 (right). Median data for 2010 and 2011 unselected, own-rooted, seedling progeny were 3.58 and 1.58 g 100 g^{-1} (left) and 3.89 and 3.02 g 100 g^{-1} (right), respectively. Fruit on the 'Muir Beauty' grafted parental tree was 5.8 and 3.8 g 100 g^{-1} in 2010 and 2011, respectively. The fruit on the 'D6N-103' grafted parental tree (left) was 4.6 and 2.7 g 100 g^{-1} in 2010 and 2011, respectively. The fruit on the 'D2N-76' grafted parental tree (right) was only tested in 2010 and had a sorbitol value of 7.3 g 100 g^{-1}.

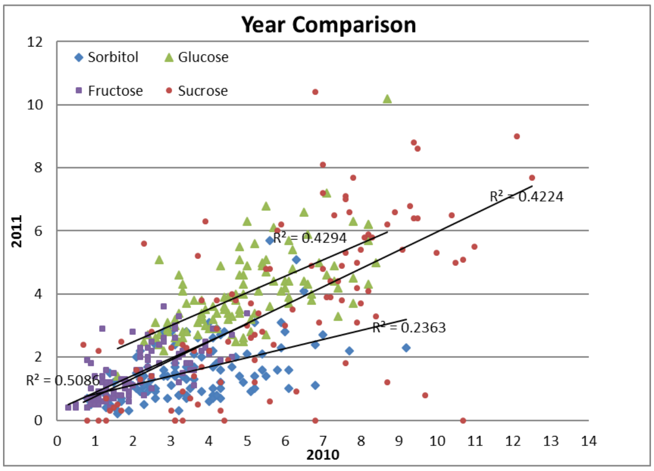

Figure 3. A year comparison of 2010 and 2011 for the 'D6N-103' × 'Muir Beauty' seedling soluble solid data. R^2 values are as follows: Sucrose=0.4224, Sorbitol=0.2363, Glucose=0.4294, and Fructose=0.5086.

Others have reported that individual sugars relative to the total sugar (sugar profile) are fairly consistent from year to year (Cantin et al., 2009; Castro et al., 2016). The frequency distribution for sorbitol when evaluating the percentage of total sugars (glucose, fructose, sucrose, and sorbitol) varied less from 2010-2011 among seedlings for both crosses. In both crosses, the parents had higher relative values of sorbitol than the majority of seedlings, but as with the concentrations, there were still a few seedlings with an increase in sorbitol as compared to the parents. Similarly, sucrose had no apparent distribution peak when evaluating the percent of total data, but despite the high variability, most of the seedlings had higher sucrose percentages than the parents. For breeders, the percent of total sugars may be a better evaluation method because the year to year variation should be less. Thus, the year to year sugar ratios within the total soluble solids appear to be relatively consistent for an individual. Therefore, there appears to be a moderate breeding potential for increasing both sorbitol and sucrose. The frequency distribution for sorbitol indicates a few items with small gains per generation, and sucrose has such variability, an item with higher sucrose than the parents appears easily attainable.

The yearly variations observed in the seedlings used in this study are likely due to annual variations in weather patterns, fruit crop loads, and fruit maturity. Fruit crop loads were not adjusted in the seedling orchards in this study but were more consistently managed in the selection/cultivar orchards. An additional factor in environmental variability might be the harvest date. When using a fruit firmness measurement to determine the ripeness, specific fruits might have already passed maturity or were not quite ready to be harvested. With large phenotypic variation among siblings, flesh textures are quite variable so flesh firmness might not be the best indicator of maturity for all genotypes. Other factors such as the initiation of fruit drop and rate of change in fruit flesh firmness might be additional indicators. As Usenik et al. (2008) showed, the harvest date is an important factor in obtaining the optimal sugar levels within the fruit. In the breeding program, we are selecting more and more for items that partially dry on the tree. These items do not soften as readily as conventional prunes. Some trees have fruit that will never get really soft because they lose fruit water and flesh pressure increases as the fruits dry on the tree. These fruit textures or differences in fruit softening patterns due to differences in flesh consistencies are not addressed in this paper but might have been an extraneous factor in determining harvest timing for some genotypes.

CONCLUSIONS

- The germplasm collection in our program contains genotypes with a wide range of sugar profiles.
- Fruit sorbitol concentrations of the breeding program parents tend to be greater than progeny means but there still appears to be some potential to select new genotypes with even greater sorbitol concentrations.
- Year to year variations in sucrose concentrations relative to total sugars is greater than for sorbitol and the other reducing sugars.
- Year to year variations in sugar profiles and concentrations complicates the process of breeding new cultivars with consistent sugar profiles.

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