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1 **Genetic and physiological analysis of tomato fruit weight and composition –**

2 **Influence of carbon availability on QTL detection**

3

4 Short running title: tomato size and sugar content

5

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1 **Abstract**

2 Throughout tomato domestication, large increase in fruit size was associated with loss of dry
3 matter and sugar contents. This study aims at dissecting the contributions of genetic variation and
4 physiological processes underlying the relationships between fruit growth and accumulation of dry
5 matter and sugars. Fruit quality traits and physiological parameters were measured on twenty
6 introgression lines derived from the introgression of *Solanum chmielewskii* into *S. lycopersicum*,
7 under high (HL, unpruned trusses) and low (LL, trusses pruned to one fruit) fruit load conditions.
8 Inter- and intra-genotypic correlations among traits were estimated and quantitative trait loci
9 (QTL) for size, composition and physiological traits were mapped. LL increased almost all traits,
10 but the response of sugar content was genotype dependent, involving either dilution effects or
11 differences in carbon allocation to sugars. Genotype x fruit load interactions were significant for
12 most traits and only 30% of the QTL were stable under both fruit loads. Many QTL for fresh
13 weight and cell or seed numbers co-localized. Eleven clusters of QTL for fresh weight and dry
14 matter or sugar content were detected, eight with opposite allele effects and three with negative
15 effects. Two genotypic antagonistic relationships, between fresh weight and dry matter content and
16 between cell number and cell size, were significant only under HL; the second could be interpreted
17 as a competition for carbohydrates among cells. The role of cuticular conductance, fruit
18 transpiration or cracking in the relation between fruit fresh weight and composition was also
19 emphasized at the genetic and physiological levels.

20 **Key words**

21 Carbon allocation, physiological processes, fruit load, genetic variability, genotype x environment
22 interaction, quality, QTL, *Solanum lycopersicum*, *Solanum chmielewskii*, sugar content

23

1

2 **Introduction**

3 Plant domestication is the genetic modification of wild species to meet human needs (Doebley *et*
4 *al.*, 2006). In tomato, wild species originated in the Andes region where their tiny sweet fruits
5 propagate the species. Tomato domestication dramatically increased fruit yield, and changes
6 mainly occurred in fruit morphology or plant development. The most obvious evolution has been
7 the massive increase in fruit size (Tanksley, 2004; Bai and Lindhout, 2007; Cong *et al.*, 2008)
8 which was associated with a reduction in sugar content and subsequently in sweet flavour. Soluble-
9 solids content, mainly constituted by sugars, is high in wild tomato species such as *Solanum*
10 *pimpinellifolium* or *Solanum chmielewskii* with more than 6% and 10% of the fruit fresh weight,
11 respectively (Rick, 1974), whereas most of fresh-market tomatoes contain less than 4% soluble
12 solids. A negative relationship between fresh weight and soluble solids content in the fruit
13 (Goldenberg and von der Pahlen, 1966; Ibarbia and Lambeth, 1971) hampers the transfer of high
14 soluble-solids content from wild species into cultivated varieties. Several reviews summarize the
15 numerous experiments of quantitative trait loci (QTL) mapping for tomato fresh weight and
16 soluble solids content, and their co-localizations (Grandillo *et al.*, 1999; Causse *et al.*, 2006;
17 Foolad, 2007). QTL for these two traits are frequently co-localized with opposite allele effects.
18 Such QTL co-localizations can be due either to genetic linkage, and recombination could modify
19 the relationships, or to fruit physiology and these relationships cannot be genetically modified.
20 Yousef and Juvik (2001) identified chromosomal segments from *S. chmielewskii* that had a
21 positive influence on fruit soluble solids while maintaining fruit size unaltered. Similarly, the
22 alleles of *S. pennellii* at the QTL *Lin5* for soluble solids content increases sugar content without
23 reducing total yield (Fridman *et al.*, 2004).

24 In breeding programs, interactions between genotype and environment limit the possibility to
25 increase sugar content. These interactions were studied in wheat through comparisons of various
26 trial locations (Robert, 1997; Matus-Cadiz *et al.*, 2003) or through impact of stresses (drought,

1 salt) (Snape *et al.*, 2007; Williams *et al.*, 2008). In tomato, QTL detection under different saline
2 conditions revealed a QTL specific to saline conditions (Villalta *et al.*, 2007), indicating strong
3 interactions between QTL and environment.

4 Understanding the relation between fruit fresh weight and sugar content in tomato fruits requires
5 identifying the key processes underlying this relationship, both from the genetic and physiological
6 points of view. The integration of physiological and genetic approaches allows a better dissection
7 of the genetic basis of complex traits as well as their interactions with the environment. For
8 instance, several co-localizations of QTL for fruit weight, fruit dry matter content or fruit sugar
9 content and QTL for ecophysiological parameters were identified in peach (Quilot *et al.*, 2005),
10 suggesting which physiological processes were involved in the genetic variation.

11 Fruit weight and composition depend on the balance between inward and outward fluxes to/from
12 fruit (mostly water and carbon), which involve many different processes. Transpiration leads to a
13 water loss (Wu *et al.*, 2003), and may decrease the fruit fresh weight and concentrate the soluble
14 compounds. Cell division and cell expansion determine final fruit size and carbohydrate dilution
15 within cells (Bohner and Bangerth, 1988; Ho, 1996). The number of cells influences the structural
16 dry matter through the amount of cell wall. The size of cells mainly affects the capacity to store
17 soluble dry matter. Finally, the number of seeds may interfere with cell division and cell expansion
18 through the production of hormones (Rylski, 1979; Gillaspay *et al.*, 1993). At the plant level,
19 characteristics of the leaves (carbon source) could affect the sugar production *via* photosynthesis.
20 Carbon supply can be modified by environmental stresses or cultural practices. For instance, fruit
21 thinning reduces the competition for carbon, and thus promotes fruit size and sugar content in
22 several species, like peach fruits (Morandi *et al.*, 2008), apple fruits (Link, 2000), mandarin fruits
23 (Kubo *et al.*, 2001), and papaya fruits (Zhou *et al.*, 2000). In tomato, this treatment has previously
24 been carried out, increasing in similar proportions fresh and dry weight (Heuvelink, 1997).

25 The aims of this study were first to determine and dissect the relative influence of genotypes and
26 fruit load on fruit weight and sugar content, then to identify which processes were underlying these
27 traits and their relationships, at both genetic and physiological levels. For this purpose, a set of

1 physiological, biochemical and morphological parameters related to the main processes of fruit
2 growth and plant development were measured in a population consisting of 20 introgression lines
3 derived from a cross between a wild tomato species (*Solanum chmielewskii*) with small green
4 fruits and a medium sized cultivar (*Solanum lycopersicum* L.). QTL mapping and interactions
5 between QTL and carbohydrate availability were examined over two years, by comparing plants
6 under two contrasted fruit loads. One fruit per truss was the condition with no limitation for
7 carbohydrate supply and corresponded to maximum genotypic potential. The second condition was
8 similar to what is currently applied for genetic studies: a free load condition (without fruit
9 pruning), with competition for assimilates among fruits.

10

11 **Materials and methods**

12 ***Plant material***

13 The study was performed using the *Solanum lycopersicum* line ‘Moneyberg’ (hereafter referred to
14 as M) and 20 indeterminate lines carrying single or multiple introgressions of the *Solanum*
15 *chmielewskii* LA1840 in the background of Moneyberg, kindly provided by Keygene (The
16 Netherlands). Each line was named by the chromosomal number and the location of the largest
17 introgression. For instance, genotype C3a was the line that contained an introgressed fragment at
18 the top of chromosome 3, while genotype C3d possessed an introgression at the bottom of
19 chromosome 3.

20 ***Growth conditions and experimental treatments***

21 Seeds were sown at the end of February, and a total of 400 plants were grown at a density of 3.6
22 plants m⁻² in a ground bed greenhouse in Avignon (Southern France) at day-night temperature set
23 points of 24/16°C during spring 2006 (March-July) and 25/15°C during spring 2007 (March-July).
24 Plants were randomly distributed in two blocks containing each 200 plants and facing respectively
25 North and South. Plant nutrition and chemical pest and disease control followed commercial

1 practices and plants were conducted on single vine. Starting from anthesis of the first truss, flowers
2 were pollinated with an electrical shaker every two to three days.
3 For each genotype, ten plants were randomly selected in the first block while nine plants were
4 randomly selected in the second block. On 12 plants of each genotype, trusses were pruned to one
5 fruit (low fruit load, LL) while on seven other plants trusses were not pruned (high fruit load, HL).
6 Under HL condition, the average number of fruit sets per truss within the population was 5.3. On
7 each inflorescence of the LL plants, all the flowers except the second one were removed just after
8 fruit set. The fruit removal experiment concerned the first nine trusses. All the plants were stopped
9 two leaves above the ninth truss.

10 ***Observations and measurements***

11 *Plant development*

12 Anthesis time, achieved as flower fully opened, was recorded three times a week in order to
13 determine fruit age and fruit development duration (**Dura** expressed in days) considered as time
14 between anthesis and red ripe stage. Plant development traits, used as indicators of plant vigour
15 and carbohydrate supply, were measured on nine contrasted genotypes (C11b, C12d, C3a, C3c,
16 C4c, C4d, C7a, C8e and C9d) and M. For each genotype and treatment, four randomly selected
17 plants (two per block) were measured for the number of leaves (**LfN**) until the ninth truss and the
18 height of the fourth truss (**H4t** expressed in cm), at the end of the growing season. The area of five
19 representative leaves were measured using a planimeter, and then the total leaf area (**LfA**
20 expressed in cm²/plant) was calculated by multiplying the mean leaf area by the number of leaves.
21 Dry weight of the five leaves was assessed after five days in a ventilated oven at 80°C and the
22 specific leaf weight (**SLW** expressed in g.cm⁻²) was calculated.

23 *Fruit cuticular conductance and cracking*

24 Fruit transpiration is an important process involved in fruit growth and water content. Fruit
25 cracking and fruit cuticular conductance were thus measured. Fruit surface conductance to water

1 vapor diffusion (**CutC** expressed in $\text{cm}\cdot\text{h}^{-1}$) was measured as described in Gibert *et al.* (2005), only
2 on fruits harvested in 2007, and at 21 days post anthesis, as this stage corresponds to the visual
3 absence of cuticular macro-cracks. Five fruits per genotype and per fruit load, located on the fifth
4 truss (flower 2), were harvested on five plants randomly selected within the two blocks. Then, fruit
5 cracking was estimated on five red ripe fruits, harvested for each genotype and treatment on five
6 different plants randomly selected within the two blocks. In order to eliminate bias due to
7 competition within and among trusses, only the second fruits of the fourth trusses were harvested.
8 On each fruit, cheek, suture and height diameters were measured using a calliper and the total
9 cuticle area was calculated assuming spherical form. The cuticular macro-crack area (**FCr**
10 expressed in percentage of cracked area relative to the total cuticle area) was calculated by drawing
11 their outlines on tracing paper, cutting the area and measuring it with a planimeter.

12 *Fruit composition, cell number and size*

13 The five red ripe fruits analyzed for **FCr** were used for fruit composition, and cell measurements.
14 Fruit fresh weight was measured (**FW** expressed in g / fruit), jelly and seeds were removed from
15 the fruits and seeds were counted (**SdN**). Then, pericarp tissue including external, internal and
16 transverse parts was weighted. One half of the fruit pericarps was ground in liquid nitrogen using a
17 blender. Powders were freeze-dried and stored at -20°C prior to sugar extraction. Pericarp dry
18 weight (**DW** expressed in g) and dry matter content (**DMC** expressed in g / 100 g FW) were
19 measured after lyophilization of tomato powders. Sugars were extracted from the pericarp
20 according to the method described in Gomez *et al.* (2002). The main sugar contents (glucose,
21 fructose and sucrose) were quantified by enzymatic assay in 96-well microplates, as detailed in
22 Gomez *et al.* (2007). Sugar contents were expressed relative to the pericarp fresh weight (**SUGfw**
23 in g / 100 g FW) or to the pericarp dry weight (**SUGdw** in g / 100 g DW). The pericarp structural
24 carbon content (**StrCfw**) was estimated as follow:

$$25 \text{StrCfw} = \text{DMC} - \text{SUGfw} \quad [1]$$

26 where **StrCfw**, **DMC** and **SUGfw** are expressed in g / 100 g FW. This relationship was

1 experimentally checked on 30 red ripe fruits, by the extraction of pericarp insoluble material after
2 sugar eliminations from lyophilized powders.

3 On the second half of the fruit pericarp, cell division and expansion were evaluated by measuring
4 the number (CIN) and mean size (CIS expressed in nL) of pericarp cells, according to the method
5 described in Bertin *et al.* (2002).

6 **DNA markers and assays**

7 A set of PCR-based markers consisting of 130 Conserved Ortholog Set II (COSII) markers (Wu *et*
8 *al.*, 2006); http://www.sgn.cornell.edu/markers/cosii_markers.pl) and 3 Simple Sequence Repeats
9 (SSR) (Frary *et al.*, 2005), covering all 12 tomato chromosomes, were used to genotype the
10 population of 20 *S. chmielewskii* LA1840 introgression lines (IL).

11 Genomic DNA of the two parent lines and of the 20 IL was extracted from leaf tissue of three
12 weeks old plants according to the protocol of Fulton *et al.* (1995).

13 The PCR of each marker was performed on both parents (Moneyberg and LA1840) in 25 µl
14 reactions containing 50 ng of template DNA, 2.5 pmol of each forward and reverse primer, 1X
15 Colorless GoTaq[®] Flexi Buffer (Promega) and, 0.2 mM dNTPs, and 0.2 U GoTaq[®] DNA
16 Polymerase (Promega). The reactions were amplified using a DNA Engine (PTC-200) Peltier
17 Thermal Cycler (Biorad). Amplification consisted of an initial denaturation for 5 min at 94° C,
18 followed by 35 cycles of amplification with denaturation at 94° C for 30 s, annealing at 55° C for
19 45 s, and extension at 72° C for 60 s, with a final cycle of 72° C for 5 min. Following
20 amplification, PCR products were analyzed by electrophoresis on 1% agarose gels in 1X TAE
21 buffer for 1-2 h at 100 V and room temperature. Direct fragment length polymorphism was
22 detected for 12% of the COSII markers. In the other cases, the PCR products of the parent lines
23 were digested with different frequent cutter restriction enzymes including *TaqI*, *HinfI*, *AluI*, *DraI*,
24 *RsaI*, *MseI* and electrophoresed through 2% agarose to identify polymorphisms. If no
25 polymorphism was detected with these enzymes, then the PCR products of the two parent lines
26 were sequenced. For this purpose, amplicons showing a single band on agarose gels were cleaned

1 up from nucleotides and residual primers using 10 µl of PCR product and 2 µl of ExoSAP-IT[®],
2 and the mixture was incubated at 37°C for 1h and then at 80°C for 15 minutes. A 10 µl sequencing
3 reaction volume was prepared using 2 µl of the cleaned PCR product, 1 µl of either the forward or
4 reverse primer, 1 µl of 5X BigDye Buffer 3.1 and 1 µl BigDye terminator v3.1 (Applied
5 Biosystems). The sequencing PCR consisted of initial denaturation at 96°C for 60s and 25 cycles
6 of 10 seconds at 96°C, 5 sec at 55°C, and 4 min at 60°C. The sequences were obtained through
7 ABI Prism 3100 Genetic Analyzer automated sequencer (Applied Biosystems). Restriction maps
8 were predicted using CAPS designer (http://www.sgn.cornell.edu/tools/caps_designer).
9 For some of the regions where polymorphic COSII markers were not found, SSR were surveyed in
10 order to increase the map saturation. PCR reactions and thermocycling were as described for the
11 COSII markers; PCR products were separated on 2.5% agarose gels in 1X TAE buffer for 1-2 h at
12 100 V and room temperature.
13 Sequences of the primers used for the COSII and SSR assays are available on the SGN website
14 (<http://www.sgn.cornell.edu>). The polymorphic COSII and SSR markers were then assayed on the
15 20 IL.

17 **Statistical analyses**

18 Altogether, data obtained in this study contained very few missing values, and all variables were
19 normally distributed. After checking that block effect was not significant, the effects of year,
20 genotype, fruit load and their interactions on each trait were analyzed using a three-way ANOVA.
21 The linear model used was:

$$22 \quad Y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + \varepsilon_{ijkl} \quad [2]$$

23 where Y_{ijkl} is the trait value for plant l on genotype i , fruit load j and year k ; μ is the general mean ;
24 α_i is the effect of genotype i , β_j is the effect of fruit load j , δ_k is the effect of year k ; $(\alpha\beta)_{ij}$ is the
25 effect of the interaction between genotype i and fruit load j ; $(\alpha\delta)_{ik}$ is the effect of the interaction

1 between genotype i and year k ; $(\beta\delta)_{jk}$ is the effect of the interaction between fruit load j and year
2 k and ε_{ijkl} is the error term.

3 The percentage of variation due to each factor was then calculated as:

$$4 \quad V_i = \frac{SS_i \times 100}{\sum_i SS_i} \quad [3]$$

5 where SS is the sum of squares of the factor i . For each trait t , the percentage of fruit load variation
6 (Δ_{FL}) from HL to LL was calculated following:

$$7 \quad (\Delta_{FL})_t = \frac{(\mu_t)_{LL} - (\mu_t)_{HL}}{(\mu_t)_{HL}} \times 100 \quad [4]$$

8 where $(\mu_t)_{HL}$ and $(\mu_t)_{LL}$ are the general means of the trait t under HL and LL, respectively.

9 For each genotype, the effect of fruit load on fruit fresh weight (FW), pericarp dry weight (DW),
10 pericarp dry matter content (DMC), and pericarp sugar contents (SUGfw and SUGdw), was
11 analyzed by a two-way analysis of variance following this model:

$$12 \quad Y_{jkl} = \mu + \beta_j + \delta_k + (\beta\delta)_{jk} + \varepsilon_{jkl} \quad [5]$$

13 where Y_{jkl} is the trait value for plant l , fruit load j and year k ; μ is the general mean ; β_j is the effect
14 of fruit load j , δ_k is the effect of year k , $(\beta\delta)_{jk}$ is the effect of the interaction between fruit load j
15 and year k and ε_{jkl} is the error term.

16 Multivariate analyses were conducted using the MANOVA function in R ([http://www.r-](http://www.r-project.org)
17 [project.org](http://www.r-project.org)). Genotypic and residual covariances were estimated for each trait pair under both fruit
18 loads by pooling the two years and all genotypes as explained in Holland (2006). Covariance
19 matrices were then used to calculate genotypic and residual correlation coefficients (hereafter
20 called inter and intra genotypic correlation coefficients).

21 QTL analysis was performed under each fruit load condition, first separately on both years by
22 using a one-way ANOVA and then by pooling the two years in a two-way ANOVA. The within-
23 genotype mean squares from these ANOVA were used to carry out Dunnett multiple comparison
24 test (Dunnett, 1980) in SAS version 9.1.3 (SAS Institute, Inc.), to determine which genotypes were

1 significantly different (at the 0.05 probability level) from the parent conferring the genetic
2 background (Moneyberg), meaning which genotypes carried a QTL. The QTL effects are
3 presented as percentages of difference from Moneyberg.

4

5 **Results**

6 ***Genotype and fruit load effect on fruit and plant trait variation***

7 As individual sugar contents (glucose, fructose and sucrose contents) were highly correlated
8 together and to total sugar content ($r \geq 0.8$, data not shown), only the total pericarp sugar content
9 was considered in the following analysis. Analysis of variance showed that the genotype
10 significantly influenced all traits (Table 1), and that fruit load significantly influenced most of the
11 traits, except seed number, cell size, leaf number and specific leaf weight. Most of the traits
12 showed higher average values under LL than under HL, except the sugar content (SUGdw), the
13 cuticular conductance (CutC), and the fruit development duration (Dura). Fruit load mostly
14 affected fruit cracking, pericarp dry weight and fruit weight which increased by about 1000%, 87%
15 and 55%, respectively, under LL condition. Genotype x fruit load interactions were also significant
16 for all traits except the seed number, the height of the 4th truss and the leaf number. Year effects
17 and interactions between genotype, fruit load and year were also found significant. Globally the
18 percentages of variations due to interactions between year and genotype or between year and fruit
19 load were lower than those of genotype or fruit load.

20 Fruit load effect was analyzed for each genotype on fruit fresh weight, pericarp dry weight, dry
21 matter content and sugar contents (Fig. 1). Linear positive relationships were found between HL
22 and LL conditions for fruit fresh weight, pericarp dry weight and dry matter content. For sugar
23 contents, the correlation between HL and LL values was lower (SUGfw) or non significant
24 (SUGdw). The fruit fresh weight and pericarp dry weight of all genotypes significantly increased
25 from HL to LL, enlarging twice their range of variation (Fig. 1B and Fig. 1C) and the dry matter

1 content (DMC) significantly increased for all the genotypes except for C3a. Five groups of
2 genotypes were identified according to their response to low fruit load. The first group contained
3 two genotypes and was characterized by significant increase in sugar contents (SUGdw and
4 SUGfw). The second group was the most represented within this population as it contained 13
5 genotypes (including M): it was distinguished by increase in SUGfw but no change in SUGdw.
6 Groups 3, 4 and 5 (three, two and one genotypes, respectively) showed no change in SUGfw,
7 associated with a decrease (Gr 3) or no change (Gr 4 and Gr 5) in SUGdw. The belonging to these
8 groups was not dependent on particular values of fruit fresh weight or sugar contents.

9 ***Inter- and intra-genotypic correlations among traits***

10 Genotypic and residual correlations among traits were evaluated by analyzing inter- and intra-
11 genotypic correlation coefficients among variables, respectively (Table 2). Some correlations were
12 common to inter- and intra- genotypic levels and to both fruit loads. Fruit fresh weight was
13 positively correlated to pericarp dry weight, seed number and cell number. Pericarp dry weight
14 was positively correlated to cell number. Dry matter content and sugar content (SUGdw) were
15 positively correlated to SUGfw and positively or negatively to the structural carbon content,
16 respectively.

17 Only few and low correlations were specific to the intra-genotypic level (Table 2). More
18 significant correlations occurred at the inter-genotypic level and one was common to HL and LL:
19 the higher the structural carbon content, the lower the seed number. Antagonisms were found
20 between fruit fresh weight and dry matter content or structural carbon content under HL. Dry
21 matter content was positively related to fruit cracking and leaf number and negatively to the fruit
22 development duration under HL. Dry matter content was also positively correlated to plant height
23 under LL. The sugar content (SUGfw) was positively related to fruit cracking and leaf number
24 under HL and to specific leaf weight under LL. It was also negatively linked to fruit development
25 duration and to cell expansion *via* cell size under LL. Moreover, a significant negative correlation
26 was found between cell number and cell size only under HL ($r=-0.58$, data not shown), suggesting
27 a competition for assimilates among cells. Finally, the structural carbon content was negatively

1 linked to fruit development duration and to cuticular conductance under HL while positively to
2 leaf number under LL.

3

4 **QTL for plant development, fruit weight and composition traits**

5 *Linkage map of introgressions*

6 The *S. chmielewskii* LA1840 population provided by Keygene had been developed using AFLP
7 markers (<http://www.keygene.com>, unpublished data). In order to enhance the rate of introgression
8 breeding, facilitate marker-assisted selection of new IL and comparisons between function maps of
9 tomato and potato, within the framework of a large European project (<http://www.eu-sol.net/>), the
10 *S. chmielewskii* IL population along with other four interspecific tomato mapping populations and
11 one potato mapping population are being anchored to a common set of COSII markers. The other
12 five mapping populations are: a potato diploid population F1840 (Gebhardt *et al.*, 2003), the *S.*
13 *pennellii* LA716 IL (Eshed and Zamir, 1995), the *S. habrochaites* LA1777 IL (Monforte and
14 Tanksley, 2000), the *S. neorickii* LA2133 backcross inbred lines (BIL) (Fulton *et al.*, 2000), and
15 the *S. cheesmaniae* LA483 recombinant inbred lines (RIL) (Paran *et al.*, 1995).

16 The *S. chmielewskii* IL population has been anchored to 130 COSII markers and 3 SSR spanning a
17 total of 1459 cM with an average interval of 11 cM between adjacent markers. One hundred and
18 five COSII markers are common to at least four of the six mapping populations. Grandillo *et*
19 *al.* were thus allowed to make comparisons among QTL mapping studies (unpublished data).
20 Fourteen genotypes (C1a, C3a, C3d, C4c, C4d, C6e, C7a, C7b, C7d, C8a, C8c, C9d, C10b, C12d)
21 were identified as carrying a single *S. chmielewskii* introgression, covering around 30% of the
22 genome, three genotypes carried two introgressions (C3c, C8e, C11b), one genotype carried three
23 introgressions (C9a) and two genotypes carried four introgressions (C5b, C9c) (Fig. 2). Altogether,
24 the 20 IL covered approximately 50% of the *S. chmielewskii* genome. The genetic length of each
25 introgression varied from 4% to 45% of the chromosome length. Some of the lines still contained

1 heterozygous fragments, either as single introgression (C4c) or as additional introgressions, on
2 chromosomes 2 (C3c), 4 (C5b), and 11 (C9a, C9c).

3 *Quantitative trait locus analysis*

4 Tables 3, 4 and 5 present QTL detected separately under each fruit load condition, and according
5 to the model based on the two-year experiment. Positive or negative QTL correspond to a location
6 where the allele of *S. chmielewskii* increased or decreased the trait, respectively, compared to
7 Moneyberg. Excluding QTL for cuticular conductance which were only identified in 2007, 84
8 QTL were detected in 2006 versus 90 in 2007. Sixty-one QTL were common to both years. Sixty
9 eight QTL were detected under HL versus 74 under LL. Only 30% of these QTL were detected
10 whatever the fruit load and hereafter called 'stable'. When stable, the sign of the QTL was the
11 same under both fruit loads, except for QTL of cuticular conductance.

12

13 **QTL for fruit weight and composition traits**

14 Fourteen QTL were detected for FW, half being stable, the other half being detected only under LL
15 (Table 3). All stable QTL were negative, with two genotypes (C11b and C3c) having a strong
16 effect (fruits were 50% smaller than M). A multiple introgressed genotype (C5b) carried a positive
17 QTL for FW under LL. Ten QTL were identified for DMC, all with positive effect and six were
18 stable. Concerning sugar contents, six QTL for SUGfw were detected: two QTL were stable, and
19 only two had negative effects. On the contrary, all the 11 QTL identified for SUGdw had low
20 negative effects. They were twice more numerous under LL than under HL and C11b was the only
21 genotype carrying a stable QTL whatever the fruit load.

22 **QTL for fruit physiological and plant developmental traits**

23 Five QTL with low effects were detected for fruit development duration and three of them were
24 stable under both fruit loads (Table 4). Eight negative QTL for seed number were identified under
25 HL, and half of them were not detected under LL. QTL for cell traits (number and size) were
26 mainly detected under HL (nine QTL under HL against three under LL), and a QTL for cell

1 number was stable (C11b). All QTL for cell number had negative effects while for cell size, two
2 positive and one negative QTL were identified. The cuticular conductance is the trait for which the
3 number of detected QTL was the largest, but also the trait for which a surprising behaviour was
4 observed between the two fruit loads. Under HL, all QTL had negative effect (except C9c), while
5 under LL all QTL had positive effect, and nine stable QTL had opposite effects under HL and LL.
6 This could be explained by the fact that Moneyberg had one of the highest values for conductance
7 under HL, and the lowest under LL (data not shown). Concerning fruit cracking, C4d carried a
8 stable positive QTL. The five other QTL were only detected under LL and had negative effects.
9 Only few QTL were detected for plant development traits (from one to five QTL per trait) as QTL
10 analysis was only carried out on ten genotypes (Table 5). Among them, less than the half were
11 stable.

12 *Co-localizations between QTL for fruit weight and composition and QTL for fruit* 13 *physiology or plant development*

14 One objective of the present study was to look for co-localizations between QTL for fruit fresh
15 weight and composition and QTL for physiological parameters under both fruit load conditions. In
16 order to avoid additive or epistatic effects due to multiple introgressions, only QTL co-
17 localizations found on genotypes carrying a single introgression were shown on Fig. 3. Eleven
18 clusters of QTL for fruit fresh weight and composition traits were found. Five regions carrying
19 QTL for fruit weight with negative effects of *S. chmielewskii* alleles carried also QTL for dry
20 matter content with an opposite allele effect, in accordance with the inter-genotypic correlation. In
21 the same way, three co-localizations between QTL for fruit fresh weight and sugar content
22 (SUGfw) were identified with opposite allele effects, while two others were identified with the
23 same negative allele effects. Finally, three co-localizations were identified between QTL for fruit
24 fresh weight and QTL for sugar content (SUGdw), with the same negative allele effects. Several
25 co-localizations between QTL for fruit fresh weight and QTL for cell number or for seed number
26 (both 3 clusters) were detected with same sign effects. Co-localizations between QTL for sugar

1 content (SUGfw) and QTL for cell size were expected according to correlations. A region located
2 on the bottom of chromosome 9 carried such co-localization with opposite allele effects (C9d). All
3 QTL for fruit cracking co-localized with QTL for fruit fresh weight. Three of them had the same
4 allele effects except on chromosome 4.

5 Some co-localizations between QTL for dry matter content and QTL for plant development did not
6 always correspond to the direction of correlations (Table 2). For example, dry matter content was
7 positively correlated to plant height at the inter-genotypic level and QTL analysis revealed one co-
8 localization for these two traits with similar allele effects (C4d), and two others with opposite
9 allele effects (C3a and C9d). Some co-localizations were also identified between QTL for sugar
10 content (SUGfw) and QTL for specific leaf weight or leaf number (C3a and C4d).

11 **Discussion**

12 ***Relationships between fruit fresh weight and composition***

13 The influence of carbon availability on the relationships between fruit weight and sugar content
14 was studied *via* fruit thinning. In peach fruits (Morandi *et al.*, 2008) or in apple fruits (Link, 2000),
15 a lower fruit load increased simultaneously fruit fresh weight and sugar content. In tomato, some
16 fruit thinning experiments were carried out and showed that fruit load reduction led to increase
17 fruit fresh and dry weights (Gautier *et al.*, 2001; Bertin, 2005; Baldet *et al.*, 2006), but none of
18 them has dealt with effects on fruit sugar content. In this tomato population, most of the genotypes
19 (15 genotypes over 21) reacted to fruit thinning by increasing the dry matter content and, for only
20 two of them, by also increasing the carbohydrate allocation to sugar metabolism. For the six other
21 genotypes, sugar content relative to fresh weight was unchanged whatever the fruit load. Finally, in
22 some cases, even if the dry matter content increased, the carbohydrate allocation to sugars was
23 stable or decreased, leading to no change in sugar content. The only significant correlation
24 between fruit fresh weight and composition was found at the inter-genotypic level, under high fruit
25 load conditions (Table 2) and concerned the antagonism between fruit fresh weight and dry matter

1 content, associated with a negative relation between fruit fresh weight and structural carbon
2 content, but not between fruit fresh weight and sugar content. From a genetic point of view,
3 antagonism between fruit fresh weight and soluble solids content has been observed during tomato
4 improvement under high load conditions, and is mainly due to co-localizations of QTL with
5 antagonistic effects (Bernacchi *et al.*, 1998; Chen *et al.*, 1999; Saliba-Colombani *et al.*, 2001;
6 Lecomte *et al.*, 2004). In the present population, markers used to locate the introgressed fragments
7 were common to four tomato mapping populations. By this way, it was possible to compare QTL
8 from the *S. chmielewskii* population with the previous works. Co-localizations between QTL for
9 fruit fresh weight and QTL for dry matter content or sugar content on fresh weight basis were
10 detected with opposite effects (Fig. 3), and corresponded to regions that were already identified in
11 other progenies under high fruit load (Grandillo *et al.*, 1999; Causse *et al.*, 2006). These co-
12 localizations of QTL for fruit fresh weight with QTL for dry matter content with antagonistic
13 effects were common to both fruit loads (C9d), specific to high load (C3a) or specific to low load
14 (C3d, C4d and C8a) in the *S. chmielewskii* population. Moreover, two co-localizations were
15 identified between QTL for fruit fresh weight and QTL for sugar content relative to fresh weight,
16 with same negative allele effects under high load (C10b) and under low load (C12d). These
17 regions could be involved in carbon allocation to cell structures or to sugar metabolism, because
18 they also co-localized with QTL for sugar content relative to dry weight. In this population, we did
19 not find any stable QTL with positive effects for sugar content which was not associated with a
20 negative QTL for fruit fresh weight.

21

22 ***Processes underlying fruit weight and composition***

23 Cuticle properties are involved in water loss by transpiration (Schönherr, 1976; Becker *et al.*,
24 1986; Kerstiens, 1996). Concerning fruit cracking, it usually occurs when fruit growth rate is high
25 (Christensen, 1973). It was thus expected that under low fruit load, when fruit growth was faster,
26 QTL for fruit fresh weight co-localized with QTL for fruit cracking. Genotype C4d showed an

1 extreme phenotype even under HL and carried a major mutation for this trait. It probably
2 corresponded to the mutation *Cwp1* described by Hovav *et al.* (2007). Three other QTL for fruit
3 cracking were mapped and co-localized with QTL for fruit fresh weight with similar effects (Fig.
4 3), while no such link was found between fruit cracking and fruit fresh weight through correlation
5 analysis (Table 2). An increase in cuticular conductance or in fruit cracking was expected to
6 increase the dry matter content or the sugar content (SUGfw). The hypothesis of a concentration of
7 dry matter by water loss was confirmed at the inter-genotypic level under HL condition, as fruit
8 cracking was positively correlated to dry matter content or sugar content. To avoid interaction with
9 fruit cracking, measurement of cuticular conductance was performed 21 days after anthesis, during
10 fruit cellular expansion, whereas all other variables were analyzed at the red ripe stage. As
11 cuticular conductance is known to decrease throughout fruit development (Gibert *et al.*, 2005),
12 conclusions may be biased. Numerous QTL for cuticular conductance were particularly susceptible
13 to fruit load change as *S. chmielewskii* alleles carried negative effects under HL and positive
14 effects under LL. This is probably linked to the extreme behaviour of Moneyberg changing from
15 HL to LL. No other trait behaved similarly, making the interpretation of these co-localizations
16 difficult.

17 Cell number is a determinant factor of fruit sink strength, usually determined during early stages of
18 tomato fruit development (Bohner and Bangerth, 1988; Joubes *et al.*, 1999). In agreement with the
19 literature (Higashi *et al.*, 1999; Jullien *et al.*, 2001; Bertin, 2005), the fruit fresh weight was
20 positively correlated with cell number and was present both at intra-genotypic and inter-genotypic
21 levels whatever the fruit load (Table 2). This relationship was also confirmed by the numerous co-
22 localizations under both fruit loads between QTL for fruit fresh weight and QTL for cell number,
23 with similar effects (Fig. 3). The increase in fruit fresh weight and cell division in the absence of
24 carbohydrate competition has also been described by Baldet *et al.* (2006) and was linked to the
25 regulation of *fw2.2*, a cell cycle-control gene. Cell expansion was not directly linked to the fruit
26 fresh weight as no significant correlation was found between cell size and fruit fresh weight and
27 QTL co-localizations of these two traits were either with similar allele effects (C9d) or with

1 opposite effects (C12d). Moreover, at the inter-genotypic level and under high fruit load condition,
2 a negative correlation between cell number and cell size suggested the existence of competition for
3 carbohydrates among cells. This competition may reduce the cell growth potential, resulting in
4 smaller cells (Bertin, 2005; Tsukaya, 2006).

5 Positive correlations between fruit fresh weight and seed number were probably due to the effect
6 of the latter on the sink strength of the fruit (Nitsch, 1970). QTL co-localizations of these two traits
7 were similar to previous QTL mapping experiments carried out on a *S. pimpinellifolium* F₂
8 population (Van der Knaap and Tanksley, 2003) or on a *S. peruvianum* BC₃ population (Fulton *et*
9 *al.*, 1997). A co-localization was common to the *S. pimpinellifolium* F₂ population and to the
10 present population at the bottom of chromosome 6. The role of seeds in the sink strength could be
11 related to hormonal signalling, hormones being strongly implicated in the control of cell division
12 rate and sustenance (Gillaspy *et al.*, 1993).

13 Plant development traits are commonly described in ecophysiological studies, and were used here
14 to estimate source strength. The main hypothesis was that the higher the number of leaves, or the
15 leaf area, the higher the sugar production *via* photosynthesis, and thus the higher the fruit dry
16 matter or sugar contents. Positive correlations between leaf number and dry matter or sugar content
17 (SUGfw) comforted this hypothesis. A balance between plant vigour and distribution of
18 assimilates to the fruit was thus found at the whole plant level, as already mentioned by Vaast *et*
19 *al.* (2006). The hypothesis was also corroborated at the genetic level, as co-localizations between
20 QTL for leaf area or leaf number and QTL for dry matter content or sugar content were identified
21 with same allele effects (C4d and C9d).

22

23 **Conclusion**

24 The present paper aimed at assessing the implication of various processes in the relationships
25 between fruit weight and its composition. Our results suggested that these relationships could be
26 mainly related to sink strength through cell division whose intensity was modulated by fruit load.

1 An antagonism between fruit fresh weight and dry matter content was only detected at the inter-
2 genotypic level, in condition of competition for assimilates. This study also revealed different
3 behaviours of genotypes with respect to changes in fruit load and it was consequently not possible
4 to deduce a general scenario for the whole population. Moreover, a lot of QTL had different effects
5 according to fruit load, suggesting that carbohydrate supply can strongly interact with the genome,
6 probably *via* sugar or hormonal sensing. Although co-localizations of QTL can hide either
7 pleiotropy or genetic linkage, this work could contribute to help in choice of candidate genes as
8 physiological hypotheses linked to quality traits were formulated at the genetic level. QTL with
9 similar behaviours under both fruit loads could also be interesting targets for breeding programs as
10 they would be more likely stable under various environments.

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Table 1: Percentage of variation attributable to the effects of genotype (G), fruit load (FL), year (Y), genotype x fruit load interaction (G x FL), genotype x year interaction (G x Y), year x fruit load interaction (Y x FL) by analysis of variance. When fruit load effect was significant, the percentage of variation from high load (HL) to low load (LL) (Δ_{FL}) was calculated (equation 4 in Material and Methods).

	<i>abbreviation</i>	Genotype (G)	Fruit load (FL)	Δ_{FL}	Year (Y)	G x FL	G x Y	Y x FL
Fruit weight and composition								
Fruit fresh weight (g)	FW	38 ***	52 ***	+ 55%	0 ns	6 ***	2 ns	1 **
Dry weight of the pericarp (g)	DW	25 ***	63 ***	+ 87%	3 *	1 *	3 *	5 ns
Dry matter content of the pericarp (g / 100 g FW)	DMC	43 ***	38 ***	+ 17%	14 ***	3 ***	3 ns	0 ns
Sugar content of the pericarp (g / 100 g DW)	SUGdw	25 ***	1 *	- 1%	45 ***	13 ***	14 ***	2 **
Sugar content of the pericarp (g / 100 g FW)	SUGfw	53 ***	17 ***	+ 17%	6 ***	9 ***	10 ***	3 **
Structural carbon content of the pericarp (g / 100 g FW)	StrCfw	19 ***	19 ***	+ 17%	45 ***	6 **	8 ***	2 **
Fruit physiology								
Development duration (days)	Dura	36 ***	9 ***	- 4%	29 ***	7 *	19 ***	1 *
Seed number	SdN	55 ***	0 ns	ns	27 ***	6 ns	10 *	1 ns
Cell number of the pericarp	CIN	60 ***	23 ***	+ 42%	0 ns	12 *	3 ns	1 ns
Cell size in the pericarp (nL)	CIS	78 ***	0 ns	ns	0 ns	16 *	4 ns	2 ns
Cuticular conductance (cm.h ⁻¹)	CutC	26 ***	18 ***	- 25%	/	49 ***	/	/
Fruit cracking	FCr	19 ***	26 ***	+ 1000%	17 ***	15 ***	9 ***	14 ***
Plant development								
Height of the 4 th truss (cm)	H4t	77 ***	1 *	+ 4%	19 ***	2 ns	2 ns	0 ns
Leaf number	LfN	22 ***	0 ns	ns	63 ***	4 ns	9 **	2 ns
Specific leaf weight (g.cm ⁻²)	SLW	18 *	1 ns	ns	50 ***	13 *	19 **	0 ns
Total leaf area (cm ²)	LfA	52 ***	8 ***	+ 43%	5 *	24 **	10 *	1 ns

'ns': not significant; *: significant at the 0.05 probability level; **: significant at the 0.001 probability level; ***: significant at the 0.0001 probability level

Table 2: Inter-genotypic (Inter) and Intra-genotypic (Intra) correlation coefficients for traits relative to weight, composition, physiology of the fruit, and plant development, under high load (HL) and low load (LL). Correlation coefficients significant at the 0.05 probability level were highlighted in grey.

	FW				DW				DMC				SUGdw				SUGfw				StrCfw			
	Inter		Intra		Inter		Intra		Inter		Intra		Inter		Intra		Inter		Intra		Inter		Intra	
	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL
DW	0.90	0.94	0.91	0.89	1	1	1	1																
DMC	-0.55	-0.31	0.06	-0.10	-0.14	0.03	0.44	0.34	1	1	1	1												
SUGdw	0.23	-0.21	0.05	0.03	0.28	-0.30	-0.01	-0.09	0.04	-0.05	-0.12	-0.25	1	1	1	1								
SUGfw	-0.29	-0.44	0.12	-0.06	0.06	-0.16	0.35	0.19	0.77	0.86	0.64	0.58	0.66	0.46	0.49	0.63	1	1	1	1				
StrCfw	-0.57	-0.17	0.05	-0.09	-0.27	0.16	0.34	0.27	0.78	0.90	0.76	0.83	-0.59	-0.47	-0.72	-0.74	0.20	0.55	-0.01	0.02	1	1	1	1
Dura	0.21	0.10	-0.08	-0.02	0.11	0.14	-0.13	-0.02	-0.47	-0.26	-0.06	0.02	0.23	-0.65	-0.07	0.02	-0.30	-0.48	-0.14	0.05	-0.48	0.04	-0.01	0.02
SdN	0.48	0.42	0.39	0.37	0.31	0.26	0.31	0.37	-0.43	-0.34	-0.06	0.11	0.46	0.36	0.14	0.05	-0.01	-0.16	0.07	0.12	-0.63	-0.45	-0.13	0.07
CIN	0.77	0.87	0.69	0.71	0.67	0.82	0.67	0.52	-0.38	-0.31	0.25	-0.06	0.11	0.19	-0.42	0.02	-0.12	-0.20	0.14	0.03	-0.40	-0.37	0.16	-0.04
CIS	0.02	0.40	0.13	0.00	-0.02	0.37	0.01	-0.05	-0.09	-0.38	-0.15	-0.15	-0.03	-0.42	0.05	0.00	-0.11	-0.53	-0.03	-0.20	0.01	0.03	-0.15	-0.15
CutC	0.15	-0.26	-0.09	0.09	0.11	-0.33	-0.19	-0.16	-0.40	0.15	-0.06	-0.14	0.03	0.13	0.03	0.06	-0.26	0.29	0.02	-0.16	-0.55	-0.05	-0.05	-0.16
FCr	0.00	0.40	0.04	0.32	0.31	0.43	0.02	0.25	0.58	0.10	-0.01	-0.12	0.25	-0.24	0.05	0.01	0.62	-0.03	0.02	-0.10	0.28	0.25	-0.04	-0.07
H4t *	0.27	0.11	0.05	0.29	0.46	0.34	0.11	0.19	0.39	0.97	0.11	0.17	0.29	0.07	-0.28	-0.07	0.67	0.58	-0.22	0.05	0.21	0.54	0.23	0.14
LfN *	-0.55	-0.42	0.09	0.09	-0.44	-0.34	0.15	-0.02	0.82	0.01	0.23	-0.07	0.06	-0.73	-0.18	0.00	0.97	-0.10	0.13	-0.30	0.60	0.93	0.22	-0.10
SLW *	0.34	0.37	-0.26	-0.08	0.45	0.07	-0.20	0.10	-0.10	-0.05	0.07	0.23	-0.33	0.07	0.14	0.13	-0.57	0.91	0.17	0.34	0.35	0.18	-0.06	0.00
LfA *	0.04	-0.20	0.61	0.17	-0.18	-0.32	0.57	0.15	-0.25	-0.10	0.01	-0.16	0.02	0.46	-0.15	0.21	0.02	0.53	-0.11	0.00	-0.02	0.01	0.06	-0.19

* : traits for which correlation coefficients were calculated only among 10 genotypes

1 **Table 3: QTL characteristics for fruit weight and composition traits.**

2 Genotypes for which significant differences were detected at the 0.05 probability level, in the model
 3 taking into account the two-year experiment. Chromosomes carrying introgressions are indicated for
 4 each genotype. Effects under HL or under LL are expressed as the average percentage of difference
 5 between the genotype and Moneyberg under high load and low load, respectively, over two years.
 6 Under each fruit load, the year when QTL was significant was indicated as '06 / 07' when the QTL
 7 was detected whatever the year, '06' or '07' when it was significant only in 2006 or 2007,
 8 respectively.

Trait	Genotype	Chr.	Effect under HL	Detection year under HL	Effect under LL	Detection year under LL
FW	C3a	3	-30	06 / 07	-17	06 / 07
	C3c	2;3	-41	06 / 07	-51	06 / 07
	C3d	3	ns	-	-29	06 / 07
	C4d	4	ns	-	-26	06
	C5b	4;5;7;11	ns	-	19	07
	C6e	6	ns	-	-16	06 / 07
	C7b	7	-27	07	-27	06
	C8a	8	ns	-	-23	07
	C8c	8	ns	-	-19	06 / 07
	C9a	7;9;11	ns	-	-16	07
	C9d	9	-30	06	-39	06 / 07
	C10b	10	-26	07	-22	06
	C11b	11;12	-49	06 / 07	-47	06 / 07
C12d	12	-18	07	-24	06	
DMC	C1a	1	13	07	10	06 / 07
	C3a	3	11	06 / 07	ns	-
	C3c	2;3	23	06 / 07	15	07
	C3d	3	9	06 / 07	12	07
	C4d	4	27	06 / 07	24	06 / 07
	C5b	4;5;7;11	ns	-	8	06 / 07
	C7d	7	11	06 / 07	ns	-
	C8a	8	14	06	15	07
	C9d	9	19	06 / 07	12	07
	C11b	11;12	10	06 / 07	ns	-
SUGfw	C3a	3	20	07	ns	-
	C3c	2;3	21	07	ns	-
	C4d	4	35	06 / 07	19	07
	C9d	9	20	06 / 07	14	07
	C10b	10	-19	07	ns	-
	C12d	12	ns	-	-15	07
SUGdw	C1a	1	ns	-	-3	06 / 07
	C5b	4;5;7;11	ns	-	-9	06 / 07
	C6e	6	ns	-	-8	06 / 07
	C7a	7	ns	-	-14	06
	C7d	7	ns	-	-3	06 / 07
	C8a	8	-8	06 / 07	ns	-
	C8c	8	-8	06 / 07	ns	-
	C9a	7;9;11	ns	-	-8	06 / 07
	C10b	10	-12	07	ns	-
	C11b	11;12	-19	07	-6	06 / 07
	C12d	12	ns	-	-3	06 / 07

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 10 'ns' means that the QTL was not significant.

1 **Table 4: QTL characteristics for fruit physiological traits.**

2 Genotypes for which significant differences were detected at the 0.05 probability level, in the model
3 taking into account the two-year experiment. Chromosomes carrying introgressions are indicated for
4 each genotype. Effects under HL or under LL are expressed as the average percentage of difference
5 between the genotype and Moneyberg under high load and low load, respectively, over two years.
6 Under each fruit load, the year when QTL was significant was indicated as '06 / 07' when the QTL
7 was detected whatever the year, '06' or '07' when it was significant only in 2006 or 2007,
8 respectively.

Trait	Genotype	Chr.	Effect under HL	Detection year under HL	Effect under LL	Detection year under LL
Dura						
	C3a	3	-8	06 / 07	ns	-
	C4d	4	-6	06	-6	06 / 07
	C7d	7	ns	-	10	06
	C9d	9	-7	07	-8	07
	C10b	10	-8	06 / 07	-7	06 / 07
SdN						
	C1a	1	-38	07	ns	-
	C3d	3	-32	06 / 07	ns	-
	C5b	4;5;7;11	-43	07	-46	07
	C6e	6	-49	07	-45	07
	C8a	8	-20	06 / 07	ns	-
	C9d	9	-49	06 / 07	-66	06 / 07
	C10b	10	-42	07	ns	-
	C11b	11;12	-71	06 / 07	-50	06
CIN						
	C1a	1	-19	06	ns	-
	C3a	3	-38	06 / 07	ns	-
	C3c	2;3	-32	06 / 07	ns	-
	C5b	4;5;7;11	-28	06 / 07	ns	-
	C9a	7;9;11	-28	06 / 07	ns	-
	C9d	9	ns	-	-39	06 / 07
	C11b	11;12	-56	06 / 07	-48	06 / 07
	C12d	12	-37	06 / 07	ns	-
CIS						
	C1a	1	ns	-	17	06
	C9d	9	-12	06	ns	-
	C12d	12	32	06	ns	-
CutC						
	C1a	1	ns	na	52	na
	C3a	3	ns	na	124	na
	C3c	2;3	-42	na	189	na
	C3d	3	-49	na	107	na
	C4c	4	-29	na	40	na
	C4d	4	-48	na	213	na
	C5b	4;5;7;11	-27	na	187	na
	C6e	6	-45	na	131	na
	C7a	7	ns	na	123	na
	C7b	7	-42	na	71	na
	C7d	7	ns	na	66	na
	C8a	8	-41	na	ns	na
	C8c	8	ns	na	107	na
	C8e	3;8	-45	na	50	na
	C9a	7;9;11	ns	na	90	na
	C9c	1;7;9;11	59	na	ns	na
	C9d	9	-17	na	ns	na
	C10b	10	-50	na	77	na
	C11b	11;12	ns	na	184	na
	C12d	12	ns	na	110	na
FCr						
	C3a	3	ns	-	-70	07
	C3c	2;3	ns	-	-100	07
	C4d	4	741	06 / 07	182	06 / 07
	C7b	7	ns	-	-74	06 / 07
	C9d	9	ns	-	-58	06 / 07
	C11b	11;12	ns	-	-63	06 / 07

1

2 'ns' means that the QTL was not significant.

3 'na' means that QTL stability cannot be deduced as the cuticular conductance was only measured in
4 2007.

5

1 **Table 5: QTL characteristics for plant developmental traits.**

2 Genotypes for which significant differences were detected at the 0.05 probability level, in the model
 3 taking into account the two-year experiment. Chromosomes carrying introgressions are indicated for
 4 each genotype. Effects under HL or under LL are expressed as the average percentage of difference
 5 between the genotype and Moneyberg under high load and low load, respectively, over two years.
 6 Under each fruit load, the year when QTL was significant was indicated as '06 / 07' when the QTL
 7 was detected whatever the year, '06' or '07' when it was significant only in 2006 or 2007,
 8 respectively.

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Trait	Genotype	Chr.	Effect under HL	Detection year under HL	Effect under LL	Detection year under LL
H4t	C11b	11;12	-21	06	-26	06 / 07
	C12d	12	-18	06	-23	06 / 07
	C3a	3	-21	06	ns	-
	C4d	4	21	06	19	06
	C9d	9	ns	-	16	06
LfN	C4d	4	14	06 / 07	ns	-
SLW	C12d	12	20	06 / 07	ns	-
	C3a	3	37	06 / 07	68	06
	C7a	7	ns	-	64	06
LfA	C3a	3	-59	06 / 07	-56	06 / 07
	C3c	2;3	ns	-	40	06
	C9d	9	35	06 / 07	ns	-

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12
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14 'ns' means that the QTL was not significant.

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1 **Figure legends**

2 **Figure 1: Effect of fruit load on several traits.**

3 A) Groups of genotypes according to fruit load effects on B) fruit fresh weight (FW), C) pericarp dry
4 weight (DW), D) pericarp dry matter content (DMC), E) pericarp sugar content relative to fresh
5 weight (SUGfw) and F) pericarp sugar content relative to dry weight (SUGdw). Arrows indicate
6 significant increase or decrease and (–) no change from high load (HL) to low load (LL). According
7 to the different combinations among trait variations, genotypes were ordered into 5 groups (Gr 1-Gr
8 5). Each point is the mean of the two years of measurements. Triangles point-up referred to group 1,
9 circles to group 2, diamonds to group 3, squares to group 4 and triangles point-down to group 5. On
10 each graph, black symbol indicate significant difference between HL and LL and white symbol
11 refers to non significant differences at the 0.05 probability level. On each graph, a linear regression
12 was fitted to all genotypes, and values of determination coefficients (R^2) and p-value (p) are shown
13 on each figure. Dotted lines referred to bisecting lines.

14
15 **Figure 2: Linkage map** showing the locations of the 110 COS markers whose names begin with
16 ‘C2_At’ and the 3 SSR makers. Numbers on the right of the marker names indicate the genetic
17 distances in cM from the top of the chromosome. Map distances are based on the tomato-EXPEN
18 F2.2000 mapping population (*S. lycopersicum* LA925 x *S. pennellii* LA716 type F2.2000)
19 (<http://www.sgn.cornell.edu/>). At the bottom of each chromosome, the total genetic length is
20 mentioned. If the genotype carried a single homozygous introgression, its location is black-filled (for
21 example C1a). If the genotype carried a single heterozygous introgression, its location is grey-filled
22 (C4c). If the genotype carried multiple homozygous introgressions, its name referred to the largest
23 introgression and the locations of all the introgressions are white-filled. The locations of the other
24 introgressions are indicated at the bottom of the fragment. For example, the C3c carried its main
25 introgression on chromosome 3 and a smaller one on chromosome 2. If the genotype carried multiple
26 heterozygous introgressions, introgressions are hatched.

1 **Figure 3: Genetic map of the QTL detected on genotypes carrying a single introgressed**
2 **fragment, and at least one QTL for fruit weight or composition.** QTL for fruit weight and
3 composition are circled; QTL only detected under high fruit load (HL) are on the left of the
4 chromosome; QTL only detected under low fruit load (LL) are on the right of the chromosome; QTL
5 detected whatever the fruit load are at the middle of the chromosome. (-) and (+) indicate if the *S.*
6 *chmielewskii* alleles had negative or positive effects on the trait, respectively.

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