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RESEARCH PAPER

Identification of growth processes involved in QTLs for tomato fruit size and composition

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Abstract

Many quantitative trait loci (QTLs) for quality traits have been located on the tomato genetic map, but introgression of favourable wild alleles into large fruited species is hampered by co-localizations of QTLs with antagonist effects. The aim of this study was to assess the growth processes controlled by the main QTLs for fruit size and composition. Four nearly isogenic lines (NILs) derived from an intraspecific cross between a tasty cherry tomato (Cervil) and a normal-tasting large fruit tomato (Levovil) were studied. The lines carried one (L2, L4, and L9) or five (Lx) introgressions from Cervil on chromosomes 1, 2, 4, and 9. QTLs for fruit size could be mainly associated with cell division processes in L2 and L9, whereas cell expansion was rather homogeneous among the genotypes, except Cervil for which the low expansion rate was attributed to low cell plasticity. The link between endoreduplication and fruit size remained unclear, as cell or fruit sizes were positively correlated with the cell DNA content, but not with the endoreduplication factor. QTLs for fruit composition reflected differences in water accumulation rather than in sugar accumulation, except in L9 for which the up-regulation of sucrose unloading and hexose transport and/or starch synthesis was suggested. This may explain the increased amount of carbon allocated to cell structures in L9, which could be related to a QTL for fruit texture. In Lx, these effects were attenuated, except on fruit size and cell division. Finally, the region on top of chromosome 9 may control size and composition attributes in tomato, by a combination of QTL effects on cell division, cell wall synthesis, and carbon import and metabolism.

Key words: Cell division and expansion, endoreduplication, fruit quality, near isogenic line, osmotic regulation, quantitative trait locus, *Solanum lycopersicum*, starch, sugar and acid contents.

Introduction

Advances in genetics and genomics in the last 10 years have boosted knowledge of the molecular control of fruit quality. At the same time, they have pointed out the complexity of the control of single fruit quality traits such as fruit size, shape, firmness, or sugar and acid content (Fernie *et al.*, 2006; Causse *et al.*, 2007). For tomato in particular, a great number of quantitative trait loci (QTLs) for these quality traits has been fine-mapped thanks to the identification of numerous molecular markers (Saliba-Colombani *et al.*, 2001). In only a few cases have the genes underlying the main QTL effect been identified, such as, for instance, *fw2.2* which is involved in fruit size regulation (Cong *et al.*, 2002), or *Lin5* controlling soluble solid content in fruit (Fridman *et al.*, 2000). Favourable alleles could be introgressed into different tomato recipient lines by marker-assisted selection (Lecomte *et al.*, 2004), providing the so-called near isogenic lines, which carry single or several regions of interest in a homogenous genetic background. Thus, the advent of new molecular techniques provided a powerful tool to optimize the use of genetic diversity, by bringing together, in one genotype, alleles that maximize a given trait (Paterson *et al.*, 1988; Bai and Lindhout, 2007). In spite of these advances, breeding programmes for fruit organoleptic quality are still facing difficulties, partly due to the fact that most quality traits are polygenic and strongly influenced by environment. In tomato, ~30 QTLs affecting fruit size and mass

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(Grandillo *et al.*, 1999; Cong *et al.*, 2002), and 23 QTLs controlling the content in soluble solids (Fridman *et al.*, 2000) have been mapped.

One of the major objectives in tomato breeding regarding quality traits is to introduce QTLs for high soluble solid content observed in small- or green-fruited cultivars or in wild species into large fruited cultivated species. Progress towards this objective is slow, first because of the co-localizations of QTLs which create some antagonist effects, and the presence of several QTLs with low or less than additive effects (Causse *et al.*, 2007), and secondly because of interactions between QTLs and the environment or genetic background (Chaïb *et al.*, 2007). Dissecting complex traits into elementary physiological processes may help identify the genetic control of quality traits and also help with the search for candidate genes. It may be especially useful in NILs differing from the parental line by only small genome regions; seeking the physiological processes involved in phenotypic variations may provide direct information on the gene functions or regulatory steps potentially introgressed, as it is expected that only a few processes drive the variation in one particular trait. The capacity of ecophysiological approaches to analyse genetic variability has been illustrated in a few studies. For instance, cell division was shown to be involved in a QTL for tomato fruit size (Bertin *et al.*, 2003), and ecophysiological models describing organ function have been successfully used to study the co-locations of QTLs for model parameters and for organ traits (Quilot *et al.*, 2005; Tardieu *et al.*, 2005). These studies elucidated some processes actually involved in genetic variations.

The aim of this study was to identify the ecophysiological processes involved in genetic variations underlying changes in tomato fruit size and composition. The main processes putatively involved in the regulation of these two traits have been quantified on four QTL-NILs derived from an intraspecific cross between a particularly good-tasting cherry tomato line (Cervil) and a large-fruited line with a common taste (Levovil). These lines were nearly isogenic to their recipient line (as defined in Van Berloo *et al.*, 2001), from which they differed by the presence of one or several QTLs for quality traits introgressed from Cervil (Causse *et al.*, 2002). These traits were related to fruit size, soluble content, sourness and sweetness, and texture. Fruit size is the result of cell division and expansion processes. In tomato, cell division is restricted to ~10–20 d after anthesis (Bertin *et al.*, 2007) and, in a given environment, the final fruit weight is highly correlated to the number of pericarp cells (Bünger-Kibler and Bangerth, 1983; Bertin, 2005). Cell growth and expansion depend on cell wall plasticity and accumulation of water and carbon compounds. Admittedly, cell expansion is driven by the turgor pressure induced by osmotic pressure, which is mostly determined by soluble sugars, acids, and potassium salts in tomato fruit (Ho *et al.*, 1987). Thus, sugar and acid contents not only determine fruit sweetness and sourness, respectively, but they also play an active role in fruit osmotic pressure and, thus, in fruit size regulation. During cell expansion, endoreduplication of DNA has been shown to be involved in growth regulation in many species (Sugimoto-Shirasu and Roberts, 2003; Bertin *et al.*, 2007). The final fruit composition, usually expressed on a fruit fresh weight basis, results from carbon import and dilution by the accumulated water (Ho, 1996). The fruit dry matter content varies >2-fold among genotypes, and consists of one-half soluble sugars, and one-quarter organic acids (Davies and Hobson, 1981). Temporal variations in composition during fruit development may indicate different causes for variations in final composition. For instance, during the first half period of fruit development, part of the sugars are stored as starch, an osmotically inactive reserve of carbon, whose size is related to the final sugar content (Ho, 1986).

In the present investigation, processes driving fruit growth and composition have been quantified during fruit development on two parental lines and four QTL-NILs grown in similar conditions. The results outlined the main processes involved in size and composition differences between cherry and large-fruit genotypes (Cervil and Levovil), and allowed the effects of QTLs for quality among the QTL-NILs to be analysed. Genes putatively involved in these QTLs were discussed further.

Materials and methods

Plant material

The initial QTL analysis was performed on a population of recombinant inbred lines (RILs) developed from an intraspecific cross, between Cervil, a cherry tomato [*Solanum lycopersicum* var. *cerasiforme* (Dun.) Gray] with 7 g fruits, good taste, and high aroma intensity, and Levovil (a *S. lycopersicum* Mill. line, hereafter called Lev) with 125 g fruits and normal taste (Causse *et al.*, 2002). Based on the QTL map, five regions (located on chromosomes 1, 2, 4, and 9, respectively) were introgressed in the Lev genetic background (Lecomte *et al.*, 2004). Two QTLs for fruit fresh weight were detected on chromosome 2. QTLs for fruit composition (soluble solid, dry matter, and sugar contents, and titratable acidity) were present on each of the five regions, as well as a QTL for fruit texture (firmness, elasticity, mealiness, and meltiness) (Fig. 1). The introgressed lines were produced as described in Chaib *et al.* (2006). They were obtained after three backcrosses on the recipient line followed by three selfing generations (Lecomte *et al.*, 2004), and they carried homozygous alleles from Cervil at the five regions. One introgressed line cumulated these five regions (Lx), and four isogenic lines carried a single introgressed region on chromosome 2 (L2), 4 (L4), and 9 (L9).

Culture conditions

Plants were grown under standard glasshouse conditions in Avignon, France (43°55′N; 4°52′E). Planting took place in February at a density of 2 plants m^{-2}, and the day–night temperature set-point was 20–18 °C. Inflorescences were pollinated by bumblebees.

The different genotypes were planted in four blocks of six plants randomly distributed over the greenhouse compartment. Trusses were pruned in order to homogenize truss size along the stem within each genotype. The maximum number of flowers left on each inflorescence was 12 for Cervil and Lx, nine for L2, eight for L9, and six for L4 and Lev. These inflorescence sizes corresponded to the average source–sink regulation by the plant itself when inflorescences are left unpruned (except Cervil which can set a larger number of fruits). The plant growth analysis performed at the end of the experiment (Table 1) suggested that the distribution of dry matter was homogenous among the genotypes, except in Cervil and to a lesser extent in Lx, for which the source–sink ratio was probaby higher than in the other lines, despite the low leaf area. In these two genotypes, the surplus of dry matter was accumulated in leaves (higher specific leaf weight).

Observations and fruit sampling

For each genotype, fruit diameter was non-invasively measured twice a week with a calliper square on one basal and one tip fruit of the first five trusses from eight plants. Fruit sampling took place from April to May, on the first seven trusses of different plants. Flower buds and fruit ages (from anthesis to red ripe stage) were related to the time of flower anthesis, which was recorded individually. To avoid unbalanced source–sink relationships, one to two fruits per truss were sampled on a given plant, and then the plant was excluded from further sampling. Fruits were weighed and sized, cut into two halves when large enough, and then distributed for the following measurements. Fruit dry matter content was assessed after 5 d in a ventilated oven at 80 °C. Pericarp cell number was measured on basal and tip fruits to account for the gradient of cell number within the inflorescence which increases with fruit size. The pericarp

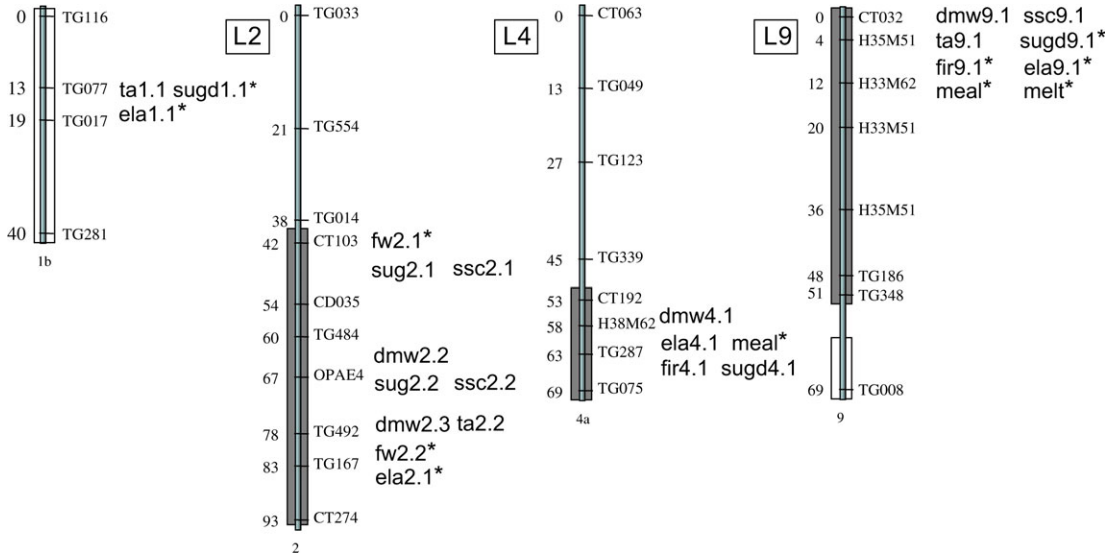

Fig. 1. Molecular map showing regions of interest on chromosomes 1, 2, 4, and 9, carrying the QTL for organoleptic quality, based on an intraspecific RIL population derived from a cross between a cherry tomato line and a large fruit line. Distances in Kosambi centiMorgans are on the left of chromosomes, and marker names are on the right. The introgressed regions in Lx are indicated by white rectangles and the regions introgressed in L2, L4, and L9 are in grey. On the right of chromosomes, QTLs are mentioned for fruit weight (fw), titratable acidity (ta), dry matter content (dmw), soluble solid content (ssc), sugar content (sug) relative to fresh weight, firmness (fir), elasticity (ela), mealiness (meal), and meltiness (melt) as described in Saliba-Colombani *et al.* (2001), and for sugar contents relative to dry weight (sugd, unpublished data). Asterisks indicate that the Levovil allele provided higher value to the trait.

Table 1. Plant growth analysis performed at the end of the experiment

At this time, ~8–10 trusses were set on plants from all genotypes, except on Cervil plants which set between 13 and 15 trusses. Data (dm, dry matter; SLW, specific leaf weight) are means of three plants per genotype, and values in parenthesis are the SEM. Lower case letters indicate statistically homogenous groups detected by pairwise multiple comparison procedures (Bonferroni *t*-test, $P \leqslant 0.05$).

	Lev	Cervil	L4	L9	L2	Lx
Total shoot dm (g)	347.6 (9.0)	284.2 (25.5)	398.8 (70.9)	355.2 (40.1)	272.1 (14.9)	277.0 (13.5)
Leaf area m^2 pl^{-1}	3.26 (0.26)	2.38 (0.02)	3.20 (0.16)	3.12 (0.22)	2.79 (0.64)	2.23 (0.25)
SLW g m^{-2}	28.4c (2.2)	42.6a (4.8)	36.6a,b,c (5.4)	34.2a,b,c (3.1)	30.3b,c (1.6)	39.6a,b (1.1)
% dm in leaf+stem	56%	82%	65%	64%	64%	69%
% dm in fruits	44%	18%	35%	36%	36%	31%

was weighed, and cell suspensions were obtained after maceration in a pectinase solution, as described in Bertin *et al.* (2002). A minimum of eight samples per fruit were counted. The mean volume of an average cell was estimated by dividing the pericarp volume by the number of cells. The ploidy level of cells from the pericarp was measured by flow cytometry, as described in Bertin *et al.* (2007). Histograms were analysed with the WinMDI software (version 2.8) to determine the relative number of nuclei containing different amounts of DNA expressed as C values (from 2C to 512C). Three measurements were made in each fruit, when allowed by its size, and the average value was considered. The mean endoreduplication factor (EF) was calculated as proposed by Coockson *et al.* (2006):

$$EF = \sum_{i=1}^{p} \frac{E_i \times p_i}{p_{tot}} \quad (1)$$

where p is the number of peaks of different DNA content (maximum=8) in the sample, E_i is the number of endocycles performed by nuclei in peak i (E_1=0, E_2=1, E_3=2….E_8=7), p_i is the number of nuclei in the peak of value E_i, and p_{tot} is the total number of nuclei in the sample. *EF* indicates the number of endocycles an average cell of the tissue has undergone.

The ploidy index (P^i) is the C level of an average cell of the tissue:

$$P^i = \sum_{i=1}^{p} \frac{C_i \times p_i}{p_{tot}} \quad (2)$$

where C_i is the C value of nuclei in peak i (C_1=2, C_2=4, C_3=8….C_8=256).

Considering that 1C is equivalent to 950 Mb, i.e. 950 pg DNA (Arumuganathan and Earle, 1991), the amount of DNA per cell (pg cell^{-1}) was calculated as:

$$\text{DNA} = P^i \times 950 \quad (3)$$

Sugar and acid composition was determined on whole fruits, immediately frozen in liquid nitrogen after sampling, ground, and stored at –20 °C. Soluble sugars (sucrose, fructose, and glucose) and organic acids (malic and citric acids) were extracted and measured by HPLC analysis, and starch content was determined enzymatically (Gomez *et al.*, 2002, 2003).

Fruit osmotic pressures due to soluble sugars or organic acids were calculated according to (Nobel, 1974):

$$P_{osm}(bars) = \frac{RT \times W_d \left(\frac{[Gluc]}{W_{Mgluc}} + \frac{[Fruc]}{W_{Mfruc}} + \frac{[Sucr]}{W_{Msuc}} \right)}{(W_f - W_d)} \quad (4)$$

where R is the gas constant (83.1 cm^3 bar K^{-1} mol^{-1}), T is temperature (293 K), W_f and W_d are the fruit fresh and dry weights (g), W_{Mi} is the molecular weight of compound i, and [*Gluc*], [*Fruc*], and [*Sucr*] are the dry matter contents (g g^{-1} dm) in glucose, fructose, and sucrose, respectively.

The total fruit osmotic pressure was probably underestimated as the contribution of potassium salts was not considered. It may be higher than the contribution of hexose (Ho *et al.*, 1987), despite some controversies (Mitchell *et al.*, 1991).

The structural dry matter was estimated as the difference between total dry matter and soluble dry matter (here estimated as the sum of soluble sugars, starch, and acids). The amount of structural dry matter per unit cell surface was calculated as an indicator of cell wall thickness. Soluble compounds and dry matter were measured on the whole fruit, whereas cell number only concerned the pericarp. However, the pericarp accounts for ~75% of the fruit weight, and it may be assumed that there was no genetic variability of the percentage structural matter in the locular tissue.

Dynamics of fruit growth, cell division, and expansion were plotted versus the number of days after anthesis (DAA) in order to outline differences in the length of these processes. Then, an index of development was calculated as the ratio between fruit age and the average time needed to ripen, in order to compare the temporal dynamics of different compound accumulation and DNA endoreduplication, on a comparable physiological time scale.

At the end of the experiment, three plants per genotype were sampled. Plant leaf area was measured with an area meter (LI-COR model 3000 Area Meter, Lincoln, NE, USA), and total leaf, stem, and fruit dry matter was measured after drying at 80 °C in a ventilated oven for 6 d. The average specific leaf weight (SLW) was measured on a sample of five leaves per plant harvested at different heights along the stem.

Statistical analysis

Fruit growth, cell number, cell volume, and ploidy curves were fitted to three-parameter sigmoid functions. A logistic function ($y = \frac{a}{1+e^{\frac{-(x-b)}{c}}}$)was chosen for the cell number and ploidy index, whereas a Gompertz function ($y = a \times e^{-e^{\frac{-(x-b)}{c}}}$) was preferred for fruit growth and cell size, because it better accounted for the slow increase of these variables at the start of the observed period. Parameter estimation was carried out using the least squares method. Differences between two (or more) treatments were tested by comparing the sum of the residual sums of squares from individual fittings (ΣSSi), with the residual sum of squares for the common fitting (SSc), considering that the statistic

$$F = \frac{\left| SSc - \sum_{i=1}^{n} SSi \right| / ((n-1) \times k)}{\sum_{i=1}^{n} SSi / (Ndata - k)}$$

follows Fisher's law with (n–1)×k and ($Ndata$–k) degrees of freedom. $Ndata$ is the total number of data, n is the number of individual regressions, and k is the number of fitted parameters for each regression.

Means and variances of the relative rates of division (cell cell^{-1} d^{-1}) were calculated using the bootstrap method ('resample' from original sample with replacement). A linear increase of the cell number was assumed between two

successive close sampling dates. A Tukey test was then performed to compare the six genotypes at the different dates.

Analysis of variance (ANOVA) was performed to analyse genotype effects for traits measured at maturity. When significant effects (P <0.05) were detected, Tukey or Bonferroni tests were performed for pairwise comparisons of mean responses. If data normality and variance equality tests failed, then ANOVAs on ranks were performed, and pairwise comparisons of mean responses were analysed by Dunnet's test. When not deduced from theoretical calculations, data were presented as averages ±95% confidence intervals (CIs). Sigmastat Jandel Scientific software (V2.03, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis.

Results

QTLs for fruit size in relation to development, cell division, and cell expansion

Differences in final fruit size were due to different lengths of development, and to different growth rates (Fig. 2). The time from anthesis to maturity differed by a maximum of 13 d between the parental lines (45 d and 58 d in Cervil and Lev, respectively). All the QTL-NILs showed a reduced rate of growth compared with Lev, and they differed in their growth timing: the fruit growth rate of L4 and L9 peaked at ~25–30 DAA, similarly to Lev, whereas L2 and Lx reached their maximum growth rate at ~20 DAA, as Cervil did. Their final fresh weights ranged from 6 g in Cervil to 148 g in Lev. Differences among genotypes were significant, except between Lev and L4.

Dynamics of the pericarp cell population are shown in Fig. 3. The parental lines presented both the maximum and minimum number of pericarp cells (Fig. 3A). Lev cells divided until ~25 DAA and reached a final cell number of 9.6×10^6. In contrast, cell division ceased at ~12 DAA in Cervil fruits, and the final cell number was $\sim1.2\times10^6$. Among the QTL-NILs, the ranks were those previously observed for fruit growth, with differences occurring in the length and rate of division. In L4 (8.7×10^6 cells) and L9 (6.4×10^6 cells) fruits, the length of the cell division period was similar to that in Lev fruits, but the division rate was lower in L9. In L2 and Lx, the short division periods and lowered division rates were both responsible for low pericarp cell number (4.2×10^6 in L2 and 2.2×10^6 in Lx). At maturity, cell numbers were significantly different among genotypes. A statistical analysis of the relative division rate (cell cell^{-1} d^{-1}) of each of the six genotypes (not shown) showed that higher division rates in large-fruited genotypes were not due to a higher relative rate of cell division, but rather to the higher number of cells compared with that in small fruit-size genotypes.

In contrast to cell division, cell expansion of one average-sized pericarp cell varied only slightly among the four NILs. Final cell size was similar in L4 and Lev, and somewhat lower in L9, L2, and Lx (Fig. 3B). Cells expanded over the whole period of fruit development, and reached an average volume of ~10.6 nl. Conversely, Cervil cells stopped growing at ~20–25 DAA, and their final volume was >3 times smaller (3 nl).

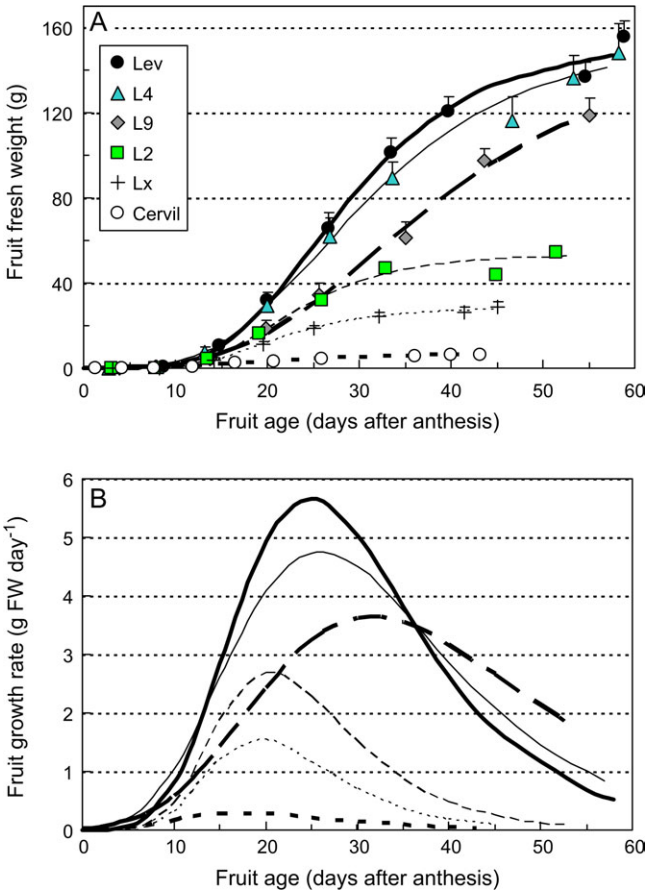

Fig. 2. (A) Fruit fresh weight increase during fruit ageing. Curves were fitted with three-parameter Gompertz functions (R^2 > 0.92). For each genotype (Lev, solid bold line; L4, solid thin line; L9, large dotted bold line; L2, large dotted thin line; Lx, small dotted thin line; Cervil, small dotted bold line) 150–300 individual fruits were sampled between anthesis and red mature stage at 5 d intervals. Each point is the mean of >20 fruits, and vertical bars show 95% CIs when larger than symbols. (B) Growth rates were deduced from the derivative curves.

Variations in fruit fresh weight were mainly correlated to variations in cell number, both among and within genotypes (Fig. 3C). No significant relationship existed between final fruit weight and final cell size among the QTL-NILs (not shown).

Is endoreduplication involved in differences in size of contrasted NILs?

The EF [Eqn (1)] is the number of endocycles an average cell has undergone. To account for differences in developmental periods among lines, an index of development was calculated as the ratio between fruit age and the average time needed to ripen. EF increased early during fruit development, to final average values of 3.0 endocycles in Lx, 3.1 in Cervil, 3.3 in Lev and L4, 3.4 in L2, and 3.5 in L9 (Fig. 4A), without significant differences among genotypes.

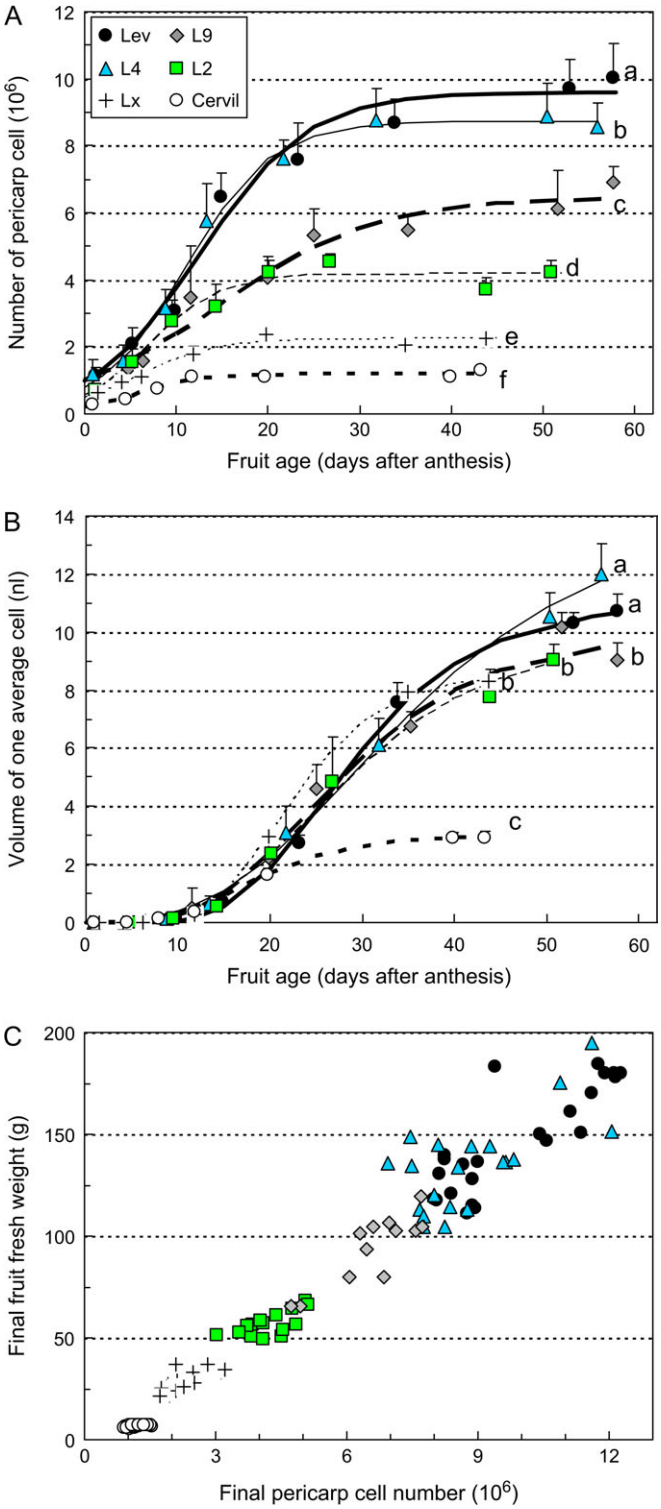

Fig. 3. Dynamics of (A) pericarp cell number and (B) volume of one mean cell during fruit ageing. Each point is the mean of 8–10 fruits, and vertical bars show 95% CIs when larger than symbols. Basal and tip fruits were pooled which explains data scattering, in particular for the large fruit genotypes. Cell number data were fitted by a three-parameter logistic function (R^2 >0.80) and cell volume data were fitted by a three-parameter Gompertz function (R^2 >0.96) (Lev, solid bold line; L4, solid thin line; L9, large dotted bold line; L2, large dotted thin line; Lx, small dotted thin line; Cervil, small dotted bold line). Lettering indicates statistically homogenous groups at maturity (P <0.05). (C) Relationships at maturity between fruit fresh weight and cell number. Each point represents an individual fruit.

The mean DNA amount accumulated in cells increased during fruit development, with slight differences among lines. Cervil, Lx, and L2 had the lowest values, L4 and L9 the highest values, whereas Lev was intermediate (Fig. 4B). Fruit fresh weight and mean ploidy index [Eqn (2); not shown] or final cell DNA content and final cell size (Fig. 4C) were positively correlated [r=0.94 (P <0.01) and r=0.86 (P=0.03), respectively].

Does osmotic regulation contribute to the QTLs for fruit size?

The temporal dynamic of fruit osmotic pressure due to both sugars and acids is shown in Fig. 5. The two parental lines largely differed in their osmotic regulation. In Cervil fruits, the osmotic pressure increased from the first third of development until maturity, to ~0.66 MPa, whereas it was rather stable in Lev fruits at ~0.3 MPa. In L9, the fruit osmotic pressure was higher than in Lev, and it linearly increased over the whole period of development to 0.5 MPa at maturity. The dynamics in Lx, L2, and L4 were close to that observed in Lev, despite small significant differences between Lx and Lev at maturity (Fig. 5). When considered separately, the fruit osmotic pressure due to organic acids was less than half the fruit osmotic pressure due to sugars, except during the very early stage (development index of 0.1) when it was slightly higher (not shown). No global correlation between fruit size and osmotic pressure was evidenced among genotypes.

QTLs for fruit composition in relation to the pattern of carbon accumulation and partitioning

Fruit dry matter content varied >2-fold between the parental lines (from 4.4% to 10.4%), and Cervil fruits had the highest values. As for the fresh weight, Lev and L4 fruits were similar (4.6%), whereas all other QTL-NILs contained more dry matter than Lev (5.3, 6.0, and 6.6% in L2, Lx, and L9, respectively), and they were significantly different from one another.

The total amount of carbon accumulated as sugars (g soluble sugar+starch per 100 g dry matter) was higher in L9 and in Cervil fruits at some periods of development (Fig. 6A), but differences among genotypes at maturity were not significant. However, the patterns of starch and soluble sugar accumulation were quite different among genotypes. Lev fruits accumulated half the starch and twice the soluble sugars of that in Cervil fruits, and the starch pool decreased much later in Cervil fruits, which contained a low amount of starch at maturity (Fig. 6B). Patterns of starch and soluble sugar contents were similar in L2, L4, and Lev (Fig. 6B, C). In L9 and Lx, these patterns were intermediate between the parental lines, but L9 was marked by a high soluble sugar content, close to that of Lev fruits. Dynamics of individual sugars (mainly glucose and fructose) were

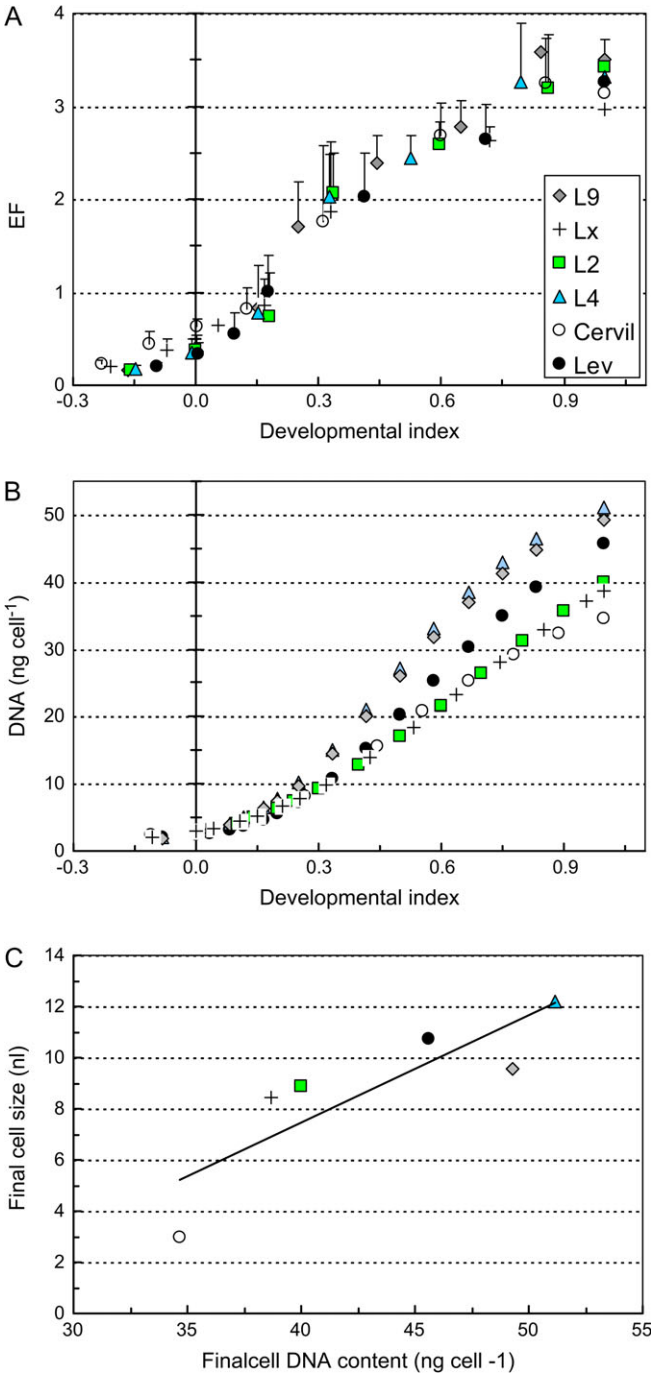

Fig. 4. (A) Endoreduplication factor measured during fruit development. Each point is the mean of 7–15 fruits, and vertical bars are 95% CIs. (B) Dynamics of cell DNA content estimated from the C level of an average cell measured during fruit development and considering that 1C is equivalent to 950 pg of DNA [Eqn (3)]. (C) Relationships between final cell size and final cell DNA content for the two parental lines and four QTL-NILs.

similar to that of the sum of sugars, and the ratio between fructose and glucose was not significantly different among the lines at maturity (not shown). Excluding L9, there was a compensative relationship between soluble sugar content and starch content during fruit development.

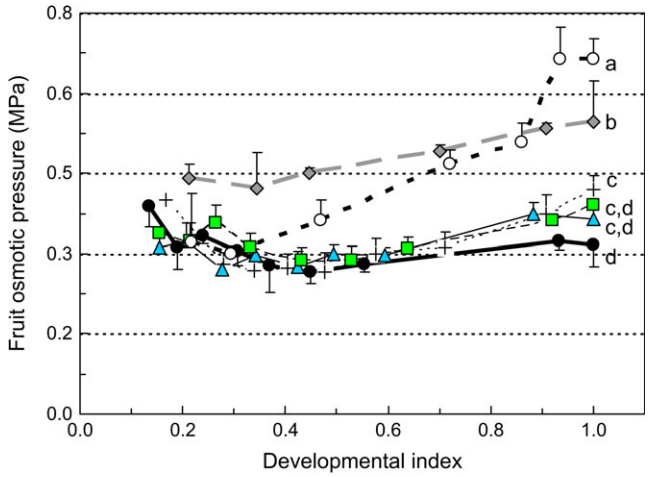

Fig. 5. Dynamics of the fruit osmotic pressure (calculated according to Nobel, 1974) due to soluble sugar and organic acid accumulation in each genotype (Lev, solid bold line; L4, solid thin line; L9, large dotted bold line; L2, large dotted thin line; Lx, small dotted thin line; Cervil, small dotted bold line). Each point is the mean of 10–15 fruits, and vertical bars are 95% CIs. Lettering indicates statistically homogenous groups at maturity (P <0.05).

Finally, Cervil fruits contained the highest soluble sugar content on a fresh weight basis (3.3% against 1.7% in Lev, 2.0% in L2 and L4, and 2.2% in Lx), but the lowest content on a dry weight basis. In contrast, L9 fruits contained among the highest soluble sugar contents on both a fresh (2.9%) and a dry weight basis (42%), which in addition to the low water content may explain the higher osmotic pressure in this line.

The patterns of citric and malic acid contents were different in the two parental lines (Fig. 6C, D). In Cervil fruits, the contents of both acids remained low in the first period of development, then increased in the second half of development and decreased again during maturation. In Lev, the citric acid content decreased in the first half of development and then increased later, in contrast to the pattern of malic acid accumulation. Lev fruits were richer in malic acid and poorer in citric acid than Cervil fruits. In the four QTL-NILs, the dynamics of acid accumulation were close to those observed in Lev. At maturity, the citric acid content was significantly increased in L2 and L9 compared with Lev, whereas the malic content was significantly decreased in all NILs, especially in Lx and L9.

Allocation of dry matter between structure and soluble storage may be involved in QTLs for quality

The percentage dry matter allocated to the structural compounds rapidly stabilized during development to between 50% and 55% of total fruit dry matter. Slightly lower proportions were observed for Cervil and L9 fruits in the first half of development, hypothetically because of the high transient storage as starch (Fig. 6B), Indeed, at maturity, whereas the pool of starch has been exhausted, differences among genotypes were no longer significant (Fig. 7A). The

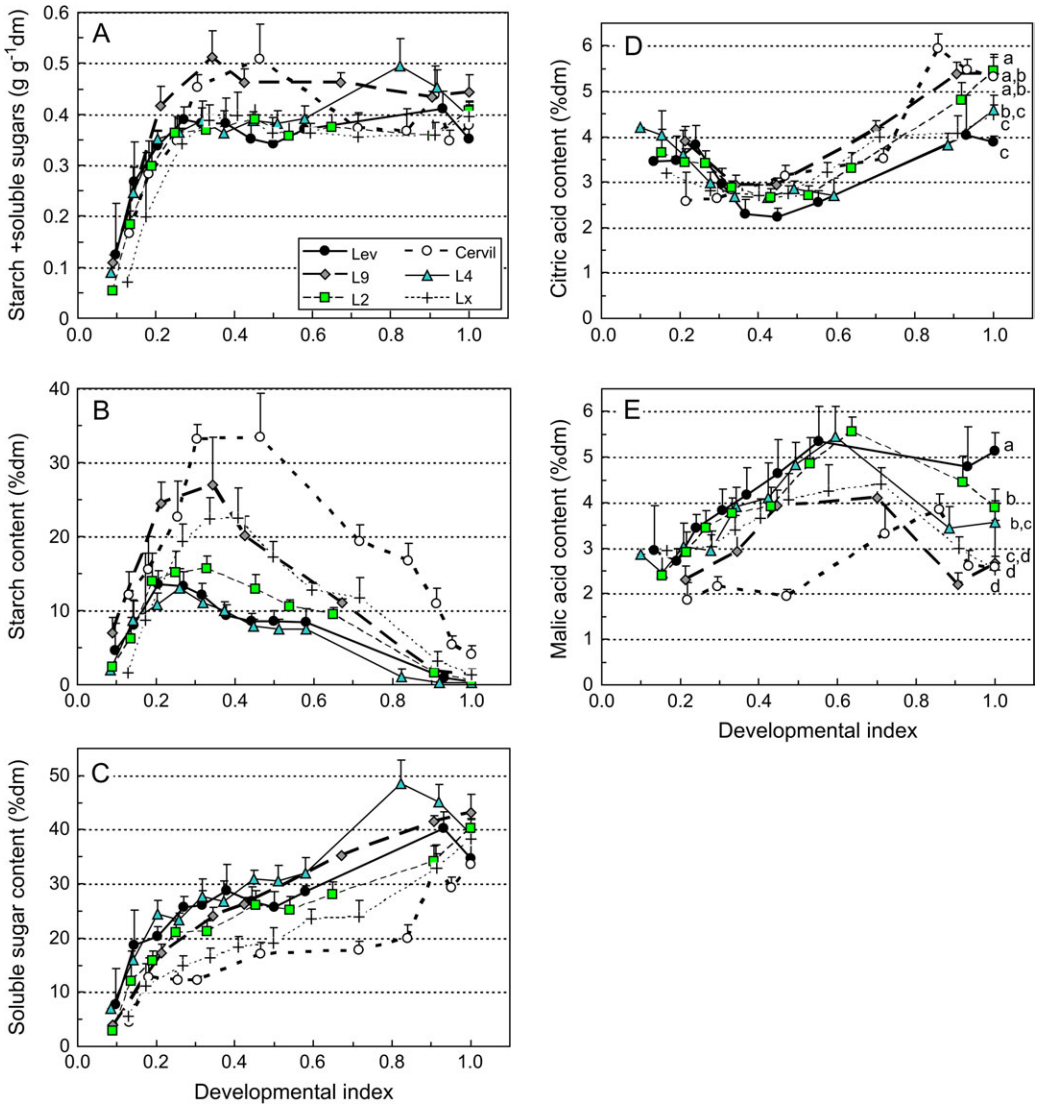

Fig. 6. Dynamics of (A) total carbohydrate, (B) starch, (C) soluble sugars, (D) citric acid, and (E) malic acid contents on a dry matter basis, during fruit development of each genotype (Lev, solid bold line; L4, solid thin line; L9, large dotted bold line; L2, large dotted thin line; Lx, small dotted thin line; Cervil, small dotted bold line). Each point is the mean of 10–15 fruits, and vertical bars are 95% CIs. Lettering indicates statistically homogenous groups at maturity (P <0.05) when the ANOVA outlined a significant genotype effect.

absolute amount of structural matter per unit cell area was calculated as an indicator of cell wall thickness, which probably plays a role in fruit texture. Compared with Lev, it was 1.5 times higher in Cervil (Fig. 7B) due to the low number of very small cells, and more than double in L9 fruit, due to their high fruit dry weight (9.03 g fruit^{-1} versus ~6.8 g fruit^{-1} in Lev or L4) combined with slightly fewer and smaller cells. L2 had the lowest value, due to the low fruit dry weight (2.7 g fruit^{-1}) and lowest proportion of structural dry matter.

Discussion

In genetics, QTL analysis allows breeders to locate genetic factors associated with variations of traits of interest, and to transfer favourable QTL alleles in recipient lines through marker-assisted selection. Seldom have ecophysiological analyses of these QTLs been performed, though they may emphasize the processes affected by these QTLs (Bertin *et al.*, 2003; Quilot *et al.*, 2005), and help bridge the gap between QTLs and genes (Quarrie *et al.*, 2006). Among the processes investigated in the present study, some of them were specifically modified depending on genotypes, and could be related to QTL effects.

QTLs for fruit size mainly reflected QTLs for cell division on chromosomes 2 and 9

In many species and organs, QTLs for yield or size have often been associated with division processes (Bertin *et al.*, 2003; Quarrie *et al.*, 2006). Differences in fruit size between wild and domesticated tomato were also attributed to differences in cell division (Kortstee *et al.*, 2007). Although organ size cannot be simply considered as the sum of cell size and number (Tsukaya, 2008), in the present study, the genetic

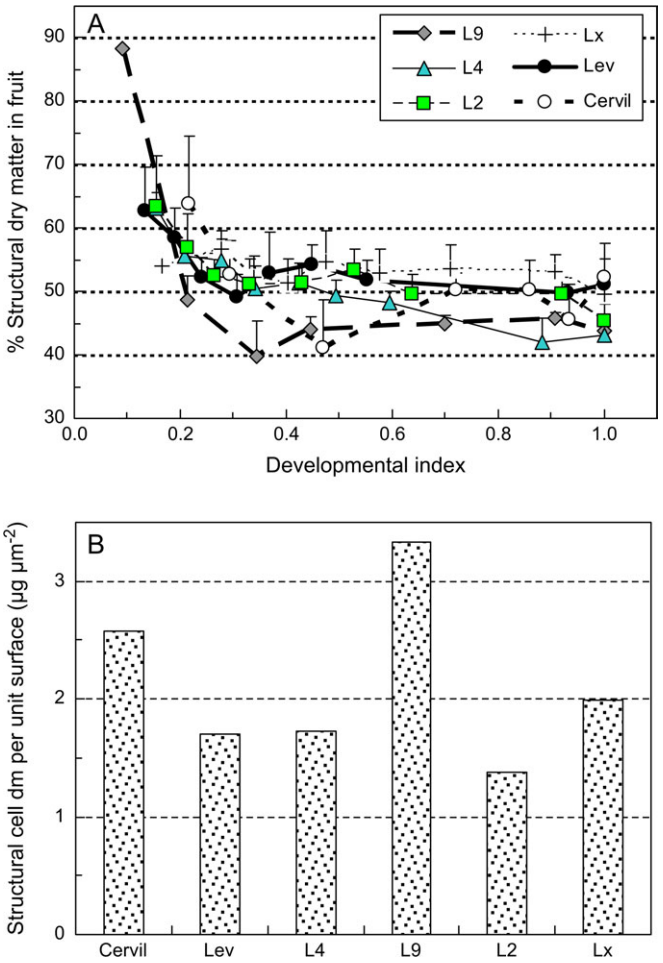

Figure 7. (A) Dynamics of the percentage of structural dry matter estimated during fruit development. Each point is the mean of 10–15 fruits, and vertical bars are 95% CIs. (B) Estimation of structural dry weight per cell surface at maturity for the different genotypes.

variation in fruit fresh weight, as well as the environmental variability within each genotype (from 5 g to 180 g), were tightly correlated to differences in cell number (Fig. 3), without compensatory effects between number and size of cells. Two types of controls could be discerned: the first observed in L2 mainly shortened the period of cell division (60% fewer cells than in Lev), whereas the second observed in L9 mainly lowered the rate of cell division (20% fewer cells than in Lev). In Lx, which contained 80% fewer cells than Lev, both effects probably co-occurred. These results are consistent with the presence of Fw2.2 in the region introgressed in L2, a QTL key for tomato fruit size, which affects the timing of gene regulation during the early phase of mitotic activity (Frary *et al.*, 2000; Cong *et al.*, 2002). In addition, the results suggest that a gene(s) regulating cell division may be present on top of chromosome 9. Interestingly, the data suggested that differences in the cell division rate at the fruit level mainly resulted from different sizes of the cell population, probably originated from genetic variations in mitotic activity in the shoot apical meristem, which can affect the floral meristem size (Gyllaspy *et al.*, 1993).

Small cells in Cervil fruits probably resulted from low cell plasticity

In contrast to division, cell expansion was hardly different in Lev and in the QTL-NILs. The lower cell size in small fruits was mostly related to the shorter period of growth, except in Cervil. Interestingly, the reduction of the length of the division period was exactly reflected in the duration of the whole period of development, supporting the idea that the first may determine the second, and might underlie fruit size (Cong *et al.*, 2002). However, in Cervil fruits, the low cell expansion could not be ascribed only to the short period of development. Other factors, such as cell plasticity or factors driving the influx of water, were probably involved. Irreversible cell expansion is powered by the turgor pressure generated by osmotic pressure within the cell (Lockart, 1965), and the inflow of water is driven by the gradient of water potential between fruit and surrounding tissues (Lee, 1989). High osmotic pressure (low osmotic potential) in fleshy fruit has been assumed to allow the development of low water potential, and thus the maintenance of a high gradient of water potential between the plant and the fruit. However, in this study, the absence of a positive correlation between osmotic pressure and cell/fruit size, and the high structural dry weight per cell surface estimated in Cervil fruits, would promote the hypothesis of low cell plasticity.

The link between endoreduplication and tomato fruit size remains unclear

Endoreduplication might be an important process involved in QTLs for fruit size (Sugimoto-Shirazu and Roberts, 2003). Among pea genotypes (Lemontey *et al.*, 2000) or contrasted tomato lines (Cheniclet *et al.*, 2005), positive correlations between endoreduplication and seed or pericarp cell size have been reported. In this study, despite a 25-fold increase in fruit fresh weight, the EF changed by less than one endocycle among genotypes, and was unrelated to cell or fruit size. However, the mean cell DNA content varied by 1.5-fold, and it was positively correlated to the final cell size or to the fruit fresh weight. It remains difficult to draw conclusions about the relevance of these relationships, as they may result from different lengths of development. However, Cervil fruits contained very small cells and low DNA content compared with Lx, whose developmental period was only a few days longer.

Differences in dry matter content and allocation between structural and storage compounds may explain QTLs for fruit sugar content and texture

In many genetic studies, the chemical composition of fruits is analysed on a fresh weight basis, as this is the most relevant to assess the perception of taste by consumers (Causse *et al.*, 2003). In the present study, the higher sugar content on a fresh weight basis in small fruit size genotypes could be attributed to their high dry matter content (low water content), rather than to the increase of sugar accumulation in the dry matter. In accordance, QTLs for sugar or soluble

solid contents (Fig. 1) co-localized with QTLs for dry matter content on the bottom of chromosome 2 and the top of chromosome 9. In contrast, the QTLs for sugar content on a dry matter basis were unexplained, and even in contradiction to the highest sugar content observed in L9 fruits.

In L9 and Cervil fruits, slightly lower proportions of fruit dry matter were allocated to structures in the first half of development, hypothetically because of the allocation of carbon for starch synthesis, whose turnover reduced the differences among genotypes at maturity. The amount of carbon allocated to cell structures may be involved in fruit texture (Bourne, 2002), a complex quality trait which implies fruit firmness, mealiness, and juiciness. QTLs for texture have been detected on chromosomes 4 and 9 (Causse *et al.*, 2002; Chaib *et al.*, 2006), but these QTLs depend on the environment (Chaïb *et al.*, 2007). These authors reported a higher firmness, and a lower elasticity and mealiness of L9 and Lx fruits compared with Lev, though differences were not always significant. If one assumes that increasing cell wall thickness probably contributes to increase fruit firmness and to decrease fruit elasticity, the present analysis agreed with the results reported by Chaib *et al.* (2007) for L9. These results are supported by a recent study which outlined different chemical composition and structure of cell walls in L9 and Lx fruits (Quemener *et al.*, 2007). In contrast, high juiciness and low mealiness of L4 fruits (Chaib *et al.*, 2007) would be related to large cells, high water content, and a low amount of structural dry matter per cell surface.

Genes involved in the control of starch synthesis are present on top of chromosome 9

In agreement with the literature (Ho, 1986) which suggests that the size of the starch pool serves as a reservoir for the synthesis of soluble sugars, the peak of starch content and the final sugar content in percentage fresh weight were positively correlated. In contrast to Cervil or L2, L9 fruits also accumulated large amounts of soluble sugars during the phase of starch accumulation, resulting in the highest content of sugars in the dry matter. Starch accumulation may be enhanced by an increase of the main enzyme activity [ADP glucose pyrophosphorylase (ADPGppase)] or of the level of synthesis of the substrate (glucose 6P) (Robinson *et al.*, 1988; Nguyen-Quoc and Foyer, 2001); it is also triggered by the amount of sucrose unloaded in the fruit (D'Aoust *et al.*, 1999; N'Tchobo *et al.*, 1999), which is higher in cherry tomatoes than in large-fruited cultivars (Islam and Khan, 2001). The presence of the apoplastic cell wall invertase gene *lin5* (Fridman and Zamir, 2003; Fridman *et al.*, 2004) and of a hexose transporter (Causse *et al.*, 2004), together with the absence of a gene regulating the ADPGppase on chromosome 9 (Schaffer *et al.*, 2000), supports the hypothesis that high starch accumulation in L9 was stimulated by the higher sucrose unloading, and hexose transport within the cells (Ruan *et al.*, 1995, 1997; Ho, 1996; Schaffer *et al.*, 1999), as observed in an other study (Li *et al.*, 2002). However, the fact that high starch, but not high soluble contents, were recovered in Lx suggested the involvement of several levels of regulation.

In cherry tomatoes, the gluconeogenesis, which leads to the refixation of CO_2 inside the fruit by photosynthesis or by dark fixation as malate, was shown to promote starch synthesis and dry matter accumulation (Ho, 1996). The hypothesis of such a regulation in Cervil and in L9 fruits may be put forward, as they contained less malate and more starch than the other genotypes.

Conclusion

By analysing the main ecophysiological processes involved in fruit growth and composition, it was possible to outline major differences among QTL-NILs involved in the genetic control of final fruit size and composition. These differences are in accordance with molecular or genetic analysis already performed on these tomato lines, but they also pointed to some new hypotheses, in particular for L9. This line presented singular patterns of growth and accumulation of dry matter and soluble solids, and it differed from the parental lines by accumulating different QTL effects, implying cell division, cell wall synthesis, and sugar and acid metabolism. The top of chromosome 9, in contrast to chromosome 2, seems an interesting region to accumulate fruit size and composition attributes, as sugar content was increased without substantial loss of fruit size, which represents a real challenge for breeders.

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