Theor Appl Genet (2006) 112: 934–944
DOI 10.1007/s00122-005-0197-7

ORIGINAL PAPER

J. Chaïb · L. Lecomte · M. Buret · M. Causse

Stability over genetic backgrounds, generations and years of quantitative trait locus (QTLs) for organoleptic quality in tomato

Received: 11 July 2005 / Accepted: 14 December 2005 / Published online: 10 January 2006

Abstract The efficiency of marker-assisted backcross for the introgression of a quantitative trait locus (QTL) from a donor line into a recipient line depends on the stability of QTL expression. QTLs for six quality traits in tomato (fruit weight, firmness, locule number, soluble solid content, sugar content and titratable acidity) were studied in order to investigate their individual effect and their stability over years, generations and genetic backgrounds. Five chromosome regions carrying fruit quality QTLs were transferred following a marker-assisted backcross scheme from a cherry tomato line into three modern lines with larger fruits. Three sets of genotypes corresponding to three generations were compared: (1) an RIL population, which contained 50% of each parental genome, (2) three BC3S1 populations which segregated simultaneously for the five regions of interest but were almost fully homozygous for the recipient genome on the eight chromosomes carrying no QTL and (3) three sets of QTL-NILs (BC3S3 lines) which differed from the recipient line only in one of the five regions. QTL detection was performed in each generation, in each genetic background and during 2 successive years for QTL-NILs. About half of the QTLs detected in QTL-NILs were detected in both years. Eight of the ten QTLs detected in RILs were recovered in the QTL-NILs with the genetic background used for the initial QTL mapping experiment, with the exception of two QTLs for fruit firmness. Several new QTLs were detected. In the two other genetic backgrounds, the number of QTLs in common with the RILs was lower, but several new QTLs were also detected in advanced generations.

Keywords Fruit quality · Quantitative trait locus (QTL) · *Solanum lycopersicum* · Genetic background · Marker-assisted selection (MAS)

Abbreviations Bgb: Vil B genetic background · Dgb: Vil D genetic background · EUG: Eugenol · FW: Fruit weight · Lgb: Levovil genetic background · LONB: Locule number · MAS: Marker-assisted selection · MYP: Orthometoxyphenol · SSC: Soluble solids content · SUC: Sugar content · TA: Titratable acidity

Introduction

Thanks to the progress in molecular marker techniques and quantitative trait locus (QTL) analysis (Asins 2002; Mohan et al. 1997; Tanksley 1993, Zeng 1994), several studies have described the genetic basis of quantitatively inherited traits. Marker-assisted selection (MAS) is often mentioned as a perspective of these QTL studies, but few applied MAS programs are reported in the literature (Dekkers and Hospital 2002). Most of the MAS experiments concern the introgression of QTLs for the improvement of a single trait in the genetic background that has been used for the QTL detection (Ahmadi et al. 2001; Robert et al. 2001; Stuber and Sisco 1992; Van Berloo et al. 2001). Such studies are still far from current breeding practices. Indeed, a breeder may have to (1) simultaneously select for several traits, (2) use several recipient parents, and (3) check that the selected QTLs are not linked to unfavorable alleles for major agronomic traits. These preoccupations have been progressively taken into account in MAS studies. On one hand, the simultaneous selection of several traits was not systematically followed by an increase in the phenotype for all the traits. For instance, the introgression of three QTLs for earliness and yield in a maize elite line proved successful in improving earliness, but important

Communicated by I. Paran

J. Chaïb · L. Lecomte · M. Causse (✉)
INRA, Unité de Génétique et Amélioration des Fruits et Légumes, Domaine Saint-Maurice, BP94, 84143 Montfavet Cedex, France
E-mail: Mathilde.Causse@avignon.inra.fr
Tel.: +33-4-32722710
Fax: +33-4-32722702

M. Buret
INRA, UMR Sécurité et Qualité des Produits d'Origine Végétale, Domaine Saint-Paul, 84914 Avignon Cedex 9, France

discrepancies were observed in the magnitude and sense of the yield QTL effects when compared to the predicted yield improvements (Bouchez et al. 2002). On the other hand, the use of several recipient parents appeared essential in investigating the interest and the usefulness of QTLs detected in a given population when they are transferred into unrelated genetic backgrounds. Optimistic results were obtained when QTLs were introgressed into a single new genetic background (Li et al. 2001; Toojinda et al. 1998), but when several recipient parents were used, the consistency of the QTL effects in the different genetic backgrounds was less obvious (Sebolt et al. 2000; Yousef and Juvik 2002). Moreover, the introgressed QTLs may be linked to unfavorable effects for major agronomic traits (Robert et al. 2001; Sebolt et al. 2000), and interactions with the environment can strongly influence the MAS results (Romagosa et al. 1999; Zhu et al. 1999).

In a previous study, QTL analysis of the progeny of a cherry tomato line (hereafter C) with a large fruited line (hereafter L) revealed that organoleptic quality of tomato fruit produced in a glasshouse was controlled by several QTLs (Causse et al. 2001; Saliba-Colombani et al. 2001). By chance, most of the QTLs for physical, chemical and sensory traits were located in a few chromosome regions (Causse et al. 2002) and most of the favorable alleles were provided by the C genotype, allowing a marker-assisted backcross scheme to be performed (Lecomte et al. 2004a). Five QTL regions were thus introgressed into three recipient lines, the L line and two other lines (hereafter B and D) that were unrelated to the population used for QTL analysis. Fruit quality of the prototypes cumulating the five QTLs is described by Lecomte et al. (2004a). A positive improvement of quality components was shown for fruit composition in sugars, soluble solids and titratable acidity (TA). The selection efficiency was confirmed by sensory profiles and hedonic assays of the improved lines and of hybrids between these lines and several large fruited lines. Nevertheless, the progress in fruit weight was much lower than expected based on the QTLs detected in RILs. Different effects of the simultaneous introgression of the five regions were observed according to the genetic backgrounds: additive effects were detected for soluble solid content (SSC) and sugar contents (SUC) in two genetic backgrounds. A partially dominant effect on TA was detected only in the L genetic background. In contrast, additive to dominant unfavorable effects of the donor alleles were observed for fruit weight and locule number (LONB) in the three genetic backgrounds, and an effect on firmness was only detected in the two firmest genetic backgrounds.

In the experiment described herein, we investigate the influence of various factors on QTL expression. QTLs for six traits of organoleptic quality in tomato were studied in order to characterize (1) their individual effect using QTL-NILs obtained from the MAS program previously described, (2) the stability of this effect over years, generations and genetic backgrounds and (3) the epistatic interactions between QTLs. The three BC3S1 populations segregating only for the four chromosomes carrying the five regions of interest, with only 30% of the donor genome, were evaluated and QTL mapping was performed in each of the three genetic backgrounds. Furthermore, QTL-NILs (BC3S3 lines) having favorable C alleles at only one region of interest, with approximately 10% of the introgressed genome, were evaluated during two successive years. Our objective was to assess QTL stability over three generations (RILs, BC3S1 populations, QTL-NILs), in three different genetic backgrounds and over years.

Materials and methods

Plant material and introgression scheme

The initial QTL analysis was performed (Causse et al. 2001; Saliba-Colombani et al. 2001) using a population of 144 recombinant inbred lines (RILs) developed from an intraspecific cross between Cervil; a cherry tomato, *Solanum lycopersicum*, var. *cerasiforme* (Dun.), Gray (hereafter C) with 7 g fruits, a good taste and high aroma intensity, and Levovil (a *S. lycopersicum* Mill. line) with 125 g fruits and a common taste. QTLs controlling organoleptic quality traits for glasshouse production were detected (Causse et al. 2002). Based on their involvement in sensory traits, five regions (hereafter 1, 2, 4, 9A and 9B, as they were located on chromosomes 1, 2, 4 and 9, respectively) were chosen to be introgressed in lines with bigger fruits. A QTL for sourness was detected in region 1, QTLs for sweetness, tomato aroma intensity, mealiness and meltiness were detected in region 2, a QTL for mealiness was detected in region 4, QTLs for sourness, tomato aroma intensity, mealiness, meltiness and flesh firmness were detected in region 9A and a QTL for pharmaceutical aroma was detected in region 9B. QTLs for physical and chemical traits were also detected in these regions (Fig. 1). The favorable alleles for fruit quality being conferred by the C parent in most of the cases, the cherry tomato alleles at the five regions were introgressed into large fruit genotypes in order to obtain QTL-NILs. A single RIL (LR134) with C alleles at the five regions was used as the donor parent of the breeding program. The same marker-assisted backcross program was performed with three different recipient lines, kindly provided by Vilmorin: Levovil, VilB and VilD, hereafter L, B and D, respectively (Table 1). As the donor parent (LR134) contained 47% of recipient genome L, the first cross between LR134 and each recipient line was considered as a BC1. The BC1 progeny was genetically homogenous; it was thus backcrossed without any selection to the recipient line to produce a BC2 population. Almost 300 plants were grown, and after an MAS step, one BC2 individual was selected and backcrossed again to produce a BC3 population. Similarly, one BC3 individual was selected and three selfing generations were performed. In each BC3S1 population (hereafter

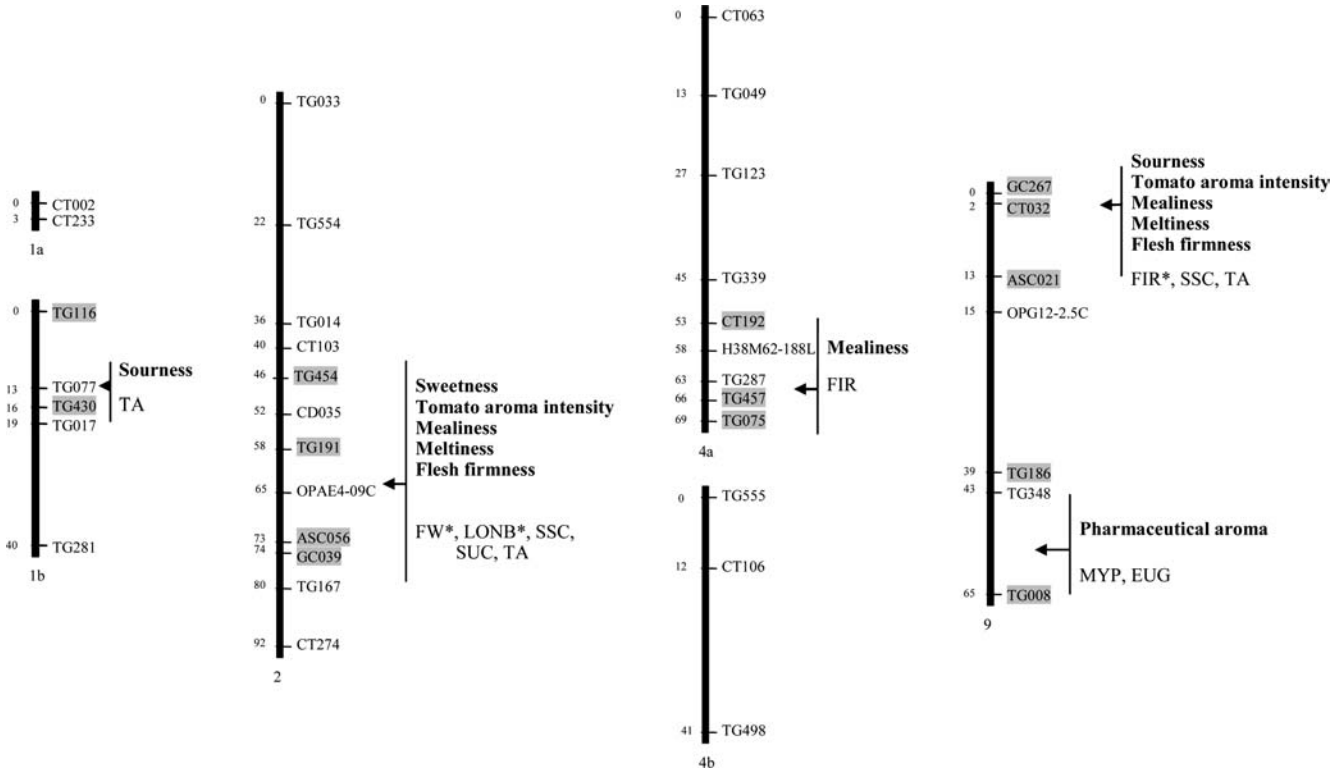

Fig. 1 Molecular map showing the five regions of interest carrying quantitative trait loci (QTLs) for organoleptic quality, based on an intraspecific RIL population derived from a cross between a cherry tomato line (*C*) and a large fruit line (*L*). Distances in Kosambi centiMorgans are on the left of the chromosomes and marker names are on the *right*. The markers used to check the introgression of the regions during marker-assisted selection are *underlined*. Arrows determine the localisation of clusters of QTLs highly involved in the organoleptic quality. To the right of the arrows, QTL detected for sensory traits (in *bold*) and for instrumental traits (see abbreviations) are mentioned. MYP and EUG (orthometoxyphenol and eugenol, respectively) correspond to quantified aroma volatiles related to the perception of pharmaceutical aroma (Causse et al. 2002). *Stars* indicate that the L allele provided higher value to the trait

BC3S1-L, BC3S1-B and BC3S1-D), the segregation of markers in the five regions of interest was comparable to that of an F2 population. Then, BC3S3 lines with homozygous alleles at the five regions were selected and BC3S3 lines carrying C alleles at a single introgressed region were evaluated. These lines were nearly isogenic to their recipient line and were thus called QTL-NILs (Van Berloo et al. 2001). The QTL-NILs were named with a letter corresponding to their genetic background and a number for the QTL region carried. For example, the line carrying a C allele at the region of interest on chromosome 2 with a genetic background L was noted NIL-L2. In each genetic background (hereafter Lgb, Bgb, Dgb), a line was obtained for each QTL region, with the exception of NIL-B9A and NIL-D9B that were not produced.

Plant genotyping

DNA was extracted according to the DNA microprep protocol (Fulton et al. 1995), and molecular markers were scored as recommended for the genetic map construction (Saliba-Colombani et al. 2000). The three MAS programs were performed until BC3S1 generations, using ten markers (RFLP and RAPD) to check for the presence of donor type alleles on QTL segments (Lecomte et al. 2004a). Several individuals had the alleles of interest for the five regions in BC2 and BC3 generations. Thus, a background selection on both carrier and non-carrier chromosomes was achieved with four to seven markers (RFLP and RAPD) to select, in each case, the individual with the genetic background the closest to the corresponding recurrent line. Three additional markers (RFLP) were scored in BC3S1 populations: one on chromosome 2 (GC039), one on chromosome 4 (TG457) and one located between regions 9A and 9B (TG186) in order to estimate the whole chromosome 9 genetic length (Fig. 1). To characterize the part of recipient genome, QTL-NILs were genotyped with 36 of the 84 RFLP markers spread over the genetic map (Saliba-Colombani et al. 2000).

Phenotypic evaluation

Quantitative trait loci were initially detected in a population of 143 RILs as described by Saliba-Colombani et al. (2001). Five trials were then performed in a heated glasshouse, four at Montfavet and one at Ledenon (Southern France).

Table 1 Variation of physical and chemical traits within the four populations: RILs, BC3S1-L, BC3S1-B and BC3S1-D

Trait	C	LR134	L	B	D	RIL				BC3S1-L				BC3S1-B				BC3S1-D			
						Min	Mean	Max	Var	Min	Mean	Max	Var	Min	Mean	Max	Var	Min	Mean	Max	Var
FW (g)	7.0	20.7	125.1	133.0	119.0	10.8	27.0	71.3	125.0	31.7	64.9	127.9	313.6	37.2	69.8	108.4	221.3	37.6	82.8	116.8	226.0
LONB	2.35	2.12	4.01	2.88	3.87	2.00	2.97	5.2	0.6	2.00	2.94	5.35	0.4	2.04	2.36	3.04	0.1	2.00	2.61	3.64	0.2
FIR	54.1	64.9	64.6	84.6	80.2	NA	NA	NA	NA	55.2	64.1	73.5	14.4	65.3	74.4	89.6	21.7	67.8	75.1	86.2	16.2
SSC(°Brix)	10.33	7.78	5.58	7.09	6.09	4.38	7.13	9.38	0.8	6.00	7.51	8.9	0.4	6.02	7.59	8.78	0.4	5.14	6.26	7.52	0.3
SUC(g/100 gfm)	4.21	4.07	3.11	3.40	3.40	2.85	4.02	5.26	0.3	2.77	3.8	4.74	0.1	2.76	3.76	4.7	0.2	2.8	3.48	4.3	0.1
TA(meqH+/100 gfm)	11.11	6.64	4.92	6.68	6.15	4.17	7.78	11.72	1.7	4.58	5.75	7.06	0.3	5.4	7.16	8.72	0.4	4.27	5.37	6.87	0.4

Average values of the parent lines (based on the three trials) are shown. In each population, the minimum, the mean, the maximum values and the overall variance of each trait are mentioned

C Cervil, *L* Levovil, *B* VilB, *D* VilD, *NA* non available

First, for each BC3S1 population, genotypic and phenotypic evaluations were performed with almost one hundred plants: 106 BC3S1-B and 103 BC3S1-L plants were evaluated from February to June 2001 and 83 BC3S1-D plants were evaluated from February to June 2002. The recipient lines, Cervil and the hybrids between the recipient lines and Cervil were grown as control in each trial. The first 25 fruits of each plant were harvested when fully ripe. Five sets of five fruits each from successive harvests were gathered for each BC3S1 plant (five independent repetitions). Fruit-by-fruit evaluations were performed for fruit weight (FW) and firmness (FIR). Fruit firmness was evaluated with a Durofel (a probe was applied at two points on the fruit equator, the movement of the probe was recorded and the average of the two measures was used). Then, fruits were cut to count the LONB and frozen (−30°C). Chemical analyses were performed on fruit powder derived from simultaneously blending five fruits with liquid nitrogen. Soluble solids content (SSC), SUC and TA were evaluated as described by Saliba-Colombani et al. (2001) for QTL analysis.

The last two trials were performed from February to June 2002 and from February to June 2003. In each trial, the recipient lines, Cervil, LR134 and 13 QTL-NILs were evaluated, each one represented by a unique plot of six plants. Ripe fruits were harvested in bulk on the six plants of each plot twice a week for 6 weeks. For the first harvest of each week, seven fruits from the bulk were kept randomly per plot in order to obtain six sets per line (six independent repetitions). A total of 42 fruits per plot were evaluated for the three physical and the three chemical traits as previously described. Chemical analyses were performed on frozen fruit powder derived from simultaneously blending the seven fruits of each crop with liquid nitrogen.

Statistical analyses

In each BC3S1 population, since all the plants were derived from a single BC3 plant, recombination was estimated in the regions of interest similar to an F2 population, using MAPMAKER/EXP version 3.0 (Lander et al. 1987; Lincoln et al. 1992). The repeated control genotypes (Cervil, the recipient parents and the F1 hybrids) allowed line and trial effects and genotype × trial interactions to be tested by analysis of variance. For each BC3S1 population, the conditional probability of being donor type at any given point of the introgressed regions, given the genotypes of the two markers flanking this point, was computed using the MDM program (Servin et al. 2002). A linear regression between MDM genotype data and phenotype data was performed and a QTL effect was detected at a $P < 0.01$ threshold. For all the traits, phenotypic data of the QTL-NILs were first compared together by analysis of variance, and then to their respective recipient line using a Dunnett test. For each genetic background, the additive effect a_i of region i

was estimated: $a_i = (R\text{-}R_i)/2$, where R and R_i are the values of the recipient parent and of the QTL-NIL carrying the region i, respectively. All the statistical analyses were performed using the SAS software (SAS Institute 1988).

Results and discussion

Phenotypic variation in RILs and BC3S1 populations

Phenotypic data for parental lines are presented in Table 1. The C accession had a fruit weight of 7 g, while the recipient lines had fruit weighing more than 120 g (Table 1). Fruits of C exhibited 2.3 locules on average, less than B (2.9 on average) and D and L (4 on average). The soluble solid content of C is much larger (10.3° Brix) than that of the recipient lines, which ranged from 5.6° Brix in L to 7.1° Brix in B. C was also much more acid (11.1 meq H+) than L (4.9 meq H+), the two other lines being intermediate with 6.1 and 6.7 meq H+ for D and B, respectively. Distributions of the phenotypic data in RILs (Saliba-Colombani et al. 2001) were compared to the distributions of each BC3S1 population.

The comparison of the two populations having the same genetic background (RILs and BC3S1-L) revealed the influence of the five segregating regions and that of the fixation of the eight chromosomes homozygous in BC3S1. Indeed, due to the backcross and MAS process, in BC3S1 the eight chromosomes carrying no QTL were almost completely homozygous for the recipient genotype as confirmed by markers (data not shown). On the contrary, most of the four chromosomes carrying the regions of interest were still segregating. Differences between RIL and BC3S1 distributions may thus be caused either by the fixation of QTLs on nonselected chromosomes, or by differences in allelic effects in B and D genotypes. Continuous phenotypic distribution was shown for each trait, suggesting the traits were under polygenic control (Fig. 2). The important variation still observed in BC3S1 populations confirmed the interest of the five selected regions for improving tomato fruit quality. Variance in the RILs was larger than in the BC3S1-L population for every trait, except for FW where BC3S1 was characterized by an increase in variance, due to the increase in average FW in BC3S1 and to the relation between mean and variance, usually observed for FW (Table 1). The increase in the FW mean in BC3S1 could be the consequence of the fixation of L alleles at the QTLs detected in RILs on chromosomes 3, 11 and 12. SSC and SUC in the RILs and in BC3S1-L ranged between the two parental values. For TA, BC3S1 were in average much closer to the recipient parent value. Two different methods were used to evaluate firmness: in the RIL population, firmness was measured with a penetrometer as the force by surface unit needed to deform the fruit by 5% of its initial diameter (Saliba-Colombani et al. 2001), whereas it was measured using a Durofel in advanced generations. Firmness distribution in BC3S1-L was centered on the value of the L parental line (Table 1).

The influence of the genetic background was investigated by comparing phenotypic data among the three BC3S1 populations, BC3S1-L, BC3S1-B and BC3S1-D. Distributions of FW in BC3S1-B and BC3S1-D were similar to BC3S1-L, and higher on average than for the RILs (Fig. 2). Favorable transgressions were observed for SUC and for FIR with each BC3S1 population and for LONB, only in BC3S1-L and BC3S1-B. As expected according to parental values, BC3S1-L displayed higher LONB values than BC3S1-B or BC3S1-D, probably because of the allelic substitution of L alleles by B or D alleles at the QTLs. On average, a very low variation was observed in the BC3S1-B population for this trait, LONB of B and C being almost similar (Table 1). In contrast, SSC and TA were on average higher than the recipient lines, except in BC3S1-D. For FW and FIR, BC3S1-D showed higher values than BC3S1-B and both were on average higher than BC3S1-L. On the contrary, for all chemical traits, BC3S1-D displayed the lowest average value, as if most of the QTLs segregating in L and B progeny were no longer segregating, or as if the D genetic background provided negative alleles. BC3S1-L and BC3S1-B showed the same range of variation for SSC and SUC, which were on average larger than the RILs. The increase in average was higher in BC3S1-B than in BC3S1-L, as expected based on the parental values. Distributions of TA were different in each genetic background. BC3S1-L values were lower than BC3S1-B values in average, and both were lower than the RIL one. Unfavorable QTL alleles must thus have been fixed in the genetic background for these BC3S1 populations.

QTL detection in the three BC3S1 populations

Thirteen markers were scored in each of the three BC3S1 populations, in order to assess the genotype of the individuals within each of the five segregating regions. A marker located between the regions 9A and 9B was also scored to construct a genetic map of the whole chromosome 9. Thus, four linkage groups were constructed (Table 2). No segregation bias was detected. The distances within these linkage groups were highly consistent with those obtained for the RIL population (Saliba-Colombani et al. 2000). Indeed, locus order was the same in all the cases and genetic distances of BC3S1 linkage groups were equivalent for the chromosomes 1 and 9, slightly reduced for chromosome 2 and increased for chromosome 4. However, the changes were not very important, particularly when compared to the reductions observed in advanced generations of tomato crosses involving wild species (Monforte and Tanksley 2000).

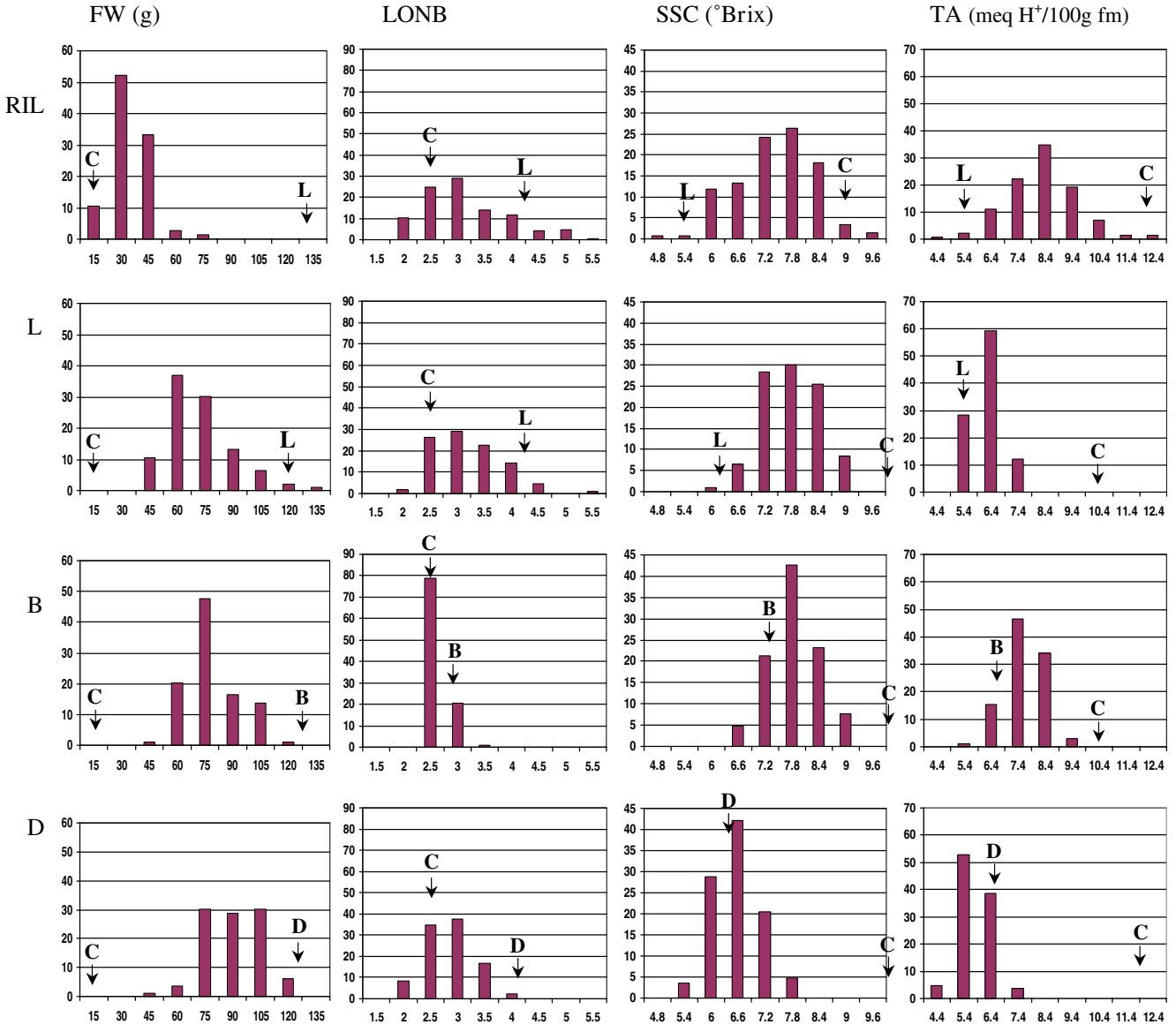

Fig. 2 Distributions of the RILs and of the three BC3S1 populations with each genetic background (B, D, L) for fruit weight (*FW*), locule number (*LONB*), soluble solids contents (*SSC*) and titratable acidity (*TA*). The population is indicated on the *left*, the trait at the *top of the graphs*. The abscissa indicates the value of the trait and distributions are given in percentage of the whole population. The values of parental lines in each population are indicated (*C* Cervil, *L* Levovil, *B* VilB, *D* VilD)

Table 2 Variation in genetic distances within the five introgressed regions among the four segregating populations

Region	Markers	Genetic length (cM)			
		RIL	BC3S1-L	BC3S1-B	BC3S1-D
1	TG116-TG430	14.1	14.2	18.9	13.7
2	TG454-TG191-ASC056-GC039	27.0	16.7	22.7	24.7
4	CT192-TG457-TG075	13.8	23.7	18.6	33.9
9A(9)	CT032-ASC021-(TG186-TG008)	10.2 (55.8)	10.9 (56.5)	16.9 (62.7)	15.4 (54.8)

Marker names are detailed, and the genetic distance of the region is indicated for each population (in Kosambi centiMorgan). For chromosome 9, the length of region 9A is given with the whole chromosome length in parentheses

Based on the multilocus genotype frequencies computed with the MDM program (Servin et al. 2002), linear regressions with phenotypic data were performed for QTL analyses in BC3S1 populations. For regions where QTLs were detected, the additive effects of the QTLs were estimated (Table 3). For FW, QTLs were detected

in the five regions, but with differences according to genetic backgrounds. For LONB, QTLs were detected on chromosome 1 and on chromosome 2. For FIR, a QTL was detected on chromosome 4, but showing a positive effect in Lgb and a negative one in the two other genetic backgrounds. Other QTLs for FIR were detected on chromosome 2 and 4. For SSC, significant effects were detected in the five regions with BC3S1 populations and a QTL was detected in the region 9A. Other QTLs were detected in regions 1, 2, 4 and 9B, the favorable alleles being conferred by the recipient parent for the QTL detected in region 4 with BC3S1-D. As no QTL for SSC was detected on the 8 noncarrier chromosomes in the RILs, the same set of QTLs is supposed to segregate in the RILs and in BC3S1. The difference between the three genetic backgrounds could thus be explained by epistatic interactions between QTLs and the genetic background. For SUC and TA, QTLs were detected in the five regions, but none of the QTLs were detected in one region simultaneously for the three populations. The BC3S1-L and BC3S1-B were evaluated in the same trial, one year before the BC3S1-D. Nevertheless, there were many more QTLs in common (11) between BC3S1-B and BC3S1-D, than between L and B or L and D populations (5 and 3 common QTLs, respectively). Another consequence of the fixation of major QTLs in the genetic background is that the segregating regions in BC3S1 populations displayed stronger effects than in the RIL population and allowed the expression of minor QTLs to be detected.

QTL detection in QTL-NILs

QTL-NILs were evaluated during two successive trials, in 2002 and 2003, and the additive effect of each introgressed region was estimated (Table 4). Significant effects were detected in all the five regions for FW and in four regions for LONB. For the other traits, the effects were strongly dependent on the genetic background. A total of 31 QTLs were detected in 2002 and 23 in 2003. About half of the significant effects (19) were significant in the two trials, whatever the genetic background, 13 for physical traits and 6 for chemical traits. Eight of them were previously detected in the RILs, five concerned physical traits (FW, LONB and FIR) and three concerned chemical traits (SSC and TA). Eleven new QTLs were stable over years. For

Table 3 Additive effect of the QTLs detected for each region in (1) the RIL population (Saliba-Colombani et al, 2001), (2) each BC3S1 population and (3) each QTL-NIL

Trait	Chromosome segments	RIL	L		B		D	
			BC3S1	BC3S3	BC3S1	BC3S3	BC3S1	BC3S3
FW (g)	1	NS	NS	−22.29	NS	−18.8	*−7.4*	−8.77
	2	-9.2 (46.2)	−23.5	−33.52	−11.3	−18.27	NS	NS
	4	NS	NS	NS	−19.8	−14.92	NS	−6.71
	9a	NS	NS	−14.68	*−7.2*	NA	−15.0	−19.65
	9b	NS	NS	NS	−11.9	−8.94	NS	NA
LONB	1	NS	NS	NS	−0.15	−0.25	−0.22	−0.5
	2	-0.57 (37.3)	−0.96	−1.12	−0.19	−0.17	−0.51	−0.92
	4	NS	NS	NS	NS	NS	NS	NS
	9a	NS	NS	NS	NS	NA	NS	−0.36
	9b	NS	NS	NS	NS	NS	NS	NA
FIR	1	NS	NS	NS	NS	NS	NS	NS
	2	NS	NS	−3.69	−3.7	−3.47	−2.0	NS
	4	2.8×10^3 (33.3)	1.8	NS	*−2.4*	NS	−2.8	−4.19
	9a	-2.0×10^3 (10.3)	NS	NS	NS	NA	−2.2	NS
	9b	NS	NS	NS	NS	NS	NS	NA
SSC (°Brix)	1	NS	NS	0.65	0.42	NS	0.36	NS
	2	0.42 (18.6)	NS	0.62	*0.26*	NS	NS	−0.37
	4	NS	NS	NS	NS	−0.49	−0.35	NS
	9a	0.26 (13.3)	0.40	0.52	0.48	NA	0.34	0.53
	9b	NS	NS	NS	0.43	NS	NS	NA
SUC (g/100 g fm)	1	NS	NS	0.32	0.27	NS	*0.19*	NS
	2	0.27 (25.3)	*0.08*	0.32	NS	NS	NS	NS
	4	NS	NS	NS	0.27	NS	−0.21	NS
	9a	NS	0.15	0.26	0.23	NA	NS	0.45
	9b	NS	0.09	NS	NS	NS	NS	NA
TA (meq H+/100 g fm)	1	0.50 (11.2)	NS	0.31	*0.28*	NS	*0.31*	NS
	2	0.49 (17.2)	NS	0.65	NS	NS	−0.49	−0.34
	4	NS	*−0.15*	NS	NS	NS	NS	NS
	9a	0.44 (22.4)	NS	0.32	0.35	NA	NS	NS
	9b	NS	NS	NS	0.56	NS	0.59	NA

The percentage of phenotypic variation explained by the QTL detected by CIM in the RIL population is given in parentheses. Additive effects are in italics when significance was observed at $P < 0.01$ but not at $P < 0.001$. For QTL-NILs, additive effects were obtained using the mean of the 2 years, 2002 and 2003, and significant effects were detected at $P < 0.05$. For FIR, another method of measure was used, explaining the difference in effect with BC3S1 and QTL-NILs

Table 4 Additive effect of the QTLs detected in QTL-NILs (BC3S3 lines) in each genetic background over 2 years, for six fruit traits

		Chromosome segments														
		1			2			4			9A			9B		
Trait	Year	L	B	D	L	B	D	L	B	D	L	B	D	L	B	D
FW (g)	2002	-33.89	−10.4	−15.43	−43.95	−19.95	−14.94	NS	−12.9	−10.18	−24.42	NA	−24.25	NS	−12.32	NA
	2003	-9.58	−26.71	NS	−23.1	−16.1	NS	NS	−16.46	NS	NS	NA	−15.04	NS	NS	NA
	Mean	−22.29	−18.8	−8.77	−33.52	−18.27	NS	NS	−14.92	−6.71	−14.68	NA	−19.65	NS	−8.94	NA
LONB	2002	-0.45	−0.25	−0.37	−1.27	−0.18	−0.78	NS	NS	0.27	NS	NA	−0.25	NS	NS	NA
	2003	NS	−0.25	−0.7	−0.89	NS	−1.1	NS	NS	−0.29	NS	NA	−0.52	NS	NS	NA
	Mean	NS	−0.25	−0.5	−1.12	−0.17	−0.92	NS	NS	NS	NS	NA	−0.36	NS	NS	NA
FIR	2002	NS	NS	NS	NS	NS	NS	NS	NS	−3.24	NS	NA	−3.07	NS	NS	NA
	2003	NS	NS	NS	−7.4	NS	NS	NS	NS	−5.14	NS	NA	NS	NS	NS	NA
	Mean	NS	NS	NS	−3.69	−3.47	NS	NS	NS	−4.19	NS	NA	NS	NS	NS	NA
SSC (°Brix)	2002	0.78	NS	NS	0.55	NS	NS	NS	−0.64	NS	0.6	NA	0.79	NS	NS	NA
	2003	0.51	NS	NS	0.69	NS	−0.58	NS	−0.38	NS	0.45	NA	NS	NS	NS	NA
	Mean	0.65	NS	NS	0.62	NS	−0.37	NS	−0.49	NS	0.52	NA	0.53	NS	NS	NA
SUC (g/100 g fm)	2002	0.46	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA	0.43	NS	NS	NA
	2003	NS	NS	NS	0.36	NS	NS	NS	NS	NS	0.23	NA	0.46	NS	NS	NA
	Mean	0.32	NS	NS	0.32	NS	NS	NS	NS	NS	0.26	NA	0.45	NS	NS	NA
TA (meq H+/100 g fm)	2002	NS	NS	NS	0.88	NS	−0.46	NS	NS	NS	0.53	NA	NS	NS	NS	NA
	2003	NS	NS	NS	0.42	NS	NS	NS	NS	NS	NS	NA	NS	NS	NS	NA
	Mean	0.31	NS	NS	0.65	NS	−0.34	NS	NS	NS	0.32	NA	NS	NS	NS	NA

Dunnett test comparing the QTL-NIL to the corresponding recipient line
NS not significant at $P < 0.05$, *NA* not available

physical traits, more QTLs were detected in 2002 (21) than in 2003 (14). In some cases, strong differences in additive effects between the 2 years were observed. Consequently, no significant effect was detected for the average of the 2 years. Additive effects for FIR were less consistent as only the QTL detected for NIL-D4 was stable over years. Stability of effects for chemical traits was comparable to that revealed for physical traits, but the number of QTLs detected in 2002 and in 2003 was similar. For SUC, two QTLs among three were only detected in 2003, and the opposite was observed for TA. Inconsistency of QTL or differences in the observed effect over years could be attributed to the interaction between QTL and the environment. Although the trials were all performed in the glasshouse in the same spring period, the climate could not be totally controlled and was not identical every year. Indeed, phenotype data of parental lines which were recorded during the four trials revealed significant differences between trials for every trait. Nevertheless, interactions between genotype and trial were only significant for FIR, SUC and TA (data not shown). When we pooled the phenotype data of the two years, 33 significant effects were detected.

Consistency of QTLs over generations

Stability of the QTLs in advanced generations and over the genetic backgrounds was investigated by comparing the QTLs detected in the RIL population to that detected in the three BC3S1 populations and in QTL-NILs (Table 3). RILs contained 50% of each parental genome (L and C), BC3S1 progeny segregated simultaneously for five regions of interest carried by four chromosomes and were fixed for the recipient genome over the 8 other chromosomes, and QTL-NILs carried approximately 10% of the C genome and differed from the recipient line in only one of the five regions. A negative effect of C alleles was expected for all the physical traits, except for FIR in region 4 (Saliba-Colombani et al. 2001). According to the QTL analysis performed with the RILs, among the five regions, a single QTL was expected on chromosome 2 for FW. This QTL was recovered in BC3S1 populations and in QTL-NILs except in Dgb. Three other QTLs were detected for FW in BC3S1-B and two in BC3S1-D. Moreover, two new QTLs were detected in Lgb. In Bgb and in Dgb, one new QTL was detected in QTL-NILs in region 1 and in region 4. For LONB, only one region of chromosome 2 was involved in the genetic control of the trait, according to RIL analysis. The involvement of region 2 was confirmed whatever the generation, the genetic background and the year (except in Bgb in 2003), but strong differences in allelic effects were detected between the genetic backgrounds. New QTLs for LONB were detected in region 1 and in region 9A. QTLs for FIR were detected in regions 4 and 9A in the RILs. The involvement of region 4 was confirmed in all the BC3S1 populations, with favorable effects provided in BC3S1-L by Cervil alleles, as in the RILs, and provided on the contrary, by the recipient type allele in BC3S1-B and BC3S1-D. At the QTL-NIL level, only NIL-D4 showed differences from the recipient line. The involvement of region 9A was only confirmed in BC3S1-D. A new QTL was detected in region 2. Surprisingly, this QTL was detected in NIL-L2 whereas none of the QTL detected in the RILs was confirmed in Lgb in the advanced generation.

Favorable alleles were provided by Cervil for all the QTLs controlling chemical traits (Saliba-Colombani et al. 2001). For SSC, positive effects were detected in RILs in regions 2 and 9A. For region 2, in BC3S1, a single effect was detected in Bgb, whereas the QTL was detected with NIL-L2 and NIL-D2. For region 9A, the QTL was confirmed in each BC3S1 population and in all the corresponding QTL-NILs available. Moreover, additional QTLs were detected, two with a favorable effect in the region 1 and in the region 9B and one with an unfavorable effect in region 4. For SUC, although only a single QTL was detected in region 2 with the RILs, significant effects were detected in the five regions with BC3S1 populations, with different regions according to the genetic backgrounds. Significant effects were detected in QTL-NILs only in NIL-L2 and three new QTLs were detected for NIL-L1, NIL-L9A and NIL-D9A. For TA, according to the RILs, Cervil alleles provided favorable effects in regions 1, 2 and 9A. The involvement of those regions was confirmed at least in one generation and/or in one genetic background. Unexpected QTLs were detected in BC3S1-L in region 4 and in BC3S1-B and BC3S1-D in region 9B.

Discrepancy in QTL expression among generations could be attributed to biological and experimental reasons. Disappearance of QTLs in advanced generations may be due to actual interactions between QTL and the genetic background, even though the usual population types and sizes do not allow the detection of such interactions. New QTLs may appear because of the fixation of the previously segregating major QTLs. This fixation may also explain the variation in the effect of the QTLs. Indeed, several QTLs detected in RILs were fixed in the advanced generations with a recipient genotype, due to the backcross process (for FW on chromosomes 3, 11 and 12; for NBLO on chromosome 12; for SUC on chromosomes 3, 10 and 11 and for TA on chromosomes 3 and 12). The methods of QTL detection, which varied according to the populations, and the power of QTL detection, which is related to the population structure, could be among the experimental reasons which might participate in the differences observed. The size of the population may influence the power of QTL detection: 144 RILs were analysed while about 100 plants was assessed per BC3S1 population. Nevertheless, only four chromosomes segregated in these populations, reducing the genetic variance and increasing consequently the power of QTL detection.

For the six traits studied, about 50% of the expected QTLs based on RIL data, were detected in at least one BC3S1: 40, 20 and 40% were detected in Lgb, Bgb and Dgb over the three generations (RIL/BC3S1/BC3S3), respectively. Several new QTLs were detected in BC3S1-B and BC3S1-D, with many QTLs common to Bgb and Dgb (Fig. 3). However, strong differences in the allelic effects were detected according to the recipient parents that could mainly be attributed to the allelic variation in the regions of interest. New QTLs were also detected during an MAS scheme for the improvement of stripe rust resistance in barley (Toojinda et al. 1998), and the authors suggested that favorable alleles were fixed at these additional loci in the original mapping population. Furthermore, new QTLs could be detected as the overall

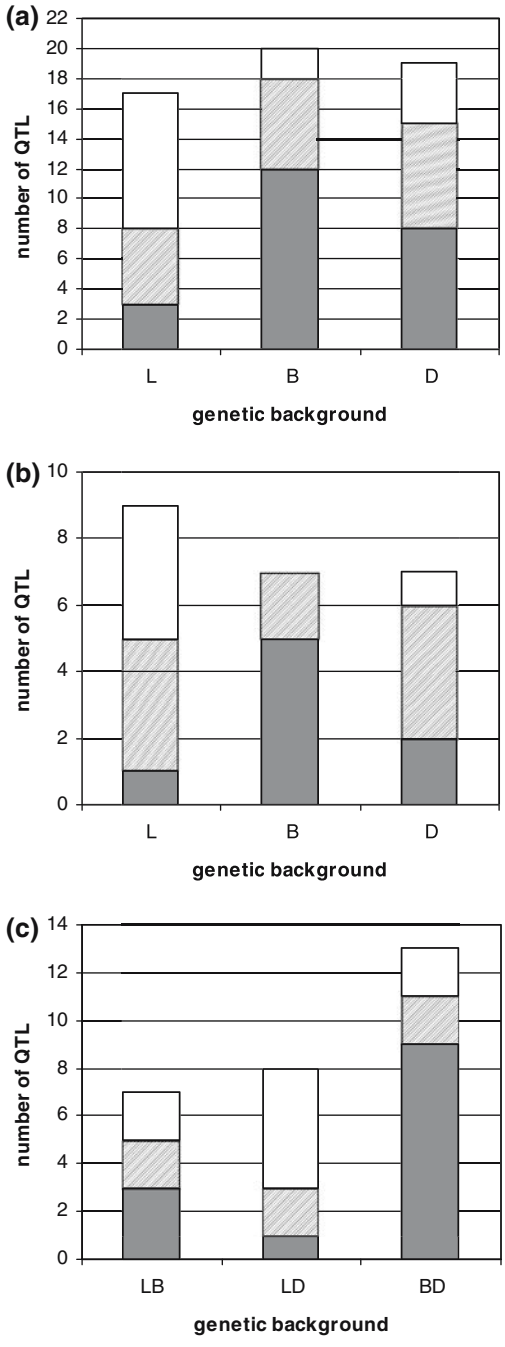

Fig. 3 Number of the QTLs detected per generation and genetic background for the six traits. **a** For each genetic background, number of QTLs detected in BC3S1 (*gray*) and QTL-NILs (*white*) and common to the two generations (*hatched*), **b** for each genetic background, number of QTLs detected in common in RILs and QTL-NILs (*white*), in RILs and BC3S1 (*gray*) and common to the three generations (*hatched*) **c** number of QTLs detected common to two genetic backgrounds in BC3S1 (*gray*), QTL-NILs (*white*) and common to both generations (*hatched*)

genetic variation decreases when advanced generations are considered.

In total, 41 QTLs were detected in the three BC3S1 populations and 33 in QTL-NILs (based on the average of the measurements in 2002 and in 2003), but only 18 were common to both BC3S1 and QTL-NILs generations and 10 were common to the three generations. For a given trait, a given region and a given genetic background, when a QTL was detected in several generations, its effect was usually larger in the more advanced generation (Table 3). Overall, for all the traits, ten QTLs were stable over generations (RIL/BC3S1/BC3S3) in at least one genetic background. QTLs were more conserved between RILs and advanced generations for Lgb (particularly between RILs and QTL-NILs) than for Bgb or Dgb. This could be due to the fact that L was used to create the RIL population in which QTL analysis was first performed. The two QTLs not recovered in Lgb concerned FIR which was measured with two different apparatus. With Dgb, three QTLs displayed allelic effects opposite to that expected (for FIR, SSC and TA). Such a lack of consistency of QTL effects even using the same genetic background has already been reported (Shen et al. 2001). This inconsistency suggests that the QTLs could be involved in gene interactions. Finally, five new QTLs compared to those detected in RILs were detected, essentially for physical traits, and were stable over generations: for FW on chromosomes 1, 4, 9A and 9B, for LONB on chromosome 1, for FIR on chromosome 2 and for SUC on chromosome 9A.

Consistency of QTLs over genetic backgrounds

QTL effects were compared in three genetic backgrounds: L, B and D. Consistency of the QTLs between Bgb and Dgb were high. Indeed, among the 17, 20, and 19 QTLs detected with at least one advanced generation in Lgb, Bgb and Dgb, respectively, 4 QTLs were common to the three genetic backgrounds, 7 to Bgb and Lgb, 8 to Dgb and Lgb and 13 to Bgb and Dgb (Fig. 3). Nevertheless, when only QTLs detected in both BC3S1 and QTL-NILs were considered, only two QTLs were common to each pair of genetic backgrounds and the effects of these QTLs were strongly dependent on the genetic background. Only two QTLs were detected in the three genetic backgrounds whatever the generation: for NBLO on chromosome 2 and for SSC in region 9A. These two QTLs probably correspond to major genes that have been previously detected in crosses involving other parental lines (Lippman and Tanksley 2001; Fridman et al. 2002). The QTL for FW on chromosome 2 detected in Lgb and Bgb in all the generations probably corresponds to fw2.2, a QTL with a large effect which has been recently cloned and which is one of the QTLs explaining the increase in fruit size from wild species to cultivated tomato that occurred during domestication (Frary et al. 2000). In studies where QTLs were transferred into genetic backgrounds unrelated to the mapping population, the lack of consistency of the QTL effects was often attributed to the allelic variation at the locus of interest (Reyna and Sneller 2001; Sebolt et al. 2000). To quote the case of firmness again, not only were two methods of measurements used, but also, both the recipient parents, B and D, are much firmer than L and QTLs for firmness detected in Bgb and Dgb on chromosome 4 had allelic effects opposite to expected.

Conclusion

This study showed that several sources of variation, such as environmental conditions, population structure and genetic background might influence QTL expression. All these factors may reduce the efficiency of MAS and explain the low progress in fruit weight observed in the lines derived by marker-assisted backcross (Lecomte et al. 2004a). As shown here, the fixation of favorable alleles at several QTLs in the genetic background is not sufficient as it has resulted in the expression of new QTLs carried by one of the five introgressed regions. The large number of QTLs detected in BC3S1, even with a small population size, underlined the interest in mapping QTLs in advanced generations, as proposed by Tanksley and Nelson (1996). QTL-NILs constitute other valuable material for a more detailed evaluation of the effect of each QTL (Van Berloo et al. 2001). They allow either screening of whole genomes for QTLs (Eshed and Zamir 1995) or focusing on a specific region of interest for fine mapping (Lecomte et al. 2004b). Lecomte et al. (2004a) showed significant differences between the three genetic backgrounds in the improvement of quality traits. The C genotype is a cherry tomato which was shown as distant from L, B and D, based on molecular markers. On the contrary, very few markers revealed differences between the three recipient lines (less than 5% polymorphic loci), so few differences could be expected. This was observed for the traits where the lines were not very different (as for FW or SSC), but when the means are different, allelic differences at the QTL may cause a lower effect or even opposite allelic effects than expected (as for FIR or TA, for instance).

Acknowledgements Many thanks to N. Baffert, A. Gautier and A. Luciani for genotypic and phenotypic works, to A.M. Cossalter for taking care of the plants and for her help during the phenotypic evaluations, to P. Duffé for plant genotyping, to R. Matthieu for chemical evaluations, to C. Dillmann for SAS programs and to Rebecca Stevens for English corrections. Laurent Lecomte was partly supported by the Conseil Régional Provence-Alpes-Côte d'Azur (France). The experiments comply with the current French laws.

References

Ahmadi N, Albar L, Pressoir G, Pinel A, Fargette D, Ghesquière A (2001) Genetic basis and mapping of the resistance to *Rice yellow mottle virus*. III. Analysis of QTL efficiency in intro-

gressed progenies confirmed the hypothesis of complementary epistasis between two resistance QTLs. Theor Appl Genet 103:1084–1092

Asins MJ (2002) Present and future of quantitative trait locus analysis in plant breeding. Plant Breed 121:281–291

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

Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Nat Rev Genet 3:22–32

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Frary A, Nesbitt TC, Frary A, Grandillo S, Van Der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88

Fridman E, Liu YS, Carmel-Goren L, Gur A, Shoresh M, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. Mol Genet Genomics 266:821–826

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

Lander ES, Green P, Abrahamson J, Barlox A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lecomte L, Duffé P, Buret M, Hospital F, Causse M (2004a) Marker-assisted introgression of five QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic backgrounds. Theor Appl Genet 109:658–668

Lecomte L, Saliba-Colombani V, Gautier A, Gomez-Jimenez MC, Duffé P, Buret M, Causse M (2004b) Fine mapping of QTLs of chromosome 2 affecting the fruit architecture and composition of tomato. Mol Breed 13:1–14

Li Z, Jakkula L, Hussey RS, Tamulonis JP, Boerma HR (2001) SSR mapping and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode. Theor Appl Genet 103:1167–1173

Lincoln SE, Daly MJ, Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP version 3.0. Whitehead Institute Technical Report

Lippman Z, Tanksley SD (2001) Dissecting the genetic pathway to extreme fruit size in tomato using a cross between the small-fruited wild species *Lycopersicon pimpinellifolium* and *L. esculentum* var. Giant Heirloom. Genetics 158:413–422

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87–103

Monforte AJ, Tanksley SD (2000) Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor Appl Genet 100:471–479

Reyna N, Sneller CH (2001) Evaluation of marker-assisted introgression of yield QTL alleles into adapted soybean. Crop Sci 41:1317–1321

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

Romagosa I, Han F, Ullrich SE, Hayes PM, Wesenberg DM (1999) Verification of yield QTL through realized molecular marker-assisted selection responses in a barley cross. Mol Breed 5:143–152

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 North Carolina (USA)

Sebolt AM, Shoemaker RC, Diers BW (2000) Analysis of a quantitative 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 93:227–228

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

Stuber CW, Sisco PH (1992) Marker-facilitated transfer of QTL alleles between elite inbred lines and responses in hybrids. In: 46th annual corn and sorghum research conference, American Seed Trade Assoc Ed, pp 104–113

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

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

Van Berloo R, Aalbers H, Werkman A, Niks RE (2001) Resistance QTL confirmed through development of QTL-NILs for barley leaf rust resistance. Mol Breed 8:187–195

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

Zeng Z B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zhu H, Briceño G, Dovel R, Hayes PM, Liu BH, Liu CT, Ullrich SE (1999) Molecular breeding for grain yield in barley: an evaluation of QTL effects in a spring barley cross. Theor Appl Genet 98:772–779