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Candidate genes and QTLs for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]

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Abstract The identification of genes involved in variation of peach fruit quality would assist breeders in creating new cultivars with improved fruit quality. Major genes and quantitative trait loci (QTLs) for physical and chemical components of fruit quality have already been detected, based on the peach [*Prunus persica* (L.) Batsch] cv. Ferjalou Jalousia® (low-acid peach) × cv. Fantasia (normally-acid nectarine) F_2 intraspecific cross. Our aim was to associate these QTLs to structural genes using a candidate gene/QTL approach. Eighteen cDNAs encoding key proteins in soluble sugar and organic acid metabolic pathways as well as in cell expansion were isolated from peach fruit. A single-strand conformation polymorphism strategy based on specific cDNA-based primers was used to map the corresponding genes. Since no polymorphism could be detected in the Ferjalou Jalousia® × Fantasia population, gene mapping was performed on the almond [*Prunus amygdalus* (*P. dulcis*)] cv. Texas × peach cv. Earlygold F_2 interspecific cross from which a saturated map was available. Twelve candidate genes were assigned to four linkage groups of the peach genome. In a second step, the previous QTL detection was enhanced by integrating anchor loci between the Ferjalou Jalousia® × Fantasia and Texas × Earlygold maps and data from a third year of trait assessment on the Ferjalou Jalousia® × Fantasia population. Comparative mapping allowed us to detect a candidate gene/QTL co-location. It involved a cDNA encoding a vacuolar H^+-pyrophosphatase (*PRUpe;Vp2*) that energises solute accumulation, and QTLs for sucrose and soluble solid content. This preliminary result may be the first step in the future development of marker-assisted selection for peach fruit sucrose and soluble solid content.

Keywords *Prunus* · Fruit quality · Sugar and organic acid metabolism · Candidate genes · QTL · Comparative mapping · Single-strand conformation polymorphism (SSCP)

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Introduction

Among temperate fruit crops, the peach (*Prunus persica* L. Batsch) breeding industry is one of the most dynamic (Fideghelli et al. 1998). Peach breeders continuously release new commercial cultivars, most of which are tasty and aromatic if ripened on the tree. However, in the last decade, the consumption of raw peaches and nectarines in the European Union and in the United States has not increased. This trend is largely due to the low quality of fruit that are harvested at an immature stage for storage and shipment reasons. Consequently, in the peach industry, the major breeding objective is to find the right compromise between quality and immaturity at harvest (Fideghelli et al. 1998). Public research organisations also have breeding programmes, using wild peach-related species, where the main objective is resistance to pests and diseases (Kervella et al. 1998). However, wild peach-related species produce fruits of low quality. Therefore, a better knowledge of the genetic and molecular basis underlying fruit quality would benefit peach breeding programmes.

Soluble sugars and organic acids are important components of fruit taste and, in combination with aromas, have an impact on the overall organoleptic quality of peaches. Fruit taste depends on the content and type of

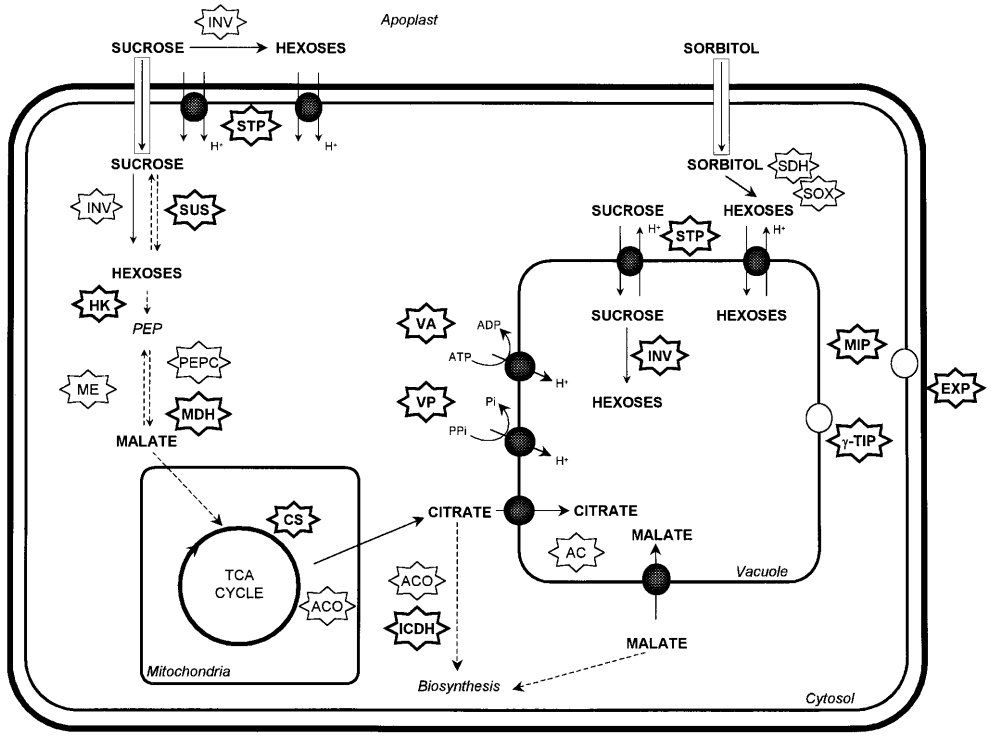

Fig. 1 A simplified model showing the involvement of the candidate genes isolated from peach fruit (*bold stars*) in the main pathways conditioning sugar and organic acid accumulation in peach fruit. Sucrose unloading from the phloem may occur (1) through the plasmodesmata, (2) by means of a plasmalemma sucrose transporter (*STP*) or (3) following hydrolysis by a cell-wall invertase (*INV*) and consecutive transport of the resulting hexoses through a plasmalemma hexose transporter (*STP*). Similar pathways are hypothesised for sorbitol unloading. In the cytosol, sucrose can be metabolised by invertase (*INV*) or sucrose synthase (*SUS*) and sorbitol by sorbitol dehydrogenase (*SDH*) or sorbitol oxydase (*SOX*). Hexokinase (*HK*), which controls the first step of glycolysis, may also be involved in sugar sensing. Malic acid synthesis mainly occurs in the cytosol by phospho*enol*pyruvate carboxylase (*PEPC*) and NAD-dependent malate dehydrogenase (*MDH*), while citric acid synthesis takes place in the mitochondria through the tricarboxylic acid cycle (*TCA*) via mitochondrial citrate synthase (*CS*) and aconitase (*ACO*). Vacuolar H^+-ATPase (*VA*) and vacuolar H^+-pyrophosphatase (*VP*) pump protons into the vacuole, providing the driving force for organic acid transport across the tonoplast via putative organic acid channels (*AC*). Soluble sugars may be stored in the vacuole through tonoplastic soluble sugar transporters (*STP*). Malic acid degradation is mainly attributed to cytosolic NADP-dependent malic enzyme (*ME*), while cytosolic aconitase (*ACO*) and NADP-dependent isocitrate dehydrogenase (*ICDH*) both participate in citric acid catabolism. Both organic acids are likely to be utilised for amino acid synthesis. Cell expansion that occurs during the accumulation of sugars and organic acids also involves expansins (*EXP*) for cell-wall loosening and plasmalemma aquaporin (*MIP*) or tonoplast aquaporin (*γ-TIP*) for the influx of water and small solutes

soluble sugars and organic acids (Pangborn 1963). In ripe peaches, as in most fleshy fruits, the main soluble sugars are sucrose, fructose and glucose. The main organic acids are malic and citric acids. Sorbitol and quinic acid are also detected at low levels (Moing et al. 1998). Although fruit pH is a trait whose phenotypic variation is continuous in peach, peach fruits can be grouped into two types according to their pH value: the normally-acid (pH below 4.0) and the low-acid phenotypes (pH above 4.0) (Dirlewanger et al. 1998; Yoshida 1970). A major dominant allele *D* from the honey-type peaches is responsible for low acidity (Monet 1979). The progeny issued from a cross between two peach varieties, a low-acid flat peach, Ferjalou Jalousia, and a normally-acid round nectarine, Fantasia, segregating for fruit quality traits, was used to map the *D* gene and quantitative trait loci (QTLs) controlling various physical and chemical components of peach fruit quality. Furthermore, QTLs for pH, titratable acidity and malic and citric acid contents mapped near the *D* gene (Dirlewanger et al. 1998, 1999). Few other QTLs controlling fruit quality have been mapped in peach. Recently, QTLs for fruit development period, fruit size, pH, soluble sugar and soluble solid contents (SSC) have been detected in this species (Abbott et al. 1998; Quarta et al. 2000). However, the genes controlling QTLs for peach fruit quality remain unknown. Peach stands as the ideal genetic system for the identification and characterisation of important genes in *Rosaceae* species (Sosinski et al. 2000), but genomic tools are still under development for this species. Therefore, among the possible strategies used to identify a gene underlying a QTL, the functional candidate gene approach still appears to be the simplest one for peach (de Vienne et al. 1999). It is based on the a priori choice of genes which may be functionally related to the trait. A correlation between the trait under study and allelic polymorphism at the candidate gene is a strong argument in favour of the candidate gene. If not, the candidate gene is ruled out as being involved in trait variation. For example, the candidate gene approach has already been successful in identifying genes controlling sucrose accu-

mulation (Klann et al. 1996) and fruit ripening (Yen et al. 1995) in tomato fruit and carotenoid accumulation in red pepper (Huh et al. 2001).

Our knowledge of soluble sugar and organic acid accumulation in fleshy fruits has considerably increased over the last decade. Critical steps for soluble sugar and organic acid accumulation in fleshy fruits are schematised in Fig. 1. They include (1) phloem unloading of sucrose or, in species belonging to the *Rosaceae* family like peach, of sucrose and sorbitol into fruit cells (Beruter 1993; Herbers and Sonnewald 1998; Ho 1996); (2) sugar metabolism (Ho 1996; Yamaki and Ishikawa 1986); (3) organic acid metabolism (Etienne et al. 2002; Sadka et al. 2000a; b); (4) solute accumulation into vacuole (Etienne et al. 2002; Martinoia et al. 2000; Müller et al. 1997; Terrier et al. 1998). In addition, other processes that enable cell expansion, such as cell-wall loosening (Cosgrove 2000) and water transport processes (Martinoia et al. 2000); may also be crucial. Moing et al. (1998, 2000) characterised fruit development, organic acid and soluble sugar accumulation in the normally-acid nectarine Fantasia and the low-acid peach Ferjalou Jalousia®. They found that Ferjalou Jalousia® fruits showed a large reduction in malic and citric acid accumulation during the first and the second rapid growth phases compared to Fantasia fruits (Moing et al. 2000). Hexose content remained significantly lower in Ferjalou Jalousia® than in Fantasia throughout fruit development (Moing et al. 2000). Sucrose accumulation during ripening was equal in Ferjalou Jalousia® fruit (Moing et al. 1998) or reduced in Ferjalou Jalousia® fruit (Etienne et al. 2002).

The objective of the present study was to interpret QTLs involved in peach fruit quality in terms of functional candidate genes. We assumed that, among the cDNAs which encode proteins involved in some of the above-mentioned mechanisms, some could be responsible for variation in fruit size and soluble sugar and organic acid contents in peach. In a preliminary step, we isolated six full-length cDNAs from peach fruit that encode key proteins in organic acid metabolism and proton pumping in the vacuole and compared their expression throughout the normal fruit development of low-acid and normally-acid peaches (Etienne et al. 2002). In the present study, we report on (1) the isolation of 12 additional cDNAs from peach fruit encoding proteins participating in sucrose metabolism, cell expansion and water transport processes; (2) the mapping of 12 out of the 18 cDNAs isolated from peach fruit; (3) the re-examination of QTLs for components of peach fruit quality; and (4) a co-location between a cDNA encoding a vacuolar H^+-pyrophosphatase and QTLs for sucrose and soluble solid contents.

Materials and methods

Plant material

Functional cDNAs were isolated from fruits of the peach cultivar Fantasia harvested at 30 and 45 days after blooming (DAB), during the first stage of rapid growth following the method described previously (Etienne et al. 2002).

For linkage mapping, two F_2 populations were used: a peach *Prunus persica*) intraspecific population, Ferjalou Jalousia® × Fantasia, and an almond [*P. amygdalus* (*P. dulcis*)] Texas × peach (*P. persica*) Earlygold interspecific population. Ferjalou Jalousia® produces low-acid flat peaches, while Fantasia produces normally-acid round nectarines. The Ferjalou Jalousia® × Fantasia progeny, including 63 trees, allowed previous QTL mapping for peach fruit quality (Dirlewanger et al. 1999). The highly polymorphic Texas × Earlygold progeny, including 75 trees, was previously used to construct a saturated linkage map for *Prunus* (Joobeur et al. 1998). In the study reported here, we used 52 out of the 75 individuals of the Texas × Earlygold Texas × Earlygold progeny for segregation analysis of the functional candidate genes that showed single-strand conformation polymorphism.

Isolation of candidate genes

Six full-length cDNAs had been isolated earlier. These encode key proteins in organic acid metabolism and solute accumulation (Table 1; Etienne et al. 2002). These genes were found to be differentially regulated during fruit development in both normally-acid (Fantasia) and low-acid (Ferjalou Jalousia®) genotypes (Etienne et al. 2002). In the present study, the transcriptase-polymerase chain reaction (RT-PCR) strategy, with degenerate primers and RNAs extracted from 45 DAB Fantasia fruit, was used to isolate additional cDNAs putatively involved in soluble sugar unloading and metabolism, water transport and cell-wall loosening (Table 1).

Determination of PCR primers

For each of the isolated cDNAs, specific primers were designed to amplify the corresponding peach genomic sequences. In order to increase the detection of polymorphism between Ferjalou Jalousia® and Fantasia peach genotypes, the following rules were applied to the choice of primers. (1) When full-length cDNA sequences were available, the reverse primers were designed in the highly divergent and gene-specific 3'untranslated regions (UTRs). (2) When only partial cDNA sequences were available, primer pairs were designed preferentially in nucleotide regions corresponding to divergent amino acid domains. The deduced amino acid sequence of each partial cDNA was compared to those of the homologous plant proteins using CLUSTAL W (Thompson et al. 1994) and BLOCKMAKER (Henikoff 1995) software. When two or more members of a multigene family were isolated (hexose transporter, hexokinase, NADP-dependent isocitrate deshydrogenase, vacuolar pyrophosphatase and membrane-intrinsic protein), gene-specific primers were designed after a comparison of their nucleotide sequences using pair-wise BLAST (Tatusova and Madden 1999). (3) Primers were further chosen to preferentially amplify genomic regions spanning one or more introns. Putative positions of the introns on peach genes were inferred from their known positions on the homologous genes in other plant species using BLAST (Altschul et al. 1990). Introns and 3' UTR regions are likely to show more polymorphism at the intraspecific level (Anneloor et al. 2001; Brunel et al. 1999).

Detection of polymorphism by single strand conformational polymorphism

The single-strand conformation polymorphism (SSCP) technique (Orita et al. 1989) was used to map the candidate genes in the Ferjalou Jalousia® × Fantasia and Texas × Earlygold F_2 progenies. Twenty nanograms of genomic DNA was amplified by PCR in a 25 µl (final volume) PCR reaction containing 100 m*M* Tris-HCl, pH 8.3, 500 m*M* KCl, 15 m*M* $MgCl_2$, 0.01% gelatin, 200 µ*M* of each dNTP, 5 pmol each primer and 0.25 U *Taq* DNA polymerase (Sigma, St. Louis, Mo.). The PCR conditions were as follows: preliminary denaturation (5 min, 95 °C), followed by 30 cycles consisting of denaturation (30 s, 95 °C); annealing (45 s, 60 °C) and

extension (90 s, 72 °C); and a final extension (5 min, 72 °C). Ten microliters of each amplification was loaded on a 2% agarose gel to control PCR amplification. PCR products were then subjected to SSCP electrophoresis (Bodenes et al. 1996). Electrophoresis was run in a 0.6 × TBE buffer at 16 V/cm and at a constant temperature of 15 °C. SSCP migrations were performed overnight except for *PRUpe;Ai1*, *PRUpe;Mip2* and *PRUpe;Mip3* which were run for 5 h and 30 min. Gels were stained with silver nitrate and dried between plastic sheets.

Inheritance and linkage analysis for SSCP markers

χ^2 tests were carried out to examine whether the observed genotypic frequencies deviated from the 1:2:1 or 3:1 Mendelian ratios. Additionally, when testing a 1:2:1 ratio with one of the observed genotypic frequencies being null, the probability of the observed segregation ratio to differ significantly from the expected codominant segregation ratio was estimated by $Pr[G \geq \chi_{df,\alpha}]$ where G is a log-likelihood ratio, $\chi_{df,\alpha}$ is a variable from the χ^2 distribution with df degrees of freedom (df = 2 for codominant markers) and α is the probability of a Type-I error.

Segregation data for each candidate gene were added to the corresponding dataset of the Texas × Earlygold markers (Joobeur et al. 1998). Linkage analysis was performed using the software package MAPMAKER/EXP v. 3.0 (Lander et al. 1987). The Kosambi function was used to convert recombination units into map distances. Given the distorted segregations, we used the mapping procedure adapted by Joobeur et al. (1998) that minimises the wrong assignment of loci to linkage groups and errors in the estimation of their genetic distances. Genetic distances were first estimated using all markers of each linkage group in the Texas × Earlygold map and then using the markers that compose the framework of each linkage group (Joobeur et al. 1998).

Integration of new RFLP and SSR markers in the Ferjalou Jalousia® × Fantasia map

The Ferjalou Jalousia® × Fantasia map was completed with additional restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers analysed on 63 individuals. RFLP probes from the European *Prunus* Mapping Project (15 probes) and from a differential screening (149 probes named PC) of a peach fruit cDNA library between two early growth stages of the Fantasia variety (Rothan et al. 1998) were analysed on the Ferjalou Jalousia® × Fantasia population. Genomic DNA extraction and hybridisation for the RFLP analysis were performed as for almond (Viruel et al. 1995). RFLP probes were tested for polymorphism using four restriction enzymes (*Hind*III, *Eco*RI, *Hpa*II, *Bgl*II). Twenty-four SSRs from peach were analysed for polymorphism: 16 were developed by Cipriani et al. (1999) (UDP 96-001, -003, -005, -008, -013, -015, -018, -019; UDP 97-401, -402, -403, UDP 98-405, -406, -407, -408, -409) and eight by Sosinski et al. (2000) (pchgms 1, 2, 3, 4 and pchcms 1, 2, 3, 4). SSRs were PCR-amplified as follows: 20 m*M* Tris-HCl, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 200 µ*M* of each dNTP, 0.2 µ*M* of each primer, 0.7 U DNA polymerase (GibcoBRL, Gaithersburg, Md.) and 20 ng of peach genomic DNA in a 15-µl final volume. PCR reactions were performed on a GeneAmp 9600 thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) using an initial denaturation step of 1 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 57 °C, 2 min at 72 °C and a final extension of 4 min at 72 °C. The PCR products were then denatured by the addition of one volume of 95% formamide/dye solution, heated for 5 min at 94 °C, and chilled on ice. Finally 1.5 µl of the denatured preparation was loaded on a 6% polyacrylamide sequencing gel containing 7.5 *M* urea in 0.5 × TBE buffer (90 m*M* Tris, 90 m*M* boric acid, 2 m*M* EDTA). The gels were run for 2 h at 80 W. Following electrophoresis, the gel was silver-stained (Cho et al. 1996).

With the intent of QTL re-examination, a new Ferjalou Jalousia® × Fantasia marker framework map was established using a selected subset of markers, including all codominant markers and the dominant markers that were found to be linked to a QTL in the previous study (Dirlewanger et al. 1999). Linkage analysis was performed as described previously (Dirlewanger et al. 1998).

Quantitative traits assessment

Quantitative traits were measured for three years – 1995, 1996 (Dirlewanger et al. 1999) and 1998 – on the parents (Ferjalou Jalousia® and Fantasia), on the F_1 hybrid and on the Ferjalou Jalousia® × Fantasia progeny. Three traits were noted after observation of the tree (blooming date, fruit maturity date and duration of fruit development) and 12 traits were measured after fruit harvest at maturity. Blooming date corresponded to tree full bloom. Maturity date corresponded to the beginning of fruit softening. The fruit development period was calculated as the number of days between blooming and maturity dates. For all fruit characteristics, four fruits per tree were analysed separately, and pH, titratable acidity, and organic acid and sugar concentrations were measured on fruit juice as described previously (Dirlewanger et al. 1999). The mean value for both parents, the F_1 hybrid and each Ferjalou Jalousia® × Fantasia genotype was calculated for each year.

QTL analysis

First, a QTL search was performed separately for each of the three years using the mean values for the corresponding year. Then, a QTL search was performed on estimated genotypic values calculated as follows. A two-way analysis of variance (ANOVA) using the SAS GLM procedure (SAS 1994) was performed to partition total phenotypic variation into genotype, year and genotype × year interaction effect. It revealed that the major effect was the year effect. Consequently, phenotypic variation was partitioned into genotype and year effects, and for each trait, the estimated genotypic value per individual was assessed from ANOVA after adjustment to the year effect. The mean for the three years of the genotypic components and errors per individual calculated for both parents, the F_1 hybrid and each Ferjalou Jalousia® × Fantasia F_2 genotype was used for trait distribution, correlation calculation and QTL detection. Distribution and Spearman correlation coefficients were calculated with the SAS statistics package.

QTL detection was first performed with simple linear regression and simple interval mapping (IM). Composite interval mapping (CIM) was also performed. We used MAPMAKER/QTL 1.1 (Lincoln et al. 1992a, b) for IM and the QTLCARTOGRAPHER software (Basten et al. 1997) for simple linear regression and CIM. A forward-backward stepwise regression was used for the selection of co-factors. For each trait, the three markers with the highest *P* values were added as co-factors in the CIM step (model 6, using a moving window size of 10 cM). The likelihood value of the presence of a QTL was expressed as the LOD score. We retained an experiment-wise error threshold of 0.05. For each trait, a significance threshold was evaluated by 1,000 permutations of each analysis performed with simple linear regression, which was approximately equivalent to a significance of the *F* statistics at the 5% level. This threshold was also chosen for declaring a putative QTL by simple linear regression, IM and CIM. The part of phenotypic variation explained by the QTL (R^2) was estimated in both IM and CIM models. The estimate of the QTL position is the point where the maximum LOD score was found by IM in the region under consideration. A one-LOD support interval was constructed for each QTL (Lander and Botstein, 1989).

Results

Isolation of partial cDNAs as candidate genes for peach fruit quality

In the present study, we isolated 12 peach fruit cDNAs likely to explain variations in fruit weight and soluble

Table 1 Characteristics of full-length and partial cDNAs isolated from peach fruit

Identity	Enzyme commission number	Accession number	Clone	Type[a]	Related sequence[b]		
					Origin	Accession number	Percentage identity[c]
Soluble sugar unloading							
Sugar transporter	–	AF367454	*PRUpe;Stp1*[d]	p	Tobacco	CAA47324.1	85
		AF367455	*PRUpe;Stp2*[d]	p	Sugarcane	AAA18533.1	92
Soluble sugar metabolism							
Sucrose synthase	2.4.1.13	AF367450	*PRUpe;Sus1*[d]	cy	Watermelon	BAA89232.1	87
Hexokinase	2.7.1.1	AF367451	*PRUpe;Hk1*[d]	cy	Orange	AAG28503.1	88
		AF367452	*PRUpe;Hk2*[d]	cy	Potato	AAF14186.1	83
Invertase	3.2.1.26	AF367453	*PRUpe;Ai1*[d]	v	Carrot	CAA53099.1	77
Organic acid metabolism							
Malate dehydrogenase	1.1.1.37	AF367442	*PRUpe;Mdh1*[e]	cy	Tobacco	CAC12826.1	93
Isocitrate dehydrogenase	1.1.1.42	AF367443	*PRUpe;Icdh1*[e]	cy	Celery	CAA73139.1	91
			PRUpe;Icdh2[e]	cy	Eucalyptus	AAB39248.1	87
Citrate synthase	4.1.3.7	AF367444	*PRUpe;Cs1*[e]	m	Pummelo	AAA82743.1	81
Solute accumulation							
Vacuolar H^+-ATPase	3.6.1.3	AF367445	*PRUpe;AtpvA1*[e]	v csu	Pummelo	BAA87891.1	95
Vacuolar H^+-pyrophosphatase	3.6.1.1	AF367446	*PRUpe;Vp1*[e]	v	Pear	Suzuki et al. 1999	95
		AF367447	*PRUpe;Vp2*[e]	v	Grape	AAF69010.1	89
Water transport							
Gamma tonoplast intrinsic protein	–	AF367456	*PRUpe;gTip1*[d]	v	Grape	AAF78757.1	85
Membrane intrinsic protein	–	AF367457	*PRUpe;Mip1*[d]	p	Cabbage	AAG30607.1	87
		AF367458	*PRUpe;Mip2*[d]	p	Ice plant	AAB18227.1	89
		AF367460	*PRUpe;Mip3*[d]	p	Radish	BAA92261.1	90
Cell wall loosening							
Expansin	–	AF367459	*PRUpe;Exp1*[d]		Sweet cherry	AAG13983.1	99

[a] cy, cytosol; m, mitochondrial; v, vacuolar; p, plasmalemma; csu, catalytic subunit
[b] Most similar amino acid sequence as identified by BLAST searches of the non-redundant sequence database
[c] Percentage sequence identity determined at the amino acid sequence
[d] Partial cDNAs isolated from Fantasia fruits by RT-PCR with degenerated oligonucleotide primers available on request
[e] Full-length cDNAs isolated from Fantasia fruits and described in Etienne et al. (2002)

sugar and organic acid accumulation between Ferjalou Jalousia® and Fantasia fruits. We used the RT-PCR strategy that previously enabled the isolation of six organic acid-related genes from peach fruit (Table 1; Etienne et al. 2002). Newly isolated peach cDNAs include hexose transporters (*PRUpe;Stp1* and *PRUpe;Stp2*), sucrose synthase (*PRUpe;Sus1*), hexokinases (*PRUpe;Hk1* and *PRUpe;Hk2*), vacuolar acid invertase (*PRUpe;Ai1*), membrane-intrinsic proteins (*PRUpe;Mip1*, *PRUpe;Mip2* and*PRUpe;Mip3*), gamma-tonoplast intrinsic protein (*PRUpe;gTip1*) and expansin (*PRUpe;Exp1*) (Table 1). Primers designed for cytosolic aconitase, NADP-dependent malic enzyme, NAD-dependent sorbitol dehydrogenase and plasmalemma sucrose transporter failed to amplify any cDNA by 30 and 45 DAB. Five of the cDNAs were homologous to genes isolated from fruit in other fleshy fruit species (*PRUpe;Icdh1*, *PRUpe;Cs1*, *PRUpe;AtpvA1*, *PRUpe;Vp1* and *PRUpe;Vp2*) (Table 1).

As shown in Fig. 1, the 18 candidate genes isolated from peach fruit and available for gene mapping in our study encode a variety of functions related to cell expansion and organic acid and sugar accumulation: (1) phloem unloading of sugars into fruit cells [hexose transporters (Ho 1996; Williams et al. 2000)]; (2) sugar metabolism and signal [sucrose synthase and hexokinase (Winter and Huber 2000)]; (3) organic acid metabolism [cytosolic NAD-dependent malate dehydrogenase, mitochondrial citrate synthase, cytosolic NADP-dependent isocitrate dehydrogenases (Sadka et al. 2000a, b)]; (4) establishment of an electrochemical gradient across the tonoplast for solute transport into the vacuole [proton pumps: catalytic subunit A of vacuolar H^+-ATPase and vacuolar H^+-pyrophosphatases; (Martinoia et al. 2000; Müller et al. 1997; Terrier et al. 1998)]; (5) hydrolysis of sucrose to hexoses in the vacuole [vacuolar invertase (Harada et al. 1995; Klann et al. 1996)]; (6) water and small solute transport [membrane intrinsic proteins and gamma-Tip (Martinoia et al. 2000); (7) cell-wall extension [expansin (Cosgrove 2000)].

Detection of candidate gene polymorphism by SSCP

We developed an SSCP strategy with gene-specific primers applied to both the Ferjalou Jalousia® × Fantasia and

Table 2 Primer-pair sequences used to amplify the genes, cDNA and genomic fragment size, annealing temperatures and population in which polymorphism was detected

Gene	Primer sequence	cDNA fragment size[a] (bp)	Genomic DNA fragment size[b] (bp)	T[c] (°C)	Segregating population
PRUpe;Mdh1	F 5′-CCG AGG GCA CCT GGG TTT CCA TGG-3′ R 5′-CCG CAG ATT GTA GAC ACC ATT ATG ACC-3′	601	700	60	Texas × Earlygold
PRUpe;Cs1	F 5′-ACG ATA CTC ATG TCA ACG GGA GTT TGC-3′ R 5′-AGA CAG AGC AGT AAA ACA CCC TGA GC-3′	546	1,200[d]	60	Texas × Earlygold
PRUpe;Icdh1	F 5′-AGG GTT CAC CAG AAA GGA GGT GAA ACC-3′ R 5′-AGG ACA AAC TTG CTT GCG GAA GC-3′	527	1,100[d]	60	Texas × Earlygold
PRUpe;Icdh2	F 5′-TTT TTG TAC CTG ATG GGC CC-3′ R 5′-GGC CCG ACC ATA ACC TCC TT-3′	295	800[d]	50	None
PRUpe;Stp1	F 5′-GCT CGT CCC CAG TGA AAT CT-3′ R 5′-GGA CAT CAC GAA CAA AGA AC-3′	151	400[d]	50	None
PRUpe;Stp2	F 5′-GAC AGT GCC AAG CGA GAT AT-3′ R 5′-CCT TGG TTC CAG GCA GGA AC-3′	188	200	50	None
PRUpe;Ai1	F 5′-CAA CGG CGT GTG GAC CGG TCT TCG-3′ R 5′-TCG GGG CAT TCC CAC ATG CCC GTA CC-3′	377	400	60	Texas × Earlygold
PRUpe;Sus1	F 5′-CGC GTC AAG ACA ATG TCT TGG GTA CC-3′ R 5′-CAT GGC TAT AAG ATC AGC TGT AAA TTG G-3′	550	1,600[d]	50	None
PRUpe;Hk1	F 5′-GAA ATG CAC GCT GGC CTC GCA TCC-3′ R 5′-TCT CCC ACC ACA TCT TGC TCA ACC-3′	438	1,500[d]	60	Texas × Earlygold
PRUpe;Hk2	F 5′-TCC GTG TGC TGA GGG TGC AAT TGG-3′ R 5′-GTA GAA GAC CAT GCC ATT TTG GTA TTG C-3′	500	>2,000[d]	60	None
PRUpe;AtpvA1	F 5′-TGT AGG AAA GGA TGC TTT GGC TGA AGG-3′ R 5′-CAA CTA GAA CAA CTG CCA CAT GAT CAA AGC-3′	549	1,500[d]	50	Texas × Earlygold
PRUpe;Vp1	F 5′-TCC ACC GGG TGC CCT TGT CAT TCT TAC C-3′ R 5′-GGA AGA GGG AAG AGA GAT ACT GGT AC-3′	528	800[d]	60	Texas × Earlygold
PRUpe;Vp2	F 5′-TGA TGC TTC CCT CAA GGA GAT GAT CC 3′ R 5′-CAT CAT GAA AGT ACT GAT TAG GGT GAA GC-3′	506	700[d]	60	Texas × Earlygold
PRUpe;Mip1	F 5′-GTT GTT GAA AAT CAC AGC AGG ACC AAA GC-3′ R 5′-GAG GTC TCG TTG ATC GAG CTG TGG C-3′	369	>2,000[d]	50	None
PRUpe;Mip2	F 5′-CTC GCA AGG TTT CAC TGC CGA GGG CAG-3′ R 5′-ACT TCT AGC AGG GTT GAT ACC AGT TCC-3′	346	500[d]	60	Texas × Earlygold
PRUpe;Mip3	F 5′-GTA TGG TGG AGG GGC CAA TGG GCT TGC-3′ R 5′-GGC TTC TTG CTG GGT TTA TGC CAG TGC-3′	238	250	60	Texas × Earlygold
PRUpe;Tip1	F 5′-GCC CAG TTG CTT GGA GCC GTG ATT GC 3′ R 5′-CCA GCT GAC GAC AGC GGG TCC GAA GG-3′	312	400	60	Texas × Earlygold
PRUpe;Exp1	F 5′-AAC AAT GGC TTG GGC TGT GGT TCT TGC-3′ R 5′-CTG GCC CCA ATT CCT GGA CAT TGC-3′	399	500[d]	60	Texas × Earlygold

[a] The distance between the primers at the cDNA level was deduced from the peach cDNA sequence
[b] The distance at the genomic level was estimated by gel electrophoresis of amplified fragments
[c] Annealing temperature in degrees Centigrade
[d] Indicates the supposed presence of introns in the amplified product as deduced from comparisons with plant genomic sequences available in GenBank database

the Texas × Earlygold mapping populations. Eighteen gene-specific PCR primer pairs were designed (Table 2). Each produced a single amplified product from the total genomic DNA of both parents and the F_1 hybrids. The length of the PCR products was similar in all parental genotypes. Most primer pairs generated products larger than expected from the transcribed sequence, indicating the presence of introns (Table 2). The length of the genomic PCR products ranged from 250 to more than 2,000 bp. No polymorphism was detected by SSCP analysis within the genomic PCR products in the Ferjalou Jalousia® × Fantasia population. In contrast, polymorphism was detected in the Texas × Earlygold population for 12 out of the 18 candidate genes tested (Table 2). Two or four bands were obtained on SSCP gels, and the segregation observed in the progeny was consistent with the patterns observed in the parents. The electrophoretic profiles could be genetically interpreted following Bodenes et al. (1996).

Assignment of candidate genes to linkage groups

The 12 candidate genes were assigned to four linkage groups of the Texas × Earlygold map. Map distances from the closest reference markers are given in Table 3 in relation with the anchor loci from the Texas × Earlygold map. *PRUpe;Mdh1* and *PRUpe;Exp1* mapped to linkage group 1. The *PRUpe;Mdh1* position on linkage group 1 was in good agreement with that of malate dehydrogenase isozyme locus MDH1 on the Ferjalou Jalousia® × Fantasia map. *PRUpe;Cs1* and *PRUpe;Ai1* mapped to linkage group 2. In contrast with *PRUpe;Cs1*, *PRUpe;Ai1* had a severely distorted ratio with a signifi-

Table 3 Observed phenotypic ratios, log likelihood ratios (G), and test probabilities [Pr(G>χ^2)] for SSCP markers for fruit quality-related genes segregating in almond the cv. Texas × peach cv. Earlygold F_2 population

Gene	cDNA	Phenotypic ratio[a]			G	Pr[G>χ^2]	Linkage group	Closest reference markers (cM)
		T/T	T/E	E/E				
Malate dehydrogenase	*PRUpe;Mdh1*	7	26	13	2.61	0.11	1	AG109 (5.0)
Expansin	*PRUpe;Exp1*	6	27	16	5.23	0.02	1	LY5B (5.1); MC001 (4.1)
Citrate synthase	*PRUpe;Cs1*	8	31	8	4.87	0.03	2	AC31 (6.0)
Invertase	*PRUpe;Ai1*	3	31	11	11.44	0.0007	2	CC125 (14.6)
Vacuolar H^+-ATPase	*PRUpe;AtpvA1*	9	16	15	3.13	0.08	3	*PRUpe;Icdh1* (21)
Isocitrate dehydrogenase	*PRUpe;Icdh1*	6	27	11	3.79	0.05	3	*PRUpe;AtpvA1* (21); CC127 (10)
Hexokinase	*PRUpe;Hk2*	11	25	8	1.30	0.25	3	AG7 (4.1); *PRUpe;Vp1* (8.7)
Vacuolar H^+-pyrophosphatase	*PRUpe;Vp1*	10	33	6	7.03	0.01	3	*PRUpe;Hk2* (8.7); CC116 (3)
Gamma tonoplast intrinsic protein	*PRUpe;Tip1*	15	20	8	2.37	0.12	6	AG54 (11.4); *PRUpe;Mip2* (7.5)
Membrane intrinsic protein	*PRUpe;Mip2*	13	22	7	1.92	0.17	6	*PRUpe;Tip1* (7.5); AC50 (3.2)
	PRUpe;Mip3	3	20	23	18.23	<0.0001	6	PC21 (10.2); *Ltp2* (13.3)
Vacuolar H^+-pyrophosphatase	*PRUpe;Vp2*	0	27	32	44.79	<0.0001	6	FG209A (0)

[a] T/T Almond cv. Texas homozygotes; T/E, heterozygotes; E/E, peach cv. Earlygold homozygotes

cant excess of heterozygotes as was observed in most skewed loci from linkage group 2 (Joobeur et al. 1998). *PRUpe;AtpvA1*, *PRUpe;Icdh1*, *PRUpe;Hk2* and *PRUpe;Vp1* mapped to linkage group 3. The *PRUpe;Icdh1* position on linkage group 3 was in good agreement with isocitrate dehydrogenase isozyme locus *Idh-2* in the same map. In contrast with *PRUpe;Hk2*, *PRUpe;Vp1* had a significant distorted ratio ($\alpha = 0.01$). Thus, *PRUpe;Vp1* was added after *PRUpe;Hk2* to the framework map, as described in Joobeur et al. (1998). *PRUpe;tip1*, *PRUpe;Mip2*, *PRUpe;Mip3* and *PRUpe;Vp2* mapped to linkage group 6. *PRUpe;Mip3* and *PRUpe;Vp2 loci* had severely distorted ratios with strong selection against almond Texas alleles (*PRUpe;Vp2* completely lacked almond Texas homozygotes). *PRUpe;Vp2* mapped at one extreme of linkage group 6, in a distorted region that was previously associated with the position of the self-incompatibility locus (SI) of almond totally linked to the RFLP marker FG209A (Joobeur et al. 1998).

Integration of codominant markers to the Ferjalou Jalousia® × Fantasia linkage map

Six polymorphic RFLP loci (PC115, PC128, PC164, PC172, FG209A) were detected using 164 probes (Fig. 4). Marker FG209 was of particular interest since it constitutes an anchor point on linkage group 6 within the Texas × Earlygold map where it was found totally linked to *PRUpe;Vp2*. Out of the 24 SSRs screened, five were polymorphic in the Ferjalou Jalousia® × Fantasia population. Each SSR locus mapped to a distinct linkage group. Four SSRs were from the $(AG/CT)_n$-enriched library (Cipriani et al. 1999). UDP98-407 showed a slight departure from the 1:2:1 segregation ratio ($P = 0.021$). Previous results indicated that the major gene controlling the non-acid trait and QTLs involved in peach fruit acidity are linked to dominant markers on linkage groups 5 (the 'non-acid' trait) and 6 (R16-1.2, AA-CAT9). SSRs UDP97-401 and UDP98-407 mapped respectively in the neighbourhood of each of these genomic regions controlling peach fruit acidity. Codominant markers (UDP97-401 and UDP98-407) and dominant markers (the non-acid trait and AA-CAT9) were used in QTL re-examination.

The Ferjalou Jalousia® × Fantasia map previously used for QTL detection included 249 markers (including 29 codominant markers) mapped to 11 linkage groups and covering 712 cM (Dirlewanger et al. 1999). In the present study, we constructed a framework map including 40 codominant (one isozyme, 34 RFLPs, 5 SSRs) and 29 dominant markers [11 RFLPs, 12 AFLPs, 1 inter-microsatellite amplifications (IMA), 1 random amplified polymorphic DNA (RAPD) and four morphological traits: flat (S), non-acid (D), peach (G), male sterility (Ps)]. All markers coalesced into eight linkage groups (G1–G8, Fig. 4), the basic chromosome number of peach. No marker was found to be independent, suggesting that the map was saturated. The eight linkage groups spanned 564 cM, and the average distance between markers was 9.2 cM. The linkage group sizes ranged from 31 cM to 117 cM.

Trait variation and correlations

A significant year effect was detected for all traits (data not shown). The distribution of the different traits was calculated using the mean over 3 years of estimation of genotypic values. Distributions of the estimated genotypic means of traits for which QTLs were detected are presented in Figs. 2, 3. Only a few traits showed a normal distribution; these were fruit fresh weight, soluble solid content, quinic acid, sucrose and glucose content. Based on the overall estimates of genotypic values, fruits from the normally-acid genotype Fantasia had significantly higher fresh weight, titratable acidity, malic acid and citric acid content, and significantly lower pH and sucrose content than that of Ferjalou Jalousia.

The Spearman correlation coefficients calculated between genetic estimates are shown in Table 4. Maturity

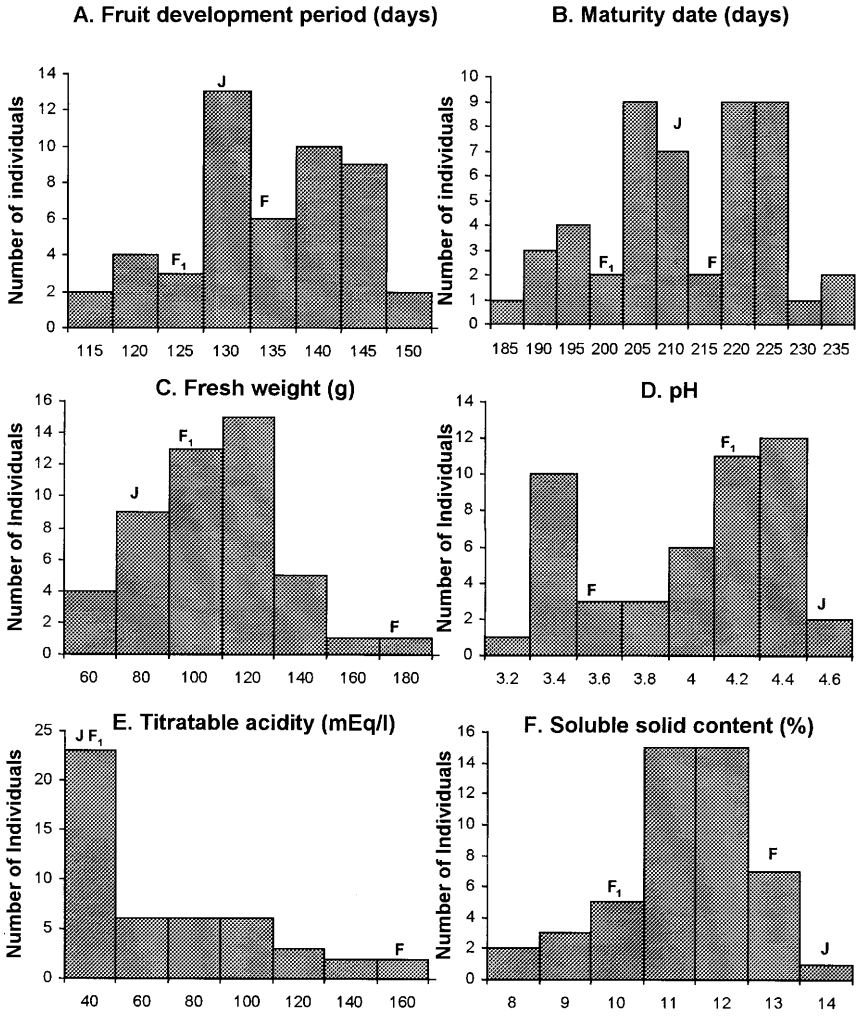

Fig. 2A–F Distribution of estimated genotype means for agronomic characters measured on the peach Ferjalou Jalousia® × Fantasia F_2 progeny for 3 years. **A** Fruit development period, **B** maturity date, **C** fruit fresh weight, **D** fruit juice pH, **E** fruit juice titratable acidity, **F** fruit juice soluble solid content. The values of the parents, Ferjalou Jalousia, and Fantasia, and of the F_1 individual are indicated by a *J*, *F* and F_1, respectively

Table 4 Spearman correlation coefficients between variables adjusted for the year effect and measured in the F_2 progeny. For fruit characteristics, the correlation coefficients were calculated with 12 replicated values per genotype; for tree characteristics, the correlation coefficients were calculated with two or three replicated values per genotype (*TA* titratable acidity, *SSC* soluble solid content, *FW* fresh weight)

Traits	Maturity date	Development	Fruit FW	pH	TA	SSC	Malic acid	Citric acid	Quinic acid	Sucrose	Glucose
Maturity date											
Development	0.988**										
Fruit FW	0.135	0.054									
pH	–0.113	–0.188	–0.128**								
TA	0.033	0.246*	0.044	–0.844**							
SSC	0.411**	0.335**	–0.052	0.133**	–0.120**						
Malic acid	0.082	0.135	0.012	–0.729**	0.746**	–0.035					
Citric acid	–0.083	–0.002	0.157**	–0.767**	0.751**	–0.309**	0.685**				
Quinic acid	0.088	–0.088	0.023	–0.037	0.070	0.241**	0.160**	0.161**			
Sucrose	0.107	0.108	–0.163**	0.334**	–0.308**	0.654**	–0.206**	–0.429**	0.145**		
Glucose	0.504**	0.457**	0.065	–0.341**	0.230**	0.275**	0.236**	0.208**	0.159**	0.263**	
Fructose	0.608**	0.601**	0.058	–0.344**	0.247**	0.251**	0.242**	0.220**	0.171**	0.211**	0.889**

*$P < 0.05$, **$P < 0.01$

date was significantly positively correlated with fruit development period, soluble solid content and glucose and fructose contents. The fruit development period was significantly positively correlated with titratable acidity, soluble solid content and glucose and fructose contents. Titratable acidity and pH were highly negatively correlated. Titratable acidity was negatively correlated with soluble solid contents and sucrose contents, and positively correlated with malic acid, citric acid, glucose and fructose contents. Acid contents were positively corre-

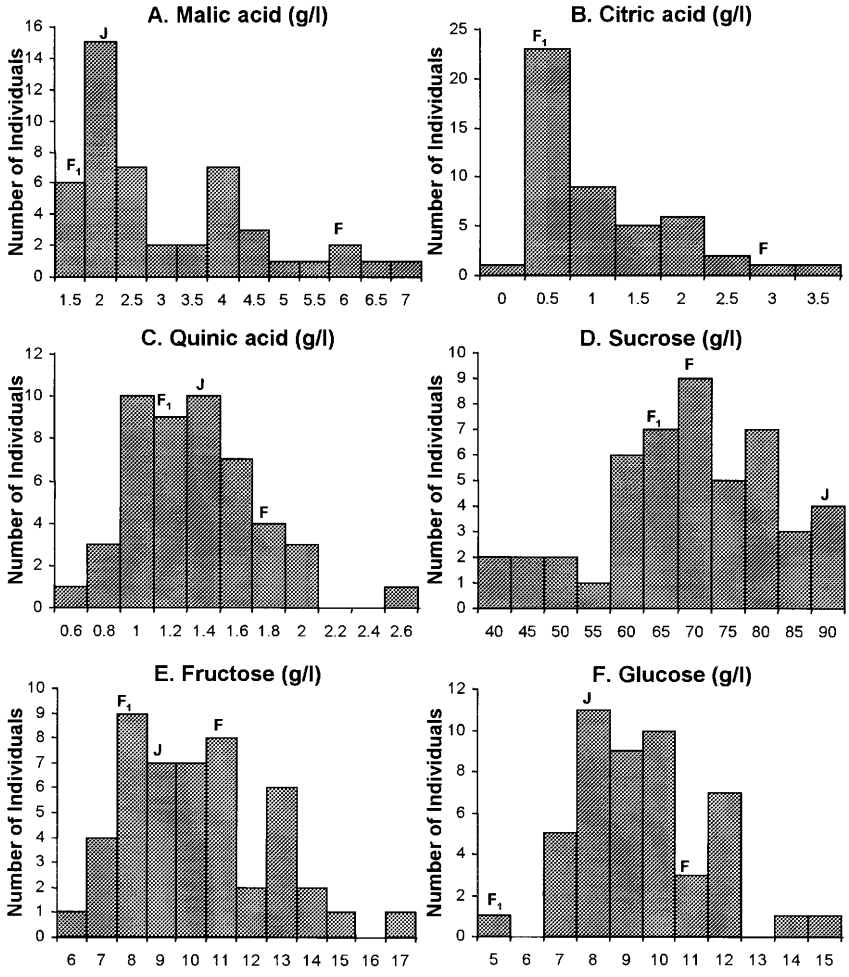

Fig. 3A–F Distribution of estimated genotype means for fruit organic acid and sugar contents measured on the peach Ferjalou Jalousia® × Fantasia F_2 progeny for 3 years. **A** Malic acid, **B** citric acid, **C** quinic acid, **D** sucrose, **E** fructose, **F** glucose. The values of the parents, Ferjalou Jalousia® and Fantasia, and of the F_1 individual are indicated by a, *J*, *F* and F_1, respectively

lated, especially malic and citric acid. Soluble solid content was highly positively correlated with quinic acid, sucrose, glucose and fructose contents, and negatively correlated with malic and citric acid contents. Soluble sugars were positively correlated, with the highest correlation observed between glucose and fructose contents. The correlations calculated using genotypic estimates were in general agreement with the correlations measured on a yearly basis [1995 and 1996 (Dirlewanger et al. 1999) and 1998, data not shown)].

QTL detection

QTL analyses were performed by (1) linear regression analysis on the genotype mean per individual from individual years (1995, 1996 and 1998), and (2) three methods, linear regression analysis, IM and CIM on the mean of the genotypic estimate over years – referred to as the 'overall estimate' (Table 5). Because of the small size of our mapping population, we only retained the most significant QTLs. QTLs detected by simple linear regression analysis based on the overall estimate were selected as being the most reliable QTLs because (1) simple linear regression is robust to a departure from normality and (2) in the overall estimate, the year effect was removed. QTLs fell into four genomic regions corresponding, respectively, to linkage groups 4, 5, 6 and 8 (Fig. 4, Table 5). QTLs detected by simple linear regression ($P < 0.0001$) based on yearly data or on the 'overall estimate' always involved the same set of linked markers, ranging from a minimum of two (UDP97-402 and CC133 on linkage group 4, FG25 and FG209A on linkage group 6 and FG215 and FG40 on linkage group 8) to seven (AG21b-AG25a interval on linkage group 5) (data not shown). For each trait, we retained the molecular marker with the highest LOD-score value that stands also for (1) the most significant effect detected in stepwise regression and (2) the peak position detected by IM. All retained markers were codominant and allowed estimation of additive and dominance statistics, except for the *D* gene. We only reported additive statistics (Table 5). Although the CIM method was impaired in our experiment by the population size, we used it in order to increase the precision of the most-likely position and R^2 evaluation of the QTLs selected (Zeng 1994). All QTLs detected by simple linear regression and IM were detected by CIM except QTLs for ripening date and fruit development period. For all traits, results by CIM did not vary when declaring 1, 2, 3, 4 or 5 cofactors, except for

Table 5 QTLs detected for peach fruit quality components based on simple linear regression, interval mapping and composite interval mapping (CIM). The most closely associated marker locus is indicated. The *F* statistic is for the hypothesis that the marker is unliked to the quantitative trait

Trait	Linkage group	Simple linear regression					Interval mapping Overall estimate			CIM Overall estimate		
		Marker	1995 *F*	1996 *F*	1998 *F*	Overall estimate						
						LOD[a]	*F*	Pos.	LOD	R^2	LOD	Effect[d]	R^2
Maturity date	4	UDP97-402	60,86****	58,883****	51,727****	9	57,205****	0	11.9	77.7	–	–	–
Fruit development period	4	UDP97-402	54,962****	59,45****	–	9	55,542****	8	11.6	76.2	–	–	–
Fresh weight	6	FG25	32,876****	12,126***	28,804****	4.9	26,507****	59.5	6.9	51	8.1	31.8	52
pH	5	D	63,399****	45,963****	47,198****	10.4	69,664****	27.4	15.9	86	16.2	–0.5	90
Titratable acidity	5	D	73,586****	52,689****	27,99****	10.8	73,618****	33.4	15.6	80	17.3	38.3	44
Soluble-solids content	4	UDP97-402	18,178****	4,153*	12,193***	4.1	21,217****	0	4.2	34	5.5	–0.9	34
	6	FG209A	–	17,908****	7,427**	2.5	12,35***	63.5	2.7	24	3.2	–0.4	17
Malic acid	5	D	84,706****	58,991****	46,046****	10.7	72,473****	31.4	12.3	72	14.2	1.3	83
Citric acid	5	D	63,19****	39,522****	40,908****	9.1	57,878****	33.4	14.2	77	14.9	0.8	39
Quinic acid	8	FG215	9,039**	–	35,241****	4.9	26,426****	0	5.7	43	4.7	0.3	33
Sucrose	5	D	16,581***	13,568***	9,482**	4.1	21,326****	23.8	4.6	39	3.9	–6.5	29
	6	FG209	4,791*	10,097**	20,535****	4.5	23,769****	63.5	4.6	39	6.0	–11.5	37
Glucose	4	CC133	7,137**	20,983****	8,3**	4.5	24,041****	10	4.6	38	5.4	–1.6	37
Fructose	4	CC133	11,061**	27,984****	8,533**	4.6	24,231****	8	4.9	41	5.6	–1.7	25

*, **, ***, ****Significance at the 5%, 1%, 0.1% and 0.01% levels, respectively

[a] LOD, Log-likelihood at the specified position

[b] Pos., distance of the QTL in centi Morgans (Kosambi) from the top of the linkage group

[c] R^2, percentage of phenotypic variation explained by the QTL

[d] Effect, additive value of the QTL expressed as the effect of substituting a cv. Jalousia allele by a cv. Fantasia allele

QTLs for pH and malic acid content for which R^2 values were over 100% using more than three cofactors (data not shown). Declaring three cofactors, six QTLs had lower R^2 values in CIM than in IM, while the other five QTLs had similar R^2 values in CIM than in IM. The QTLs retained explained from 17% to 90% of the phenotypic variance, with an average of 43%.

On linkage group 4, QTLs for ripening date and fruit development period were detected from the dataset available for individual years (1995, 1996 and 1998 for ripening date; 1995 and 1996 for fruit development period) as for the overall estimate. In the same genomic region, QTLs were detected for glucose, fructose and SSC. This inference between QTLs for ripening date, fruit development period, fructose, glucose and SSC is supported by the correlation between these traits (Table 4).

On linkage group 5, a genomic region containing the *D* gene was associated with a variation of 90% for pH, 44% for titratable acidity, 83% for malic acid, 39% for citric acid and 29% for sucrose content. The year effect was highly significant for sucrose content ($P<0.0001$, data not shown). This could account for low LOD scores (below threshold) observed when dealing with yearly data (data not shown). All QTLs for peach fruit acidity were in the direction predicted by the phenotype. Alleles from the normally-acid parent Fantasia increased titratable acidity by 38.3 mEq l^{-1}, malic acid content by 1.3 g l^{-1}, citric acid content by 0.8 g l^{-1} and quinic acid content by 0.3 g l^{-1} and lowered pH by 0.5 unit. In contrast with the phenotypic data, alleles from the normally-acid parent Fantasia decreased sucrose content by 6.5 g l^{-1}.

On linkage group 6, another genomic region containing marker FG209A was found to explain 37% of the variation in sucrose content. As for the QTL for sucrose content mapped on linkage group 5, the LOD scores were lower for each year data than for the overall genotype estimate (data not shown). A QTL for SSC (R^2 = 17%) was also found near the marker FG209A. Alleles from the normally-acid genotype Fantasia were associated with a decrease in sucrose content and SSC in mature fruit by 11.5 g l^{-1} and 0.4° Brix. Near the FG25 marker (totally linked to the peach flat marker), a QTL accounting for 52% variation in peach fruit fresh weight was detected. Alleles from the round nectarine Fantasia increased fresh weight by 31.8 g, which is consistent with phenotypic data.

On linkage group 8, a QTL for quinic acid (R^2 = 33%) was detected near the FG215 marker.

Co-location

The ordered interval PC60-*Pgl1*-FG209A is found in both the Texas × Earlygold and Ferjalou Jalousia® × Fantasia linkage group 6 with respective map distances of 6.0–5.0 cM and 10.0–4.5 cM. This allowed us to consider it to be an homologous fragment (Fig. 5). In the Ferjalou Jalousia® × Fantasia map, marker FG209A was

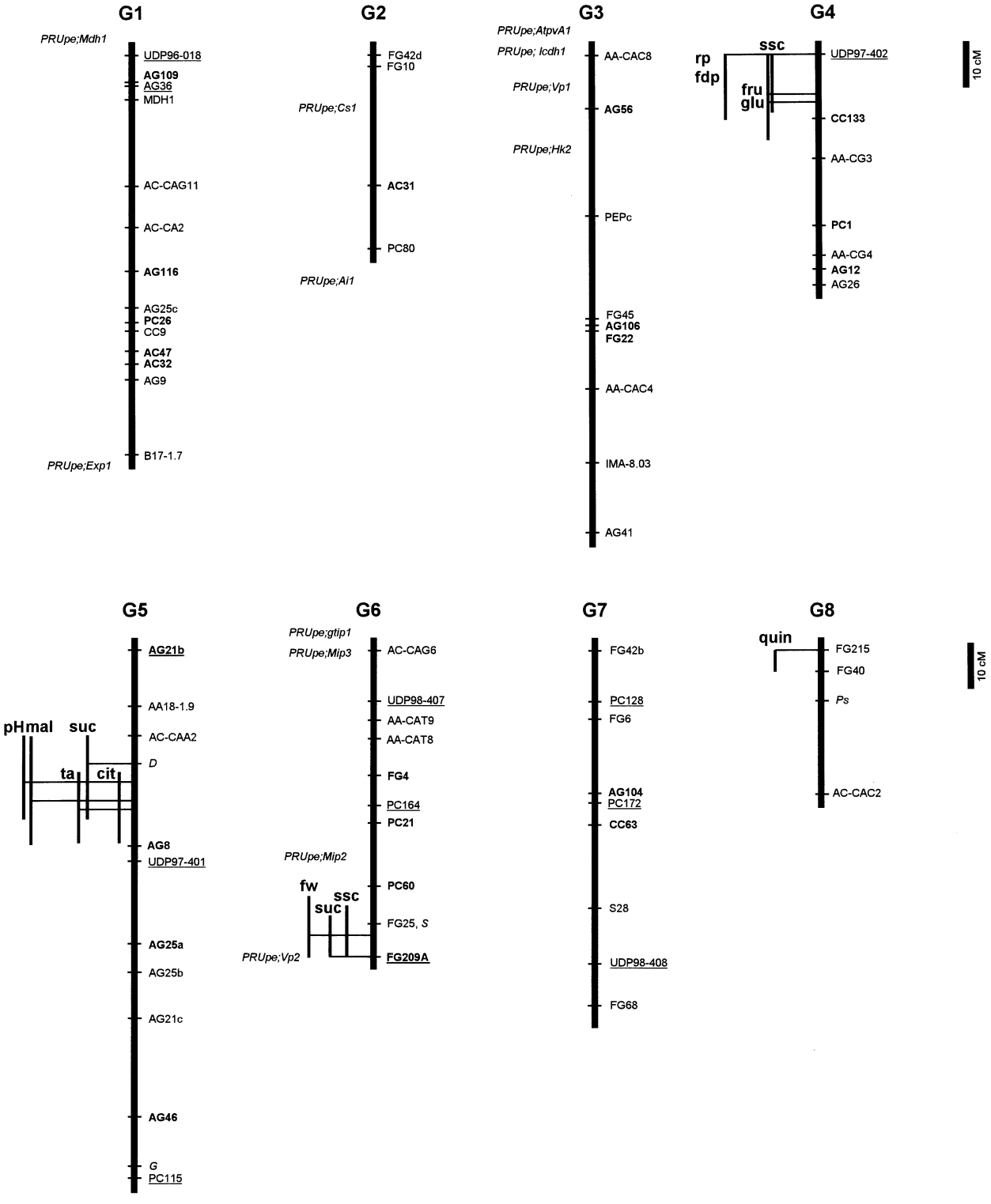

Fig. 4 Linkage map of peach chromosomes based on the intraspecific F_2 population of a cross between a low-acid peach (Ferjalou Jalousia) and a normally-acid nectarine (Fantasia). Linkage groups have been labelled as *G1–G8*. Names of the markers are listed on the *right* of each linkage group. *Underlined* loci are the RFLPs and SSRs markers that were integrated to the map. Loci in bold characters are the anchor points with the almond Texas × peach Earlygold (Texas × Earlygold) map. Loci in *italics* are the morphological markers (*D* low-acid, *G* peach; *ps* male sterility, *S* flat). Loci seperated by a *coma* were placed at the same position. Candidate genes are placed on the linkage groups (*right side*) they were assigned to by comparative mapping with Texas × Earlygold map. The most likely positions of QTLs detected by MAPMAKER/QTL for fruit quality components (overall estimate) are indicated: *cit* citrate, *fdp* fruit development period, *fru* fructose, *fw* fresh weight, *glu* glucose, *mal* malate, *quin* quinate; *rp* ripening date, *ssc* soluble solid content, *suc* sucrose, *ta* titratable acidity. The most likely position for a QTL is indicated by a *horizontal line* proportional to the effect (R^2). The one-LOD support confidence interval is indicated by a *vertical line*

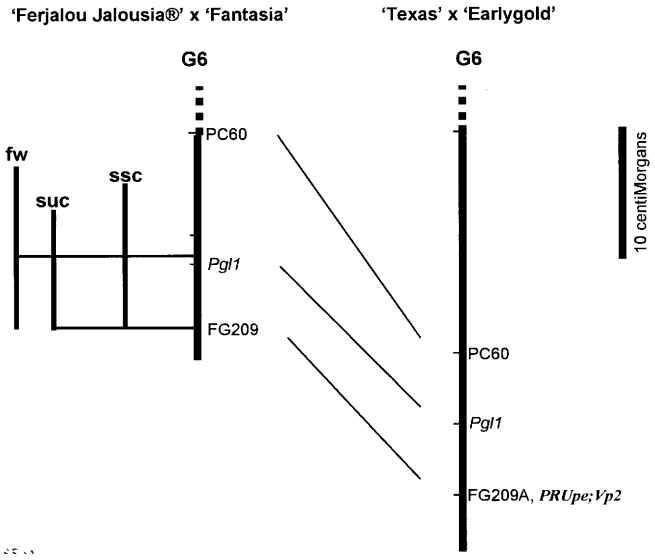

Fig. 5 Alignment of the homologous fragments at the end of linkage group 6 from the Ferjalou Jalousia® × Fantasia and the Texas × Earlygold maps. Shared markers are indicated. Loci separated by a *coma* were placed at the same position. *Numbers* at the *bottom* of each linkage group indicate the cumulative mapping distance in Kosambi centiMorgans from the 'top' of the linkage group. QTLs indicated in Fig. 4 are recalled. *PRUpe;Vp2* is indicated in *bold letters*

found to be significantly linked to two QTLs for sucrose and soluble solid contents. FG209A being a codominant marker, we could estimate the dominance/additivity statistic: –1.6 for SSC and –0.6 for sucrose content, indicating, respectively, underdominance (the heterozygous genotype is lower than parental homozygotes) and partial underdominance. In the Texas × Earlygold map, marker FG209A was found to be totally linked to the candidate gene *PRUpe;Vp2* isolated from Fantasia fruits and encoding a vacuolar H^+-pyrophosphatase. We concluded that *PRUpe;Vp2* was co-located to QTLs for sucrose and soluble solid contents.

Discussion

QTL accuracy

Peach is a self-fertile fruit species with a very low genetic variability (Scorza and Okie 1990), as confirmed by the low polymorphism revealed using DNA markers for the development of intraspecific linkage maps (Chaparro et al. 1994; Dirlewanger et al. 1998; Rajapakse et al. 1995; Warburton et al. 1996). This low level of polymorphism has slowed down the development of a saturated linkage map based on the Ferjalou Jalousia® × Fantasia population. A previous QTL detection using the Ferjalou Jalousia® × Fantasia progeny was achieved with mostly dominant markers. We used additional RFLP markers and new recently developed multi-allelic peach microsatellites (Cipriani et al. 1999; Sosinski et al. 2000) to construct a more unified map that enhanced QTL detection. Among the RFLP markers, cDNAs isolated from peach were selected as candidate genes for fruit quality, and these complemented the map with co-dominant markers. The addition of one-third more codominant loci to the Ferjalou Jalousia® × Fantasia map lowered by 21% the size of the map (564 cM versus 712 cM). Based on the interspecific saturated map for *Prunus* [Texas × Earlygold, 491 cM (Joobeur et al. 1998)], our framework map covers most of the peach genome over a relatively short distance (10 cM) suitable to QTL detection. All the QTLs detected in this study were consistent with those observed in a previous study (Dirlewanger et al. 1999) except for the QTLs for pH, titratable acidity, malic and citric acid and sorbitol on linkage group 6, which could be explained by the replacement of the dominant RAPD marker R16–1.2 by the SSR marker UDP98-407.

Genetic basis of variation in peach fruit quality

In several fleshy fruit species [peach (Yoshida 1970), pummelo (Cameron and Soost 1977), apple (Maliepaard et al. 1998) and tomato (Stevens 1972)], fruit acidity appears to be conditioned by alleles with major phenotypic effects [called macromutations as defined by Tanksley (Tanksley 1993)] at single loci. Genetic studies conducted in species which accumulate both malic and citric acids (tomato) or which accumulate only one major organic acid, either malic acid (apple) or citric acid (pummelo), have shown that malic and citric acids are each conditioned by a single gene (Fang et al. 1997; Maliepaard et al. 1998; Stevens 1972). However, in processing (Paterson et al. 1988) as well as in fresh market tomato (Saliba-Colombani et al. 2001), components of fruit acidity are also conditioned by allelic variation at several genetic loci (QTLs), each with a relatively small effect.

In the present study, we confirmed that at least one macromutation responsible for the low-acid trait in peach is located on linkage group 5 along with QTLs for pH, titratable acidity, malic and citric acid contents and sucrose content. Furthermore, these four traits are those that discriminate normally-acid Fantasia fruits from low-acid Ferjalou Jalousia® fruits. In contrast, QTLs involved in the variation of quinic acid content did not map into the macromutation genomic region. In tomato, the two major genes that were reported to be involved in malic and citric acid contents were also found to be linked (Stevens 1972).

With respect to soluble sugars, QTLs conditioning hexose content were previously detected using an intraspecific peach Suncrest × Bailey cross (Abbott et al. 1998). However, comparative QTL mapping between these QTLs and QTLs detected in the present study is impeded by the absence of common markers. In contrast, the development of linkage maps for *Prunus* in Europe, with a set of common RFLP markers (Joobeur et al. 1998), enabled a comparison of QTL assignment to the linkage group. For instance, using a population derived from a peach × *Prunus ferganensis* cross, one QTL for

pH was detected on linkage group 2 and QTLs for SSC were detected on linkage groups 1 and 2 (Quarta et al. 2000). These QTLs are not assigned to the same linkage groups as those detected in the present study. However, in sour cherry, a QTL for SSC was also located on linkage group 6, indicating that this QTL might be conserved between two *Prunus* species (Wang et al. 2000).

Using a map-based cloning approach, Frary et al. (2000) observed that a previously unknown gene controlled cell division in tomato ovary and was responsible for the effect of the *fw2.2* QTL that changes fruit weight by up to 30% in tomato. In contrast, a QTL for tomato SSC cloned using a similar approach resided in an apoplastic invertase (Fridman et al. 2000). Apoplastic invertases, which cleave sucrose into hexoses further transported into the sink cell (Fig. 1), were already well-known candidates in the control of sugar for growth and storage in sink tissue (Weber et al. 1995). In the present study, we assumed a priori that structural genes involved in soluble sugar and organic acid accumulation in fruit might be responsible for the variation in peach quality traits.

Mapping candidate genes in *Prunus*

In the model fruit tomato, the availability of a range of mapping populations segregating for specific fruit ripening loci allowed the mapping of each member of multigene families by RFLP (Giovannoni et al. 1999). In contrast to RFLP assays, PCR-based assays require almost complete identity between the primer and the target sequence and, therefore, are more gene-specific. In peach, due to the low level of polymorphism of the Ferjalou Jalousia® × Fantasia progeny, we developed a two-step strategy to map our candidate genes: (1) PCR with specific cDNA-based primers and (2) consecutive SSCP to identify sequence variants. Using PCR with specific cDNA-based primers we could expect to detect sequence variants differing in fragment length. Again, no polymorphism was observed by PCR with specific cDNA-based primers and by SSCP in the Ferjalou Jalousia® × Fantasia population. In contrast, polymorphism was detected in the Texas × Earlygold population for 12 out of the 18 genes. Furthermore, PCR-SSCP with specific cDNA-based primers enabled the discrimination of each member of multigene families. The cDNA-based SSCP genetic markers developed in the present study should be transferable to peach-related species and will be useful for synteny studies as proposed in *Pinus* species (Plomion et al. 1999).

Genes involved in fruit development, soluble sugar and organic acid metabolism and accumulation are generally not clustered

Mapped genes can be grouped on the basis of their function in the cell (eg. organic acid metabolism, soluble sugar metabolism, water and solute accumulation and cell expansion) and on the basis of their assignment onto linkage groups. The cDNAs encoding vacuolar proton-translocating pumps fell into two distinct linkage groups: linkage group 3 (*PRUpe;AtpvA1* and *PRUpe;Vp1*) and linkage group 6 (*PRUpe;Vp2*). Four cDNAs related to solute accumulation mapped on linkage group 6 (aquaporin: *PRUpe;Tip1*; *PRUpe;Mip2*; *PRUpe;Mip3* and a V-PPase *PRUpe;Vp2*). However, despite the low precision in our genetic mapping experiment, there was no apparent clustering of loci related to fruit quality, contrary to the clustering of disease resistance genes (Pflieger et al. 1999). Similarly, in tomato, genes involved in ripening and/or ethylene response were generally not found to be clustered (Giovannoni et al. 1999). According to these authors, the functional clustering of loci tends to represent biological systems in which divergence and selection of related genes is required to maintain or maximize plant viability, whereas mechanisms involved in the control of fruit development might be more diverse.

Candidate gene function based on genetic linkage

In the present study, map positioning enabled us to discard all the functional candidate genes that we selected from the control of the *D* gene (Fig. 4). However, in our experiment, the limited size of the F_2 progeny implies that only QTLs with large effects could reach statistical significance (Andersson et al. 1994). Thus, they still remain candidate genes for the other traits analysed. One of the proton pump-encoding genes, *PRUpe;Vp2*, was found to be co-located with QTLs controlling fruit SSC and sucrose content on linkage group 6 (Fig. 5).

PRUpe;Vp2 encodes a vacuolar pyrophosphatase involved in the establishment of an electrochemical gradient across the tonoplast. In a recent study, we suggested that it encodes a particular isoform of vacuolar pyrophosphatase that is highly expressed in fruit (Etienne et al. 2002). Experiments in tomato (Milner et al. 1995) and pear fruit (Shiratake et al. 1997) suggested that sucrose and hexose transportation into the vacuole of fruit cells occurs through facilitated diffusion. However, the existence of an energised membrane transport involving sugar/proton antiport, as described in other storage organs (Martinoia et al. 2000), can not be ruled out in peach fruit. In that hypothesis, *PRUpe;Vp2* might provide the proton motive force for sugar transport across the tonoplast. Thus, the co-location of the *PRUpe;Vp2* proton pump and QTLs controlling SSC and sucrose accumulation warrants further investigation to confirm the putative implication of vacuolar pyrophosphatase in the control of these traits. At the genetic level, a more precise mapping of this region will be undertaken on an extended F_2 population issued from the Ferjalou Jalousia® × Fantasia cross. At the physiological level, the effect of tonoplastic proton pumps on the transport and storage of sucrose in peach fruit cells should be investigated using

cell-free vacuoles of both normally-acid and low-acid peach fruits. It will be only after the candidate gene/QTL co-location has passed these tests that one could start predictably modifying traits via selection of candidate gene allele.

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