Europ.J.Hort.Sci., **78** (2). S. 56–66, 2013, ISSN 1611-4426.

Genetic Parameters for Sugar Content in an Interspecific Pear Population

W. Viera Arroyo[1)], P. Alspach[2)], L. Brewer[2)], J. Johnston[3)] and C. Winefield[4)]
([1)]Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP), Quito, Ecuador, [2)]The New Zealand Institute for Plant and Food Research Limited (PFR), Motueka Research Centre, Motueka, New Zealand, [3)]PFR, Mt Albert Research Centre, Auckland, New Zealand and [4)]Lincoln University, Lincoln, New Zealand)

Summary

Fruit quality and flavour are important targets in all pear breeding programmes. Perceived sweetness is directly influenced by the amount and type of sugar accumulated in fruit. Limited information is available on sugar composition in pear fruit and published studies have been completed using cultivars rather than breeding populations. The objective of this research was to determine the quantitative genetic parameters of sugar content in fruit of interspecific hybrids from families making up a pear breeding population. Glucose, fructose, sucrose and sorbitol contents were measured in mature fruit. Most of the sugars, except for sorbitol, showed genetic variability and a relatively high (i.e., > 0.5) ratio between the estimated additive genetic variance and the total variance. Sorbitol showed a high negative genetic correlation (−0.65) with fructose. It could be suggested that the main product of sorbitol conversion was fructose. Sucrose showed a negative genetic correlation with glucose (−0.37) and fructose (−0.16), which would be expected given that sucrose is metabolised into fructose and glucose. Two parents with 100 % European parentage showed the highest empirical breeding values (eBV)s for fructose and total sugars. The parent with 100 % Asian parentage showed the lowest eBV for sorbitol. The mean percentages of the sugars across the entire population were: glucose 13 %, fructose 59 %, sucrose 8 % and sorbitol 20 %, indicating fructose was the main sugar with sucrose less prominent.

Key words. fructose – genetic correlation – glucose – sorbitol – sucrose

Introduction

Pears are cultivated principally for the fresh market and the canning industry (Jackson 2003), with about 80 % of the total production destined for fresh consumption (Itai 2007). Pear cultivars have been categorized into two principal groups: European (*Pyrus communis*) and Asian types (Barbosa et al. 2010) which in this study included Japanese pear (*P. pyrifolia*) and Chinese pear (*P.* × *bretschneideri*). Worldwide, there are numerous pear breeding programmes that aim to improve fruit quality (Hancock and Lobos 2008; Brewer and Palmer 2011). Sugars, organic acids, amino acids and aromatic compounds all influence the flavour of fruit (Itai et al. 2010). The sugar composition (e.g., sucrose, glucose, fructose and sorbitol) and amount accumulated in fruit directly influence the perceived sweetness (Doyon et al. 1991; Itai et al. 2010). Within the Rosaceae family, studies about genetic parameters of sugars (principally heritability) have been carried out in crops such as apple (Kouassi et al. 2009), peach (Brooks et al. 1993) and plum (Nikolic et al. 2007), but only limited research has been carried out for pears. In fact, there is little information about estimates of genetic parameters of sugars in pear fruit (Kajiura et al. 1979) and there is also limited knowledge on sugar composition of phloem sap and unloading into pear fruit. The most closely studied system is apple (Suni et al. 2000; Klages et al. 2001; Zhang et al. 2004; Gao et al. 2005; Teo et al. 2006). Related available information in pear fruit refers to sugar accumulation (Richmond et al. 1981; Wrolstad and Shallenberger 1981), fruit sweetness (White et al. 2000) and soluble solids contents (Machida and Kozaki 1976; Shi et al. 2007; Shin et al. 2008). Moreover, pear studies about sugar accumulation have been carried out on individual cultivars only. Differences in sugar composition have been reported between cultivars of Japanese (Moriguchi et al. 1992; Shi et al. 2007; Choi et al. 2009; Itai et al. 2010), Chinese (Moriguchi et al. 1992) and European pears (Hudina and Stampar 2000b). Although interspecific hybrids are widely

used in pear breeding programmes to introduce novel traits (White et al. 2000; Costes et al. 2005; Shin et al. 2008; Brewer and Palmer 2011), to date there have been no studies investigating sugar content in interspecific hybrids between European and Asian pears. Some studies have shown that estimates for interspecific populations can differ markedly from those for intraspecific ones (Alspach and Oraguzie 2002; Connor et al. 2005).

Genetic parameters of the different sugars accumulated in the pear fruit are relevant given the importance of sugar composition to fruit quality, in particular perceived sweetness. A marker has been developed for Japanese pear linked to the *PpAIV2* gene, which is associated with the sucrose content of ripening fruit in their later stages of development, specific to cultivars with low sucrose content. This marker could be used for selection of high sucrose types with good taste (Itai et al. 2010). Fructose in equal amounts is sweeter than both glucose and sucrose (Harker et al. 2002; Rizkalla 2010). Fructose has received considerable negative media attention because of its links to health risks and obesity, especially when used as a bulk sweetener in drinks because it bypasses the intake regulatory system. However, there is no evidence linking amounts of less than 50 g of fructose per day to harmful effects or obesity in humans (Rizkalla 2010). Moreover, this sugar is acceptable for diabetics (Hudina and Stampar 2000b). The objective of this research was to determine the quantitative genetic parameters of sugar content and composition in fruit of interspecific hybrids from two pear breeding populations. These populations provide a unique genetic resource consisting of interspecific crosses from three *Pyrus* species to create a degree of diversity not apparent in crosses involving individual *Pyrus* species (White et al. 2000; Brewer et al. 2008). This approach will result in a more generic understanding of sugar composition in pears that spans three *Pyrus* species and their hybrids, and advances information from previous studies that used small numbers of genotypes and genotypes from individual species.

Materials and Methods

Pear population

Two adjacent breeding populations, one with 17 families planted in 2007 and the other with 20 families planted in 2008, were used for this research. The number of seedlings per family varied considerably (3-1291 in the 2007 population; 10-591 in the 2008 population). For each population, a subset of the seedlings from as many families as possible was planted in a randomised block design with ten full-sib plants per plot and between one and six (four in 2008) plots per family. These subsets were embedded in the planting of the remaining trees. The seedlings were planted at the Motueka Plant & Food Research Station (41° 6' S 172° 58' E) on their own roots at 3 m between rows and 0.75 m within rows. The aim was to evaluate ten fruit from each of 10 to 15 seedlings, chosen from the designed subset, per family. However, given the close planting and age of the seedlings, it was not possible to include all families nor achieve the desired number of seedlings in all cases, although all families had at least 90 fruit evaluated (Table 1). When there were more than 15 usable seedlings, a random selection was chosen from each plot.

The 20 chosen families were derived from interspecific hybrids between European, Japanese and Chinese pears (Table 1). Each parent was related to at least one of the others and 58 % of possible pairings showed some degree of relationship (data not shown). Thus, almost all the families assessed had some degree of relationship between them, except family C10 with families C8 and C9. Although only six families showed no inbreeding, the degree of inbreeding of the others was generally low (median inbreeding coefficient = 0.10, maximum 0.30).

For each selected seedling, the trunk circumference was measured (cm) and the total number of fruit counted to calculate the number of fruit per trunk cross-sectional area (TCA). The target crop load was set at 2.5 fruit per TCA (cm^2) and excess fruit were removed in mid-December. After thinning, the range for fruit per TCA for the selected seedlings varied from 0.3 to 2.5. Harvesting was carried out weekly from the end of January to March. When fruit were deemed mature according to a combination of indicators (skin colour, skin finish, seed colours and fruit firmness), 10 to 20 fruit from each seedling were harvested into labelled paper bags (one bag per seedling). The paper bags containing fruit were then placed in a cool store for 30 days at 0 ± 0.5 °C.

Preparation of samples for chemical analyses

After the 30 days of storage, each fruit was cut in half horizontally about the region of maximum diameter where the sugar content is considered to be more stable (Wang and Sheng 2005), and a 1-cm slice was cut from the upper half. Using a stainless steel corer (1.1 cm in diameter), two subsamples of flesh (without skin) were taken from the first slice, one from each side to account for blush/shade side effects on carbohydrate composition. The subsamples from all the fruit from a seedling were immediately placed in small plastic containers (50 ml) that were filled with liquid nitrogen. Frozen samples were ground using a batch mill chilled with liquid nitrogen (A11 basic analytical mill). Four 2-second pulsations (28000 rotations per minute) were made per sample resulting in the production of a fine powder, in frozen form. Finally, approximately 0.2 g of powder was placed in tubes containing 5 ml of ethanol (80 %) and stored at −20 ± 1 °C.

Sugar analyses

Sugar analyses were carried out using high performance liquid chromatography (HPLC) (Wasik et al. 2007). The

Table 1. Parentage and percentage of European, Japanese and Chinese pear for the families evaluated in the pear population, and the numbers of plots, seedlings and fruit assessed for each family.

Family	Parents (Mother × Father)	Parentage (%)			Number of		
		European	Japanese	Chinese	Plots	Seedlings	Fruit
C1	P9 × 'Conf.'	75	0	25	2	7	140
C2	P3 × P11	69	13	18	3	10	94
C3	P13 × 'Conf.'	63	25	12	1	5	99
C4	P13 × P1	63	25	12	2	10	98
C5	P1 × P13	63	25	12	4	15	150
C6	P12 × P1	63	25	12	6	15	144
C7	P4 × P11	56	25	19	2	8	154
C8	P6 × P7	50	38	12	2	7	137
C9	P2 × P17	50	25	25	3	11	110
C10	P17 × 'Conf.'	50	25	25	2	8	150
C11	P8 × P11	44	13	43	3	16	154
C12	P11 × P8	44	13	43	2	14	137
C13	P12 × P5	38	50	12	4	15	148
C14	P12 × P9	38	25	37	5	15	150
C15	P9 × P13	38	25	37	3	6	106
C16	P14 × P11	31	38	31	1	5	93
C17	P12 × P11	31	38	31	5	15	150
C18	P15 × P11	31	38	31	2	8	157
C19	P13 × P11	31	38	31	3	15	149
C20	P16 × P10	31	25	44	2	6	120
Mean		48	26	26			
Total					57	211	2640

'Conf.' = 'Conference'

stored samples were filtered through a 0.45 μm nylon membrane to obtain a filtrate. After filtration, 1200 μl of adonitol (Sigma-Aldrich, USA, ≥ 99.0 %) at a concentration of 400 μg ml^{-1} was added as an internal standard to 400 μl of each sample to make a final volume of 1600 μl in a vial. Finally, samples were analysed by HPLC. The HPLC analysis system consisted of a LC-20 AD Prominence Liquid Chromatograph (Shimadzu Corp., Kyoto, Japan), a SiL-10 AF Auto Sampler (Shimadzu Corp., Kyoto, Japan), a CTO – 10 Asvp Column Oven (Shimadzu Corp., Kyoto, Japan) and an Alltech 3300 ELSD Detector (Grace Division Discovery Sciences, Australia). A Prevail Carbohydrate ES 5 μ analysis column (250 mm × 4.6 mm) (Grace Division Discovery Sciences, Australia) and a Prevail Carbohydrate ES 5 μ guard column (Grace Division Discovery Sciences, Australia) were used. A multi-point calibration approach was applied to obtain standard calibration curves for the sugars. A calibration curve was generated extending from 10 to 1000 μg ml^{-1}. The standards used for the sugar analyses were: D-glucose (Merck, USA, ≥ 99.5 %), sucrose (Fisons, England, ≥ 99.0 %), D-fructose (Univar, New Zealand) and D-sorbitol (Sigma-Aldrich, USA, ≥ 99.5 %). Finally, chromatograms were registered for each sugar, and the result was expressed in mg g^{-1} of fruit fresh weight.

Variables

The different sugars (glucose, fructose, sucrose, sorbitol and total sugars) constituted the variables assessed. Variables were measured on individual seedlings. Square root transformations were applied to glucose and sorbitol measurements before analysis to achieve normality of the residuals.

Statistical analysis

The linear mixed model approach was used to fit the plant model (Lynch and Walsh 1998), with the general mean as the only fixed effect:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Zu} + \mathbf{e},$$

where y is the 211 × 1 vector of observations on individual seedlings, **X** and **Z** are the incidence matrices for the fixed and random effects respectively (dimensions 211 × 1 and 211 × q assuming n observations) and **e** is the 211 × 1

vector of residuals. **e** is distributed with mean 0 and variance-covariance matrix $\mathbf{R} = \sigma^2\mathbf{I}$ where σ^2 is the residual variance and **I** is the identity matrix. **u** is distributed, independently of **e**, with mean 0 and variance-covariance matrix $\mathbf{G} = \sigma_A{}^2\mathbf{A}$ where $\sigma_A{}^2$ is the additive genetic variance and **A** is the numerator relationship matrix (i.e., twice the coancestry matrix). For simplicity, the design was considered to be completely randomised, which is not an unreasonable approximation given the selection process undertaken for the evaluated seedlings. Two random effects models were tested: both had genotypes (seedlings) with pedigree information but one incorporated family to check for specific combining ability effects whereas the other did not. Since the two models had the same fixed effects and were nested, the likelihood ratio test (LRT) was used to compare the two models, in conjunction with graphical comparisons (e.g., scatter plots of progeny mean empirical breeding values (eBV) on the mid-parental eBV). Separate univariate analyses were undertaken for each variable. Variance components for the random effects and empirical breeding values (i.e., best linear unbiased predictors, BLUP) were estimated directly from the model. The former were used to compute the ratio between the estimated additive genetic variance and the total variance ($R_{G:T}$). In balanced experiments without correlation between relatives this would estimate the narrow sense heritability. However, in unbalanced situations with correlation between relatives this is not the case (Piepho and Möhring 2007). Phenotypic correlations were computed as the Pearson's correlation coefficients between the raw data (transformed where appropriate) and genetic correlations were approximated as the correlation between the eBV. Standard errors for the estimates of $R_{G:T}$ were obtained using the jack knife method (Buzas 1997), which involved successively dropping each family from the calculations.

All statistical analyses and graphs were carried out using R 2.13.0 (R. Development Core Team 2011), and the mixed models were fitted using the asreml-r package (Butler 2009).

Results

The simple family means of the untransformed variables showed a high degree of variability between the families (Table 2). The range was between 26 % (total sugars) and 196 % (sucrose) of the overall mean.

Table 2. Mean values per pear family for sugars.

Family	Glucose	Glucose*	Fructose	Sucrose	Sorbitol	Sorbitol*	Total Sugars
				(mg g^{-1})			
C1	14.3	3.77	65.5	2.84	26.0	5.05	109
C2	13.7	3.66	55.0	11.19	18.5	4.22	98
C3	10.6	3.22	64.9	11.49	16.2	4.01	103
C4	12.3	3.48	64.3	9.65	25.7	4.99	112
C5	11.7	3.38	66.9	16.56	18.0	4.11	113
C6	**10.2**	**3.17**	75.1	12.51	19.5	4.29	**117**
C7	10.6	3.23	60.6	7.93	22.5	4.68	102
C8	15.7	3.92	56.0	3.41	17.3	4.10	92
C9	16.0	3.95	**75.4**	5.86	18.5	4.20	116
C10	12.6	3.52	72.0	4.75	**15.8**	**3.91**	105
C11	13.2	3.60	55.0	12.56	**27.9**	**5.23**	109
C12	12.7	3.52	**50.1**	**17.91**	23.8	4.81	104
C13	12.8	3.54	64.0	7.55	23.6	4.79	108
C14	18.0	4.22	67.2	4.54	24.9	4.93	115
C15	18.0	4.17	55.2	3.02	16.4	4.00	93
C16	13.8	3.67	56.4	**2.30**	18.1	4.22	**91**
C17	12.6	3.53	53.2	9.08	18.9	4.32	94
C18	12.6	3.53	50.6	6.22	25.9	5.07	95
C19	18.1	4.23	63.7	2.88	23.6	4.77	108
C20	**18.9**	**4.33**	56.4	6.86	23.6	4.81	106
Mean	13.9	3.68	61.4	7.96	21.2	4.53	104

* Square root transformed before computing the means.
Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Random effects

For glucose, the likelihood ratio test indicated that the simpler model (e.g., that without family in the random effects) was adequate (P > 0.05). For fructose, sucrose, sorbitol and total sugars, the LRT indicated that including family in the model resulted in a statistically significant improvement (P = 0.005, 0.008, 0.023 and 0.020 respectively). However, for the simpler model (e.g., excluding family) there was a strong correlation between mid-parent eBV and the progeny mean eBV (Fig. 1). Furthermore, the family variance was less than one fifth of the additive genetic variance in most of the cases. Therefore, specific combining affects were not considered important for breeding and the simple model was deemed adequate for the analysis of all variables.

Empirical breeding values

The range for the parental eBV was between 21 % (total sugars) and 281 % (sucrose) of the overall mean (Fig. 2).

Variance components

Values for genetic variances were larger than the residual ones for most of the variables except for sorbitol and total sugars (Table 3). The ratio $R_{G:T}$ (Table 3) varied widely from 0.18 (sorbitol) to 0.84 (glucose).

Genetic and phenotypic correlations

In two-thirds of the cases, genetic correlations were larger than the corresponding phenotypic ones (Table 4).

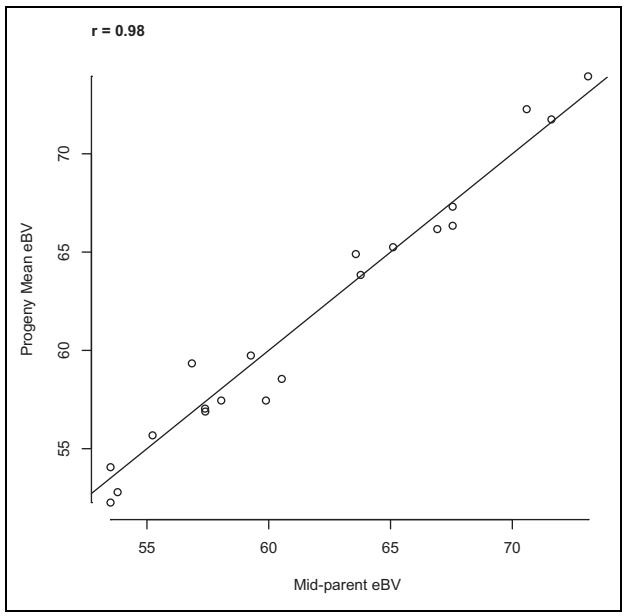

Fig. 1. Scatterplot showing the relationship between the pear progeny means eBV and the mid-parent eBV for fructose using the model without family in the random effects. Each point presents a family and the solid line is that of equality. The correlation coefficient is 0.98.

The two sets of correlations (e.g., genetic and phenotypic) showed a relatively high correlation (0.64). The highest positive genetic correlation (0.76) was between fructose and total sugars whereas the highest negative correlation (−0.65) was between fructose and sorbitol.

Discussion

Combining ability

The pear seedlings evaluated in this study constituted a breeding population. All the parents were related to at

Table 3. Variance components and the ratio $R_{G:T}$ for the variables evaluated in the pear population. The jack knife standard errors for $R_{G:T}$ are also shown, along the minimum and maximum values obtained on excluding successive families.

	Glucose*	Fructose	Sucrose	Sorbitol*	Total Sugars
Variance					
Total	0.44	146	70.6	0.83	274
Genetic	0.37	79	53.7	0.15	84
Residual	0.07	67	16.9	0.68	190
Ratio between genetic and total variance, $R_{G:T}$					
$R_{G:T}$	0.84	0.54	0.76	0.18	0.31
s.e.	0.20	0.24	0.27	0.17	0.31
Min $R_{G:T}$	0.74	0.49	0.66	0.07	0.23
Max $R_{G:T}$	0.94	0.74	0.96	0.26	0.57

* Square root transformed before analysis.

Table 4. Genetic correlations (upper triangle) and phenotypic correlations (lower triangle) between all pairs of pear traits.

	Glucose*	Fructose	Sucrose	Sorbitol*	Total Sugars
Glucose*	–	−0.03	−0.37	0.13	−0.06
Fructose	0.21	–	−0.12	−0.65	0.76
Sucrose	−0.21	−0.26	–	0.10	0.36
Sorbitol*	0.01	−0.03	−0.01	–	−0.25
Total Sugars	0.33	0.67	0.24	0.48	–

* Square root transformed before analysis.

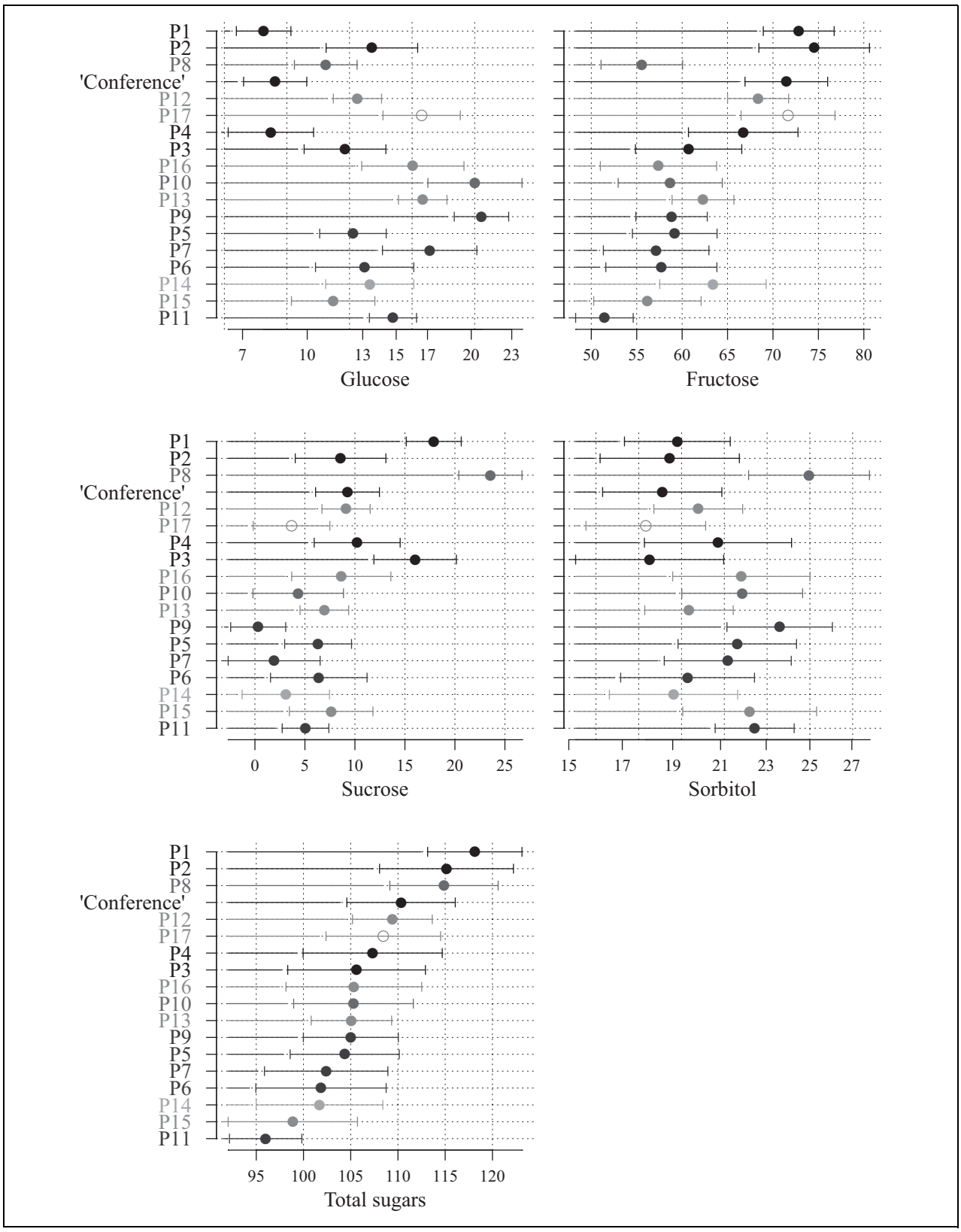

Fig. 2. Parental pear eBV for the four individual sugars and total sugars. Parents have been sorted by total sugars. Grey scale represents the parentage from 100 % European (black) to 100 % Asian (white; e.g., the open circle of P17). All sugars measured in mg g^{-1}; note the square-root scale for glucose and sorbitol. Horizontal bars show one standard error each way.

least one of the others and most families were slightly inbred. Although, for some of the sugars, there was a statistically significant difference in the LRT test comparing the models with and without family, the magnitude of the specific combining effect was low. For all variables examined, at least one of the top ranked parents was a parent of the top ranked families and similarly for the bottom ranked parents and families.

Sugars

Sugars are distributed by a system of sieve elements (phloem) to the fruit (Teo et al. 2006) and they are mainly accumulated (more than 80 %) in vacuoles in the cells of the pear fruit (Yamaki et al. 1993). Nevertheless, sugars sequestered in vacuoles easily efflux into the cytosol and the free space, where they are also accumulated, because of the leakiness of the membrane encouraged by fruit maturation and ripening (Yamaki et al. 1993; Yamaki 1995). Sugar accumulation is important for sweetness and fruit quality (Yamaki 2010). Values for genetic variances were superior to the environmental ones for all the sugars, except for sorbitol. The latter is supported by Hudina and Stampar (2000a), who stated that sorbitol content is influenced by various environmental factors such as stress (water stress and defoliation), foliar fertilization and regular water supply. The relatively high $R_{G:T}$ for fructose, glucose and sucrose may indicate that good progress could be made breeding for high concentrations of these sugars, although there may be a trade-off between sucrose and glucose. However, as indicated by Piepho and Möhring (2007), this should be verified by estimating genetic gain directly. Nevertheless, these results suggested that glucose, fructose and sucrose contents are influenced by genetic conditions. The genetic variability observed in this dataset for accumulation of the different sugars analysed offers significant opportunities for selection and recombination of these desirable sugars such as sorbitol, sucrose and fructose, which particularly contribute to the flavour of the fruit and are important for diabetic nutrition when sorbitol and fructose dominate the sugar types (Hudina and Stampar 2000b). Sorbitol, sucrose, fructose and glucose are the major storage sugars in mature pear fruit (Kajiura et al. 1979; Yamaki and Moriguchi 1989), with fructose and sorbitol the most abundant in this study. While these four sugars also occur in the fruit of other *Rosaceae* species such as apple, the relative abundance of sorbitol and sucrose differs. In apple fruit, sorbitol is the least abundant of these sugars, while sucrose is the second most abundant (Suni et al. 2000; Klages et al. 2001; Beruter 2004; Gao et al. 2005). In the present study, the opposite was found for pears, where sucrose was the least abundant and sorbitol the second most abundant (Table 2). The results for fructose and glucose are similar between apples and pear, with fructose being the most abundant sugar in both systems, and glucose concentrations being second lowest. These results highlight important differences in carbohydrate metabolism within the Rosaceae, in particular in sorbitol and sucrose metabolism.

Sorbitol was the only one of the four sugars to have a $R_{G:T}$ of less than 0.5. The difference between the highest parental eBV for sorbitol (e.g, P8 and P9 at $\approx$ 24 mg g^{-1}) and the lowest (e.g., P3 and P17 at $\approx$ 19 mg g^{-1}) was small relative to the other sugars. Sorbitol is the principal transport sugar that occurs in leaves of the Rosaceae family where it represents around 80% of the total sugars (Choi et al. 2009). It is loaded into phloem and then translocated to the fruit (Zimmermann and Ziegler 1975), where it is unloaded into the parenchyma tissue (Yamaki 2010). Sorbitol is actively converted into other sugars after unloading in the fruit (Yamaki and Moriguchi 1989; Moriguchi et al. 1992). Sorbitol taken up in the cytosol is converted to fructose, facilitated by the action of the enzyme sorbitol dehydrogenase (Yamaki 1995, 2010; Beruter 2004; Kanayama 2009), while sorbitol unloaded apoplastically is metabolised to glucose by sorbitol oxidase localized on the cell wall (Yamaki 1995). The genetic correlation between sorbitol and fructose (−0.65) suggests that sorbitol dehydrogenase activity was present. Moreover, given the negligible genetic correlation between sorbitol and glucose (0.13), it could be suggested that the main product of sorbitol conversion was fructose, as has been reported in apple (Beruter 2004) and other fruit crops (Bieleski and Redgwell 1980, as cited in Hu et al. 1997).

The amounts of sorbitol measured in the present study for pears were three times higher than those reported for apple (Suni et al. 2000) where excessive sorbitol accumulation is associated with the core tissue developing a glassy appearance (Gao et al. 2005). The greater accumulation of sorbitol in pears relative to apples could be for many reasons, including: 1) more sorbitol being transported to the fruit in the phloem; 2) greater capacity to unload sorbitol into the fruit; and 3) less capacity to metabolise sorbitol in the fruit. Further research is needed to determine the sugar composition of phloem exudate and to determine how much of the sorbitol is located in the apoplast relative to the vacuole. Yamaki and Moriguchi (1989) found that the conversion of translocated sorbitol to fructose in Japanese pear fruit showed a fluctuating pattern. It was high in young fruit, decreased with fruit enlargement and increased again with fruit maturation.

Another sugar translocated to the pear fruit is sucrose (Zimmermann and Ziegler 1975; Yamaki 2010). It is generated in leaves, translocated to fruit flesh through the phloem and unloaded into the parenchyma tissue. Its accumulation in fruit is driven by specific sucrose-metabolic pathways (Yamaki 2010). The highest parental eBVs for sucrose (> 16 mg g^{-1}) were registered by P1, P3 and P8 while the lowest (< 3.5 mg g^{-1}) were found for P7, P9 and P14. Sucrose is converted into fructose and glucose by the action of invertase or to fructose and UDP-glucose

by sucrose synthase (SS) before being taken into the cell (Yamaki 1995, 2010; Rolland et al. 2006). An important source of UDP-glucose for sucrose synthesis can occur from starch degradation (Beruter 2004). Thus, it is likely that the final sucrose concentration is a combined contribution from starch degradation and from sucrose stored in the soluble form. At harvest, Asian pear fruit have completed starch hydrolysis in contrast to both apples and European pears which are harvested with high starch content commonly determined from a starch pattern index. In this research, harvest was undertaken at the green-yellow skin colour change, which is later than normal harvest timing for a European pear for long-term storage, and the starch would have been partially hydrolysed. In the European cultivar 'La France', starch conversion was almost complete after 13 days of storage at 1 °C before ethylene production began (Murayama et al. 2002). The families in this study that include European parentage are expected to be segregating for fruit starch content at harvest, but the starch would have all been hydrolysed within the 30-day storage period. The biochemical breakdown products of starch are initially glucose then sucrose, as during this process sucrose-synthesizing enzymes are at maximum concentrations and sucrose is depleted (Franck et al. 2006).

European parents were grouped with higher eBVs for sucrose accumulation. Sucrose showed a negative phenotypic and genetic correlation with glucose (−0.21 and −0.37 respectively), which would be expected given that when the amount of glucose increases in the fruit, sucrose decreases because of its conversion to glucose. Fructose is also produced during sucrose conversion but phenotypic and genetic correlations with sucrose, whilst still negative (−0.26 and −0.12 respectively), were minor probably because of the added fructose generated by sorbitol conversion. Moriguchi et al. (1992) stated that sucrose accumulation is regarded as a function of SS and sucrose phosphate synthase (SPS) formation, since the reduction of the activity of invertase is not correlated with sucrose accumulation in mature fruit of Japanese pear. On the other hand, the same author suggested that little sucrose is accumulated in Chinese pear fruit because a reduction of the activity of invertase occurs. In this study, the overall percentage of sucrose registered in the fruit was low (8 %), which is supported by Fourie et al. (1991), who found that European and Asian pears do not accumulate sucrose. Reasons for lower sucrose accumulation in pears (Table 2) compared with apples (Suni et al. 2000) are yet to be established, but differences in the amounts of starch accumulation and activity of enzymes and transporter proteins related to sucrose metabolism are likely candidates. However, Yamaki (1995) suggested that SS and SPS seem to be the key enzymes for sucrose accumulation in some Japanese and Chinese pear.

Fructose positively influences the perception of sweetness in the fruit (Itai et al. 2010) because it has a higher sweetness index than sucrose and glucose (Harker et al. 2002; Rizkalla 2010). It may also contribute to human health through enhancing the growth of colonic flora and has a lower glycaemic potential than glucose or sucrose (Suni et al. 2000). Parents P1, P2, P17 and 'Conference' had the highest eBVs (> 75 mg g^{-1}) for this sugar, whereas P11 showed the lowest (≈ 50 mg g^{-1}). We suggest that the major proportion of this sugar in the fruit came from the sorbitol conversion rather than sucrose conversion. This sugar showed the highest percentage (59 %) in the fruit, which corresponds with the findings made by Fourie et al. (1991), who stated that high amounts of fructose are found in European and Asian pears.

Glucose influences plant development and metabolism through its sugar signalling effects (Rolland et al. 2006). Furthermore, this sugar enhances human health by improving fructose absorption (Suni et al. 2000). For this sugar, the highest eBVs (> 17 mg g^{-1}) were estimated for parents P9 and P10 while the lowest (≈ 7 mg g^{-1}) were showed by 'Conference', P1 and P4. Glucose showed a negative genetic correlation with sucrose, which suggests that the major proportion of this sugar in the fruit came from the sucrose conversion rather than sorbitol conversion. Overall, the genetic correlations estimated between sugars in this study (except between sucrose and fructose) were similar to the correlations reported by Kajiura et al. (1979).

For total sugars, parents P1 and P2 showed the highest eBVs (> 115 mg g^{-1}), while parents P11 and P15 had the lowest eBV (< 100 mg g^{-1}). We assume that fructose was the main sugar influencing the amount of total sugars in the fruit, because of the high genetic correlation (0.76) observed between fructose and total sugars.

Although the parents of the populations studied here were not intended to be representative of European or Asian types, some interesting differences were observed. Parents with both European and Asian pear heritage showed the highest eBVs for sorbitol (P8 and P9), sucrose (P8) and glucose (P9 and P10) while similar parents showed the lowest eBVs for sucrose (P7 and P9), fructose (P8 and P11), and total sugars (P11 and P15). P1 and P2, both with 100 % of European parentage, showed the highest eBVs for fructose and total sugars. The parent with 100 % Asian parentage showed the lowest eBV for sorbitol; however, it was within the top three parents for fructose and within the top five parents for glucose. It is noticeable that P8 registered high sorbitol and sucrose contents and low fructose content, which may suggest that the conversion of both sugars to fructose was not prevalent in this particular parent. Kajiura et al. (1979) defined some varieties of Japanese pear as high-sucrose-accumulating types and some varieties of Chinese pear as low-sucrose-accumulating types. In this study, parents P1 and P3, which showed the highest eBV for sucrose had 100 % European parentage but parent P8, which also showed a high eBV for this sugar, had more Chinese than

Japanese parentage. One of the bottom three parents that showed the lowest eBV for sucrose had more Chinese parentage (P9), another one had more Japanese parentage (P14) and the third one (P7) had the same proportions of Japanese and Chinese parentage. Therefore, the tendency observed by KAJIURA et al. (1979) cannot be applied to these results.

MORIGUCHI et al. (1992) and HUDINA and STAMPAR (2000b) assessed different cultivars of European, Japanese and Chinese pears for sugar content. In comparison to the results obtained in this study, both sets of researchers found lower percentages of fructose (44 % and 49 % cf. 59 %), higher sucrose (25 % and 14 % cf. 8 %) and similar glucose (13 % and 11 % cf. 13 %). However, MORIGUCHI et al. (1992) reported lower sorbitol (18 %) than either HUDINA and STAMPAR (2000b) (23 %) or our study (20 %). Although CHOI et al. (2009) suggested that sorbitol appears to be degraded once it has been transported to the fruit, the percentage of this sugar registered by HUDINA and STAMPAR (2000b) and in our research was relatively high. YAMADA et al. (2006) studied the cultivar 'La France' and reported similar amounts to those found in this study. Overall, fructose was the predominant sugar in the pear fruit, as has also been reported in apple (SUNI et al. 2000).

Conclusion

Despite the diverse genetic material included in this study (three species), the relative abundance of each sugar was consistent in that fructose was always the most abundant sugar and sucrose the least abundant sugar. Larger differences in relative abundance occur once comparisons are made with other Rosaceae species. In particular, major differences in relative abundance of sucrose and sorbitol occur between apple and pear. Empirical breeding values for all the sugars were highest for those parents with 100 % European parentage and some parents with a mixture of Asian and European parentage. The results indicate that interspecific hybrids between European and Asian species will not be an impediment to breeding for high sugar contents. However, further research on fundamental aspects of carbohydrate translocation, storage and metabolism are needed to advance knowledge of pears and to contribute to a wider understanding in *Rosaceae* species.

Acknowledgments

The authors thank Joy Jiao and Roger Cresswell from Lincoln University for their assistance in the sugar analyses, and Chris Morgan and Marlene Aldworth from Plant & Food (Motueka) for helping in the sample preparation. The first author thanks the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) for funding his studies at Lincoln University.

References

ALSPACH, P.A. and N.C. ORAGUZIE 2002: Estimation of genetic parameters of apple (*Malus domestica*) fruit quality from open-pollinated families. N.Z. J. Crop Hort. Sci. **30**, 219–228.

BARBOSA, W., I.M. MURATA, A.F.C. TOMBOLATO, L.M.M. MELETTI, R.F.A.VEIGA and C.V. POMMER 2010: Asian pear breeding for subtropical areas of Brazil. Acta Hort. **864**, 81–86.

BERUTER, J. 2004: Carbohydrate metabolism in two apple genotypes that differ in malate accumulation. J. Plant Physiol. **161**, 1011–1029.

BREWER, L.R., C. MORGAN, P.A. ALSPACH, R.K. VOLZ and A.G. WHITE 2008: Interspecific pear breeding for flavour and texture. Acta Hort. **800**, 461–468.

BREWER, L.R. and J.W. PALMER 2011: Global pear breeding programmes: Goals, trends and progress for new cultivars and new rootstocks. Acta Hort. **909**, 105–119.

BROOKS, S.J., J.N. MOORE and J.B. MURPHY 1993: Quantitative and qualitative changes in sugar content of peach genotypes (*Prunus persica* (L.) Batsch.). J. Am. Soc. Hort. Sci. **118**, 97–100.

BUTLER, D. 2009: asreml: asreml() fits the linear mixed model. R. package version 3.00.

BUZAS, J.S. 1997: Fast estimators of the jackknife. Am. Stat. **51**, 235–240.

CHOI, J., J. CHOI, C.J. BANG, J. LEE, D. CHOI, H. CHO and J. AHN 2009: Changes of sugar composition and related enzyme activities during fruit development of Asian pear cultivars 'Niitaka' and 'Whangkeumbae'. Hort. Environ. Biotechnol. **50**, 582–587.

CONNOR, A.M., T.K. MCGHIE, M.J. STEPHENS, H.K. HALL and P.A. ALSPACH 2005: Variation and heritability estimates of anthocyanins and their relationship to antioxidant activity in a red raspberry factorial mating design. J. Am. Soc. Hort. Sci. **130**, 534–542.

COSTES, E., A. ACOU, A. BELOUIN, M.L. LEZEC and M.H. SIMARD 2005: Heritability of morphological and architectural characters in three pear progenies. Acta Hort. **671**, 223–227.

DOYON, G., G. GAUDREAU, D. STGELAIS, Y. BEAULIEU and C.J. RANDALL 1991: Simultaneous HPLC determination of organic-acids, sugars and alcohols. Can. Inst. Food Sci. Technol. J. **24**, 87–94.

FOURIE, P.C., C.F. HANSMANN and H.M. OBERHOLZER 1991: Sugar content of fresh apples and pears in South Africa. J. Agric. Food Chem. **39**, 1938–1939.

FRANCK, C., N. SCHEERLINCK and B.M. NICOLAI 2006: Modelling metabolite changes during 'Conference' pear fruit development. Acta Hort. **707**, 261–268.

GAO, Z.F., S. JAYANTY, R. BEAUDRY and W. LOESCHER 2005: Sorbitol transporter expression in apple sink tissues: Implications for fruit sugar accumulation and watercore development. J. Am. Soc. Hort. Sci. **130**, 261–268.

Hancock, J.F. and G.A. Lobos 2008: Pears, In: Hancock, J.F. (ed.): Temperate fruit crop breeding. Springer, Michigan, United States. 299–335.

Harker, F.R., K.B. Marsh, H. Young, S.H. Murray, F.A. Gunson and S.B. Walker 2002: Sensory interpretation of instrumental measurements 2: Sweet and acid taste of apple fruit. Postharvest Biol. Technol. **24**, 241–250.

Hu, H.N., S.G. Penn, C.B. Lebrilla and P.H. Brown 1997: Isolation and characterization of soluble boron complexes in higher plants: The mechanism of phloem mobility of boron. Plant Physiol. **113**, 649–655.

Hudina, M. and F. Stampar 2000a: Free sugar and sorbitol content in pear (*Pyrus communis* L.) cv. 'Williams' during fruit development using different treatments. Acta Hort. **514**, 269–274.

Hudina, M. and F. Stampar 2000b: Free sugar and sorbitol content of pear cultivars (*Pyrus communis* L. and *Pyrus serotina* Rehd.). Zbornik Biotehniske Fakultete Univerze v Ljubljani Kmetijstvo **75**, 109–114.

Itai, A. 2007: Pear, In: Kole, C. (ed.): Genome mapping and molecular breeding in plants: Fruits and nuts. Springer, Berlin, 157–170.

Itai, A., H. Irie, R. Hatanaka, T. Tanahashi and K. Ikeda 2010: Development of DNA markers for fruit sugar composition in pear. In: XI International Pear Symposium (Abstracts). ISHS, Patagonia, Argentina, 14.

Jackson, J.E. 2003: Biology of apples and pears. Cambridge University Press, Cambridge, England.

Kajiura, I., S. Yamaki, M. Omura, T. Akihama and Y. Machida 1979: Historical changes in the sugar composition of East Asian pears and varietal classification by principal component analysis of sugar composition. Jpn. J. Breed. **29**, 1–12.

Kanayama, Y. 2009: Physiological roles of polyols in horticultural crops. J. Jpn. Soc. Hort. Sci. **78**, 158–168.

Klages, K., H. Donnison, J. Wünsche and H. Boldingh 2001: Diurnal changes in non-structural carbohydrates in leaves, phloem exudate and fruit in 'Braeburn' apple. Aust. J. Plant. Physiol. **28**, 131–139.

Kouassi, A.B., C.E. Durel, F. Costa, S. Tartarini, E.D. Weg, K. Evans, F. Fernandez-Fernandez, C. Govan, A. Boudichevskaja, F. Dunemann, A. Antofie, M. Lateur, M. Stankiewicz-Kosyl, A. Soska, K. Tomala, M. Lewandowski, K. Rutkovski, E. Zurawicz, W. Guerra and F. Laurens 2009: Estimation of genetic parameters and prediction of breeding values for apple fruit-quality traits using pedigreed plant material in Europe. Tree Genet. Genomes **5**, 659–672.

Lynch, M. and B. Walsh 1998: Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, England.

Machida, Y. and I. Kozaki 1976: Quantitative studies on fruit quality in Japanese pear breeding: Statistical analysis of a hybrid seed population. J. Jpn. Soc. Hort. Sci. **44**, 325–329.

Moriguchi, T., K. Abe, T. Sanada and S. Yamaki 1992: Levels and role of sucrose synthase, sucrose-phosphate synthase, and acid invertase in sucrose accumulation in fruit of Asian pear. J. Am. Soc. Hort. Sci. **117**, 274–278.

Murayama, H., S. Ikai and T. Fukushima 2002: Changes in starch content in 'La France' pears during storage. Acta Hort. **596**, 871–874.

Nikolic, D., V. Rakonjac, M. Milutinovic and M. Fotiric 2007: Variability and heritability of morphological and chemical fruit properties in cherry plum (*Prunus cerasifera* Ehrh.). Vocarstvo **41**, 45–49.

Piepho, H.P. and J. Möhring 2007: Computing heritability and selection response from unbalanced plant breeding trials. Genetics **177**, 1881–1888.

R. Development Core Team 2011: The R project for statistical computing (Version 2.13.0). R Foundation for Statistical Computing, Vienna, Austria.

Richmond, M.L., S.C.C. Brandao, J.I. Gray, P. Markakis and C.M. Stine 1981: Analysis of simple sugars and sorbitol in fruit by high-performance liquid chromatography. J. Agric. Food Chem. **29**, 4–7.

Rizkalla, S.W. 2010: Health implications of fructose consumption: A review of recent data. Nutr. Metab. **7**, 82–98.

Rolland, F., E. Baena-Gonzalez and J. Sheen 2006: Sugar sensing and signaling in plants: Conserved and novel mechanisms. Annu. Rev. Plant Biol. **57**, 675–709.

Shi, Z., M. Dai, T. Sun and S. Zhang 2007: Comparative studies on the accumulation process of the pigments, sugar and acid in fruit of Cuiguan and Yuguan pear cultivars. J. Fruit Sci. **24**, 833–836.

Shin, I.S., Y.U. Shin and H.S. Hwang 2008: Heritability of fruit characters of interspecific hybrids between *Pyrus pyrifolia* and *P. ussuriensis* or *P. breschneideri*. Acta Hort. **800**, 535–540.

Suni, M., M. Nyman, N.A. Eriksson, L. Björk and I. Björck 2000: Carbohydrate composition and content of organic acids in fresh and stored apples. J. Sci. Food Agric. **80**, 1538–1544.

Teo, G., Y. Suziki, S.L. Uratsu, B. Lampinen, N. Ormonde, W.K. Hu, T.M. Dejong and A.M. Dandekar 2006: Silencing leaf sorbitol synthesis alters long-distance partitioning and apple fruit quality. Proc. Natl. Acad. Sci. USA **103**, 18842–18847.

Wang, J. and K.C. Sheng 2005: Variations in firmness and sugar content in 'Huanghua' pear (*Pyrus pyrifolia* 'Nakai'). J. Hort. Sci. Biotechnol. **80**, 307–312.

Wasik, A., J. McCourt and M. Buchgraber 2007: Simultaneous determination of nine intense sweeteners in foodstuffs by high performance liquid chromatography and evaporative light scattering detection – Development and single-laboratory validation. J. Chromatogr. A. **1157**, 187–196.

White, A.G., L.R. Brewer and P.A. Alspach 2000: Heritability of fruit characteristics in pears. Acta Hort. **538**, 331–337.

Wrolstad, R.E. and R.S. Shallenberger 1981: Free sugars and sorbitol in fruits – a compilation from the literature. J. Assoc. Off. Anal. Chem. **64**, 91–103.

Yamada, K., Y. Suzue, S. Hatano, M. Tsukuda, Y. Kanayama, K. Shiratake and S. Yamaki 2006: Changes in the activity and gene expression of sorbitol- and sucrose-related enzymes associated with development of 'La France' pear fruit. J. Jpn. Soc. Hort. Sci. **75**, 38–44.

Yamaki, S. and T. Moriguchi 1989: Seasonal fluctuation of sorbitol-related enzymes and invertase activities accompanying maturation of Japanese pear (*Pyrus serotina* Rehder Var Culta Rehder) fruit. J. Jpn. Soc. Hort. Sci. **57**, 602–607.

Yamaki, S., M. Ino, S. Ozaki, Y. Tsuchimoto and J. Ofosu-Anim 1993: Cellular compartmentation and transport into tonoplast vesicles of sugars with ripening of pear fruit. Acta Hort. **343**, 12–17.

Yamaki, S. 1995: Physiology and metabolism of fruit development – Biochemistry of sugar metabolism and compartmentation in fruits. Acta Hort. **398**, 109–120.

Yamaki, S. 2010: Metabolism and accumulation of sugars translocated to fruit and their regulation. J. Jpn. Soc. Hort. Sci. **79**, 1–15.

Zhang, L.Y., Y.B. Peng, S. Pelleschi-Travier, Y. Fan, Y.F. Lu, Y.M. Lu, X.P. Gao, Y.Y. Shen, S. Delrot and D.P. Zhang 2004: Evidence for apoplasmic phloem unloading in developing apple fruit. Plant Physiol. **135**, 574–586.

Zimmermann, M.H. and H. Ziegler 1975: List of sugars and sugar alcohols in sieve-tube exudates. In: Zimmermann, M.H. and J.A. Milburn (ed.): Transport in plants: Phloem transport. Springer-Verlag, Berlin, 480–503.

Received 09/27/2012 / Accepted 12/06/2012

Addresses of authors: William Viera Arroyo (corresponding author), Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP), Av. Eloy Alfaro 30-350 y Av. Amazonas, 4th floor, Quito, Peter Alspach and Lester Brewer, The New Zealand Institute for Plant & Food Research Limited (PFR), Motueka Research Centre, Old Mill Road, R D 3, Motueka 7198, New Zealand, Jason Johnston, The New Zealand Institute for Plant & Food Research Limited (PFR), Mt Albert Research Centre, Private Bag 92169, Auckland Mail Centre, Auckland 1142, New Zealand, and Christopher Winefield, Lincoln University, P O Box 84, Lincoln 7647, New Zealand, e-mail (corresponding author): william.viera@iniap.gob.ec