

# Marker-Assisted Introgression of Favorable Alleles at Quantitative Trait Loci Between Maize Elite Lines

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## ABSTRACT

This article reports the marker-assisted introgression of favorable alleles at three quantitative trait loci (QTL) for earliness and grain yield among maize elite lines. The QTL were originally detected in 1992 by means of ANOVA in a population of 96 recombinant inbred lines (RILs). Introgression started from a selected RIL, which was crossed three times to one of the original parents and then self-fertilized, leading to BC<sub>3</sub>S<sub>1</sub> progenies. Markers were used to assist both foreground and background selection at each generation. At the end of the program, the effect of introgression was assessed phenotypically in agronomic trials, and QTL detection was performed by composite interval mapping among BC<sub>3</sub>S<sub>1</sub> progenies. The marker-assisted introgression proved successful at the genotypic level, as analyzed by precision graphical genotypes, although no emphasis was put on the reduction of linkage drag around QTL. Also, QTL positions were generally sustained in the introgression background. For earliness, the magnitude and sign of the QTL effects were in good agreement with those expected from initial RIL analyses. Conversely, for yield, important discrepancies were observed in the magnitude and sign of the QTL effects observed after introgression, when compared to those expected from initial RIL analyses. These discrepancies are probably due to important genotype-by-environment interactions.

**M**OLECULAR markers have offered new possibilities in plant and animal breeding. Among those is the possibility to detect quantitative trait loci (QTL) that control the genetic variability of the complex traits of interest and the possibility to select on the detected QTL in marker-assisted selection (MAS) breeding programs. This has received considerable attention in the past 10–15 years.

Since the benchmark article of LANDER and BOTSTEIN (1989), numerous theoretical works have addressed the improvement of the methodology of QTL detection, including the development of efficient software packages. Concomitantly, these methodologies have been applied experimentally to QTL detection in various species, traits, and environments, providing enough data for synthesis and initial general conclusions. As a result, the scientific community involved in QTL detection has become more and more aware of the pitfalls linked to QTL detection, in particular the power and the accuracy of the methods, and the sustainability of the effects detected across environments, time, and genetic background (MELCHINGER *et al.* 2000).

During the same period, a series of works have ad-

ressed the use of molecular markers in plant and animal breeding programs. Here, we mention only the use of markers for introgression in backcross programs. Backcross breeding is a well-known procedure for the introgression of a *target gene* from a *donor line* into the genomic background of a *recipient line*. The objective is to increase the *recipient genome content* (RGC) of the progenies, by repeated backcrosses to the recipient line. In this context, markers can be used to control the target gene (*foreground selection*) and (or) to hasten the return to the recipient genotype on chromosomal regions outside the target gene (*background selection*). The efficiency of such *marker-assisted introgression* programs has been analyzed in a series of theoretical works (for a review, see, for example, VISSCHER *et al.* 1996). The results indicate that marker-assisted introgression is expected to permit a gain of time of about two backcross generations, compared to conventional backcross programs, which is economically important. One particular case of marker-assisted introgression is when the target locus is a QTL, which poses additional problems:

- i. Foreground selection is more difficult for a gene for which exact chromosomal location is estimated with only a given imprecision than for a well-known gene (VISSCHER *et al.* 1996). This requires using more markers and optimizing the positions of these markers with respect to the uncertainty of the true QTL location (HOSPITAL and CHARCOSSET 1997).
- ii. Once the introgression is achieved, it must be

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checked that the effect of the QTL in the new genetic background is the same as the effect estimated originally.

- iii. If the trait of interest is a complex polygenic trait, as is often the case in plant and animal breeding, then it is unlikely that one single QTL for that trait could explain enough genetic variation to justify the economic effort corresponding to the marker-assisted introgression program. In such a case, several QTL should be introgressed simultaneously. This necessitates using larger population sizes of foreground selection and reduces the possibilities of background selection. However, such multiple introgression programs seem in theory feasible with reasonable population sizes for up to three or four QTL (HOSPITAL and CHARCOSSET 1997; KOUDANDÉ *et al.* 2000).

Here, we report and discuss the results of an experiment initiated in 1992. The experiment included: (i) detection of QTL for three quantitative traits in a cross between two elite maize inbred lines, (ii) marker-assisted introgression of the favorable alleles at three detected QTL from one inbred parent into the genomic background of the other parent, and (iii) agronomic evaluation of the effect of introgression and reestimation of the individual QTL effects in the new genetic background. Few experimental results of marker-assisted selection are available (for a review, see DEKKERS and HOSPITAL 2002). Despite some articles addressing parts of these aspects (*e.g.*, STUBER and SISCO 1992), no comprehensive study has been published so far.

It is important to note that this report has a historical dimension: Theory and practice of QTL detection have greatly evolved during the corresponding period. New and more powerful methodologies of detection have emerged, along with the appropriate softwares, that were not available at the time the experiment was started and the initial QTL detection was performed. Throughout the article, we tried to take this historical dimension into account. To do so, the article is organized in a chronological way.

## QTL DETECTION AND INTROGRESSION

**Summary of initial QTL results:** Earliness and yield are the two major traits of interest for maize breeding in northern Europe. Two elite inbred lines were chosen for their complementarity with respect to these traits: one flint line (F2) for its earliness and one indent line (Io) for its high yield potential. These two lines were crossed, and then 96 F<sub>5</sub> recombinant inbred lines (RILs) were developed through successive self-fertilizing generations, to identify QTL for these traits.

**Agronomic evaluation:** RILs were crossed to two inbred testers (F252 and Co255). Hybrid families were evaluated in 1992 at two locations in France: Gif-sur-Yvette (northern France) and Clermont-Ferrand (central France).

At each location, hybrid families were planted in two neighbor trials, corresponding each to one tester. Each trial was organized following a complete block design with three replicates. Grain moisture (GM, in percentage of the fresh grain weight) and dry grain yield (DGY, in quintals per hectare at 0% of grain moisture) were evaluated for all trials. Silking date (SD, in days after the first of July) was measured only in Gif-sur-Yvette. SD and GM both define the earliness of hybrid families.

**Genetic maps:** In 1992, a genetic map with 108 restriction fragment length polymorphism (RFLP) loci was constructed on the basis of the genotyping data available at that time for the RIL population, using MAPMAKER software (LANDER *et al.* 1987) for linkage analysis. This map (termed RIL108 herein) had a total length of 1751 cM (Haldane distance) and an average interval between 2 loci of 16 cM. It was used for initial QTL detection and to choose markers to assist introgression. However, while the marker-assisted introgression program was on its way, the RIL population continued to be used as a panel for the reference genetic map of maize in our lab (CAUSSE *et al.* 1996). As a consequence, by the end of the introgression program, a new genetic map of the RIL population (termed RIL165 herein) was available, with more individuals genotyped (145), more marker loci (165), and thus a better precision. The map RIL165, used as a reference, is presented in Figure 1 for chromosomes 5, 8, and 10. Markers from map RIL108 are included in map RIL165 with the exception of *gys7* in chromosome 10.

**Initial QTL analyses of RIL data:** In 1992, the detection of QTL was achieved for each trial on the progeny mean performances by means of ANOVA at each marker position on map RIL108, considering a 1% type I error. Analyses were performed using SAS (SAS INSTITUTE 1990). In case several neighbor markers displayed significant effects, the marker with the highest *F* value was retained.

QTL detected for the three traits of interest were located on chromosomes 1, 3, 5, 6, 8, and 10. Most QTL were specific to one location and some of them were specific to one tester. A total of five QTL were detected for yield. Among these, a single QTL had a significant effect in more than one trial. Favorable high-yielding alleles originated from the Io parent for QTL on chromosomes 1, 3, and 6, while favorable alleles originated from the F2 parent on chromosomes 5 and 10. QTL for earliness traits (five for SD and eight for GM) were located on chromosomes 1, 5, 6, 8, and 10. Among these, six QTL had significant effects in at least two trials or for both traits. Favorable alleles originated from genotype F2 for all earliness QTL, except the one on chromosome 5. Several colocations could be observed between yield and earliness QTL. High yield was associated with late flowering, except on chromosome 10, where the F2 allele at *umc44b* increased yield (+7.0 qx/ha) and decreased SD (-1.0 days).

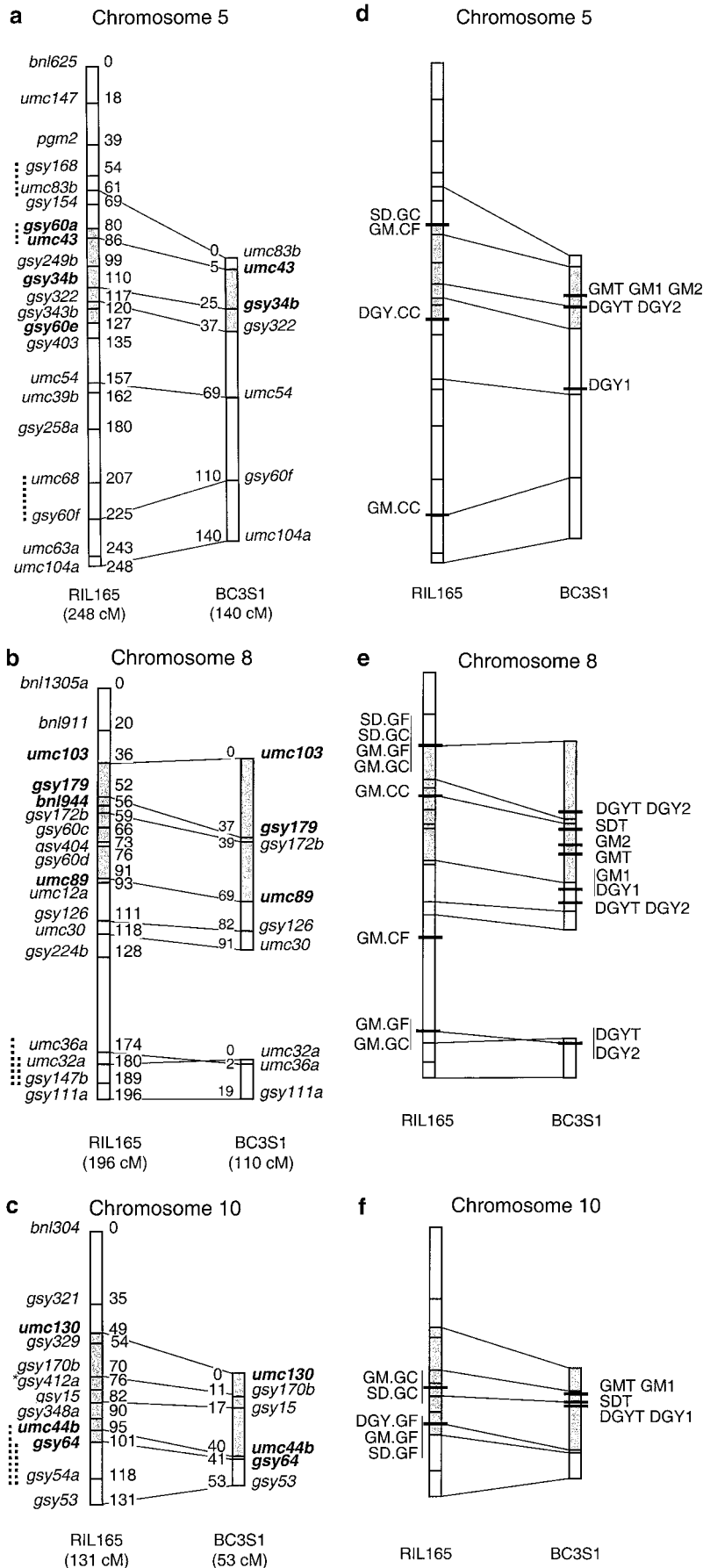


FIGURE 1.—Genetic maps and QTL location for chromosomes 5, 8, and 10. Two maps of the study (RIL165, BC3S1) are compared, respectively, for marker positions and for QTL positions for chromosomes 5 (a and d), 8 (b and e) and 10 (c and f). Introgression segments are shaded. (a–c) Loci positions are indicated in centimorgans (Haldane units). Markers used to control segment introgression are shown in boldface type. Dotted lines identify loci that are in inverted position on map RIL108. (d–f) QTL detected by ANOVA on the map RIL108 are reported at corresponding markers on the map RIL165 (see trait names in Table 1), and QTL detected by CIM are indicated on the map BC3S1 (see trait names in Table 4). (\*) marker *gsy87* (Table 1) is close to marker *gsy412a* on map RIL165.

**TABLE 1**  
**Initial QTL detection**

QTL position		SD (days)			GM (%)			DGY (qx/ha)			FQTA
Chromosome	Marker	Trial	Effect	$R^2$	Trial	Effect	$R^2$	Trial	Effect	$R^2$	
5	<i>gsy60a</i>	GC	+1.2 <sup>a</sup>	14.3	CF	+1.2 <sup>a</sup>	14.1	CC	+5.4 <sup>a</sup>	9.9	Io
	<i>gsy60e</i>						F2				
	<i>gsy60f</i>				CC	-1.2	10.4				F2
8	<i>umc103</i>	GF	-1.8 <sup>a</sup>	24.5	GF	-1.6 <sup>a</sup>	16.0				F2
		GC	-1.2	15.8	GC	-1.0	8.8				F2
	<i>gsy172b</i> <i>gys224b</i> <i>umc36a</i>				CC	-1.2	13.3				F2
					CF	-1.6	25.9				F2
					GF	-1.4	11.0				F2
					GC	-1.8	18.0				F2
10	<i>gsy87</i>	GC	-1.0	13.5	GC	-1.6	15.6				F2
	<i>umc44b</i>	GF	-1.0 <sup>a</sup>	10.8	GF	-1.2 <sup>a</sup>	9.8	GF	+7.0 <sup>a</sup>	11.6	F2

QTL detected in the RIL population (map RIL108), in chromosomes 5, 8, and 10, by ANOVA (1% type I risk level), for three traits [SD (silking date), GM (grain moisture), DGY (dry grain yield)] in four agronomic trials: GF (location, Gif-sur-Yvette; tester, F252), CF (location, Clermont-Ferrand; tester, F252), GC (location, Gif-sur-Yvette; tester, CO255), and CC (location, Clermont-Ferrand; tester, CO255). For each QTL, its position (chromosome, marker name), the corresponding trial, the additive effect of the F2 allele (defined as the difference between homozygous genotypes crossed to the tester), the part of explained variation ( $R^2$ ) in percentage, and the parental origin of the favorable allele (FQTA) are shown. Note that some QTL display significant effects in two trials.

<sup>a</sup>This QTL effect was taken as a reference to evaluate expected introgression effect.

A general problem for maize breeding in northern Europe is to increase yield without earliness decrease or, alternatively, to increase earliness with no yield cut. In this context, alleles conferring yield or (and) earliness are considered favorable. In the case of opposite effects, we considered that a 1% GM decrease is economically equivalent to a 2.5 qx/ha DGY increase, which is the assumption for hybrid maize evaluation in northern France. This approach makes it possible to decide which allele is favorable for each locus. For example, at one locus on chromosome 6, the Io allele increased both DGY (+6.4 qx/ha) and GM (+1.2%). Applying this weighting, the Io allele brings an overall favorable effect equivalent to +3.4 qx/ha of DGY.

Several QTL had close positions. Favorable effects were therefore reasoned in terms of chromosomal segments rather than single loci. Favorable segments were brought by Io for chromosomes 1, 3, and 6 and by F2 for chromosomes 1, 5, 6, 8, and 10. Identification of the favorable parent for chromosomes 5 (marker interval *gsy60a-gsy60e*), 6, and 10 was based on the economical weight index.

**Definition of an ideotype and choice of introgression segments:** A molecular ideotype was designed subsequently to maximize yield while maintaining, or even improving, the earliness level. For DGY, most favorable alleles that were detected originated from the Io parental line. On the basis of the phenotypic difference between Io and F2 for DGY, most favorable alleles at undetected QTL should also originate from Io. Therefore, we chose to keep the Io background and to introduce

F2 favorable segments. To restrain experimental cost, we chose to limit the introgression to three segments on chromosomes 5, 8, and 10. The segment positions are presented in Figure 1 (shaded boxes) and the corresponding QTL effects are presented in Table 1. Favorable earliness alleles on chromosomes 1 and 6 were therefore not considered for introgression. The F2-type segment on chromosome 10 is expected to improve yield and earliness (*i.e.*, decrease SD and GM) simultaneously. The F2-type segment on chromosome 8 is expected to confer a major decrease in SD and GM (-1.8 days and -1.6%, respectively, with tester F252). The F2-type segment on chromosome 5 is expected to improve yield but to increase SD and GM. For each trait, we estimated the maximum genetic gain expected over environments in hybrid combination with tester F252, which was further considered as the reference value throughout the experiment. The chromosome 5 segment displayed significant effects (i) with tester F252 for GM and (ii) with tester Co255 for SD and DGY. We assumed that these results were indicative of a possible effect of this segment on SD and DGY with tester F252, undetected because of limited power. We therefore considered in this case the effect with tester Co255 in the reference values (see Table 1).

Introgression of these three F2 segments in an Io background should provide an elite line of Io type with increased yield (up to +12.4 qx/ha), decreased silking date (down to -1.6 days), and decreased grain moisture (down to -1.6%).

**Marker-assisted QTL introgression:** *Introgression scheme:*



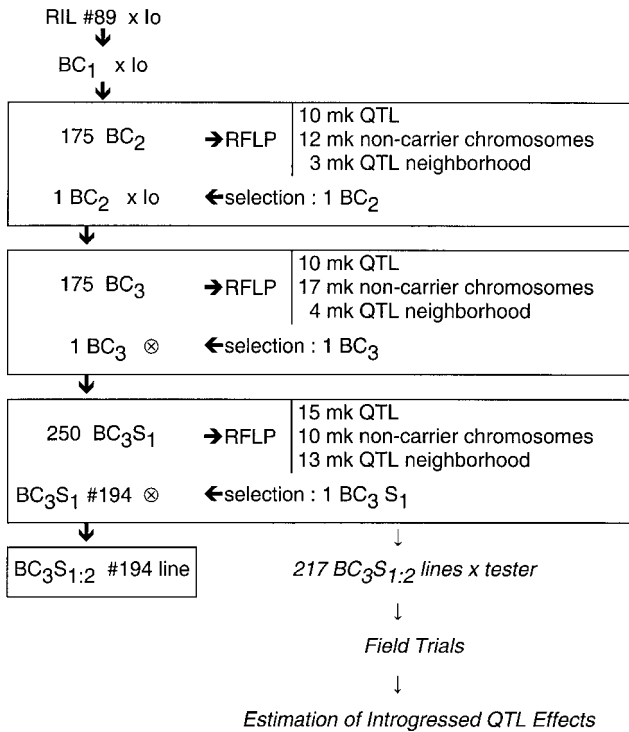


FIGURE 2.—Marker-assisted backcross introgression scheme.

The breeding scheme used for marker-assisted introgression is described in Figure 2. In the RIL population, we looked for the best genotype to start the introgression with, on the basis of the ideotype (see below). This selected RIL (no. 89) was then crossed with the recipient line (Io). This progeny was considered equivalent to a backcross (BC)<sub>1</sub> because the RIL already contains an expected 50% of the recipient genome. This BC<sub>1</sub> progeny was backcrossed to the recipient line to produce a BC<sub>2</sub> population. One selected BC<sub>2</sub> individual was backcrossed again to produce a BC<sub>3</sub> population. Finally, one selected BC<sub>3</sub> individual was selfed to fix the QTL segments in homozygous donor state, producing the BC<sub>3</sub>S<sub>1</sub> population. Within this population, we looked for the progeny closest to the defined ideotype. Note that the BC<sub>3</sub>S<sub>1</sub> population was also used as a whole to redetect QTL (see below). Therefore, a genetic map of the chromosome segments still segregating in the BC<sub>3</sub>S<sub>1</sub> population was built. This map termed BC3S1 is presented in Figure 1.

**Foreground selection:** Introgression of donor-type alleles on three QTL segments was controlled using genetic markers chosen on the basis of ANOVA results (Table 1). Markers were selected to delimit a zone of 30–35 cM around each QTL to take into account the confidence interval around the estimated position, consistent with known works at this time (LANDER and BOTSTEIN 1989; STUBER and SISCO 1992). Each chromosomal segment was checked by three markers: one near the QTL (ANOVA position) and the two others at right- and left-hand sides. Indeed, HOSPITAL and CHARCOSSET (1997)

showed that three markers are sufficient to control such intervals and minimize the risk of “losing” the target allele at the QTL. On the basis of the computations of HOSPITAL and CHARCOSSET (1997), these foreground selection markers ensure a probability >96% of “not losing” the donor allele at the QTL for each controlled segment at each generation. For the three segments simultaneously this probability is >90%. This set of markers was used throughout successive generations (markers in boldface type on map RIL165, Figure 1). Minor changes occurred through introgression steps, due to technical reasons (marker quality most of the time). On chromosome 5, *gsy60a* and *gsy60e* were discarded in the last generation. On chromosome 8, marker *bnl944* used for the RIL was replaced by *gsy179* at other generations. In the last generation, donor-type control on QTL segments was increased with five additional markers. The genotypes of the individuals selected at each generation are presented in Figure 3. The markers genotyped for foreground selection are indicated by the blue stars inside the QTL boxes.

**Background selection:** For noncarrier chromosomes, background selection was achieved with one to three markers on each chromosome, to control the return to homozygous recipient type. For carrier chromosomes, no strong background selection was applied, because we had little confidence on QTL positions. Only a few markers were checked on carrier chromosomes and a return to recipient type in the QTL vicinity was never favored with regard to a return to recipient type in parts of the genome unlinked to the QTL segments. The markers genotyped for background selection are indicated by the blue stars outside the QTL boxes in Figure 3.

**Population sizes and selections:** Among the RIL populations, applying foreground selection for donor type at markers on controlled segments, the individual RIL 89 was the only one that was of F<sub>2</sub> type at all markers on controlled segments. Hence, this line was the only one that could be selected to begin with, and no background selection was then possible. For the BC<sub>2</sub> and BC<sub>3</sub> population, 175 individuals were genotyped. On the basis of the computations of HOSPITAL and CHARCOSSET (1997), with this population size, the risk of not obtaining at least 1 individual carrying donor alleles at all foreground selection markers controlling the QTL segments is below 1% (minimum population size at 1%: 115 individuals). Within each BC<sub>2</sub> and BC<sub>3</sub> population, eight progenies were found heterozygous for all markers on controlled segments, which was consistent with expectations. At these generations, background selection was possible (1 in 8 individuals). With selfing the expected frequency of the target genotype at each locus is only 1/4 compared to 1/2 with backcross. Also, background selection proved to be more efficient in later generations (HOSPITAL *et al.* 1992). Therefore, we tried to increase population size in BC<sub>3</sub>S<sub>1</sub> as much as possible, in the limit of kernel production on the selected ear. An enlarged population of 250 BC<sub>3</sub>S<sub>1</sub> individuals was

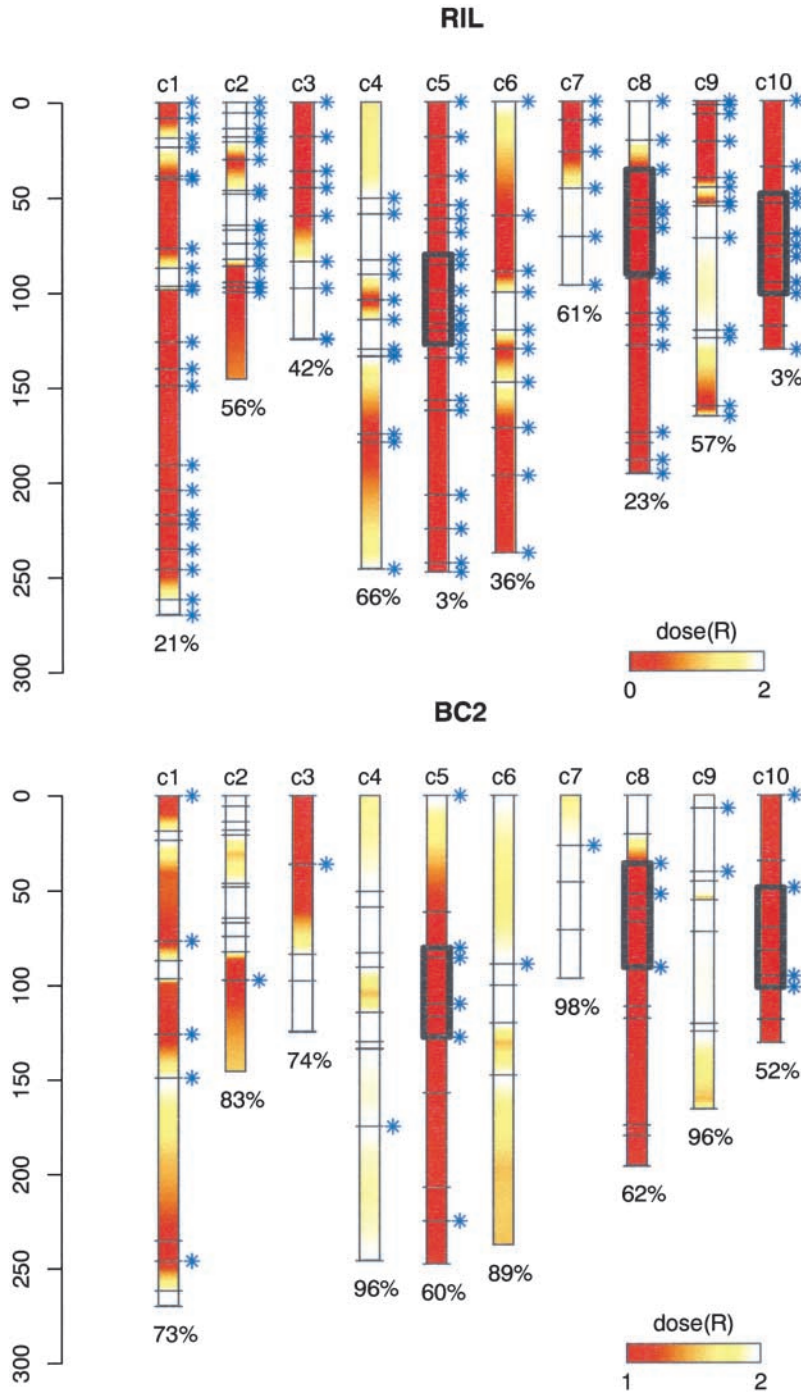


FIGURE 3.—Precision graphical genotypes. The genotypes of the individuals selected at each generation of the marker-assisted introgression scheme (RIL, BC<sub>2</sub>, BC<sub>3</sub>, BC<sub>3</sub>S<sub>1</sub>, respectively, from top to bottom) are depicted with colors indicating the expected dose of recipient (R) alleles on each chromosomal location, for the 10 chromosomes c1–c10. The size scale on the left indicates chromosome lengths in Haldane centimorgans. Colors range from red (minimal possible dose) to white (maximal possible dose). Note that maximal possible dose is always 2, but minimal possible dose is 0 for inbreds RIL and BC<sub>3</sub>S<sub>1</sub> and 1 for BC individuals. Expected allele dose is computed for each individual using the MDM program, on the basis of pedigree and marker genotype information. The locations of the informative markers used in the computation are indicated by horizontal bars across the chromosomes. These markers are informative either because they were really genotyped at the given generation (such markers are indicated by an additional blue star on the right of the bar) or because their genotypes were deduced from genotyping data available at previous and/or the following generation. Note that in some cases, deduction from genotyping data at the following generation includes the genotypes of the whole population (not shown); *e.g.*, if a marker is of unknown genotype at generation BC<sub>*t*</sub>, but not segregating in a progeny of 150+ at generation BC<sub>*t+1*</sub>, then the marker is assumed homozygous at BC<sub>*t*</sub>. Finally, individual recipient genome contents (RGC%) are given below each chromosome. These sum up to give the values in Table 2.

genotyped. Nevertheless, only 1 individual (BC<sub>3</sub>S<sub>1</sub> no. 194) was homozygous for donor type at all markers on QTL segments. No selection on genetic background was then possible in the BC<sub>3</sub>S<sub>1</sub> population.

#### Graphical genotypes and recipient genome contents:

To estimate as precisely as possible the RGC of the individuals selected at each generation of the introgression scheme, *precision graphical genotypes* (PGG) were derived, using the program MDM (SERVIN *et al.* 2002). Here, the probability of each possible genotype at any given point on a chromosome is estimated precisely on the basis of

all genotyping information available for the complete breeding scheme (genotypes at flanking and nonflanking markers at the generation considered and at previous and/or following generations, if informative). This extends the concept of *graphical genotypes* introduced by YOUNG and TANKSLEY (1989) and provides a better estimate of genome contents, because all possible recombination events in complex pedigrees are taken into account. The PGG for the individual selected at each generation of the introgression scheme are shown in Figure 3. Averaging these data over all chromosomes

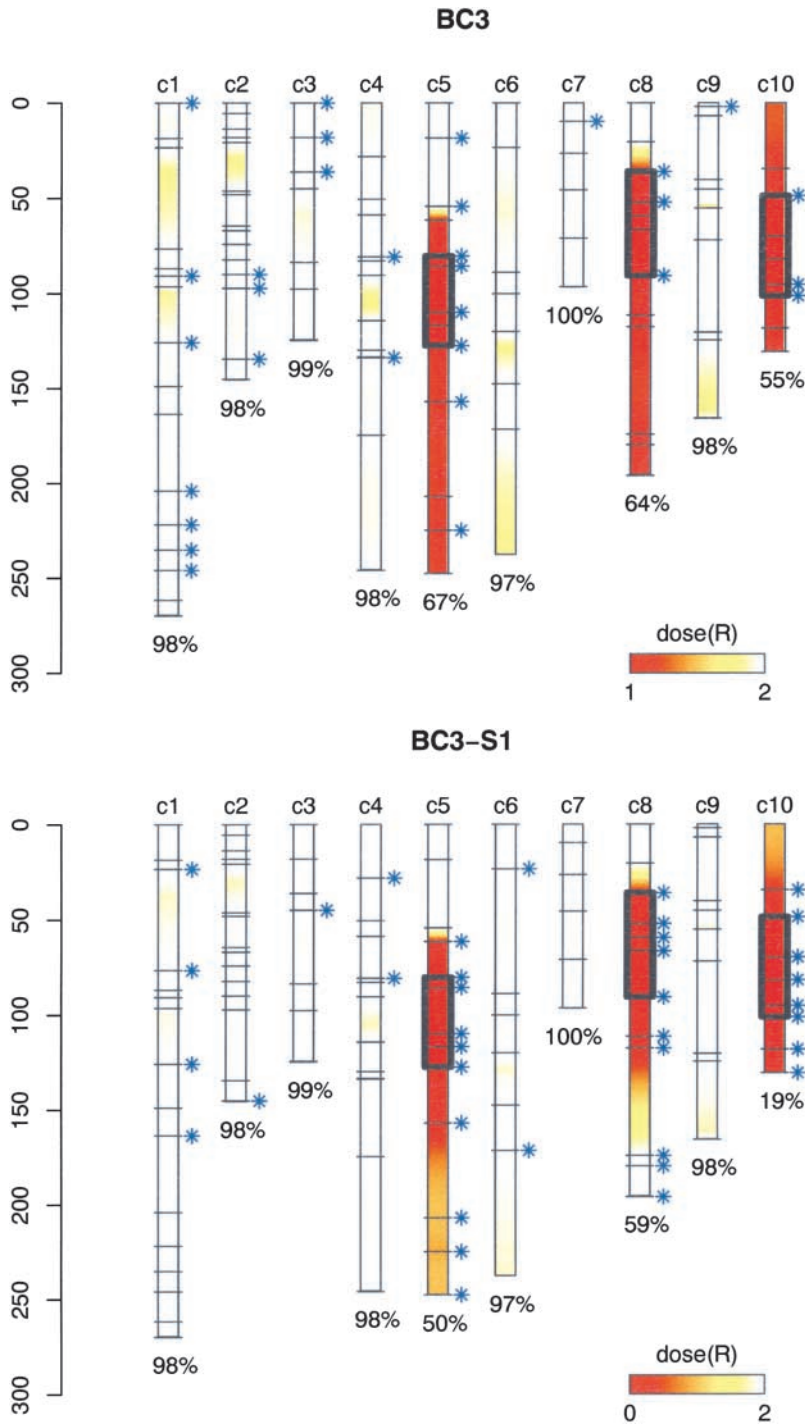


FIGURE 3.—Continued.

provides an estimate of RGC for the complete genome. This is given in Table 2 for carrier, noncarrier, and all chromosomes, respectively. Note that RGC is always computed *outside* the controlled QTL segments (Figure 3, boxes).

For carrier chromosomes, the data in Table 2 indicate that the RGC of the individuals selected at each generation of the introgression scheme is much lower than expected without background selection. The selected RIL has 22 RGC(%) less than expected, which could

relate to the fact that the population size for the RIL was small, and this individual was the only possible selection. To take this fact into account, the expected RGCs on carrier chromosomes in Table 2 for generations BC<sub>2</sub> to BC<sub>3</sub>S<sub>1</sub> were computed from the actual RGC of the selected RIL (9.4%), not from the original parents. Nevertheless, the RGC is again lower than expected at these generations. The difference increases in BC<sub>2</sub> and BC<sub>3</sub>, but slightly decreases in BC<sub>3</sub>S<sub>1</sub>, despite that no selection for RGC could be applied at this last generation.

**TABLE 2**  
**Recipient genome contents (%)**

	Carrier chromosomes		Noncarrier chromosomes		All chromosomes	
	PGG	Expected	PGG	Expected	PGG	Expected
RIL	9.4	31.4 <sup>a</sup>	46.0	50.0 <sup>a</sup>	37.0	45.4 <sup>a</sup>
BC <sub>2</sub>	59.2	65.4 <sup>b</sup>	86.3	86.5 <sup>b</sup>	79.6	81.3 <sup>b</sup>
BC <sub>3</sub>	63.4	73.5 <sup>b</sup>	98.0	93.3 <sup>b</sup>	89.5	88.4 <sup>b</sup>
BC <sub>3</sub> S <sub>1</sub>	47.3	58.2 <sup>b</sup>	98.0	93.3 <sup>b</sup>	85.5	84.7 <sup>b</sup>

Percentages of recipient genome on chromosomes either estimated from observed data using the precision graphical genotypes (PGG) of Figure 3 or expected. Percentage for carrier chromosomes does not include the QTL segments controlled during introgression. In all cases (carrier or noncarrier chromosomes) expected values assume that no background selection was applied. For the carrier chromosomes, expected values assume that QTL segments are of donor type; *i.e.*, linkage drag is taken into account by using Equation 5 of STAM and ZEVEN (1981), where  $r$  was replaced by  $R = 2r/(1 + 2r)$  for the RIL generation. Results for all chromosomes are averaged from the previous columns with total genome lengths of 420, 1288, and 1708 cM, for carrier, noncarrier, and all chromosomes, respectively.

<sup>a</sup> For the RIL, expected values are based solely on the breeding scheme.

<sup>b</sup> At other generations, expected values were computed on the basis of the breeding scheme and the real genotype of the selected RIL.

Obviously, deviations from expectation could be due to random variation, because only a few individuals were selected. However, these reduced RGCs on carrier chromosomes compared to expectations may have other possible causes:

- i. Some results indicate that recombination might be reduced on heterozygous chromosomal segments (PATERSON *et al.* 1990). This effect was not taken into account when computing expected values (see Table 2 legend).
- ii. In general, genotyping errors lead to an overestimation of distances on genetic maps. If such errors took place in the reference map used to derive the PGG of Figure 3, then RGC values of Table 2 are overestimated, but the overestimation is more important for expected values than for PGG values, because PGG values are computed for each (relatively small) interval between markers and not for the whole chromosome.
- iii. Finally, because we did not want to favor selection for background markers at the edges of QTL segments, with respect to selection for markers on noncarrier chromosomes, we might have increased linkage drag around these segments, rather than reducing it.

For carrier chromosomes, the precision graphical genotypes of Figure 3 permit us to track the most likely positions of the crossovers that took place during the introgression scheme on the individuals that were selected at each generation and illustrate the hazards of the return to recipient type on carrier chromosomes, when population size does not allow for a specific selection for these regions.

On noncarrier chromosomes, return to the recipient genome is quite satisfactory. RGC in BC<sub>2</sub> is not higher

than the expected value, which is consistent with theoretical results showing that background selection in the first backcross is not very efficient (HOSPITAL *et al.* 1992). But RGC in BC<sub>3</sub> (98%) is almost 5 RGC(%) above expected value. Note that a RGC of 98% would have been reached only in BC<sub>5</sub> if no background selection on markers had been applied. Hence, the gain is about two BC generations, which is again consistent with theoretical results (HOSPITAL *et al.* 1992; VISSCHER *et al.* 1996). There is no gain in BC<sub>3</sub>S<sub>1</sub> because no background selection could be performed at that generation.

Overall, despite the low RGC of carrier chromosomes, the total recipient genome content of the BC<sub>3</sub>S<sub>1</sub> individual selected is above expectation. Compared to the selected RIL, the marker-assisted introgression scheme leads to a gain of +48.5 RGC(%), which is satisfactory.

The PGG methodology was not available at the time selection and genotyping decisions were made during the breeding scheme. The *a posteriori* analyses of the genotypes of the individuals selected indicate that, at least on noncarrier chromosomes, probably less markers could have been genotyped to reach the same efficiency of background selection. Actually, in addition to a better estimate of genome contents, this is another advantage of using PGG, to help decision making in marker-assisted selection and reduce genotyping cost.

#### INTROGRESSED QTL EFFECTS

The whole BC<sub>3</sub>S<sub>1</sub> population was evaluated to estimate (i) the agronomic effect of introgression and (ii) the individual effect of each QTL introgressed in the final genetic background.

**Agronomic evaluation:** Among BC<sub>3</sub>S<sub>1</sub> plants, 217 were selfed to produce BC<sub>3</sub>S<sub>1:2</sub> families. These were crossed to tester line F252, yielding a total of 217 hybrid families



(Figure 2). These were planted in 1997 and 1998 at three French locations adapted to population earliness: Clermont-Ferrand (central France, C97, C98), Gif-sur-Yvette (northern France, G97, G98), and Mons (northern France, M97, M98). Among hybrid families, 204 could be planted in all trials, and all were experimented on in at least two trials. The two parental lines (F2, Io) crossed to tester line F252 were used as checks. Within each trial, hybrid families and checks were arranged following a two-block experimental design, with two replicates for families and at least six replicates for checks. Plot observations for traits SD, DGY, and GM were made in all trials. Two trials had specific problems. In G97, plant development was very limited, due to a severe aphid attack (Metopolophium). In C97, drought conditions after planting caused intraplot heterogeneity at flowering stage, which tended to disappear afterward.

Statistical analyses were performed for each trial with a mixed model. Genetic variance ( $\sigma_G^2$ ) and error variance ( $\sigma_e^2$ ) were estimated using the GLM procedure of SAS (SAS INSTITUTE 1990). Means of progenies, adjusted for fixed-block effects, were estimated for each trial. A multitrial analysis was performed to estimate the magnitude of genotype-by-environment (*GE*) effects using the mixed model,

$$Y_{ijk} = \mu + E_k + b_{jk} + G_i + GE_{ik} + e_{ijk},$$

where  $Y_{ijk}$  is the performance of family  $i$  in block  $j$  of trial  $k$ ,  $\mu$  is the mean performance of the population,  $E_k$  is the fixed environmental effect for trial  $k$ ,  $b_{jk}$  is the fixed effect for block  $j$  within trial  $k$ ,  $G_i$  is the random genetic effect for family  $i$ ,  $GE_{ik}$  the random effect for interaction between genotype  $i$  and trial  $k$ , and  $e_{ijk}$  is the residual error term.

Trials were grouped to minimize intragroup *GE* interaction variance, using the Ward clustering algorithm (SAS INSTITUTE 1990; see CORSTEN and DENIS 1990 and L. MOREAU, A. CHARCOSSET and A. GALLAIS, unpublished results). Family means, adjusted for block and/or trial effects, were estimated for the complete multitrial design and for each group of trials, using the LSMEAN procedure of SAS (SAS INSTITUTE 1990). These means were used for genetic gain evaluation and QTL analyses. Statistical components of variation for groups and for the complete set of trials were estimated through the restricted maximum-likelihood (REML) method using the VARCOMP procedure of SAS (SAS INSTITUTE 1990).

Individual trial analyses generally showed a low error variation, when compared to local reference data for the same traits (data not shown). The single exception was SD in trial C97, which displayed both a high residual variance and a nonsignificant genetic effect within the BC<sub>3</sub>S<sub>1</sub> population. This could be explained by heterogeneity in emergence. This trial was discarded for further analyses of SD. No significant genotype  $\times$  trial interaction was observed for SD, so that all trials could

be grouped into a single set (Table 3, SDT). Both GM and DGY displayed a significant genotype  $\times$  trial interaction effect, which could be explained by the definition of two different groups for these two traits (GM1 and GM2 for GM, DGY1 and DGY2 for DGY; see Table 3). Only the group of stressed trials, DGY2, still displayed a highly significant genotype  $\times$  trial interaction variance.

As expected, the Io parental line displayed later flowering than the F2 line (Table 3). It also displayed on average a higher yield, with the exception of the group of stressed trials, DGY2. Io performance was particularly low in trial G97, due to a high sensitivity to Metopolophium. Both parental lines displayed close average values for grain moisture at harvest, but contrasted values in individual trials (results not shown). F2 displayed lower moisture than Io for trials harvested at high average moisture (trials G98, M97, and M98), whereas Io displayed lower moisture than F2 for trials harvested at low average moisture (overmaturity, trials C97, C98, and G97). This inversion can be explained by (i) the earlier flowering time of F2 and (ii) the higher drying potential of dent kernels of Io.

Mean of the BC<sub>3</sub>S<sub>1</sub> population was highly significantly different from Io for all traits. Introgressed families displayed earlier flowering than Io (−2.3 days on average) and slightly lower grain moisture at harvest (−0.9% on average). Concerning yield, introgression was unfavorable in most locations (−5.6 qx/ha on average), with the exception of trial G97 (+2.8 qx/ha), which was affected by the aphid attack. Genetic effects within the BC<sub>3</sub>S<sub>1</sub> population were highly significant for all traits and conditions. Despite being significant, genetic variances were lower than those observed in the RIL population, consistent with the fixation of a large fraction of the genome (>70%).

Finally, the selected BC<sub>3</sub>S<sub>1</sub> family (BC<sub>3</sub>S<sub>1</sub> no. 194) showed a major decrease in flowering time when compared to recipient line Io (−4.1 days, highly significant) and a lower decrease in grain moisture at harvest (−1.9%, highly significant). Yield of BC<sub>3</sub>S<sub>1</sub> no. 194 was lower than that of Io (−12.9 qx/ha). Taken as a whole, these results confirm that the three introgressed segments have a major impact on the variation of the three traits of interest.

**BC<sub>3</sub>S<sub>1</sub> map:** The BC<sub>3</sub> selected plant remained heterozygous for three genomic regions, corresponding to large zones around the three introgressed QTL (see Figure 3). A genetic map of these three regions was made for segregating loci in the BC<sub>3</sub>S<sub>1</sub> population. Since all plants were derived from a single BC<sub>3</sub> plant, we considered that recombination within this population was comparable to that within an F<sub>2</sub> population. A map (termed BC3S1 herein, Figure 1) was therefore completed for an F<sub>2</sub> population, using MAPMAKER software (LANDER *et al.* 1987).

Four linkage groups were obtained, as the linkage between markers *umc30* and *umc32a* on chromosome 8

TABLE 3  
Agronomic evaluation of BC<sub>3</sub>S<sub>1</sub> hybrid families

	Silking date (days): SDT	Grain moisture (%)			Dry grain yield (qx/ha)		
		GMT	GM1	GM2	DGYT	DGY1	DGY2
Io mean	28.2	27.9	27.9	27.7	80.6	85.4	67.2
F <sub>2</sub> mean	20.6	27.7	27.5	28.2	76.5	74.1	84.3
BC <sub>3</sub> S <sub>1</sub> mean	25.9	27.0	26.8	27.4	75.0	79.5	65.9
BC <sub>3</sub> S <sub>1</sub> no. 194	24.1	26.0	26.2	25.8	67.7	73.0	54.9
$\sigma_c^2$	0.80**	0.36**	0.27**	0.72**	8.14**	5.89**	27.95**
$\sigma_{c \times E}^2$	0.06 (NS)	0.12**	0.06*	0.05 (NS)	10.17**	1.58 (NS)	13.40**
$\sigma_c^2$	1.67	0.87	0.59	1.42	30.52	29.97	31.35
$h^2$ plot <sup>a</sup>	0.32	0.27	0.29	0.33	0.17	0.16	0.38
$h^2$ means <sup>b</sup>	0.82	0.79	0.75	0.65	0.66	0.59	0.66

BC<sub>3</sub>S<sub>1</sub> hybrid families were evaluated for three traits, in groups of individual trials SDT (M97, G97, M98, G98, C98), GMT (M97, G97, C97, M98, G98, C98), GM1 (G97, C97, M98, G98), GM2 (M97, C98), DGYT (M97, G97, C97, M98, G98, C98), DGY1 (C97, M98, G98, C98), and DGY2 (M97, G97). Each group is described by adjusted means for parental checks, hybrid families, and ideotype BC<sub>3</sub>S<sub>1</sub> no. 194; variance components estimations ( $\sigma_c^2$ ,  $\sigma_{c \times E}^2$ ,  $\sigma_e^2$ ); and heritabilities at plot and means levels ( $h^2$  plot,  $h^2$  means). NS, not significant type I risk levels. \*\*1% type I risk level. \*5% type I risk level.

<sup>a</sup> Heritability at plot level:  $h^2$  plot =  $\sigma_c^2 / (\sigma_c^2 + \sigma_{c \times E}^2 + \sigma_e^2)$ .

<sup>b</sup> Heritability at means level ( $l$ , number of trials;  $p$ , number of plots per trial):  $h^2$  means =  $\sigma_c^2 / (\sigma_c^2 + \sigma_{c \times E}^2 / l + \sigma_e^2 / p \times l)$ .

was not significant (Figure 1). Note that these two markers were already far apart (63 cM) on map RIL165. The two linkage groups of chromosome 8 are further referred to as “8a” and “8b” for the “upper” and “lower” parts of the chromosome, respectively. The map obtained for these linkage groups was highly consistent with that obtained for the RIL population (RIL165). Locus order was maintained in all cases, except for tightly linked markers *umc32a* and *umc36a*. Genetic distances for map BC3S1 decreased in general, compared to map RIL165, except between markers *umc103* and *gsy179* on linkage group 8a, where distance increased from 16 cM to 37 cM. This tendency toward a decrease in genetic distance is consistent with observations by PATERSON *et al.* (1990), who found a lower recombination rate for an advanced backcross generation than for the initial BC<sub>1</sub> population. As proposed by these authors, this may be related to a lower recombination rate in heterozygous chromosomal regions, when compared to homozygous regions.

**QTL detection:** QTL were detected first by simple interval mapping (SIM) for all trials and groups of trials (results not shown) and then by composite interval mapping (CIM), as proposed by ZENG (1994) and JANSEN and STAM (1994). QTL analyses were performed using the PLABQTL 1.1 software (UTZ and MELCHINGER 1996), which involves a linear-regression approach (HALEY and KNOTT 1992). To study linkage between QTL, cofactors for CIM were selected with a backward multiple regression (B. MANGIN, personal communication). This regression was performed for each chromosome starting with (i) all markers on this chromosome and (ii) cofactors selected by forward regression on the

other chromosomes, considering an  $F$ -to-enter value of 3.5. Markers on the chromosome of interest were discarded sequentially, removing at each step the marker with the lowest partial  $R^2$ , until all remaining markers displayed significant effects at  $\alpha < 0.5\%$ . QTL positions were estimated subsequently by composite interval mapping, using all selected cofactors except those that delimited the interval of interest. A LOD threshold of 2.0 was considered for declaring a putative QTL significant, which corresponds to an individual type I risk of 0.25% and a genome-wide risk of 5%. QTL effects and  $R^2$  were estimated using a simultaneous multiple regression on all detected positions. QTL position-support intervals (computed as LOD max - 1) were determined only when a single position was detected on the chromosome, by discarding cofactors of this chromosome (COV-option of PLABQTL). The presence of significant epistatic effects between QTL was also tested.

Results of CIM are presented in Table 4 and summarized in Figure 1. When compared to SIM, use of cofactors proved useful to detect an additional minor QTL for yield (DGY1) on chromosome 5. On linkage group 8a, use of the backward procedure proved useful to show that yield variation (DGYT and DGY2) was affected by two linked QTL rather than a single QTL. No significant epistatic effect between detected QTL was found.

**Silking date:** No significant effect was observed on chromosome 5 whereas two QTL were detected on linkage group 8a and chromosome 10. These two QTL were detected within the controlled segments (zones initially identified for introgression). All trials being highly correlated for SD, precision on the mean is very high (heritability of 0.82, Table 3), which contributes to high

**TABLE 4**  
**QTL detection in introgression segments**

Chromosome	Marker interval		SD			GM			DGY					
	Left	Right	Group of trials	Position (cM)	Effect (days)	R <sup>2</sup> (%)	Group of trials	Position (cM)	Effect (%)	R <sup>2</sup> (%)	Group of trials	Position (cM)	Effect (qx/ha)	R <sup>2</sup> (%)
5	<i>umc43</i>	<i>gsy34b</i>					GMT	20 (10-34)	+0.56	12.8				
	<i>gsy34b</i>	<i>gsy322</i>					GMI	20 (6-38)	+0.44	9.1				
	<i>gsy322</i>	<i>umc54</i>					GM2	20 (8-34)	+0.84	9.9	DGYT	26 (18-38)	-2.2	7.6
8a	<i>umc103</i>	<i>gsy179</i>									DGY2	26 (18-36)	-2.2	5.7
	<i>gsy172b</i>	<i>umc89</i>	SDT	42 (38-50)	-2.6	28.2	GMT	54 (46-66)	-1.62	38.8	DGY1	66 (46-104)	-4.2	8.3
	<i>umc89</i>	<i>gsy126</i>					GM2	50 (44-60)	-2.78	33.8	DGYT	34 <sup>a</sup>	-1.9	4.1
8b	<i>umc36a</i>	<i>gsy11a</i>					GM1	72 (50-78)	-0.84	27.3	DGY2	34 <sup>a</sup>	-3.0	3.3
	<i>gsy170b</i>	<i>gsy15</i>									DGYT	78 <sup>a</sup>	-2.8	9.1
	<i>gsy15</i>	<i>umc44b</i>	SDT	16 (10-26)	-1.0	16.9	GM1	12 (10-18)	-0.82	14.9	DGY2	78 <sup>a</sup>	-5.0	8.6
10											DGY1	72 (46-82)	-2.8	9.8
											DGY2	2 (0-12)	+3.0	13.1
											DGY2	2 (0-12)	+9.2	30.0
											DGYT	18 (12-30)	-3.2	13.9
											DGY1	18 (10-28)	-4.0	18.4

QTL detected on the BC<sub>3</sub>S<sub>1</sub> population (map BC3S1), in introgression segments on chromosomes 5, 8, and 10, by composite interval mapping (cofactors selected with backward procedure), for three traits SD (silking date), GM (grain moisture), DGY (dry grain yield), in groups of agronomic trials (SDT, GMT, GMI, GM2, DGYT, DGY1, and DGY2) are shown. For each QTL, its location (chromosome, flanking markers), the corresponding group of trials, the position in centimorgans (Haldane units), the support interval on QTL position (LODmax - 1), the additive effect of the F<sub>2</sub> allele, and the contribution of the QTL to the variation of the trait, adjusted by that of other QTL (R<sup>2</sup> in percentage) are shown.

<sup>a</sup> Unavailable support interval.

precision estimates for QTL positions (12- and 16-cM support intervals, respectively). Both QTL explained a large part of phenotypic variation (28.2 and 16.9%, respectively; 38.9% for the global model), and F<sub>2</sub> alleles led to a total decrease in silking date of -3.6 days. According to the high heritability of the trait and the small segregating part of the genome, one would have expected that detected QTL explain a higher part of the phenotypic variation. LOD curves showed two extra peaks with a LOD of 1.4 (corresponding to a type I risk of 1.2%) on chromosome 5 and linkage group 8b, but the introduction of these QTL in the model did not substantially increase the fraction of variation explained (41.5%). The fraction of variation explained by the model would probably have been increased by a higher marker density in the vicinity of SD QTL on linkage group 8a, as suggested by the high individual genetic variance calculated from the additive effect of this QTL (0.845).

**Grain moisture:** The three introgressed chromosomal segments displayed significant effects for GM. One QTL was detected on chromosome 5 within the controlled segment. It had a minor effect in all groups of trials, associated with a large support interval for position estimate. One QTL was detected on linkage group 8a and had a major effect. However, its position showed a large support interval, due to low marker density. This region showed contrasted results in the two groups of trials. In group GM2, the most likely position for the QTL fell within the controlled segment and close to the QTL identified for SD. In group GM1, despite a complex SIM curve (not shown), which suggested two linked QTL, use of backward regression concluded to a single QTL 22 cM apart from that identified for group GM2. However, QTL detected for both groups of trials displayed overlapping support intervals for position estimates. CIM on the average of all trials concluded to a unique location, close to that identified for group GM2. One QTL was detected on chromosome 10 and displayed a large effect. Its position showed a small support interval, due to high marker density. This QTL affected variation in group GM1 only.

QTL on linkage group 8a explained a large fraction of GM variation (38.8%), whereas QTL on chromosomes 5 and 10 explained a smaller fraction (12.8 and 14.9%, respectively). The global model with three QTL explained 49.9% of total variation. F<sub>2</sub> alleles led to a decrease in GM on linkage group 8a and chromosome 10 (-1.6 and -1.2%, respectively), and to a moderate increase on chromosome 5 (+0.4%).

**Dry grain yield:** Significant effects were detected for the three controlled segments, when tested by both SIM and CIM. A minor QTL was detected inside the controlled segment on chromosome 5, but with a large support interval. It showed a significant effect in both groups of trials. On linkage group 8a, for group DGY1, a QTL was detected outside the controlled segment, with a large support interval for position. For group

DGY2, backward regression allowed the detection of two separate QTL, one located inside the controlled segment and one outside. Backward regression on the average of all trials (DGYT) also concluded to the presence of two linked QTL. An additional QTL was detected on linkage group 8b (12-cM support interval). This QTL affected variation within stressed trials only (group DGY2). One QTL was detected on chromosome 10 with a large support interval (18 cM). This QTL affected variation for DGYT and group DGY1.

Observed  $R^2$  values for yield were medium on linkage group 8b and chromosome 10 (13.1 and 13.9%, respectively) and low on chromosome 5 (7.6%) and linkage group 8a (4.1 and 9.1% for the two QTL, respectively). The global model with five QTL explained 42.5% of yield variation. F<sub>2</sub> alleles decreased yield for all QTL (effects ranging from -1.9 qx/ha to -3.2 qx/ha), except for QTL of linkage group 8b in the two stressed trials of group DGY2 (+9.2 qx/ha). These two trials experienced constraining agronomic conditions, so that this QTL should be considered as a stress tolerance QTL.

## DISCUSSION

**Introgression of chromosomal segments:** As suggested by theoretical studies (HOSPITAL and CHARCOSSET 1997), experimental introgression of three QTL-carrying chromosomal segments has proved to be possible by marker-assisted selection with reasonable population sizes. For each segment, introgression control by means of three to four marker loci has been appropriate, despite two marker changes within segments during the course of the program. As expected, successive genotyped populations have always been large enough to allow foreground selection and to keep the three QTL segments entirely of donor type (as far as we can judge on the basis of available marker information). Marker-assisted background selection was achieved on BC<sub>2</sub> and BC<sub>3</sub> progenies. All noncarrier chromosomes returned rapidly to recipient type in the first two backcross generations, thanks to marker control. No emphasis could be put on background selection on the three QTL-carrying chromosomes. The scheme was therefore not entirely successful in reducing linkage drag. As some markers in the QTL vicinity were fixed to donor type in the BC<sub>3</sub>S<sub>1</sub>, further backcrossing would be required to complete background selection on carrier chromosomes.

Recipient genome content on carrier chromosomes is still below expected values with no selection on markers (Table 2, see also STAM and ZEVEN 1981). However, more stringent cuts around QTL segments would require using larger populations or more backcross generations (HOSPITAL 2001). As a result, experimental costs would be increased. Another way to reduce linkage drag more drastically is to introgress QTL segments one by one in separate backcross schemes and ultimately "pyramid" them (HOSPITAL and CHARCOSSET 1997; KOU-



DANDÉ *et al.* 2000). This would increase the efficiency of background selection, in particular on carrier chromosomes. This strategy was not applied here, to limit the total duration of the program. This program was put to its end with RFLP markers, as a consequence of historical investment of the lab in this technique for mapping projects (CAUSSE *et al.* 1996). Surely it could be achieved nowadays more easily with PCR-based markers such as simple sequence repeat.

**Effect of introgression on performance:** Comparison of the introgressed family that was the closest to the ideotype (BC<sub>3</sub>S<sub>1</sub> no. 194) with recipient line Io illustrates a major effect of introgression for the three traits of interest (Table 3). For earliness traits, introgression effect is consistent in sign with that expected from initial QTL results. The magnitude of introgression effect is close to that expected for GM (−1.9 *vs.* −1.6%) and higher for SD (−4.1 *vs.* −1.6 days). On the contrary, introgression had a negative effect on DGY (−12.9 qx/ha), opposite to what was expected (+12.4 qx/ha). Considering the change in genetic background of introgressed QTL from initial RIL to the final recipient Io, epistatic interactions between such QTL and genetic background could be a first explanation of the discrepancy between observed and predicted values. No evidence for epistasis between introgressed and other QTL was found in the initial RIL population (results not shown). However, QTL × QTL interaction tests within a given population have generally a low power, so that the effect of the evolution of genetic background deserves further investigation.

**Consistency of QTL effects in RIL and BC<sub>3</sub>S<sub>1</sub> populations:** QTL analysis of the BC<sub>3</sub>S<sub>1</sub> population showed that each introgressed chromosomal segment contributed significantly to the modification in performance that was observed. Individual contributions were examined by comparing QTL results obtained for RIL and BC<sub>3</sub>S<sub>1</sub> populations (Tables 1 and 4, Figure 1). To take into account a possible effect of the different statistical methods that were used in each case, RIL results were reanalyzed using environment clustering and CIM (A. BOUCHEZ, unpublished results). These results are not presented in this article for the sake of simplicity. In any case, it did not substantially modify the comparison between the two populations. In general, contributions of QTL to the phenotypic variance ( $R^2$  values) in the BC<sub>3</sub>S<sub>1</sub> were higher than those observed in the initial RIL population. This should be due mostly to the lower phenotypic variation that was observed in BC<sub>3</sub>S<sub>1</sub> when compared to the RIL population, due to the fixation of QTL outside the introgressed segments, either detected or not in the initial RIL experiment.

**Chromosome 5:** An overall positive economic effect was expected for the introgressed region, yield increase balancing a moderate decrease in earliness. In the BC<sub>3</sub>S<sub>1</sub> population, a single QTL is clearly located inside the controlled segment. This QTL had a significant effect

on both GM and DGY. Surprisingly, it had no significant effect on SD, contrary to what was expected from the initial RIL experiment. The F<sub>2</sub> allele at this QTL increased moisture (+0.56%), but less than was expected (+1.2%). Its effect on DGY (−2.2 qx/ha) is opposite in sign to what was expected (+5.4 qx/ha). This QTL therefore seems to have a pleiotropic effect on grain moisture and yield. Its effect on yield appears to depend on environmental and (or) genetic conditions. Results of 1992 trials also suggest a possible pleiotropic effect on SD, the magnitude of which depends on environmental conditions. Finally, a new QTL was detected outside the introgressed segment for DGY, the effect of which was significant only in group DGY1.

**Chromosome 8:** Favorable F<sub>2</sub> alleles for SD and GM had been initially detected at several positions, which is consistent with literature results (VLATUDU *et al.* 1999; L. MOREAU, A. CHARCOSSET and A. GALLAIS, unpublished results). CIM analyses of the introgressed segment in BC<sub>3</sub>S<sub>1</sub> confirmed the presence of two linked QTL for GM. These QTL both show pleiotropic effects on earliness and yield, the F<sub>2</sub> allele decreasing GM and SD as expected (which is favorable), but also decreasing yield. The QTL in the *gsy179* and *gsy172b* vicinity show a significant effect on DGY and all earliness traits (SD, GM). The other QTL, in the *umc89* and *gsy126* vicinity, show a significant effect on DGY and GM (GM1), but no significant effect on SD. These unfavorable pleiotropic effects on DGY were not observed in 1992 and lead to a total decrease in performance of −4.7 qx/ha for DGYT. This effect of environmental conditions on QTL effects observed for this region was also observed by L. MOREAU, A. CHARCOSSET and A. GALLAIS (unpublished results). Concerning linkage group 8b, the QTL for DGY2 and DGYT detected in the BC<sub>3</sub>S<sub>1</sub> population was not detected in initial trials and appears specific to stressed conditions.

**Chromosome 10:** A favorable effect of the F<sub>2</sub> allele was expected for all traits, with a strong positive effect on yield. In the BC<sub>3</sub>S<sub>1</sub> population, a single QTL is clearly located inside the controlled segment. As anticipated, the F<sub>2</sub> allele decreases SD and GM. However, the pleiotropic effect on yield performance is negative, contrary to what was estimated initially. Further RIL QTL analyses (A. BOUCHEZ, unpublished results) have revealed opposite allelic effects for yield in different environments. RIL and BC<sub>3</sub>S<sub>1</sub> results therefore show that the effect of this QTL on yield is highly dependent on environmental conditions. Such inversions in signs of QTL effects in different environments have been infrequently reported in maize (STUBER *et al.* 1992; L. MOREAU, A. CHARCOSSET and A. GALLAIS, unpublished results).

Finally, the phenotypic difference between individual BC<sub>3</sub>S<sub>1</sub> no. 194 and recipient line Io was compared to that expected from the sum of QTL effects in either RIL or BC<sub>3</sub>S<sub>1</sub> (Table 5). For SD and GM, the observed phenotypic difference was consistent in sign with that

**TABLE 5**  
**Summary of introgression effects**

	Results	Trait			
		SD (days)	GM (%)	DGY (qx/ha)	Economic index (qx/ha)
Predicted from QTL effects (RIL)	Table 1	-1.6	-1.6	+12.4	+16.4
Estimated from QTL effects (BC <sub>3</sub> S <sub>1</sub> )	Table 4	-3.6	-1.9	-7.1	-2.3
Observed phenotypic mean for BC <sub>3</sub> S <sub>1,2</sub> no. 194	Table 3	-4.1	-1.9	-12.9	-8.1

Summary of predicted, estimated, and observed introgression effects for SD (silking date), GM (grain moisture), DGY (dry grain yield), and economic index (DGY - 2.5 GM, see QTL DETECTION AND INTROGRESSION). Overall additive effects of F2 alleles were obtained from reference effects in Table 1 and from effects for groups of all trials in Table 4.

expected from QTL effects. For SD, the magnitude of the difference was higher than expected. This difference can be put in relation to the fact that the unfavorable effect of F2 on chromosome 5 (+1.2 days in RIL, Table 1) was not confirmed within the BC<sub>3</sub>S<sub>1</sub> population. For GM, the magnitude of the difference is very close to that expected, consistent with the fact that all RIL QTL detected by ANOVA were confirmed after introgression. For DGY, the observed effect of introgression was different in sign and effect with that predicted from RIL QTL results. For chromosomes 5 and 10, QTL effects expected to be positive according to RIL results turned out to be negative after introgression and therefore negatively affected the yield performance of individual BC<sub>3</sub>S<sub>1</sub> no. 194. For chromosome 8, earliness QTL that showed no pleiotropic effect on yield within the RIL population finally displayed a negative pleiotropic effect on yield within the BC<sub>3</sub>S<sub>1</sub> population and also negatively affected the yield performance of individual BC<sub>3</sub>S<sub>1</sub> no. 194. Due to the high contribution of yield to the economic index, the program finally led to a decrease in economic performance (Table 5).

These results illustrate that (i) DGY is very sensitive to GE effects and (ii) the pleiotropic effect of earliness QTL on yield is highly dependent on environmental conditions. The magnitude of these phenomena calls for further research on GE interaction modeling for maize grain time. It would first be important to determine whether variation in QTL effects that was observed during this program can be related to the variation of environmental parameters such as water availability and (or) temperature at critical periods of the plant cycle. Such modeling also appears necessary to evaluate, *a priori*, the effect of the introgression of a given segment across the range of climatic conditions that can be expected over time. Finally, the large magnitude of GE interaction makes it necessary to reevaluate RIL and BC<sub>3</sub>S<sub>1</sub> populations in the same environments to investigate more accurately the possible role of genetic background evolution in the modification of QTL effects.

## CONCLUSION

Our results concur with the general tendency outlined by the few results of marker-assisted selection for QTL of polygenic traits published so far, although in different contexts (STUBER and SISCO 1992; LAWSON *et al.* 1997; ZHU *et al.* 1999; SHEN *et al.* 2001; RIBAUT *et al.* 2002). They indicate that, at the genotypic level, use of markers as simple marks to improve background selection is efficient, even with few markers, especially on noncarrier chromosomes. Foreground selection on markers to control the three target regions without the help of a phenotypic assay was also efficient. However, results of the phenotypic evaluation of introgressed progenies depend upon the complexity (defined as a combination of the number of QTL involved and the presence of interactions between QTL and environmental factors) of the trait under control: For the simpler traits (silking date and grain moisture at harvest), QTL effects in the progenies were in general accordance with those expected from the initial detection in the parental lines, while for the more complex trait (yield) results were in general not as good as expected, and one high-yielding allele putatively detected from the low-yielding parent finally exhibited an effect opposite to the expectation. These findings call for new programs, using different genetic materials, to evaluate their generality. However, for such complex traits controlled by QTL with pleiotropic effects highly affected by environment it appears necessary to have an accurate evaluation of QTL effects in varying environments before initiating an introgression program of favorable alleles. It might also be risky to perform selection solely on the basis of markers, without confirming the estimated effects by phenotypic evaluation at some step during the introgression process.

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