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ORIGINAL PAPER

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Marker-assisted introgression of five QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic backgrounds

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Abstract The evaluation of organoleptic quality of tomato fruit requires physical, chemical and sensory analyses, which are expensive and difficult to assess. Therefore, their practical use in phenotypic selection is difficult. In a previous study, the genetic control of several traits related to organoleptic quality of fresh-market tomato fruit was investigated. Five chromosome regions strongly involved in organoleptic quality attributes were then chosen to be introgressed into three different recipient lines through marker-assisted selection. A marker-assisted backcross (MABC) strategy was performed, as all the favorable alleles for quality traits were provided by the same parental tomato line, whose fruit weight (FW) and firmness were much lower than those of the lines commonly used to develop fresh market varieties. Three improved lines were obtained after three backcrossing and two selfing generations. The implementation of the MABC scheme is described. The three improved lines were crossed together and with the recipient lines in a half-diallel mating scheme, and the simultaneous effect of the five quantitative trait locus (QTL) regions was compared in different genetic backgrounds. Significant effects of the introgressed regions and of the genetic backgrounds were shown. Additive effects were detected for soluble solid and reducing sugar content in two genetic backgrounds. A partially dominant effect on titratable acidity was detected in only one genetic background. In contrast, additive to dominant unfavorable effects of the donor alleles were detected for FW and locule number in the three genetic backgrounds. Recessive QTL effects on firmness were only detected in the two firmest genetic backgrounds. Comparison of the hybrids in the half-diallel gave complementary information on the effects of: (1) the alleles at the selected regions, (2) the genetic backgrounds and (3) their interaction. Breeding efficiency strongly varied according to the recipient parent, and significant interactions between QTLs and genetic backgrounds were shown for all of the traits studied.

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Introduction

Molecular markers constitute an efficient tool for indirect selection in plant breeding. They have been widely used for following the introgression of monogenic traits such as disease resistance (Yu et al. 2000; Singh et al. 2001). For polygenic traits, several quantitative trait loci (QTLs) have been mapped and their individual effects estimated (Kearsey and Farquhar 1998). Marker-assisted selection (MAS) has been particularly efficient in investigations on traits with low heritability or traits that are expensive or difficult to assess, and various MAS strategies have been proposed. When all of the positive alleles come from a distant and unadapted line, the marker-assisted backcross (MABC) of QTLs into an elite line can be performed (Bouchez et al. 2002). Hospital and Charcosset (1997) have optimized the theoretical conditions required to use markers for introgressing several QTLs through MABC.

In fresh-market tomato, selection for sensory traits requires complex and expensive evaluation, with the sensory profiling relying on the judgment of several trained panelists. While some traits such as sweetness and sourness may be related to the sugar and acid content of the fruit, respectively (Jones and Scott 1983; Stevens et al.

1979), aroma and texture characteristics are not easily predicted by instrumental measures (Petro-Turza 1987; Causse et al. 2003). Furthermore, most of the quality traits exhibit a polygenic inheritance and are strongly influenced by environmental conditions. A negative relationship of sugar content with fruit size or yield also limits genetic progress (Stevens 1986). MAS could thus be proposed as an alternative to phenotype-based breeding for improving sensory quality of tomato.

In a previous study, our group investigated the genetic control of several traits involved in the organoleptic quality of tomato in progeny derived from a cross between a common line with large fruit and a cherry tomato line with fruit having a very good taste and a high aroma intensity (Causse et al. 2002). QTLs were mapped for physical components [fruit weight (FW), firmness (FIR), locule number (LONB), color], chemical components [soluble solid content (SSC), reducing sugar content (SUC), titratable acidity (TA), pH] and sensory attributes (descriptive profiles of flavor, aroma and texture characteristics). Eight clusters of QTLs were detected that controlled most of the variation of the organoleptic quality traits (Causse et al. 2001a; Saliba-Colombani et al. 2001). The favorable alleles were conferred by the cherry tomato line for all of the quality traits. QTLs for FW with opposite allele effects were detected in four of these clusters. Thus, we identified five chromosome regions that showed promise for improving fruit quality. As only one unfavorable QTL for FW was located within these regions—among the four detected—we predicted that FW should be increased through backcrossing with a large-fruit recipient line. We consequently designed a MABC strategy to introgress the five regions into three large-fruit varieties. Three backcrossing and two selfing generations were performed, and three improved lines were obtained with homozygous favorable alleles at the five regions of interest and recipient alleles for most of the genetic background. We then crossed the three recipient lines and three improved lines derived by MABC following a half-diallel mating scheme in order to estimate the efficiency of the improved lines when used as parents of hybrids and to analyze QTLs by genetic background interactions. Fruits of the lines and hybrids were evaluated for several quality traits. Each QTL genotype was compared in different genetic backgrounds and, vice versa, different QTL genotypes were compared in each background. The implementation of the selection scheme is reported, and the phenotypic effect of the introgressed regions is compared between the genetic backgrounds in order to analyze: (1) the simultaneous effect of the five selected regions in the three genetic backgrounds, (2) the genetic background and linkage drag effects on these QTLs and (3) the potential of the improved lines as parents of the hybrids.

Materials and methods

Plant material and introgression scheme

A population of 144 recombinant inbred lines (RILs) was developed from an intraspecific cross between a cherry tomato line [Cervil, *Lycopersicon esculentum* var. *cerasiforme* (Dun.) Gray, referred to subsequently in this article as C] with 5- to 7-g fruits, a good taste and a highly intense aroma and Levovil (a *L. esculentum* Mill. line, referred to subsequently as L) with 120- to 150-g fruits and an ordinary taste. QTLs were detected for physical components (FW, FIR, LONB, color), chemical components [dry matter weight, SSC, sugar content, TA, pH (Saliba-Colombani et al. 2001] and sensory attributes (descriptive profiles of flavor, aroma and texture, Causse et al. 2001a). Five chromosome regions (noted 1, 2, 4, 9A and 9B, located on chromosomes 1, 2, 4 and 9, respectively) were retained for MAS. A single RIL (LR134) with C alleles at the five regions was identified and used as the donor parent of the breeding program. The same MABC program was performed with three recipient lines kindly provided by the Vilmorin seed company: Levovil, Vil B and Vil D, referred to subsequently as L, B and D, respectively. As the donor parent (LR134) contained 47% L alleles, the first cross between LR134 and the recipient line was considered to be the BC_1. The BC_1 progeny was genetically homogeneous and consequently backcrossed without any selection to the recipient line to produce a BC_2 population. Almost 300 plants were grown per recipient line, and after a MAS step, a single selected BC_2 plant was backcrossed again to produce a BC_3 population. Similarly, a single BC_3 plant was selected, and two selfing generations were performed to fix the C alleles at all five QTL regions (Fig. 1). Thus, BC_3S_2 improved lines, denoted L5, B5 and D5, with homozygous alleles at the five regions were obtained. The six lines (L, B, D, L5, B5 and D5) were crossed together in a half-diallel mating scheme (Table 1).

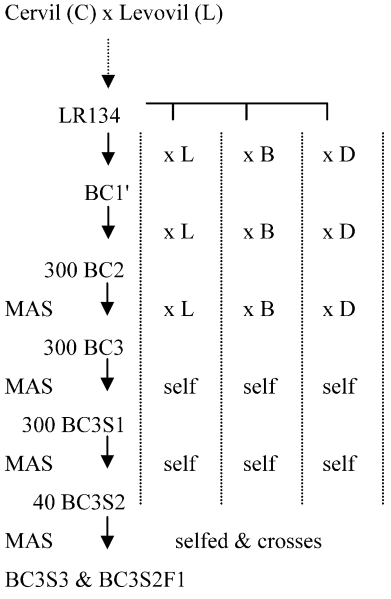

Fig. 1 Marker-assisted backcross scheme. Following QTLs detection in the progeny of Cervil [*C*, *Lycopersicon esculentum* var. *cerasiforme* (Dun.) Gray] × Levovil (*L*, *L. esculentum* Mill.), a donor line (LR134) was chosen as QTL donor and crossed with the three recipient lines Levovil (*L*), Vil B (*B*) and Vil D (*D*). Marker-assisted selection was thus applied to the BC_2, BC_3, BC_3S_1 and BC_3S_2 progenies. The selected BC_3S_2 lines were then selfed and crossed. Sample size indicated corresponds to the maximum number of plants analyzed for one recipient line

Table 1 The half-diallel mating scheme. The three recipient parents (L, B and D) and the three improved lines (B5, D5 and L5) were all crossed together. For the six lines and the 15 hybrids resulting from the mating scheme, the alleles at both the introgressed regions and in the genetic background were written as: genotype at the introgressed regions/genotype of the genetic background[a], [b] where B, C, D and L are the VilB, Cervil, VilD and Levovil alleles, respectively

Lines	B	B5	D	D5	L	L5
B	**BB/BB**					
B5	**CB/BB**	**CC/BB**				
D	DB/DB (a)	DC/DB (a)	**DD/DD**			
D5	CB/DB (a)	CC/DB (a)	**CD/DD**	**CC/DD**		
L	LB/LB (b)	LC/LB (b)	LD/LD (c)	LC/LD (c)	**LL/LL**	
L5	CB/LB (b)	CC/LB (b)	CD/LD (c)	CC/LD (c)	**CL/LL**	**CC/LL**

[a]All of the genotypes having the same alleles in their genetic background are followed by the same low-case letter (in parenthesis)
[b]Genotypes with homozygous genetic background are shown in bold

Table 2 Characteristics of the QTLs detected for physical, chemical and sensory traits in the RIL progeny within each of the five regions introgressed in the MABC scheme (Saliba-Colombani et al. 2001; Causse et al. 2001b)

Chromosome region	Confidence interval length[a,b,c] (cM)	Markers used[b]	Distance between the extreme markers (cM)	Trait[c]	R^{2d}	Favorable allele[e]
1	31	TG116 (f)	16	TA	11.2	C
		OPB12-0.7C (o) *TG430* (*f*)		SOU	11.3	C
2	31	OPB08-1.5C (o) *TG454* (*f*)	23	SSC	18.6	C
		OPAE4-0.9C (o) *TG191* (*f*)		SUC	25.3	C
		GC039 (f) *ASC056* (*p*)		TA	17.2	C
				SWE	18.3	C
				ARO	26.2	C
				FIT	9.3	C
				MEA	25.5	C
				MEL	13.9	C
				FW	46.2	L
				LONB	37.3	L
4	19	CT192 (f)	16	FIR	33.3	C
		TG075 (f)		MEA	29.7	C
9A	15	CT032 (f)	12	SSC	13.3	C
		ASC21 (p)		TA	22.4	C
				SOU	24.7	C
				ARO	24.3	C
				MEA	22.1	C
				MEL	45.5	C
				FIT	41.1	C
				FIR	10.3	L
9B	17	TG008 (f)	–	*PHA*	20.6	C

[a]The overall length of the confidence interval (in centiMorgans) included the confidence intervals of all the QTLs in the region, estimated applying a 1.5 LOD score decrease
[b]The markers used to monitor the introgression of the regions are indicated (marker type between brackets). The additional markers used to perform the selection during selfing generations are in italics. *f*, RFLP marker; *o*, RAPD marker; *p*,codominant PCR marker
[c]QTLs for sensory attributes are in italics. *ARO*, Tomato aroma intensity; *FIR*, instrumental firmness; *FIT*, flesh firmness; *FW*, fruit weight; *LONB*, locule number; *MEA*, mealiness; *MEL*, meltiness; *PHA*, pharmaceutical aroma; *SOU*, sourness; *SSC*, soluble solid content; *SUC*, reducing sugar content; *SWE*, sweetness; *TA*, titratable acidity
[d] R^2 is the percentage of phenotypic variation explained by a QTL based on composite interval mapping. The favorable alleles for fruit quality improvement are described in detail elsewhere
[e]C, Cervil [*Lycopersicon esculentum* var. *cerasiforme* (Dun.) Gray]; L, Levovil (*L. esculentum* Mill.)

Foreground selection

DNA of all the plants studied was extracted following the DNA microprep protocol (Fulton et al. 1995), and molecular markers were scored as recommended for genetic map construction (Saliba-Colombani et al. 2000). Ten markers were used to monitor the presence of donor-type alleles in QTL segments (Table 2). The three random amplified polymorphic DNA (RAPD) markers were dominant for the C alleles and were used to rapidly and easily detect heterozygous individuals of the backcross populations but could not be used to distinguish between heterozygous and homozygous individuals in the selfing generations. Thus, to monitor introgression in selfing generations, we replaced OPB12-0.7C on chromosome 1 and OPB08-1.5C and OPAE4-0.9C on chromosome 2 with restriction fragment length polymorphism (RFLP) markers TG430, TG454 and TG191, respectively. In contrast, a RFLP marker (GC039 on chromosome 2) was replaced by ASC056, which is a new codominant PCR-based marker. The same markers were used in the three MABC programs, as the same polymorphisms were detected between C and each of the recipient lines (except for the OPB12-0.7C RAPD locus on chromosome 1, which was not polymorphic between C and D and was replaced by TG430, a RFLP locus).

Background selection

Several plants of the BC_2 and BC_3 generation in each of the three MABC programs had the donor alleles at the five selected regions. Background selection was performed in each case to select the plant with the genetic background the closest to that of the recipient line. This selection was performed with 13 RFLP and RAPD markers located on both carrier and non-carrier chromosomes (Fig. 2). For carrier chromosomes, no strong selection was applied, and only one marker on chromosome 1 and one marker on chromosome 4 were used. As these two chromosomes correspond to two linkage groups (Saliba-Colombani et al. 2000), the markers used for background selection were distant from the regions of interest. For non-carrier chromosomes, the markers were used to monitor the return to the recipient genome on regions where LR134 had C alleles.

To estimate the recipient genome length at the end of the MABC scheme, the BC_3S_2 improved lines were genotyped with 36 RFLP markers distributed over the regions where LR134 had C alleles and chosen on the basis of the genetic map of Saliba-Colombani et al. (2000).

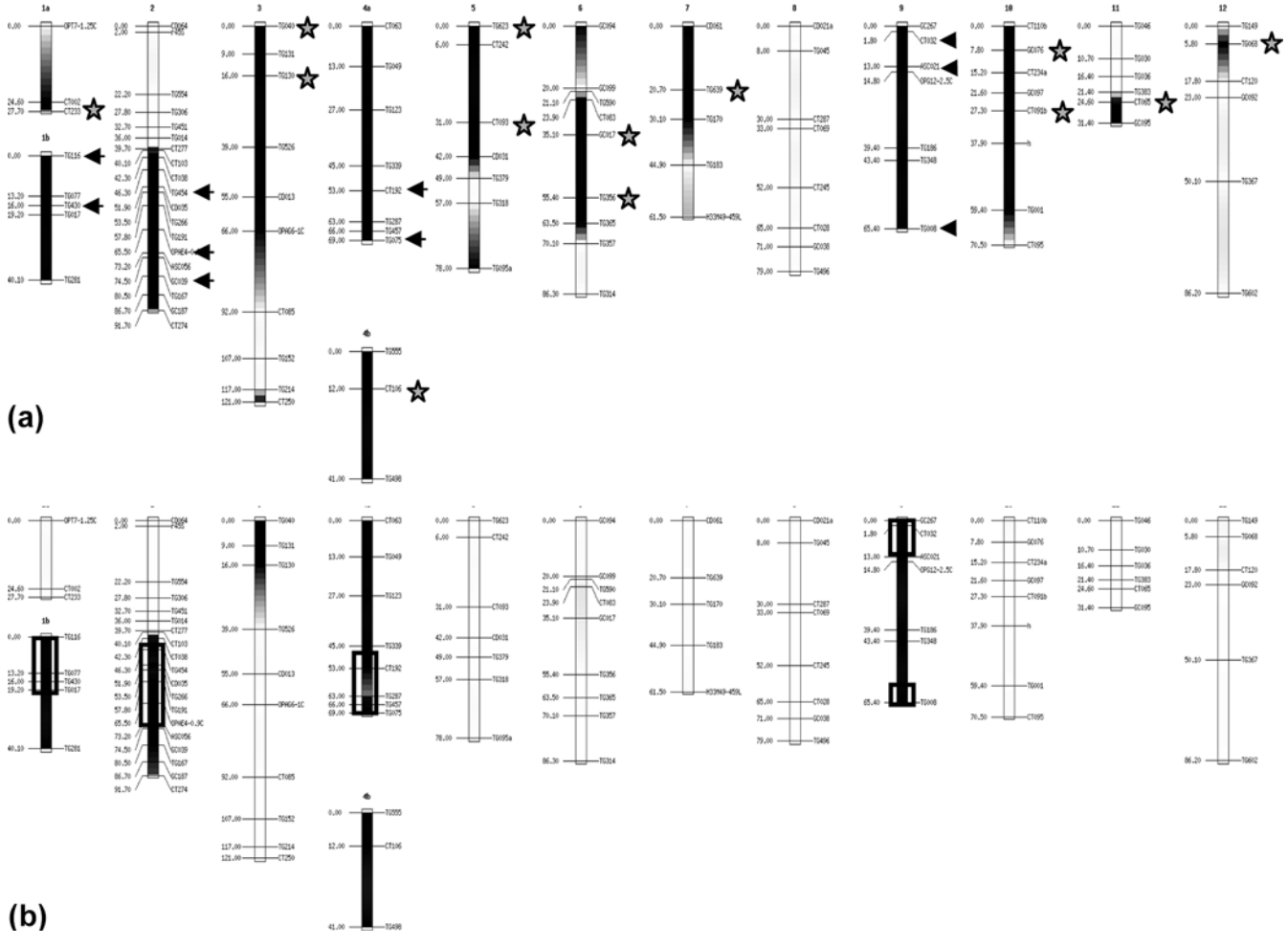

Fig. 2 Graphical genotypes of the donor LR134 (**a**) and the improved L5 (**b**) lines. For all 12 chromosomes the genotypes are depicted with *colors* indicating the expected dose of recipient alleles at each chromosomal location. The *horizontal bars* indicate chromosome lengths in Kosambi centiMorgans on the *left* and marker names on the *right*. Colors range from *black* (minimal possible dose) to *white* (maximal possible dose). The expected allele dose was computed using the MDM program (Servin et al. 2002) based on the pedigree and marker genotype information. The introgressed regions are *boxed*. *Arrows* and *stars* indicate the locations of markers used for QTLs and background selection, respectively

Phenotypic evaluation

Lines and hybrids were evaluated in a trial performed in a heated glasshouse from February to June 2002 at INRA Montfavet (southern France). The six lines (L, B, D, L5, B5 and D5) and the 15 F_1 hybrids obtained from the half-diallel cross (Table 1) were evaluated, each represented by a plot of six plants. Red ripe fruits were harvested in bulk from the six plants of each genotype, twice a week for 6 weeks. A total of 42 fruits per plot were then evaluated (seven fruits per week) for physical and chemical traits. Fruit-by-fruit evaluation was first performed for FW and FIR. Fruit FIR was evaluated with a Durofel. Fruits were cut to count the LONB and frozen (−30°C). Chemical analyses were performed on frozen fruit powder derived from blending the seven fruits of each crop with liquid nitrogen. SSC, SUC and TA were evaluated as for QTLs analysis (Saliba-Colombani et al. 2001). The pH was also measured, but no variation was observed.

Statistical analysis

The global confidence interval lengths (which included the individual confidence intervals of all the QTLs of a region) were evaluated applying a 1.5 LOD score decrease on interval mapping QTL data (Causse et al. 2001a; Saliba-Colombani et al. 2001). Based on analytical computations (Hospital and Charcosset 1997), the probability that the QTLs had the same alleles as the markers used for foreground selection were calculated for each region and extended to the case where the five regions were simultaneously considered. Thus, the minimum number of individuals that should be genotyped in the backcross generations in order to select at least one individual with the required genotype, with a 1% or 5% risk, was calculated. The percentage of recipient-type genome was estimated over all carrier and non-carrier chromosomes (the selected regions were not taken into account for the carrier chromosomes). The percentage of genetic background for all the chromosomes was defined as the percentage of total genome except for the five confidence intervals. Graphical genotypes were drawn using the GRAFGEN program (http://moulon.inra.fr/~servin/grafgen.html). The other statistical analyses were performed using SAS software (SAS Institute 1988). For all the traits, the effects of the improved lines and improved hybrids were evaluated by analysis of variance, and mean comparisons were performed using Tukey's test with a significance level of $P \leq 0.01$. When a significant difference was observed between the recipient line mean and the improved line mean, the additive and dominant effects were estimated. Additivity (a) was calculated as one-half of the difference between the recipient line value and the improved line value. Dominance (d) was estimated as the difference between the hybrid (derived from the cross between an improved line and the corresponding recipient line) value and the average of the recipient line value and the improved line. An analysis of variance was performed on the diallel genotypes with two factors—genetic background (six different genotypes) and number of C alleles at the QTLs (0, 1 or 2)—and the interaction between the two factors.

Results

Implementation of the MABC

The five regions introgressed by MABC carried favorable alleles at three QTLs for acidity and sourness, two for SSC and sweetness, two for aroma intensity, three for texture traits and one for pharmaceutical aroma (Table 2). The donor parent C carried unfavorable alleles only for FW and LONB on chromosome 2 and for FIR on chromosome 9A. One to three markers were selected to monitor each of the five regions of 15–31 cM long, taking into account the confidence intervals of all the interesting QTLs of the regions (Table 2, Fig. 2). On the basis of the analytical computations (Hospital and Charcosset 1997), the foreground selection markers guaranteed a probability of 94–99% that the QTLs had the same alleles as the markers for each monitored segment in the BC_2 plants. For the five regions simultaneously, this probability was above 81%. A single marker was used to monitor the introgression of the 9B region, as it was the only polymorphic marker available in this region. For each of the regions 1, 4 and 9A, the two markers giving the highest associated probability were retained. Indeed, as the length of the regions was shorter than 20 cM for chromosomes 4 and 9A, it was not necessary to use more than two markers (Causse et al. 2001b). For chromosome 1, despite a length of 31 cM, the effect of increasing the number of markers from two to three proved to be inefficient, as the markers were not uniformly distributed over the region. On the contrary, for the region of chromosome 2, which contained several QTLs with strong effects within 31 cM, three markers were selected to minimize the risk of losing the target alleles at the QTLs. The low molecular polymorphism among *L. esculentum* cultivars enabled us to use almost the same markers in the three programs. The risk of not obtaining at least one plant carrying donor alleles at all foreground selection markers was lower than 5% and 1% when at least 184 or 282 plants were studied, respectively. Therefore, 300 seeds were sown at each generation, leading to population sizes that varied between 225 plants and 297 plants because of variations in seed emergence between populations. PCR-based markers enabled a quick and sequential selection with fewer than 800 genotype data points; this is contrast to the about 3,000 genotype data points necessary if only RFLP markers were used and all the plants had been kept during the MABC scheme. Each population was first genotyped for a RAPD marker of region 2; thus only one-half of the population was selected and genotyped for the RAPD marker of region 1. Similarly, one-half of the remaining individuals were selected and genotyped for the PCR-based marker of region 9A, leading to less than 35 individuals that were genotyped for the other RFLP markers. Overall, the MAS was successfully performed with 846, 875 and 847 plants in the L, B and D MABC programs, respectively. These population sizes were large enough to find at least one plant carrying donor alleles at all of the foreground selection markers monitoring the QTL regions, but only four to eight plants remained for background selection at each generation. However, it did not seem worthwhile to work with a larger population size, as the number of selected plants would stay low, leading to a small increase in background selection intensity but to a large increase in marker cost. Six or eight markers were used for background selection at the BC_2 and BC_3 generations, respectively. These markers were selected inside large regions carrying C alleles in the previous generation (Fig. 2). A single BC_3S_1 plant with four, four and five homozygous regions was obtained, for the L, B and D

genetic background, respectively, and no background selection could be performed. These plants were selfed, and plants with the five homozygous regions were selected for L, B and D backgrounds.

The final recipient genome content of BC_3S_2 lines was high for the eight non-carrier chromosomes: 95%, 98% and 100%, for L5, B5 and D5, respectively. For the four QTL carrier chromosomes, it was only 23%, 49% and 49.3%, for L5, B5 and D5, respectively. The overall recipient genome recovery was therefore about 67%, 75% and 77% for L5, B5 and D5, respectively. As the five selected regions covered 12% of the whole genome, a background selection over 21%, 13% and 11% of the whole genome could still be carried out for the L5, B5 and D5 lines, respectively.

Phenotypic effect of the selection

Table 3 shows the mean values of the three recipient lines and the donor line. For each trait, the simultaneous effect of the five selected regions was evaluated at : (1) homozygous recipient, (2) heterozygous and (3) homozygous donor (c) states in the L, B or D homozygous genetic backgrounds (called Lgb, Bgb and Dgb, respectively). Additive and dominance effects were estimated and the genotypes compared among the genetic backgrounds (Fig. 3, Table 3). Three heterozygous genetic backgrounds were produced by the half-diallel: BL, DL, and BD (Table 1), each being represented by four genotypes at the QTLs based on their allele combination: (1) control hybrids produced by the hybridization of the two parental lines (B×D, B×L and D×L), called RR′; (2) hybrids produced by the hybridization of the two improved lines (B5×D5, B5×L5 and D5×L5), called CC; (3) hybrids produced by the hybridization of an improved line with a parental line (B5×D, B5×L and D5×L), called RC; (4) hybrids produced by the hybridization of the second parental line with the second improved line (B×D5, B×L5 and D×L5), called R′C (Fig. 4). Different effects of the QTLs and interactions between QTL regions and genetic background were obtained for all the traits. The simultaneous analysis of the 21 genotypes of the half-diallel also revealed significant effects of the QTL regions, of the genetic backgrounds and of interactions among the two factors for all the traits (data not shown).

For the chemical traits, higher means were conferred by the C alleles, but the improvement was strongly dependent on the genetic background. For SSC and SUC, three QTLs were transferred (two on chromosome 2 and one on chromosome 9) that controlled about 50% of the variation in the RILs. Differences between recipient and improved lines were significant in Lgb and Bgb, with similar additive effects of the QTLs. The L and D lines were not significantly different from each other, whereas L5 values for these traits were much higher than D5 values. No significant effect of the QTLs was detected in Dgb. SSC was higher in the B line than in the L or D lines, but no significant difference was observed between B5 and L5.

Table 3 Average values of physical and chemical traits for parental lines and the cumulative effect of the five introgressed regions in each homozygous genetic background

Trait[a]	C	Donor parent	Recipient parents[b]			Additive effect			*d*/a[c]		
		LR134	L	B	D	L	B	D	L	B	D
SSC (°Brix)	11.4 (0.6)	9.5 (0.9)	5.3b (0.1)	6.6a (0.4)	5.2b (0.6)	1.62	0.84	NS	−0.08	−0.01	NS
SUC (g/100 g fm)	5.8 (0.5)	4.8 (0.7)	2.3a (0.5)	2.8a (0.3)	2.4a (0.5)	1.27	0.63	NS	0.01	0.01	NS
TA (meq H^+/100 g fm)	10.0 (0.8)	6.0 (0.5)	4.6b (0.3)	6.2a (0.8)	5.7a,b (0.6)	0.57	NS	NS	−0.74	NS	NS
FW (g)	5.8 (0.5)	22.2 (4.2)	136.1b (15.2)	139.0b (8.3)	153.5a (9.4)	−53.43	−46.28	−52.15	−0.40	−0.39	−0.04
FIR	54.9 (9.0)	60.4 (6.2)	63.0b (6.2)	80.1a (4.3)	80.4a (5.7)	NS	−3.42	−5.25	NS	−1.90	−1.25
LONB	2.3 (0.5)	2.1 (0.3)	4.6a (0.8)	2.8c (0.7)	3.8b (0.8)	−1.18	−0.33	−0.86	−0.27	−0.36	−0.56

[a]See Table 2 for definition of traits
[b]Standard deviations are between brackets. Means of the three recipient parents were compared using Tukey's test at a significance level of $P \leq 0.01$. Means followed by the same letter are not significantly different. When a significant difference was observed between the RR mean and the CC mean, the additive (*a*) and dominant (*d*) effects were estimated, and *d*/*a* was calculated
[c]Additive effect for $-0.2 < d/a < 0.2$; partially dominant effect of C alleles for $-0.8 < d/a < -0.2$; dominant effect of C alleles for $-1.2 < d/a < -0.8$; overdominant effects for $d/a < -1.2$

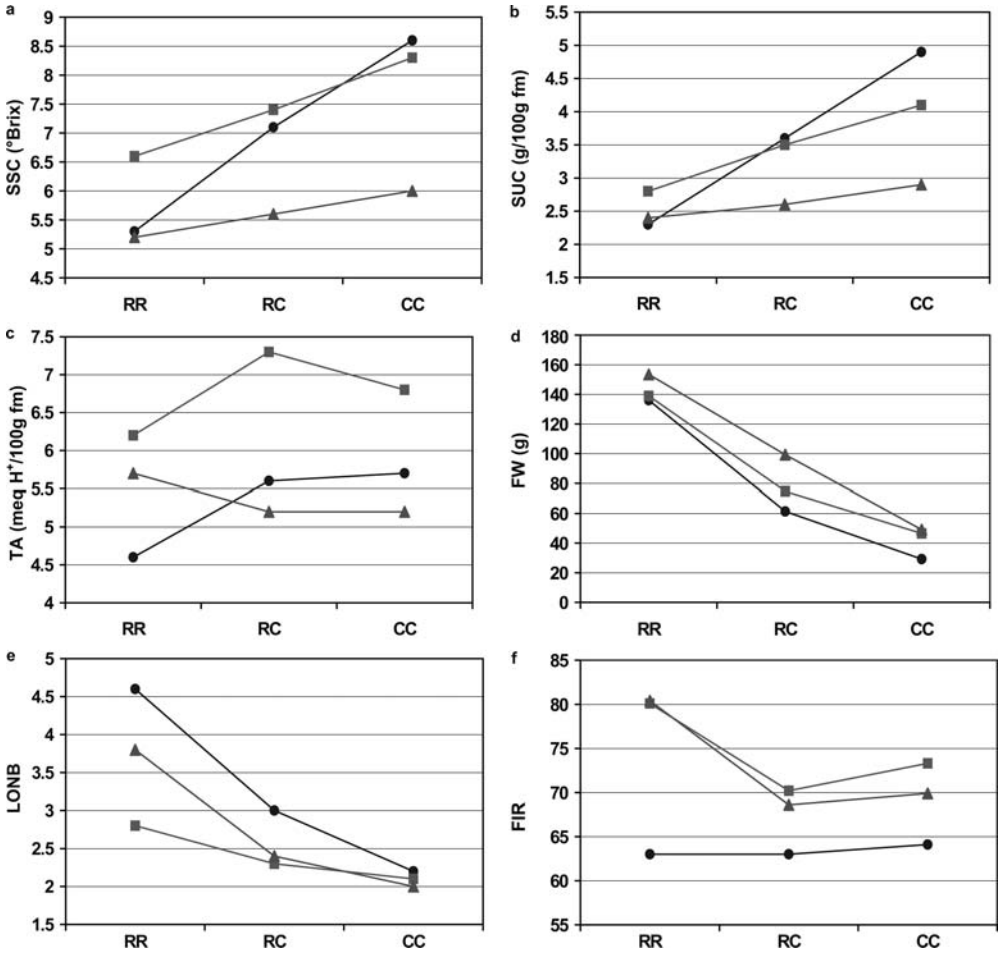

Fig. 3a–f Effect of the five introgressed regions in the three genetic backgrounds in the homozygous state. Phenotypic values of the improved lines (*CC*), the recipient lines (*RR*) and the hybrids between improved lines and recipient lines (*RC*) for each homozygous genetic background. *C* (Cervil) and *R* (recipient) illustrate the allelic combination at the five introgressed regions. Each genetic background is represented by a symbol: *circles* Lgb, *squares* Bgb, *triangles* Dgb. Six traits are shown: SSC (**a**), sugar content (**b**), TA (**c**), FW (**d**), LONB (**e**), FIR (**f**)

B alleles in the introgressed regions thus seemed to be responsible for the differences between B and L. At the hybrid level, no difference was observed for SSC between the CC hybrids nor between the RR′ hybrids. As observed in the homozygous genetic backgrounds, C alleles only showed an effect in hybrids of the B5 and L5 lines. Hybrids between improved and recipient lines involving D5 lines were not significantly different from control hybrids, but they had a significantly lower SSC than hybrids of the B5 and L5 lines (which were not significantly different from the CC hybrids). The absence of improvement provided by D5 was confirmed at the heterozygous level. For SUC, the only genetic background in which significant differences were observed at the hybrid level is BL, with B×L hybrids having a significantly lower sugar content than B5×L5, B5×L or L5×B hybrids. The effect of the C alleles was partially dominant, nevertheless B5×D and L5×D were not significantly different from B×D and D×L, respectively. Moreover, hybrids involving D5 lines were not improved, suggesting unfavorable interactions between Dgb and C alleles. The difference in recipient genome recovery of D5 in the distal extremity of chromosome 2 could also be involved as QTLs with large effects on soluble solid and sugar contents have been mapped in this region (Lecomte et al. 2004).

For TA, two QTLs were transferred, one on chromosome 1, the other on chromosome 2. C alleles provided higher values of TA in the Lgb line and displayed a partially dominant effect, but the response to the selection was not significant in Bgb and Dgb. As TA was greater in Bgb than in Lgb or Dgb, alleles providing higher acidity could be located in Bgb at a distance from the QTL regions. At the hybrid level, C alleles reduced TA in Dgb. Indeed, no significant difference was observed between control hybrids, whereas B5×L5 was significantly more acid than B5 × D5 or D5 × L5, and among RC and R'C hybrids, hybrids involving D5 lines were significantly less acid than hybrids involving B5 or L5 lines. Furthermore, D5 had a negative effect on acidity, as D5×B and D5×L were significantly less acid than D×B and D×L. This could be attributed to unfavorable epistatic interactions between the genetic background and the introgressed regions in D5. As this effect was not observed in hybrids involving B5 or L5 lines, it could only involve the regions having C alleles in D5 lines.

For FW, two QTLs were detected on chromosome 2, one in the middle region, the other in the distal part. Unlike L5 and B5, D5 did not carry C alleles at the second QTL. Significant and important FW reductions between the homozygous and heterozygous recipient at the QTL regions, and between heterozygous and homozygous C at

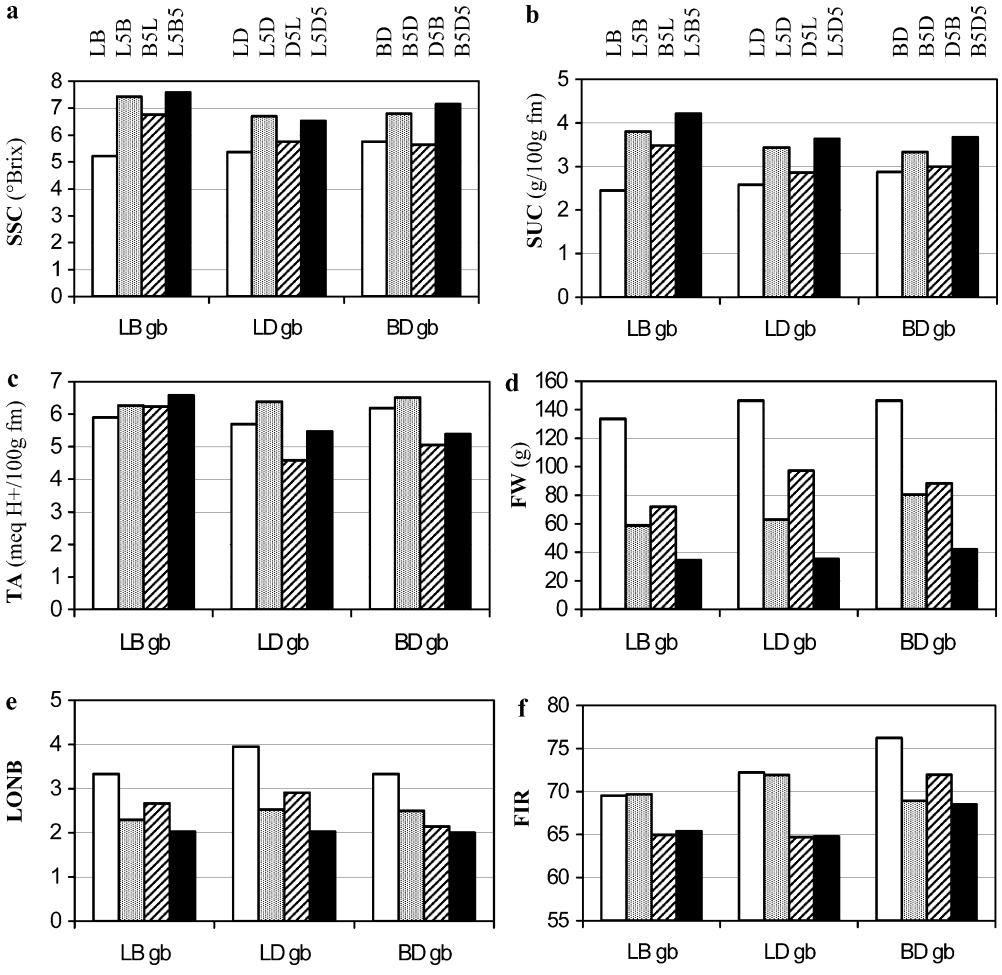

Fig. 4a–f Effect of the five introgressed regions in each of the three heterozygous genetic backgrounds (*LBgb*, *LDgb*, *BDgb*) The hybrid combinations are indicated at the *top* of the histograms. The genotypes at the QTLs are heterozygous recipient (*white column*), heterozygous recipient × C (*grey column* and *striped column*) and homozygous C (*black*). Six traits are shown: SSC (**a**), SUC (**b**), TA (**c**), FW (**d**), LONB (**e**) and FIR (**f**)

the QTL regions were observed whatever the genetic background. The selected alleles displayed an additive effect in Dgb, and a partially dominant effect in Lgb and Bgb. B5 and D5 were not different, so the difference between B and D could be attributed to allelic variation between these two lines in the QTL regions. In contrast, FW of B and L were not different, whereas B5 fruits were heavier than L5 fruits. Thus, Bgb could carry different QTL alleles than L, ones that interact with C alleles. At the hybrid level, as observed in homozygous genetic backgrounds, the effect of C alleles at the selected regions is highly significant and unfavorable for FW. For each heterozygous genetic background, RR′, RC, R′C and CC hybrids were significantly different from each other, thereby confirming that the choice of the improved line in a given cross may be important. Among the RR′ hybrids, the hybrids involving the D line had significantly heavier fruits than the B×L hybrid. FW was more influenced by the improved line than by the parental line in hybrids between improved and recipient lines: the average FW was significantly higher in hybrids involving D5 than in hybrids involving B5, and it was significantly higher in hybrids involving B5 than in hybrids involving L5. However B5 and D5 FW was not significantly different despite the difference in recipient genome recovery in the distal region of chromosome 2, where a major FW QTL, fw2.2, segregated (Lecomte et al. 2004).

Only one QTL was detected for LONB in the middle of chromosome 2. LONB was significantly greater in homozygous recipient lines than in lines heterozygous at the QTL regions and was also greater in lines heterozygous at the QTL regions than in homozygous introgressed lines. The selected alleles displayed partially dominant effects. As LONB was different between recipient lines and not between the improved lines, the differences between the recipient lines could be attributed only to allelic variation in the selected regions, with the L alleles increasing LONB the most, followed by D alleles and lastly by B alleles. The partially dominant effect of C alleles on LONB was also confirmed in heterozygous genetic backgrounds. All CC hybrids had, on average, two locules, whereas control hybrids had between 3.3 locules and 3.9 locules on average, with the RC and R′C hybrids showing intermediate values. In contrast to FW, LONB was more influenced by the recipient line than by the improved line in hybrids between improved and recipient lines, illustrating that the selected regions controlled most of the trait variation.

For FIR, three QTLs were transferred on chromosomes 2, 4 and 9. Homozygous recipient genotypes were significantly firmer than those heterozygous or

homozygous C at the QTL regions in Bgb and Dgb, and alleles for firmer fruits displayed a recessive effect, whereas no difference was observed in Lgb whatever the genotype of the QTLs. Lines with Bgb or Dgb were not different, thus B and D QTLs could be allelic for the corresponding QTLs. As FIR was systematically greater in Bgb or Dgb than in Lgb, other alleles conferring fruit FIR and located in the genetic background may also be involved. The favorable effects of B or D alleles on FIR were confirmed in heterozygous genetic backgrounds. In a BD genetic background, RC, R′C and CC hybrids were not significantly different, but they were all significantly less firm than B×D, thereby confirming the dominance of C alleles over the B and D alleles. L alleles were also dominant over B and D alleles based on the observation that there was no difference in a BL genetic background and D×L was not significantly different from L5×D. However, in a DL genetic background unfavorable interactions occurred as D×L and L5×D fruits were significantly firmer than D5×L and D5×L5 fruits.

Discussion

Recipient genome recovery

Three backcrosses were sufficient to recover most of the recipient genome for non-carrier chromosomes, even with a low selection pressure, as has been theoretically demonstrated by Hospital and Charcosset (1997). Among the three improved lines, only two segments of non-carrier chromosomes were not transferred to the recipient genome, one 20-cM-long and one 10-cM-long in L5 and B5, respectively. Overall, L5, B5 and D5 carried between 12% and 23% C alleles in their genetic background, mostly on chromosome segments linked to the QTLs. Almost no selection for genetic background was performed on carrier chromosomes, leading to a total proportion of C alleles in the improved lines that was still rather large. It is advisable that background selection be applied cautiously on carrier chromosomes when the QTL location is not accurate and the QTL could be lost (Han et al. 1997). Furthermore, as we were able to map several QTLs for quality traits in four of the five regions of interest (Table 2), we decided to introgress segments covering all of the corresponding confidence intervals. In particular, on chromosome 9, where two segments were selected at the two extremities of the chromosome, two of the three improved lines were homozygous over the whole chromosome. In B5, one optimal genotype with two recombination points was obtained, with the middle of chromosome 9 carrying B alleles. As the introgressed lines differed from each other by 80–100 cM, these large C regions could be associated with unfavorable genetic factors, leading to unfavorable linkage drag effects (Robert et al. 2001; Yousef and Juvik 2002). Nevertheless, among these regions, the only one where unfavorable QTLs have been previously detected was the distal region of chromosome 2. B5 and L5 had C alleles in this region, whereas D5 had recipient alleles: C alleles are favorable for SSC and SUC but unfavorable for FW (Lecomte et al. 2004). These results confirm the need to precisely map regions to be introgressed before starting the introgression.

Differences in allele effects in the three recipient lines

Differences in allelic effects were detected for all of the traits studied in the three recipient lines both within and outside the introgressed regions. C is a cherry tomato line (*L. esculentum* var. *cerasiforme*) so distant (both from a phenotypic and a molecular point of view) from large-fruit cultivars that it was first supposed that QTLs found in the progeny of a cross with the L line should have the same effects in progeny from crosses with other *L. esculentum* lines. Indeed, among the 40 RFLP probes screened, only three and four showed some differences between L and B or D, respectively. If the three lines had the same alleles at all QTLs, the same effects in introgressed lines were expected. In fact, the same additive effects were only detected for FW, although partially dominant effects were only detected in Lgb and Bgb. For SSC and SUC, at least three different alleles were detected, with C and D having the same effect and L having a higher effect than B, although the means of the L and D lines were not different. For TA, the only significant additive effect was observed within the Lgb. The means of B and D lines were higher than that of L and closer to the C value, which may explain the absence of improvement in D5 and B5. For FIR, effects were only detected in Bgb and Dgb, with the fruit of the B and D lines being firmer than those of L and C. We could thus deduce that these two lines carried different alleles than L and C within the introgressed regions. These lines also differed with respect to the QTLs carried by the other chromosomes, as the lines with Bgb and Dgb were systematically the firmest. Sebolt et al. (2000) made similar observations following the introgression of a QTL for soybean seed protein concentration in three genetic backgrounds: no effect was detected with the recipient line having the highest seed protein concentration. The authors suggested that this failure could be attributed to an absence of allelic variation between the donor and the recipient alleles in the introgressed region. The three recurrent lines differed with respect to LONB but reached the same level once QTLs were introgressed. In the present investigation, the QTL segments could carry all of the genes controlling the trait variation but the three recipient lines may have carried three different alleles.

In conclusion, differences in allele effects were observed for all of the traits studied, with at least three or four alleles (or different QTLs) being detected for each trait. The very low polymorphism observed among cultivars at the molecular level, either for anonymous probes (Miller and Tanksley 1990) or for gene sequences (Nesbitt and Tanksley 2002), are contradictory with such allelic diversity. Allelic variation or interactions between QTLs and the genetic background could explain differences arising as a result of the genetic backgrounds, as

proposed by Yousef and Juvik (2002). While differences in recipient genome recovery rate between the improved lines could also cause these discrepancies, these differences could only explain the differences in FW and SUC.

Interactions between QTLs and genetic backgrounds

Epistatic interactions between QTL regions and genetic backgrounds were shown in the half-diallel cross design for all the traits, except for TA. Most of the comparisons of different genotypes (CC, RC, R′C and RR′) in the three heterozygous genetic backgrounds were in agreement with allelic effects detected in the homozygous backgrounds. Our comparisons of one QTL genotype in several backgrounds frequently revealed differences: for example, L5×B and D5×B, both lines with the same genotype at the QTL, differed for SSC, SUC, TA and FW, which also suggests epistatic interactions. Epistatic interactions have often been proposed to explain such interactions (Toojinda et al. 1998; Shen et al. 2001) but require specific experimental designs to be shown (Eshed and Zamir 1996; Liao et al. 2001). The differences we observed in the three genetic backgrounds illustrates the need—from a breeding point of view—to introgress QTLs in several recipient lines given that the effects of the genetic backgrounds on the trait of interest could vary independently of the introgressed regions. Nevertheless interactions limit the efficiency of MAS and thus require the screening of QTL effects in each genetic background.

In conclusion, our investigation has shown that MABC can be easily performed for the introgression of up to five chromosome regions as long as sequential selection is possible and, consequently, PCR markers available. As we did not precisely map the QTLs, large regions of donor chromosomes were transferred, leading to unfavorable linkage drag. Nevertheless, MABC was clearly efficient in improving quality traits in the genetic background used for QTL detection, even though QTL-by-genetic-background interactions were shown to hamper progress. Furthermore, previously undetected QTLs restricted the improvement of fruit size.

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References

Bouchez A, Hospital F, Causse M, Gallais A, Charcosset A (2002) Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. Genetics 162:1945–1959

Causse M, Saliba-Colombani V, Lesschaeve I, Buret M (2001a) Genetic analysis of organoleptic quality in fresh market tomato. 2. Mapping QTLs for sensory attributes. Theor Appl Genet 102:273–283

Causse M, Lecomte L, Baffert N, Duffé P, Hospital F (2001b) Marker-assisted selection for the transfer of QTLs controlling fruit quality traits into tomato elite lines. Acta Hortic 546:557–564

Causse M, Saliba-Colombani V, Lecomte L, Duffé P, Rousselle P, Buret M (2002) QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. J Exp Bot 53:2089–2098

Causse M, Buret M, Robini K, Verschave P (2003) Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. J Food Sci 68:2342–2350

Eshed Y, Zamir D (1996) Less-than-additive epistatic interactions of quantitative trait loci in tomato. Genetics 143:1807–1817

Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13:207–209

Han F, Romagosa I, Ullrich SE, Jones BL, Hayes PM, Wesenberg DM (1997) Molecular marker-assisted selection for malting quality traits in barley. Mol Breed 3:427–437

Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485

Jones RA, Scott SJ (1983) Improvement of tomato flavor by genetically increasing sugar and acid contents. Euphytica 32:845–855

Kearsey MJ, Farquhar AGL (1998) QTL analysis in plants: where are we now? Heredity 80:137–142.

Lecomte L, Saliba-Colombani V, Gautier A, Gomez-Jimenez MC, Duffé P, Buret M, Causse M (2004) Fine mapping of QTLs of chromosome 2 affecting the fruit architecture and composition of tomato. Mol Breed 1:1–14

Liao CY, Wu P, Hu B, Yi KK (2001) Effects of genetic background and environment on QTLs and epistasis for rice (*Oryza sativa* L.) panicle number. Theor Appl Genet 103:104–111

Miller JC, Tanksley SD (1990) RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. Theor Appl Genet 80:437–48

Nesbitt TC, Tanksley SD (2002) Comparative sequencing in the Genus *Lycopersicon*: implications for the evolution of fruit size in the domestication of cultivated tomatoes. Genetics 162:365–379

Petro-Turza M (1987) Flavor of tomato and tomato products. Food Rev Int 2:309–351

Robert VJM, West MAL, Inai S, Caines A, Arntzen L, Smith JK, St Clair DA (2001) Marker-assisted introgression of blackmold resistance QTL alleles from wild *Lycopersicon cheesmanii* to cultivated tomato (*L. esculentum*) and evaluation of QTL phenotypic effects. Mol Breed 8:217–233

Saliba-Colombani V, Causse M, Gervais L, Philouze J (2000) Efficiency of AFLP, RAPD and RFLP markers for the construction of an intraspecific map of the tomato genome. Genome 43:29–40

Saliba-Colombani V, Causse M, Langlois D, Philouze J, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. Theor Appl Genet 102:259–272

SAS Institute (1988) SAS users guide: statistics. SAS Institute, Cary, N.C.

Sebolt AM, Shoemaker RC, Diers BW (2000) Analysis of a quantitatI've trait locus allele from wild soybean that increases seed protein concentration in soybean. Crop Sci 40:1438–1444

Servin B, Dillmann C, Decoux G, Hospital F (2002) MDM: a program to compute fully informative genotype frequencies in complex breeding schemes. J Hered 3:227–228

Shen L, Courtois B, McNally KL, Robin S, Li Z (2001) Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. Theor Appl Genet 103:75–83

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS (2001) Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *xa21*) using marker-assisted selection into indica rice cultivar PR106. Theor Appl Genet 102:1011–1015

Stevens MA (1986) Inheritance of tomato fruit quality components. Plant Breed Rev 4:273–311

Stevens MA, Kadre AA, Albright M (1979) Potential for increasing tomato flavor via sugar and acid contents. J Am Soc Hortic Sci 104:40–42

Toojinda T, Baird E, Booth A, Broers L, Hayes P, Powell W, Thomas W, Vivar H, Young G (1998) Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. Theor Appl Genet 96:123–131

Yousef GG, Juvik JA (2002) Enhancement of seedling emergence in sweet corn by marker-assisted backcrossing of beneficial QTL. Crop Sci 42:96–104

Yu K, Park SJ, Poysa V (2000) Marker-assisted selection of common beans for resistance to common bacterial blight: efficacy and economics. Plant Breed 119:411–415