# Selection for Resistance to Southwestern Corn Borer Using Marker-Assisted and Conventional Backcrossing

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## ABSTRACT

Two maize (*Zea mays* L) lines, susceptible and resistant to first-generation southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar, were hybridized then backcrossed to the susceptible parent to form a population that was selected over three backcross generations by either marker-assisted or conventional selection for resistance to first generation SWCB leaf feeding. Quantitative trait loci (QTL) mapping was done by $BC_1F_1$ genotyping and $BC_1F_2$ infestation with SWCB. Three putative QTL were identified on chromosomes 7, 9, and 10 accounting for 28% of the phenotypic variance. Marker-assisted selection (MAS) proceeded by selecting plants heterozygous at the QTL regions and homozygous for the recurrent parent genotype outside the QTL regions in the $BC_1F_1$ and $BC_2F_1$ generations. $BC_2F_2$ individuals were selected for the homozygous donor genotype in the QTL regions. Conventional selection initiated from the most resistant 30 $BC_1F_2$ lines. Conventional trials of $BC_2F_2$ and $BC_2F_3$ families were infested with SWCB and based on leaf damage ratings selected selfed progeny of the former generation formed the subsequent trial entries. A comparative trial of $BC_2F_3$ lines, selected by the two methods, was evaluated under SWCB infestation at three locations. Leaf damage ratings were taken at all locations and larvae weight was taken at one location. No significant differences for leaf damage ratings or larvae weight were found between lines selected by the two methods. Both methods produced lines significantly improved over the susceptible parent for SWCB leaf feeding damage indicating that the methods were equivalent as conducted in this experiment.

SUBSTANTIAL RESOURCES have been invested in developing maize with host-plant resistance to tropical insects by the International Maize and Wheat Improvement Center (CIMMYT) (Mihm, 1985, 1997; Smith et al., 1989; Thome et al., 1992). Germplasm known to be resistant to single insect species were recombined into populations that were then selected for resistance to multiple maize insect pest species (Smith et al., 1989). The emphasis was on identifying maize germplasm that displayed antibiosis to the first generation of tropical borers and fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] to reduce the number of breeding insects and the effect of subsequent generations on the maize crop.

Resistant lines identified through this effort have been released and also used in QTL mapping studies to identify regions of the genome responsible for resistance (Bohn et al., 1997; Groh et al., 1998; Khairallah et al., 1998).

Two of these studies have mapped QTL for resistance to first-generation SWCB using the same resistant parent that we used, CML67, but a different susceptible parent. Bohn et al. (1997) used an $F_2$ mapping population of 171 individuals from the cross CML131 × CML67 with line characterization of SWCB resistance in two environments and identified six QTL for first-generation SWCB resistance on chromosomes 1(3 QTL), 5, 7 and 9. Groh et al. (1998) evaluated 170 recombinant inbred lines (RIL) descended from the same $F_2$ population, in four environments. Nine QTL were identified on chromosomes 1 (4 QTL), 5, 7, 8 (2 QTL), and 9.

Khairallah et al. (1998) mapped QTL for first-generation SWCB resistance in an $F_2$:$F_3$ population derived from a cross using different resistant and susceptible lines than we used. In this population consisting of 472 lines, seven QTL were identified on chromosomes 3, 5 (2 QTL), 6 (2 QTL), 8, and 9. Groh et al. (1998) evaluated 135 RIL derived from this population and identified five QTL on chromosomes 1, 6, 8, and 9 (2 QTL).

Our study uses molecular markers to transfer regions associated with resistance to the first generation of SWCB into an elite line via backcrossing. This was an appealing prospect since the adoption of insect resistant materials has been limited because of negative characteristics of the resistant germplasm that proved difficult to improve through conventional breeding. To evaluate the relative merits of marker-assisted backcrossing versus conventional backcrossing, the same population used for MAS was improved for SWCB leaf resistance by conventional selection (CS) methods.

Our study was considered a pilot study for MAS. At the time this study was initiated, there was little published information on MAS other than simulation studies (Hospital et al., 1992). Recently, more studies have been published using MAS, but most report results of only one round of selection (Lindhout et al., 1994; Stromberg et al., 1994; Moneforte et al., 1996; Han et al., 1997; Toojinda et al., 1998; Concibido et al., 1996; Schneider et al., 1997; Romagosa et al., 1999). There are few published studies that describe MAS over multiple

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**Abbreviations:** BC, backcross; CIM, composite interval mapping; CIMMYT, Centro Internacional de Mejoramiento de Maiz y Trigo; cM, centimorgan; CML, CIMMYT maize line; CS, conventional selection; LOD, Likelihood of Odds; MAS, marker-assisted selection; NIL, near isogenic lines; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; SWCB, southwestern corn borer.

generations with the evaluation of the effect of the selected QTL regions taking place in a genetic background substantially different than the material used for QTL identification.

Two studies exemplify this category. Lawson et al. (1997) used molecular markers to backcross five QTL regions for acylsugar content from *Lycopersicon pennellii* (Corr.) D'Arcy to cultivated tomato over three generations. $BC_2F_1$ and $BC_3F_1$ plants were selected that had two and three of the QTL regions but these lines did not produce higher acylsugars than the recurrent line. $BC_3F_1$ plants were sib mated to recover plants with all five regions The resulting $BC_3F_1$ tomato lines selected by MAS had higher acylsugars than the original line, but lower than the levels of acylsugars in the $F_1$. Stuber et al. (1999) improved maize lines B73 and Mo17 using MAS. The identification of QTL regions was based on the evaluation of a large number of near isogenic lines (NIL) with isolated overlapping segments from two lines that showed promise of improving the performance of B73 and Mo17. The lines containing the introgressed segments identified as enhancing yield were evaluated as hybrids both with a heterotic tester (B73 or Mo17) and with improved lines from the opposite heterotic group. There were more than three times as many lines that yielded at least one standard deviation above the yield of the original hybrid (B73 × Mo17) than below it.

Fewer studies have attempted to compare results of CS and MAS. Van Berloo and Stam (1999) found MAS and phenotypic selection to be equivalent in selection for early flowering in *Arabidopsis thaliana* Heynh. The lines they produced by both methods were earlier than both parents. Stromberg et al. (1994) also found MAS and phenotypic selection in early generations to be equivalent in maize; however, neither method exceeded the unselected population for yield, the trait selected.

The study described herein addresses the use of molecular markers for selection of QTL regions associated with resistance to first-generation SWCB leaf feeding over multiple generations of backcrossing into an elite maize line and the comparison of this methodology with conventional selection from the same population.

## MATERIALS AND METHODS

### Population Development

Inbred CML67 was used as the donor parent for resistance to first-generation SWCB. This late maturity tropical line is highly resistant to leaf feeding by first generations of SWCB, and sugarcane borer (*Diatraea saccharalis* Fabricius), and moderately resistant to fall armyworm. CML67 has some severe agronomic problems such as low yield, yellow-red grain color, an undefined heterotic pattern, as well as susceptibilities to rust (*Puccinia sorghi* Schwein.), *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs (= *Helminthosporium turcicum* Pass.), and *Maize streakvirus* (MSV).

The recurrent parent, CML204, was released in 1993 by the CIMMYT Maize Program in Harare, Zimbabwe and is adapted to southern Africa. It is a subtropical, late maturity, white dent line that is moderately susceptible to SWCB and highly resistant to MSV, rust, and *H. turcicum*. The insect pests of the most concern in the region are *Chilo partellus*

Swinhoe (spotted stem borer) and *Busseola fusca* Fuller (African maize stalk borer). The CIMMYT research station in Zimbabwe does not have facilities for rearing insects nor does it have facilities for molecular marker work. Our reason for transferring SWCB resistance to such an elite African line is that SWCB is an aggressive leaf feeder, and SWCB resistance has been correlated with resistance to other maize borers (Thome et al., 1992), including *Chilo partellus* and *Busseola fusca* (Smith et al., 1989). This correlation of SWCB resistance with resistance to other maize stem borers, particularly less aggressive species, permitted the use of artificial infestation in a location geographically accessible to the molecular genetics laboratory, which was crucial for this study to take place.

The selection scheme for MAS is diagramed in Fig. 1. The $F_1$ was made using the recurrent parent CML204 as the female and CML67 as the pollen parent. The first backcross $(BC_1)$ was made with the $F_1$ hybrid as the female and 300 $BC_1$ seed were planted. This number was sufficient for a 99% probability of recovering at least one plant with all regions required for resistance if up to six regions were involved in conferring resistance (Sedcole, 1977). Each of the 287 plants that germinated was self pollinated to form $BC_1F_2$ ears, and pollen from each plant was used to manually pollinate a plant of the recurrent parent, to form $BC_2$ ears. Of the 287 plants, 277 selfed ears and 224 $BC_2$ ears were harvested that had at least the 40 seeds required for subsequent evaluation.

## QTL Detection

### Linkage Mapping

Leaf tissue samples were taken for restriction fragment length polymorphism (RFLP) genotyping before flowering from the two parental lines and each of the 287 $BC_1F_1$ plants by means of the methods described by Khairallah et al. (1998). Eighty-nine probes were used to genotype the $BC_1F_1$ population resulting in 105 polymorphic loci. A morphological marker, grain color, locus *y1* was recorded on the selfed ears produced on $BC_1F_1$ plants.

The segregation at each locus was checked for deviations from the expected Mendelian ratio in a backcross population (1:1) by standard Chi square tests. Those loci that did not significantly deviate from the Chi square ratio were used to construct a linkage map by the same methods employed by Khairallah et al. (1998).

### Phenotypic Evaluation of the $BC_1F_2$

SWCB leaf damage ratings from an artificially infested trial of $BC_1F_2$ families were used as trait data for QTL analysis, and the same data were used to select the best families for conventional selection. The $BC_1F_2$ trial was conducted at the CIMMYT Experiment Station in Tlaltizapán, Morelos, Mexico, (subtropical environment, 18.41° N, 99° W, 940-m elevation, 830-mm average rainfall; soil type, silty clay isothermic Udic Pellusert) during the winter cycle of 1994 (TL94A). The 277 $BC_1F_2$ families and 12 check entries were planted in a 17 × 17 alpha (0,1) lattice (Patterson and Williams, 1976) with two replications. The check entries were the two parents and three other susceptible lines, Ki3, CML215, and CML216. The trial was over-planted and was thinned to 10 plants per 2.5-m row after emergence. At the six-leaf stage, the trial was artificially infested with an average of 44 neonate SWCB larvae per plant by means of the method described by Mihm (1983). Two leaf damage ratings were taken approximately 28 d after infestation with a rating scale of 1 to 10, where 1 = no visible damage and 10 = a dead plant (modified 1–9 scale of Davis and Williams, 1989). Ratings were taken on each of the 10
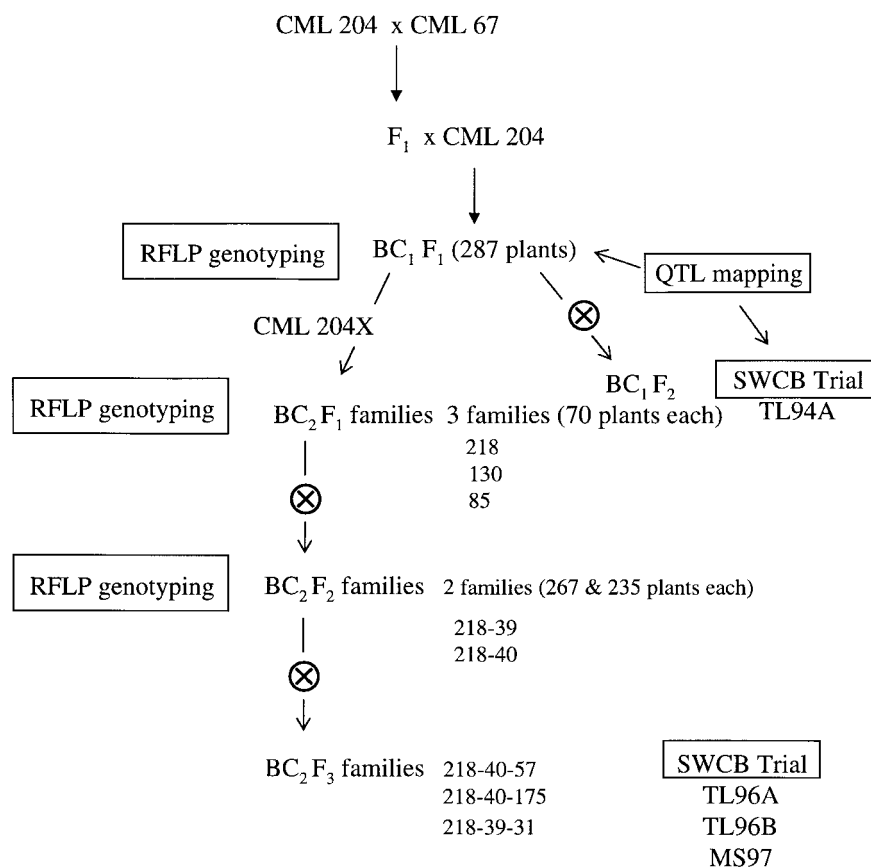
**Fig. 1. Diagram of the marker-assisted backcrossing procedure employed to transfer SWCB resistance from CML67 to CML204.**

plants within a plot and were then averaged to give plot leaf damage. The plot leaf damage from the two different ratings were analyzed separately by ProcMixed (SAS, 1988) to calculate adjusted means. The adjusted means for the two ratings were then averaged to give the leaf damage rating entry mean per $BC_1F_2$ family.

## QTL Analysis

The composite interval mapping (CIM) procedure (Jansen and Stam, 1994; Zeng, 1994) was used for QTL mapping. The analysis was performed in three steps as described by Groh et al. (1998) and a likelihood ratio threshold value of 11.5 was used for QTL detection, equivalent to LOD score of 2.5. The phenotypic variation ($R^2$) explained by each QTL and the genetic effects were estimated as part of the third step. For each QTL, the marker closest to the peak was used in a multiple regression model for the calculation of $R^2$ for all QTL.

## Marker-Assisted Selection

### $BC_1F_1$ Selection

Using the information from the QTL analysis, we initiated selection in the $BC_1F_1$ population on the basis of the genotypic profile of the individuals. This selection was based on defining a target genotype heterozygous at the QTL regions and homozygous for the CML204 (recurrent) genotype in all other regions of the genome. Genotypic data from the entire $BC_1F_1$ population were ranked according to closest fit to the target genotype. Individual plants were selected from the $BC_1F_1$ population on the basis of whether their determined genotype was heterozygous at five RFLP loci, *bnl14.07, umc81, umc153,*

*umc114,* and *umc155.* These were the peaks of the QTL on chromosomes 7 and 10 and three closely linked markers around the QTL peak on chromosome 9. Three individuals were selected from the 287 $BC_1F_1$ plants in the population.

### $BC_2F_1$ Selection

Seventy-two seeds were planted from each of the three $BC_2$ selected ears corresponding to the three selected $BC_1F_1$ plants. This number of seed gave 99% probability (Sedcole, 1977) of encountering two or more individuals with all three of the regions associated with insect resistance. At flowering all plants were self-pollinated.

Leaf samples for molecular genotyping were taken from individual plants before flowering. RFLP probes were selected on the basis of the following criteria: (i) all loci associated with the QTL regions and (ii) for each chromosome, four to six evenly spaced loci that were heterozygous in the $BC_1F_1$ individual. The population was scanned and two individuals were selected on the basis of closest fit to the target genotype, which was the same as that used for the $BC_1F_1$.

### $BC_2F_2$ Selection

Seed from the self-pollinated ears of the two selected $BC_2F_1$ individuals were planted for the third round of selection during the summer cycle of 1995. In this case, the target genotype was homozygous for the donor genotype at the QTL regions and homozygous for the recurrent genotype in the rest of the genome. The fixation of the QTL regions for the donor genotype was desired for subsequent testing of these lines under infestation and mandated a larger population size to recover the desired genotype (Sedcole, 1977); 267 and 235
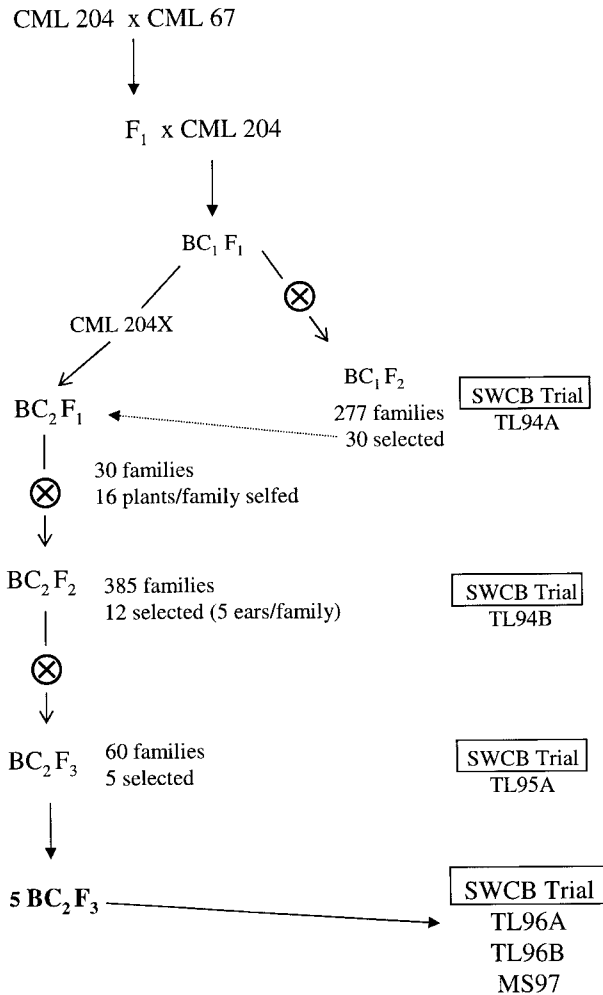
CML 204 x CML 67

F$_1$ x CML 204

BC$_1$F$_1$

CML 204X $\qquad$ ⊗

BC$_2$F$_1$ ← BC$_1$F$_2$
277 families | SWCB Trial
30 selected | TL94A

⊗ 30 families
16 plants/family selfed

BC$_2$F$_2$ 385 families | SWCB Trial
12 selected (5 ears/family) | TL94B

⊗

BC$_2$F$_3$ 60 families | SWCB Trial
5 selected | TL95A

5 BC$_2$F$_3$ ————→ SWCB Trial
TL96A
TL96B
MS97

**Fig. 2. Diagram of the conventional backcrossing procedure employed to transfer SWCB resistance from CML67 to CML204.**

seeds were planted from the selfed ears of the two selected BC$_2$F$_1$ plants. All plants were genotyped and self-pollinated as previously described. Loci used were those in the QTL regions and all those that were still heterozygous in the previous generation.

## Conventional Selection

The scheme for conventional selection is diagramed in Fig. 2. The BC$_1$F$_1$, BC$_2$F$_1$, and BC$_1$F$_2$ generations were common for the conventional and MAS procedures. Divergence in the selection methods occurred after the BC$_1$F$_2$ infested trial.

### BC$_1$F$_2$ Selection

The first stage of conventional selection used the entry means for the BC$_1$F$_2$ families from the TL94A artificially infested SWCB trial. Thirty BC$_1$F$_2$ families that had the least leaf damage from SWCB were selected.

### BC$_2$F$_2$ Selection

BC$_2$ ears produced from the BC$_1$F$_1$ population were grown and self-pollinated to form BC$_2$F$_2$ families corresponding to the selected families. One row was planted from each of the 30 selected BC$_2$F$_1$ ears. The number of selfed ears produced ranged from 14 to 16. Four of the 30 entries selected in the TL94A BC$_1$F$_2$ trials did not have corresponding BC$_2$ ears suc-

cessfully produced in TL93B so backcrosses were made by hand pollinating the selected BC$_1$F$_2$ entries within the TL94A trial with CML204 pollen to produce BC$_2$F$_2$ ears. BC$_2$F$_2$ families produced by both methods were evaluated in an infested trial, planted in Tlaltizapán in June 1994 (TL94B) in an alpha (0,1) lattice with 35 blocks × 11 entries per block and two replications (Patterson and Williams, 1976). CML204 and CML67 were used as checks. This planting suffered a heavy natural infestation of fall armyworm. A second planting of the same trial was done a month later with the same type of design. The entries in the second trial included selfed ears from the 29 BC$_1$F$_2$ families that had the lowest leaf damage ratings of the 30 selected. Both trials were planted and infested as described for the BC$_1$F$_2$ trial.

Trials were infested, rated for leaf feeding damage and adjusted entry means were calculated as described for the BC$_1$F$_2$ trial. The adjusted entry means of each of the 29 entries common between the two plantings were averaged to give an entry mean across plantings. Twelve entries with the lowest leaf damage were selected. All entries in the trial were self-pollinated.

### BC$_2$F$_3$ Selection

The selected selfed progeny from the BC$_2$F$_2$ trial were evaluated under SWCB infestation in the winter cycle of 1995 (TL95A) in the same manner as described previously. The trial was conducted as an alpha (0,1) lattice with two repetitions, 60 BC$_2$F$_3$ entries, and parental checks. Using the average leaf damage ratings for the 60 BC$_2$F$_3$ entries, we selected five entries with the lowest leaf damage. All entries in the trial were self-pollinated.

## Evaluation of Conventionally Selected and Marker Selected Lines

### BC$_2$F$_3$ Infested Combined Trial

BC$_2$F$_3$ lines from the marker-assisted and conventional selection schemes were compared in a trial artificially infested with SWCB in three environments. The marker-selected BC$_2$F$_3$ entries were selfs of individuals selected from the BC$_2$F$_2$ population as previously described. Conventional BC$_2$F$_3$ lines used in this experiment were those selected from the BC$_2$F$_3$ trial, prioritized by their performance in the conventional BC$_2$F$_2$ trial. The combined trial was planted at the CIMMYT experiment station, Tlaltizapán, Morelos, Mexico, in both the winter and summer cycles of 1996 (TL96A and TL96B) and at the Mississippi State University Experiment Station, Mississippi State, Mississippi (33.27° N, 88.49° W; 56 m above sea level; 1418-mm average rainfall, and soil type, fine montmorillonitic, non-acid, thermic Vertic Haplaquept) in the summer of 1997 (MS97). The TL96A trial was a 7 × 7 row column design (John and Eccleston, 1986; Patterson and Robinson, 1989) with three repetitions. The row column design is a further modification of the alpha lattice with directional blocking. The TL96B trial was conducted as an alpha (0,1) lattice (7 blocks × 7 entries per block) (Patterson and Williams, 1976) with three repetitions. Both trials were planted in 2.5-m rows, 0.75 m apart with 20 seeds, and later thinned to 10 plants per row. The MS97 trial was conducted as a randomized complete block with three replications. All trials consisted of 49 entries; 10 entries were parental checks (CML204 and CML67), five entries were conventionally selected BC$_2$F$_3$ lines, 10 entries were conventionally selected BC$_2$F$_4$ lines selfed from the BC$_2$F$_3$ lines, and three entries were the marker-selected BC$_2$F$_3$ lines, homozygous for the resistant genotype (CML67) at the three QTL regions. The remaining 21 entries were lines from the

MAS population that were included for comparative purposes: six entries were homozygous for the CML67 genotype at only one QTL region, six were homozygous for the resistant genotype at two regions, and the remaining nine were individuals homozygous for the resistant genotype at two of the three regions, with the third region being heterozygous.

Infestations and ratings of the trials conducted in Mexico were as previously described. The TL96A and TL96B trials were infested with averages of 45 and 35 neonate larvae of SWCB, respectively. The MS97 trial was infested with an average of 24 larvae at the 7- to 8-leaf stage, and leaf damage ratings were taken 14 d after infestation. On that same day, five plants within each row were dissected and the larvae were counted and weighed (Davis et al., 1991).

Ratings for plants within plots were averaged and adjusted means were calculated for the row-column and alpha lattice design trials. The entry means were calculated for the trials conducted in Mexico as previously described. The entry means for the MS97 trial were calculated from the plot averages by Proc Means (SAS). Analyses of variance were performed on leaf damage rating entry means for each trial and on the combined data of the three trials. In the 1996A trial, rows and columns were considered random effects for the analysis of variance. Analysis of variance was conducted on the mean weight of larvae found in five plants per plot. Linear contrasts were calculated between the three lines selected by MAS that were homozygous for the donor genotype at all QTL regions and three conventionally selected $BC_2F_3$ lines (that had the least leaf damage by SWCB in the $BC_2F_2$ trial conducted in 1994B). The marker-selected lines used in the contrasts were $BC_2F_3$ 218-40-175, 218-40-57, and 218-39-31. The conventional lines used for contrasts were $BC_2F_3$ 82(1)-5, 38-4-4, and 95-11-3. Linear contrasts were made between the recurrent parent, CML204, and the lines selected by the two methods.

### RFLP Genotyping of Conventionally Selected Lines

Seed of the conventionally selected $BC_2F_3$ lines, produced by sib-mating plots in the TL96A trial, were planted and leaf tissue was collected from 10 plants per family for RFLP genotyping as previously described. Most of the probes used to produce the $BC_1F_1$ map were used on the five conventional lines; a total of 89 of the 103 loci were scored. Graphical genotypes were drawn on the basis of positions and distances of the $BC_1F_1$ map.

### Analysis of the QTL Effects in Marker-Selected $BC_2F_3$ Lines

In total, 24 lines from the marker-assisted program were evaluated in the combined trial, since all of these lines originated from the same $BC_1F_1$ individual (218) their genotype was similar outside the QTL regions. For these marker-selected $BC_2F_3$ lines, an indicator variable, 0 or 1, was assigned to each QTL to identify the presence or absence of the donor genotype in the QTL region. For the few heterozygous regions

a fraction was used. The leaf feeding scores of each line in three environments, and the average over the three environments, and the larvae weight per plant from the Mississippi trial were then regressed on the indicator variable to estimate the effects of each QTL from the resistant line.

## RESULTS

### Map Construction and QTL Detection

#### RFLP Linkage Map

The constructed linkage map consisted of 10 chromosomes comprised of 103 RFLP loci (from 89 probes) and one morphological marker, $y1$. This map spanned a distance of 1433 centimorgans (cM) with an average density of 15.4 cM. The map was quite consistent with other maize RFLP maps, including those in the Maize Genetics Cooperative Newsletter (1994), and three maps of tropical maize done at CIMMYT (Khairallah et al., 1998; Bohn et al., 1996; Ribaut et al., 1996).

#### $BC_1F_2$ Infested Trial

The leaf damage ratings of the $BC_1F_2$ entries in the TL94A infested trial exhibited near normal distribution with no transgressive segregation. The means of the two parental lines were 4.1 for CML67 and 7.5 for CML204 on the 1 to 10 scale previously described. The average leaf damage rating for the $BC_1F_2$ population was 6.0 with a range of 4.9 to 7.3. The correlation between the two ratings of the trial minus checks was 0.86. The heritability on an entry mean basis from the mean of the two ratings was 0.52 0.06 (Lynch and Walsh, 1997). The phenotypic variance on an entry mean basis of the mean of the ratings was 0.25.

#### QTL Analysis

Three QTL regions, on chromosomes 7(c7), 9 (c9), and 10 (c10) were significant for resistance to first-generation SWCB feeding (Table 1, Fig. 3). The LOD score for the QTL on c9 was more than three times larger than that of the other two QTL. The genetic effect estimated that substituting the CML67 allele at the c9 QTL would decrease leaf damage by a half a point (−0.47) on the 1-to-10 leaf damage rating scale. The total phenotypic variance explained by the three QTL according to the CIM analysis was 27.7% (Table 1).

### Marker-Assisted Selection

Twenty individuals from the $BC_1F_1$ population met the criteria of the target genotype. Of those, three indi-

**Table 1. Putative QTL detected for SWCB resistance and their genetic effects estimated in the backcross population CML204 × (CML 204 × CML67) of 277 families by composite interval mapping (CIM, Model I, 30-cM window size).**

| Chromosome | QTL position | | | Genetic effect† 1–10 scale | % Phenotypic variance |
| | Marker interval | cM | LOD | | |
|---|---|---|---|---|---|
| 7 | *bnl14.07-umc80a* | 99 | 3.41 | −0.23 | 4.9 |
| 9 | *umc81-umc153* | 58 | 12.38 | −0.47 | 17.3 |
| 10 | *umc155-umc44a* | 40 | 2.85 | −0.24 | 6.8 |
| | | | | **Total phenotypic variance‡** | 27.7 |

† Genetic effects expressed as the change in the SWCB leaf damage rating resulting from the contribution of an allele from the resistant parent, CML67.
‡ Estimate obtained from a simultaneous fit of all putative QTL affecting the trait. Markers selected as cofactors: *umc80a* on c7, *umc153* on c9, and *umc155* on c10.
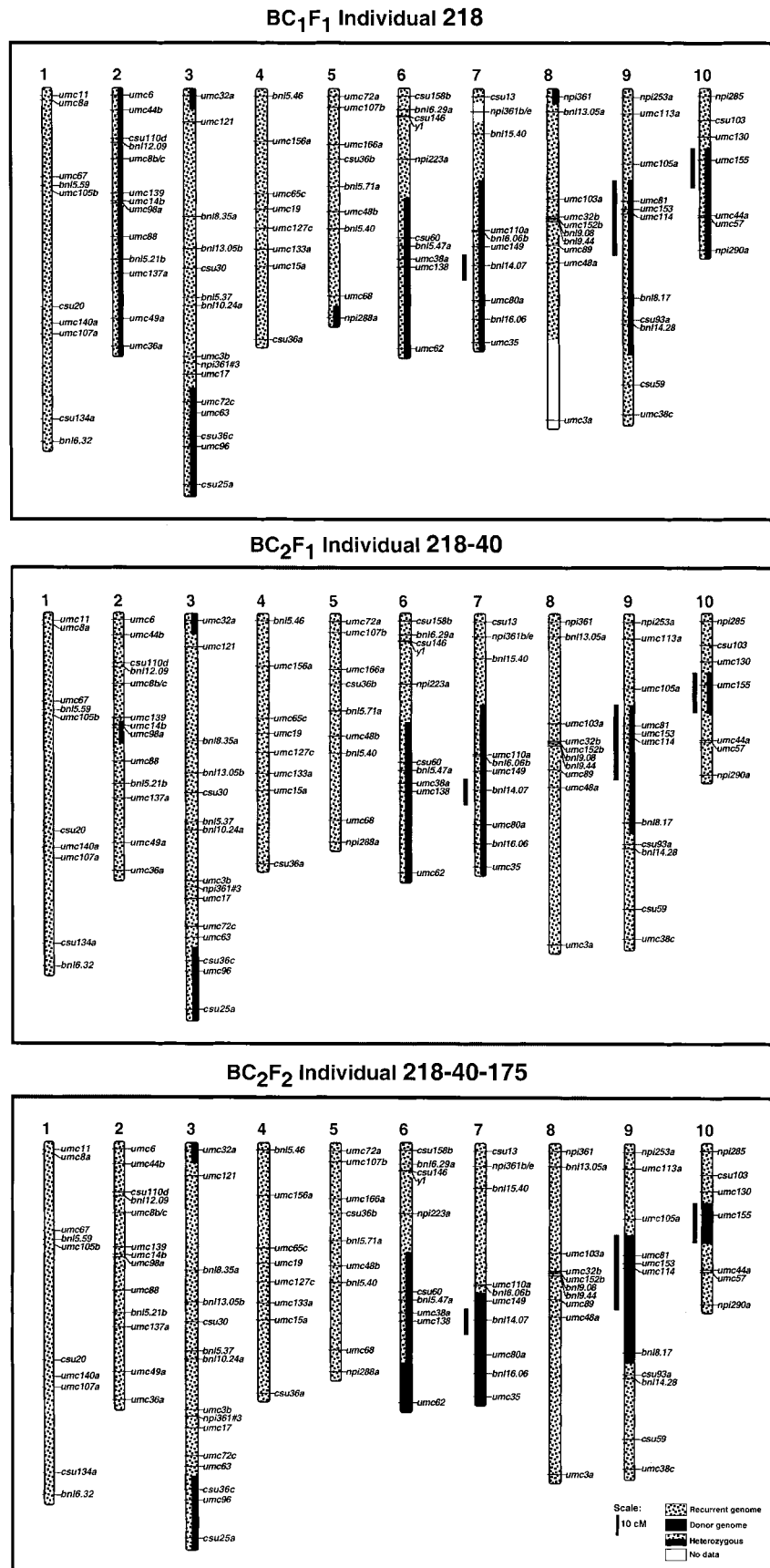
Fig. 3.  Graphical genotypes of three sequential marker-assisted selections in the BC$_1$F$_1$-218 lineage of the CML204 (recurrent) $\times$ CML67 (donor) population. Bars on the left of chromosomes 7, 9, and 10 indicate the position of the QTL for SWCB resistance.

**Table 2. Leaf damage ratings for SWCB infested trials in the conventional selection scheme.**

| Family structure | Families tested | Leaf damage ratings† | | Parental Means | | Selected fraction | | |
| | | Mean‡ | Range | CML204 | CML67 | Mean | Number of families | % of population |
|---|---|---|---|---|---|---|---|---|
| | | Population | | | | | | |
| BC$_1$F$_2$ | 277 | 6.0 | 4.9–7.3 | 7.5 | 4.1 | 5.1 | 30 | 11 |
| BC$_2$F$_2$ | 375 | 7.1 | 6.3–8.3 | 7.3 | 3.8 | 6.4 | 12 | 3 |
| BC$_2$F$_3$ | 60 | 6.8 | 6.0–7.6 | 7.4 | 4.7 | 6.2 | 5 | 8 |

† Leaf damage rating units on 1–10 scale; 1 = no visible damage, 10 = dead plant.
‡ LSD for BC$_1$F$_2$ = 0.94, BC$_2$F$_2$ = 1.04 (first planting) and 0.897 (second planting), BC$_2$F$_3$ = 0.88.

viduals were selected that had the highest percentage of the recurrent parent genotype at all other loci. The selected individuals, BC$_1$F$_1$-85, 130, and 218, were homozygous for the recurrent parent genotype in 58, 65, and 62% of the genome, respectively.

Two individuals were selected from the BC$_2$F$_1$ population derived from BC$_1$F$_1$ individual 218. These two individuals, BC$_2$F$_1$ 218-39 and 218-40, were homozygous for the recurrent parent in 70 and 71% of the genome, respectively. The decision to select within this individual only was based on the favorable recombination in individual 218 on the short arm of c10 that separated the putative QTL region from rust susceptibility (*rp1*, *rp5*, *rp6*, *rpp9*) (Maize Genetics Coop, 1995).

Three individuals in the BC$_2$F$_2$ population (218-40-57, 218-40-175, and 218-39-31) were selected that were homozygous for the donor parent genotype in the QTL regions. They were homozygous for the recurrent genotype in 75, 78, and 82% of the genome, respectively. The progression of MAS is illustrated in Fig. 3 by the BC$_1$F$_1$ 218 lineage.

### Conventional Selection

The results of the trials conducted as part of the conventional selection scheme are displayed in Table 2. The range of leaf damage ratings observed in the three trials narrowed with successive generations of selection and backcrossing. The BC$_2$F$_2$ and BC$_2$F$_3$ trial means, selected fraction means, and ranges demonstrated greater leaf feeding damage than the BC$_1$F$_2$ trial, while parental check means stayed relatively steady across the three seasons of the trials. The BC$_2$ F$_2$ trial, which was planted twice, had a correlation between the plantings of 0.70. The mean leaf damage ratings were 7.3 and 6.8 for the first and second plantings respectively. The second

planting of the trial was infested with fewer SWCB per plant because of a shortage of larvae. The LSD of the first planting of the BC$_2$F$_2$ trial was higher than that of the second planting and the highest of the conventional trials (Table 2).

## Comparison of Conventional and Marker-Selected Lines

### SWCB Resistance

The TL96A trial was not significant for row or column effects, thereby, displaying no directional partitioning of error variance. In the absence of directional effects, either rows or columns can be selected to substitute for blocks within replications and the analysis is the same as that for an alpha (0,1) lattice.

In the three environments where these lines were compared, the SWCB leaf damage ratings of the MAS and CS BC$_2$F$_3$ lines were not significantly different (Table 3). There was a numerical trend toward lower leaf damage scores in the CS lines. Both the conventionally and marker selected lines were significantly improved over the original recurrent line, CML204, for leaf damage in all but the TL96B trial. When the results of the three trials were combined across locations (Table 3), there was no significant difference between the marker and conventionally selected lines for leaf damage ratings under artificial infestation and both methods produced lines that were significantly ($P < 0.001$) improved over the original line.

The weight of SWCB larvae per plant was not significantly different between the marker selected and conventionally selected BC$_2$F$_3$ lines (Table 3). Numerically, the larvae on the conventionally selected BC$_2$F$_3$ lines weighed 10.7 mg/plant less than those collected on

**Table 3. Means and linear contrasts of SWCB leaf damage ratings and SWCB larvae weight per plant of three conventionally (CS) and three marker-selected (MAS) BC2F3 lines and recurrent parent, CML 204. Leaf damage ratings are from three locations: Tlaltizapán, Mexico, 1996 winter and summer cycles (TL96A and TL96B); Starkville, MS, 1997 (MS97) and SWCB larval weight per plant is from Starkville, Mississippi, 1997 (MS97).**

| Environment | SWCB leaf damage ratings† | | | Linear contrasts $P > T$ | | |
| | CS BC$_2$F$_3$ lines | MAS BC$_2$F$_3$ lines | CML 204 | CS vs. MAS | CS vs. CML 204 | MAS vs. CML 204 |
|---|---|---|---|---|---|---|
| TL96A | 6.94 | 7.24 | 7.81 | 0.3099 | 0.0067 | 0.0556 |
| TL96B | 6.53 | 6.66 | 7.08 | 0.6941 | 0.0724 | 0.1694 |
| MS97 | 5.89 | 6.55 | 7.33 | 0.1263 | 0.0003 | 0.0471 |
| Across locations | 6.37 | 6.74 | 7.41 | 0.1024 | 0.0001 | 0.0014 |
| | Larval weight (mg/plant) | | | | | |
| MS97 | 22.80 | 33.49 | 33.80 | 0.3352 | 0.2679 | 0.9749 |

† Leaf damage rating units on 1–10 scale; 1 = no visible damage, 10 = dead plant.

**Table 4. Post facto analysis of effects of QTL genotype in MAS BC$_2$F$_3$ lines on observed leaf damage ratings (LDR) and larval weight in SWCB artificially infested trials conducted in Tlaltizapán, Mexico, 1996 A and B cycles (TL96A and TL96B) and Starkville, Mississippi, 1997 (MS97).**

| Trait | Trial | QTL regions selected | | |
| | | c7 | c9 | c10 |
| | | Numeric decrease when donor genotype present | | |
|---|---|---|---|---|
| LDR (units 1–10 scale) | TL96A | −0.37† | −0.34† | 0.01 |
| | TL96B | 0.09 | −1.05** | 0.04 |
| | MS97 | −0.11 | −0.64† | −0.40 |
| | Across trials | −0.13 | −0.68** | −0.12 |
| Larval weight (mg/plant) | MS97 | −7.78 | −17.99† | 0.32 |

** Significance levels = 0.01.
† Significance levels = 0.10.

the marker-selected lines. This is a large difference in weight, which is surely of biological significance, but because of the high variation for the larvae weight (LSD = 37.9) the comparison was not significant. The comparisons of weight of SWCB larvae collected from the recurrent parent CML204 with larvae from the lines selected by both methods was also not significant on the basis of this LSD.

## Genomic Composition

**QTL regions selected**. The results of regressing the genotype of MAS lines in QTL regions on their leaf damage ratings in the combined BC$_2$F$_3$ trial are shown in Table 4. Of the three QTL regions identified as reducing leaf damage in the BC$_1$F$_1$/BC$_1$F$_2$ mapping, only the region on c9 was significant in this post facto analysis for reduction of leaf damage ratings across the three locations of the combined trial. Assessment of larvae weights on infested plants was not part of the original mapping study, but the effects of the three putative QTL regions follows the same trend as that for leaf damage ratings with c9 being the only one significantly decreasing the larvae weight per plant.

The graphical genotypes representing the conventionally selected lines are shown in Fig. 4. The donor genotype is present in the QTL region on c9 as heterozygous or homozygous in all three selected lines. On the other hand, in the QTL regions on c7 and c10, the donor genotype is present in only one of three cases.

**Recurrent parent recovery**. Comparisons of the re-

covery of the recurrent genotype, CML204, as a percentage of total genome size demonstrate that marker selection fixed an average of 8% more CML204 genotype over the rounds of selection (Table 5). Conventionally selected lines had a higher percentage of heterozygous regions, but as mentioned previously these regions may be heterogeneous. The percentages of fixed recurrent genome changed over the course of MAS from 61% for the BC$_1$F$_1$ 218 selection to 78% for the BC$_2$F$_2$ 218-40-175 selection.

Figure 5 shows the distribution in the three MAS selection generations of individuals in relation to the percentage of homozygous recurrent parent genome. In the BC$_1$F$_1$ population, individual 218 was selected above the mean for percentage homozygous recurrent parent genotype. In the BC$_2$F$_1$ and the BC$_2$F$_2$ populations, individuals were identified that met the selection criteria, but the selected individuals were below the mean of the population for percentage recurrent parent genotype.

## DISCUSSION

The success of marker-assisted selection for quantitative traits is dependent on the accurate identification of QTL positions as well as on the size of the effect of the QTL. A number of factors can lower the power to detect QTL, inflate QTL effects, or misidentify QTL positions. Such factors have been thoroughly discussed in reviews by Dudley (1993) and Kearsey and Farquhar (1998).

**Table 5. Percentages of donor (CML 67) and recurrent (CML 204) parent genotype in conventionally (CS) and marker-selected (MAS) BC$_2$F$_3$ lines estimated using RFLP markers. Leaf damage ratings (LDR) and genotypes within the QTL regions are indicated for each line included in the linear contrasts.**

| Selection method | Line | Genotypic classes as a percentage of total genome size† | | | | | Genotype in QTL region§ | | |
| | | Fixed CML 67 | Fixed CML 204 | Heterozygous | Missing Data | LDR‡ | c7 | c9 | c10 |
|---|---|---|---|---|---|---|---|---|---|
| CS | | | | | | | | | |
| | 38-4-4 | 14.0 | 70.0 | 14.4 | 0.9 | 6.13 | A | H | A |
| | 82-1-5 | 1.2 | 65.3 | 32.0 | 1.5 | 6.34 | H | H | H |
| | 95-11-3 | 12.3 | 75.9 | 10.3 | 1.4 | 6.65 | A | B | A |
| MAS | | | | | | | | | |
| | 218-39-31 | 9.4 | 82.0 | 5.1 | 3.5 | 6.76 | B | B | B |
| | 218-40-57 | 8.8 | 75.3 | 12.4 | 3.5 | 6.70 | B | B | B |
| | 218-40-175 | 11.3 | 77.7 | 7.1 | 3.5 | 6.78 | B | B | B |

† Based on 1432.6 cM genome length. cM of a genotype were calculated as 1/2 the distance to the adjacent markers.
‡ Leaf damage rating: average from three locations; units on 1–10 scale, 1 = no visible damage, 10 = dead plant.
§ Genotypic designations: A, CML204 (susceptible); H, heterozygous; B, CML67 (resistant).
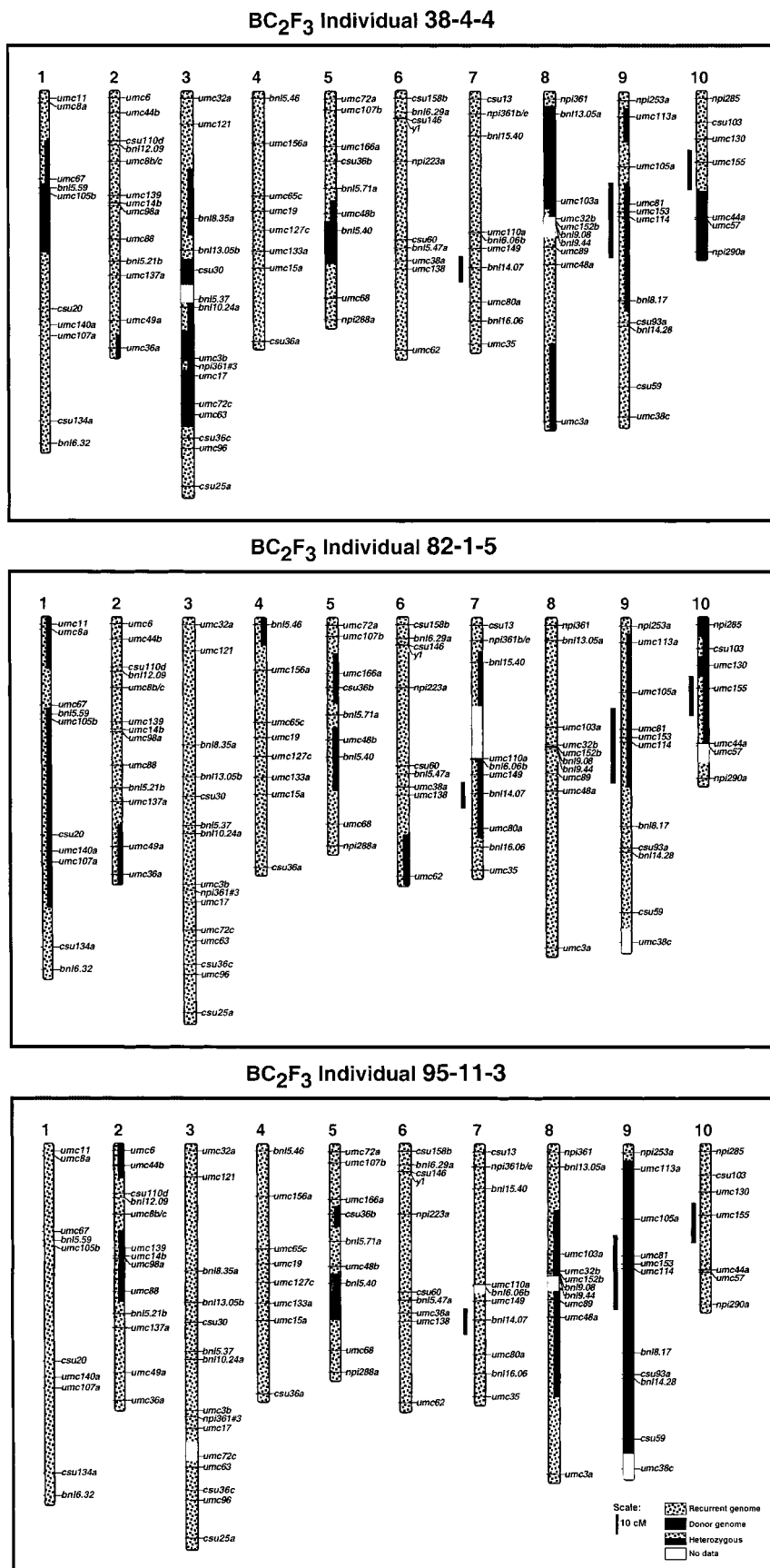
Fig. 4. Graphical genotypes of three conventionally selected BC₂F₃ lines from the CML204 (recurrent) × CML67 (donor) population. QTL regions for SWCB resistance used for MAS are indicated as bars on the left of chromosomes 7, 9, and 10. These QTL were not used in selecting the lines; they are, however, indicated to aid visual comparison of the regions selected in the two schemes.
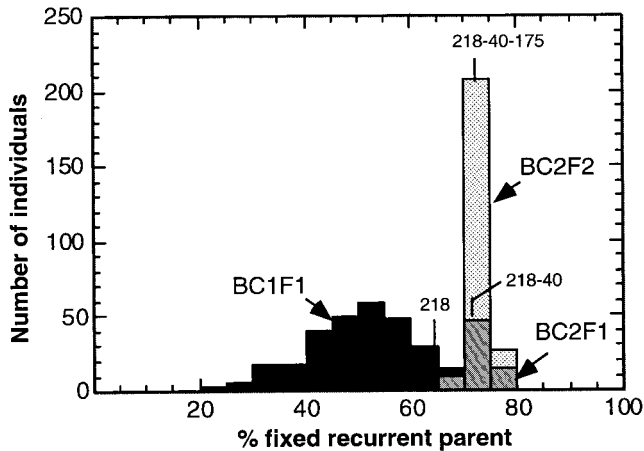
**Fig. 5. Distributions of BC$_1$F$_1$, BC$_2$F$_1$, and BC$_2$F$_2$ populations for percentage fixed recurrent parent with selected individuals indicated for each generation. Three histograms represent individuals within the BC$_1$F$_1$, BC$_2$F$_1$, and BC$_2$F$_2$ populations on the basis of their percentage of homozygous recurrent parent genotype. One selected plant for each generation is indicated by a line at its respective percentage of homozygous recurrent genome on the histogram of the population from which it was selected.**

Fewer QTL were identified in this study than in other studies of first-generation SWCB resistance (Bohn et al., 1997; Groh et al., 1998; and Khairallah et al., 1998). QTL regions with large effects in these studies, such as those identified on c1, were not significant in our population. Using the same enzyme–probe combinations, we found our population was monomorphic for these regions. More enzymes would have perhaps uncovered polymorphism in these areas. Another explanation relates to the level of susceptibility of the parents. CML131, the susceptible parent of the populations used by Bohn et al. (1997) and Groh et al. (1998), is extremely susceptible to SWCB. The recurrent parent used in the current study, CML204 is moderately susceptible and may contain the resistant allele at the QTL on c1.

Considering the QTL regions identified on common chromosomes between the current study and those of Bohn et al. (1997) and Groh et al. (1998), the QTL identified on c9 (bins 9.03-9.04, 9.04, and 9.04-9.05, respectively) can be considered common between the three studies based on their presence within common bins and on loci common to the populations. The QTL on c7 (bins 7.04, 7.03-7.04, and 7.02-7.04, respectively) may also be common between the three studies. Groh et al. (1998) found a QTL for leaf toughness in bin 7.04-7.05 on c7 that could be considered common with the QTL found in this study for SWCB resistance. They also found a QTL for protein content on c9 in bin 9.04 that is common with the previously mentioned QTL on c9. No QTL for SWCB were identified on c10 in either study. However, Bohn et al. (1997) identified a QTL for sugarcane borer at the same c10 locus (*umc155*).

The QTL we identified as conditioning resistance to first-generation SWCB also explain less of the phenotypic variance than some of the previously conducted studies. The three QTL regions identified explained 28% of the phenotypic variance, as compared with the 32% explained by Bohn et al. (1997) and the 52% ex-

plained by Groh et al. (1998) using the same resistant parent. The study by Khairallah et al. (1998) explained 30% of the phenotypic variance using a different resistant parent. The RIL study (Groh et al., 1998) explained more of the phenotypic variance because of the fact that RIL account for additive variation more efficiently than BC families (Luo and Kearsey, 1991).

The QTL were identified with a single season's data used for the trait analysis, which we recognize as being insufficient (Schneider et al., 1997). Our intent was not to rely exclusively on these data but to base selection on the QTL identified in another population that used the same recurrent parent and evaluated the segregating population over several environments (Bohn et al., 1997). Unfortunately, these data were not available in the time frame anticipated and could not be used for selection. QTL for resistance to first generation SWCB were shown to be partly germplasm specific (Groh et al., 1998) so mapping at the level done in this study would have been indicated even if data from other studies had been available at the time. Without additional information on QTL from other studies another season's data would have improved our ability to detect QTL. The concern over the power of QTL detection motivated the decisions to do post facto QTL analysis of the effects of the QTL regions singly and in combination in the relatively uniform genetic background of the MAS lines and to genotype the conventionally selected lines.

Markers representing peaks of QTL were used for selecting two of the three QTL regions instead of flanking markers. The potential for losing the QTL-marker association is greater when flanking markers are not used (Dudley, 1993). The desire to eliminate as much CML67 genotype as possible to produce an agronomically superior line influenced our decision. In retrospect, selection with single markers at QTL peaks was feasible in the BC$_1$F$_1$ generation when linkage blocks were large. Further mapping work could have identified flanking markers closer to the QTL peaks before selection was done on the BC$_2$F$_1$ generation. However, with only RFLP available at the time of the experiment this was not feasible in the time frame between generations. New marker technologies, such as single sequence repeats (SSRs), are faster and could be used to identify flanking markers in a specified region between the first and second round of selection.

The post facto analysis of the MAS lines showed no significance of the QTL on c7 and c10. This could be due to the fact that a single marker was used for the selection of these QTL, therefore, the marker-QTL association may have been lost. What is more probable, however, is that these QTL have relatively small effects, not detected by the analysis because the number of lines limited the power of detection. Moreover, these QTL may represent false or environmentally influenced QTL based on one trial. Resistance to SWCB and other borers is known to be influenced by environment (Groh et al., 1998)

The genotyping of the CS lines was done to determine

if there was similarity in loci where the donor genotype consistently appears between the CS lines and the identified QTL regions. The CS lines could not be chosen for preplanned comparisons of isolated regions as in the post facto analysis of the MAS lines, for this reason no analysis of donor genotype loci in CS lines was attempted. However, the genotypes of the conventionally selected lines do support the QTL mapping in the importance of the region on c9. All of these data indicate that the QTL region on c9 is consistently expressed with a large effect and that the regions on c10 and c7 are less significant.

The significance of the QTL on c9 has now been established in three mapping studies as well as in our post facto analysis. This QTL might be a good candidate for gene isolation and functional genomics work. The effect seen in the post facto analysis was due almost exclusively to the c9 QTL. Whether this region alone will be sufficient to cause an impact in farmers fields would depend greatly on the germplasm into which it was introgressed. If introgressed into an elite line that was at the very susceptible end of the rating scale (near 8 and above) it could provide economic benefit. However, greater benefit could be derived by focusing on the many QTL identified by Groh et al. (1998) that were not significant in this study and including those with the largest and most stable effects.

Comparing the outcome of our study with other MAS studies requires examining several benchmarks of success: (i) recovery of donor parent resistance, (ii) recovery or improvement of recurrent parent genotype or phenotype, and (iii) effectiveness of MAS as compared with CS. The studies of Chen et al. (2000), Sanchez et al. (2000), and Huang et al. (1997) have demonstrated great success in transferring resistance to bacterial blight [caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings et al.] in rice (*Oryza sativa* L.). The greatest difference between these studies and our study was the accuracy with which target regions were identified and the ability to detect marker-gene recombinations because these are qualitative traits. These studies demonstrate the use of MAS to pyramid several bacterial blight genes into a single line; however, the marker-gene associations are facilitated by the fact that these resistance genes are single genes with large effects. The phenotypic evaluation of this resistance also follows qualitative trait patterns with plants being scored as plus or minus resistance. Insect resistance is complicated by the fact that it is a multigenic trait, influenced by environment, and its phenotypic screening is dependent on a progressive and subjective rating scale. The comparison of our study with these bacterial blight studies clarifies the crux of the problem in MAS; accurate identification of QTL position and selecting QTL with relatively large effects determines success or failure of MAS. The use of molecular markers to aid in selection of identified regions as they are introgressed into desired germplasm is straightforward.

The greatest success in the use of molecular markers to improve a quantitatively inherited trait was reported by Stuber (1998). Isolation of sequential segments of the maize genome as near isogenic lines in an unrelated background (Stuber, 1998) may be the key to such success. This approach avoids complicated gene interactions, which are expected in quantitatively controlled traits. Graham et al. (1997), using this approach for fine mapping a single QTL region for heterotic response, found that two dominant QTL for yield were linked in repulsion phase exhibiting a pseudo-overdominance effect. Closely linked positive and negative loci for a trait would generally cancel out, when traditional techniques such as $F_2$:$F_3$ mapping is used with large linkage blocks, and thereby go undetected. Graham et al. (1997) pointed out that QTL are much more complex regions than originally imagined. Quantitative traits are often defined as traits affected by many genes each having a small effect. Stuber's approach of isolating regions in NIL addresses another aspect of quantitative traits which may make identifying individual factors difficult: the fact that trait expression is the combined effect of the interaction of these multiple factors.

Figure 5 helps to illustrate areas where changes in our selection strategy might have enhanced recovery of the recurrent parent genotype. In the $BC_1F_1$ population, it was possible to select above the mean for percent homozygous genome and fulfill the constraint of selecting for the heterozygous donor genotype at the QTL regions because of the large population size and the fact that this population had not been selected previously. On the other hand, the size of the $BC_2F_1$ population was too small to make great progress in recovering the recurrent parent genotype outside the QTL regions. The individuals selected from the population that fulfilled the constraint of the target genotype were below the population mean for percent homozygous recurrent parent genotype. The size of the $BC_2F_2$ population was large, but since it followed two rounds of selection, the variation for percent homozygous recurrent genome was limited. This demonstrates that both population size and previous selection influence the efficiency of marker selection.

Our study demonstrates the statistical equivalence of MAS and CS, similar to the results of Stromberg et al. (1994) and Van Berloo and Stam (1999). However, both MAS and CS produced lines significantly improved for insect resistance over the recurrent parent. This is in contrast to the results of Stromberg et al. (1994) in which neither CS nor MAS produced lines superior to the base population. But, in the current study, neither method produced lines that equaled the resistance of the donor parent. Van Berloo and Stam (1999) produced lines that were earlier than the donor parent, as well as the recurrent parent, using CS and MAS. Estimates by Groh et al. (1998) on the relative efficiency of MAS for SWCB on the basis of the additive variance explained by markers identified and the heritability of resistance to first generation SWCB leaf feeding damage were close to 1 indicating that on the basis of the RIL study, MAS and CS were predicted to be equally effective under the same selection intensity.

Another important comparison of MAS and CS relates to their relative costs. Dreher et al. (2000) com-

pared the costs of MAS and CS for the quality protein maize trait, where markers are available for the *opaque2* locus. This study compared four theoretical selection schemes, two conventional and two marker-assisted that were designed by maize breeders and molecular geneticists at CIMMYT. The economic analysis was based on actual costs at CIMMYT of laboratory and field supplies and labor. Dreher et al. (2000) provide a basis for comparing the costs of the MAS and CS selection for insect resistance. Two major components must be added to estimate costs of selection for insect resistance: the cost of insect infestations and the cost of QTL mapping. Insect rearing is estimated at US$ 0.006 per SWCB larvae (D. Bergvinson, 2000, personal communication) and the infestation and individual plant leaf damage ratings require a larger time commitment from the scientist and a BS level field technician which are the most expensive labor fraction. Therefore an additional US$ 4.23 per row was added to field costs by tripling the labor cost of scientist and technician. These two factors increased field costs for conventional selection to three times that estimated by Dreher et al. (2000) on a per row basis. QTL mapping as we did it would cost US $37 000 assuming the cost per marker from Dreher's study. This was a substantial proportion of the US $70 000 estimated as the cost of the MAS as we did it. This can be compared with US$ 11 000 for the cost of CS. Both these figures ignore the costs of population development and the multilocation trial in which the two methods were compared, as they were common to both methods. Changes in marker technology since this study was initiated have made sequential section of populations possible which lowers the costs of marker selection substantially. Had we genotyped the population for only the markers associated with the QTL regions and then done full genotyping on those that had the desired genotype in the QTL regions, the cost of the MAS would have been US$ 42 000. Had the markers-QTL associations been known without QTL mapping, the costs of the marker selection alone would have been US$ 70. The MAS process alone is not prohibitively costly or difficult for qualitative or previously mapped traits. The accurate identification of QTL position and the cost involved in producing this data as the first step in the MAS process for quantitative traits makes the comparison to CS selection less favorable.

This study is important in that it adds to the limited base of experimental data on MAS. It is a large and inclusive study which incorporates QTL detection, multigeneration selection, and comparison of results with phenotypic selection in the same population. We found that MAS and CS do not differ in their ability to improve lines for first-generation SWCB resistance. The level of precision in QTL mapping that seems to be necessary for making MAS more effective than conventional selection was not achieved. At this point, the effort required for this level of accuracy may be beyond the reach of many researchers. However, molecular methods and our understanding of the genome are constantly improving, so the comparison of these methods will certainly not remain static.

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