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Saturation of two chromosome regions conferring resistance to SCMV with SSR and AFLP markers by targeted BSA

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Abstract Quantitative trait loci (QTLs) and bulked segregant analyses (BSA) identified the major genes *Scmv1* on chromosome 6 and *Scmv2* on chromosome 3, conferring resistance against sugarcane mosaic virus (SCMV) in maize. Both chromosome regions were further enriched for SSR and AFLP markers by targeted bulked segregant analysis (tBSA) in order to identify and map only markers closely linked to either *Scmv1* or *Scmv2*. For identification of markers closely linked to the target genes, symptomless individuals of advanced backcross generations BC5 to BC9 were employed. All AFLP markers, identified by tBSA using 400 *Eco*RI/*Mse*I primer combinations, mapped within both targeted marker intervals. Fourteen SSR and six AFLP markers mapped to the *Scmv1* region. Eleven SSR and 18 AFLP markers were located in the *Scmv2* region. Whereas the linear order of SSR markers and the window size for the *Scmv2* region fitted well with publicly available genetic maps, map distances and window size differed substantially for the *Scmv1* region on chromosome 6. A possible explanation for the observed discrepancies is the presence of two closely linked resistance genes in the *Scmv1* region.

Keywords AFLP · Fine mapping · Maize · SSR · Sugarcane mosaic virus · Targeted BSA

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Introduction

Sugarcane mosaic virus (SCMV) is an important pathogen of maize (*Zea mays* L.) in Europe, causing yield losses in susceptible cultivars (Fuchs and Grüntzig 1995). It is transmitted by aphids in a non-persistent manner. Since the use of insecticides for control of the aphid vectors is ineffective, cultivation of resistant maize varieties is the only way to control SCMV.

Kuntze et al. (1997) identified three dent inbreds (D21, D32 and FAP1360A) displaying complete resistance to SCMV and maize dwarf mosaic virus (MDMV) under both field and greenhouse conditions in early maturing germplasm. Segregation and QTL analyses uncovered two genomic regions on chromosomes 6S and 3L with major effects on SCMV resistance in two independent populations. This led to the assumption of one major resistance gene in each region: *Scmv1* on chromosome 6 and *Scmv2* on chromosome 3 (Melchinger et al. 1998; Xia et al. 1999; Dußle et al. 2000).

Xu et al. (1999) applied 54 AFLP primer combinations in a bulked segregant analysis (BSA) and identified 23 markers clustering in either of both regions on chromosomes 3 and 6, confirming oligogenic inheritance of SCMV resistance in cross F7 × FAP1360A. This was also in agreement with studies demonstrating that BSA can be applied to oligigenic inherited traits (Chagué et al. 1997). Bulked segregant analysis (BSA) has been proven to be very effective for identification of closely linked markers in target regions (Michelmore et al. 1991), especially if combined with AFLPs (Ballvora et al. 1995; Thomas et al. 1995; Cervera et al. 1996). However, if hundreds or thousands of AFLP primer combinations are employed, a large number of markers are identified and must subsequently be mapped to determine their position relative to target genes. Therefore, we applied a new targeted BSA (tBSA) approach to select AFLP markers within a narrow genetic window surrounding the target genes.

The objectives of our study were to: (1) saturate the SCMV resistance regions surrounding the *Scmv1* (chromosome 6) and *Scmv2* (chromosome 3) regions with AFLP and SSR markers, and (2) perform fine-scale mapping of SCMV resistance genes relative to the SSR and AFLP markers.

Materials and methods

Plant materials, SCMV inoculation and scoring

In order to reduce the size of donor segments of symptomless individuals employed in tBSA, advanced backcross generations (BCi, i = 5 to 9) were produced. The early maturing European maize inbreds, FAP1360A, resistant to SCMV, and F7, highly susceptible to SCMV (Kuntze et al. 1997), were crossed to produce the F1 generation and were then backcrossed nine times to F7 with two generations per year. SCMV evaluation was performed during the summer seasons at Hohenheim in 1995, 1996, 1997, 1998 and 1999 in the generations BC1 (backcross 1), BC3, BC5, BC7 and BC9, respectively. BCi individuals without SCMV symptoms were employed to produce BC2, BC4, BC6 and BC8 seeds, which were randomly chosen to produce the next BC generation in the winter nursery. BC6, BC8 and BC9 families were planted and again evaluated during the summer of 2000. In order to identify escapes (i.e. symptomless but genetically susceptible BCi plants) and exclude these false positives from mapping, BCi-S1 families, produced by self-fertilizing symptomless BC6 to BC9 individuals, were planted and inoculated at Hohenheim 2000 (see Table 1). Inbreds F7 and FAP1360A were included as controls in each field trial.

All families were planted in a randomized block design with one-row-plots of 25 plants in two replications. BCi plants as well as BCi-S1 families were artificially inoculated with SCMV at the three- to four-leaf stage twice at a 1-week interval by the air brush technique described by Fuchs et al. (1996). First, scoring of mosaic symptoms was conducted 2 weeks after initial inoculation. Virus symptoms were recorded in weekly intervals at seven dates in years 1995 to 1998. In 1999 and 2000, symptoms were recorded at four dates in 2-week intervals. In addition, BC1 plants were grown in the greenhouse in 1997 to produce plant materials unselected for SCMV resistance. Two BC1 individuals, BC1-1 and BC1-2 were employed for chromosome assignment of AFLP markers putatively linked to SCMV resistance genes.

Leaf collection and DNA extraction

Leaf material was harvested individually at flowering time after SCMV symptoms were fully developed. Harvested leaves were freeze-dried and ground to a powder. DNA extraction was performed according to the CTAB method (Hoisington et al. 1994) with one additional purification step using chloroform/isoamylalcohol to obtain high quality DNA.

SSR analyses

A total of 81 simple-sequence repeat (SSR) markers mapping to the short arm of chromosome 6 (Bin 6.00 and 6.01, http://www.agron.missouri.edu/ssr.html) and near the centromere region of chromosome 3 (Bin 3.04 and 3.05, http://www.agron.missouri.edu/ssr.html) were chosen to screen the parental lines FAP1360A and F7 for polymorphism. SSR markers polymorphic between the parental lines FAP1360A and F7 were employed in assays of the BC5, BC7 and BC9 individuals chosen for tBSA. Sequences of all SSR markers were obtained from the maize database (http://www.agron.missouri.edu/ssr.html) and synthesized by Metabion (München, Germany). PCR amplification and MetaPhor gel-electrophoresis were performed as described by Lübberstedt et al. (1998).

AFLP analyses

We followed the AFLP protocol by Vos et al. (1995) with minor modifications. Genomic DNA (250 ng) was restricted with 2.5 units of *Eco*R1 and *Mse*I at 37 °C for 3 h according to the manufacturers' instructions (Gibco BRL, Life Technologies). After complete digestion, 5 pmol of the *Eco*RI adapter (5′-CTCGTAGACTGCGTACC-3′; 3′-CATCTGACGCATGGTTAA-5′), 50 pmol of the *Mse*I adapter (5′-GACGATGAGTCCTGAG-3′; 3′-TACTCAGGACTCAT-5′) (Zabeau and Vos 1993), 1 unit of T4 DNA ligase and 1 × ligation buffer (Gibco BRL, Life Technologies) were added and the mixture incubated for 2 h at 23 °C. Pre-amplification was performed with *Eco*RI and *Mse*I primers each having one selective nucleotide. The 25-µl pre-amplification reaction was conducted with 10 pmol of *Eco*RI (5′-GACTGCGTACCAATTC+N-3′) and *Mse*I (5′-GATGAGTCCTGAGTAA+N-3′) single-nucleotide primers, 5 µl of 1:10-diluted ligated DNA, 1 unit of *Taq* polymerase, 10 × PCR-buffer and 0.2 mM of dNTPs (Amersham Pharmacia Biotech, Freiburg, Germany). Pre-amplification PCR-cycle profiles were performed as described by Vos et al. (1995) with a final extension cycle at 72 °C. The *Eco*RI primers were end-labeled with γ^{33}P-ATP for the selective amplification of the restricted fragments. Five microliters of a 1:50-diluted pre-amplified DNA was selectively amplified in a 20-µl reaction using 1 pmol of *Eco*RI and 10 pmol of *Mse*I primers with three selective nucleotides, 1 unit of *Taq* DNA polymerase, 10 × PCR buffer and 0.2 mM of dNTPs using the PCR-cycle profile described by Vos et al. (1995). AFLP markers were named according to the standard list for AFLP primer nomenclature (Keygene, The Netherlands, http://wheat.pw.usda.gov/ggpages/keygene/AFLPs.html).

Targeted bulked segregant analysis (tBSA) and mapping

AFLP analyses for tBSA were conducted in four successive steps (2002) (Fig. 1). The symptomless BC7 individual 7R-7 was selected for Step 1 due to short donor regions in both target regions including *Scmv1* and *Scmv2* (Fig.1, Step 1). The resistant parent FAP1360A was included as a control in Step 1. Step 2 was included to analyze these linked AFLP bands with BC1 individuals, known to carry the donor segments for either the chromosome 3 (BC1-1) or the chromosome 6 (BC1-2) region (Fig.1, Step 2). Based on the AFLP and SSR markers identified by BSA (Xu et al. 1999) all donor alleles were present on chromosome 3 but absent on chromosome 6 for BC1-1, and vice versa for BC1-2. Consequently, BC1-1 and BC1-2 allowed assignment of AFLP markers to the *Scmv1* or the *Scmv2*, or none of both regions.

All symptomless BC7 individuals were selected with the flanking SSR markers *phi029* and *phi073* on chromosome 3, and *phi126* and *phi077* on chromosome 6, according to the genetic map of Xu et al. (1999). Due to the iterative process of tBSA, symptomless BC8 and BC9 individuals were evaluated with SSR markers closer linked to the target genes than the SSR markers employed for BC7 individuals. BC9 individuals were selected with the SSR markers *bmc1600* (distal to *Scmv1*), *phi126* (proximal to *Scmv1*) and *bmc1432* (cosegregating with *Scmv1* in the symptomless BC5 individuals) for chromosome 6. The donor region for chromosome 3 was evaluated with SSR markers *bnlg420* (distal to *Scmv2*), *bmc1113, bmc1035* (proximal to *Scmv2*) and *bmc1456* (cosegregating with *Scmv2* in the symptomless BC5 individuals). AFLP bands identified in Step 1 and 2 were analyzed with two different DNA pools for each target region consisting of symptomless BCi individuals (Fig.1, Step 3). Pool A contained symptomless BC5 to BC9 individuals with the donor allele present at the SSR locus closest linked proximal to the target gene but absent at the SSR marker distal to the target gene. Pool B consisted of symptomless BCi individuals lacking the donor allele at the

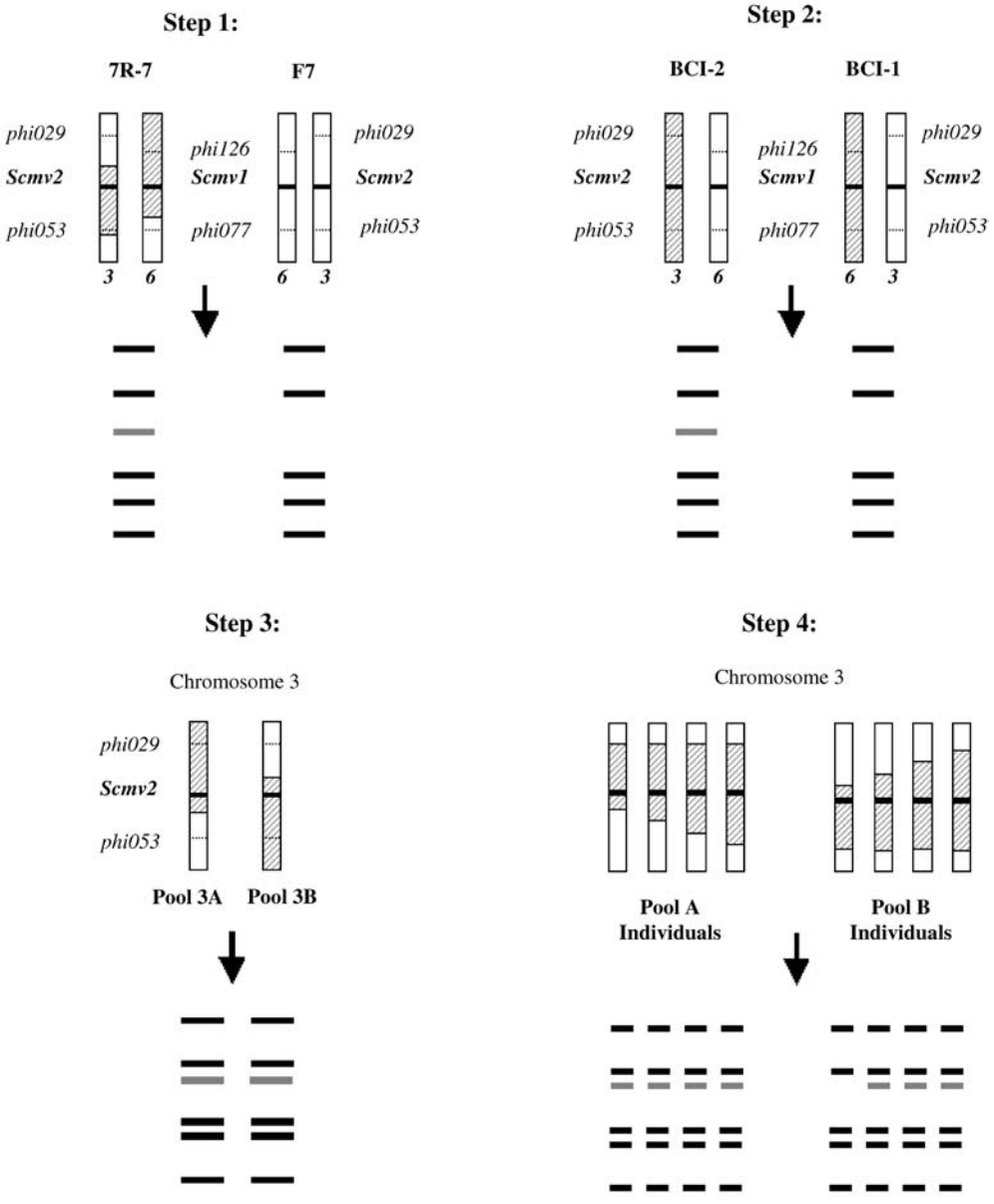

Fig. 1 Steps 1–4 Scheme of the four steps for evaluation and mapping of markers closely linked to *Scmv1* and *Scmv2* applying a modified targeted BSA (t-BSA). **Step 1**: AFLP primer combinations were screened for polymorphism in susceptible parent F7 and the resistant backcross 7 (BC7) individual 7R-7. **Step 2**: assignment of AFLP marker bands present only in individual 7R-7 (step1) to either chromosome 3 or chromosome 6 with genotypes BC1-1 (donor region on chromosome 3) and BC1-2 (donor region on chromosome 6). **Step 3**: identification of AFLP markers closely linked to the resistance genes *Scmv1* and *Scmv2*. Markers with bands present in both pools for the respective chromosome region are of interest and were mapped with the individuals of the respective pools (**Step 4**). *Shaded area* denotes the donor region of the resistant parent in the BC7 individuals pre-selected with SSR markers *phi029, phi053, phi126*, and *phi077*

proximal, but present at the distal SSR marker. Based on the results of the SSR analysis, 4, 10, 10 and 11 symptomless BC5 to BC9 individuals were selected for pools 3A, 3B, 6A and 6B, respectively. AFLP bands present in both pools for one chromosome were expected to map within the interval of both flanking SSR markers employed in Step 3. The individuals of pools 3A and 3B, or pools 6A and 6B, were individually genotyped in Step 4 of the tBSA for mapping (Figs. 2, 3.

Six AFLP markers (E32M62-1, E33M61-2, E38M47-1, E38M47-2, E38M47-3 and E38M47-4) previously mapped on chromosome 3, and three AFLP markers (E33M61-1, E35M62-1 and E38M60-2) previously identified on chromosome 6, in a BC5 population of F7 × FAP1360A (Xu et al. 1999) were mapped with the respective Step-4 individuals in order to join both linkage maps. Vice versa, all markers identified in the present study were additionally mapped in the BC5 population of cross F7 × FAP1360A previously employed by Xu et al. (1999).

Statistical analyses

Fine-scale mapping of SCMV resistance genes was done using the software Cri-Map (Green et al. 1990), taking into account the meiotic interdependence of progenies and ancestors within a population of BC individuals from different generations. Based on the results of segregation analysis (Melchinger et al. 1998), QTL analysis (Dußle et al. 2000) and BSA (Xu et al. 1999), a gene model with two complementary dominant genes was assumed for the present study. Map distances were calculated using the mapping function of Kosambi (1944). Graphical genotyping of individuals of Pool6A and 6B was conducted with GGT software (Van Berloo 1999).

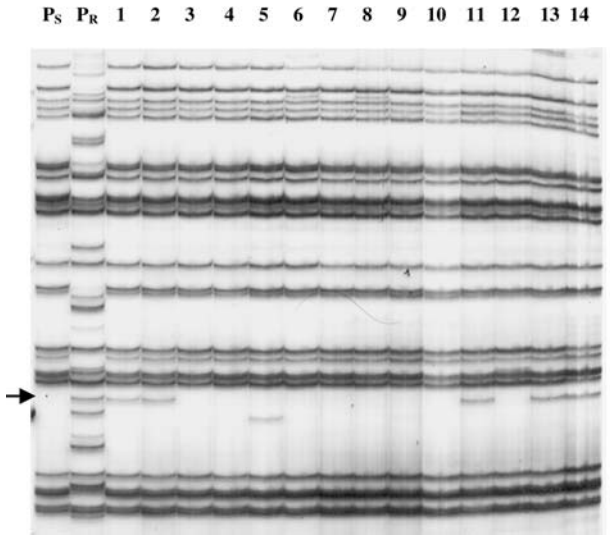

Fig. 2 Analysis of AFLP markers putatively linked to SCMV resistance genes using primer E15M7 (E45M61) with resistant BC5, BC7 and BC9 individuals of pools 3A and 3B. The putatively linked AFLP marker was identified by the first three steps of the tBSA strategy. *Lanes*: P_S susceptible parent FAP1360A, P_R resistant parent F7, *1–4* BC individuals of pool 3A, *5–14* BC individuals of pool 3B

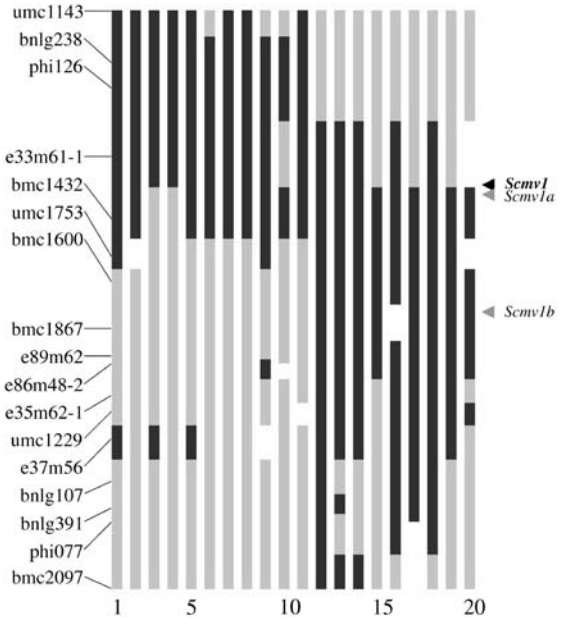

Fig. 3 Graphical genotypes of 11 BC7 to BC9 individuals of Pool 6A (1–11) and 9 BC7 to BC9 individuals of Pool 6B (12–20). *Black* = segments, heterozygous for the resistant parent FAP1360A and the susceptible parent F7, *gray* = segments homozygous for the susceptible parent F7, *white* = unknown segment because of missing marker data at these loci. ◀ = position of *Scmv1* as mapped with CriMap in the BCi population of cross F7 × FAP1360A, ◀ = positions of *Scmv1a* and *Scmv1b* as mapped by QTL analysis of 118 F_3 lines of cross F7 × FAP1360A (Yuan et al. 2002)

Results

Evaluation of SCMV resistance in field trials

The infection level of the susceptible parent F7 in 1998 (100% susceptible individuals) and 2000 (100% susceptible individuals) was higher than in 1999 (95% susceptible individuals). In 2000, an average of 1.8% of the resistant FAP1360 individuals were infected, whereas in 1995 to 1999 all plants of the resistant parent FAP1360A remained symptomless. In addition, the 27 segregating BC6 families of symptomless BC5 individuals, evaluated in 1998 and 1999, showed a smaller proportion of symptomless individuals in 1998 (2%) than in 1999 (8%).

The mean proportion of symptomless individuals within a segregating BC family was 10.4%, calculated over BC generations BC6 to BC9, and ranged from 6.9% to 15.4% (Table 1). The proportion of symptomless individuals within the 17 segregating BC7-S1 families was 40.0%, varying from 32.0% to 47.8% (Table 1). Eight of the 80 BC6-S1, BC8-S1 and BC9-S1 families were completely SCMV susceptible in 2000 (Table 1). In addition, 13 BC6 families of symptomless BC5 individuals employed for mapping by Xu et al. (1999) were completely susceptible, as well as BC8 families from two symptomless BC7 genotypes. Consequently, these symptomless BCi individuals with fully susceptible BCi or BCi-S1 progenies were excluded for Steps 3 and 4 of tBSA.

Identification of polymorphic SSR markers linked to SCMV resistance genes

Out of 81 SSR markers (41 SSRs for chromosome 3, 40 SSRs for chromosome 6) screened for the susceptible parent F7 and the resistant parent FAP1360A, 25 SSR markers were polymorphic: *bmc1432, bmc1433, bmc1600, bmc1867, bmc2097, bnlg107, bnlg161, bnlg238, bnlg391, phi077, phi126, umc1143, umc1229* and *umc1753*, on the short arm of chromosome 6; *bmc1035, bmc1113, bmc1456, bmc1638, bnlg420, phi053, umc1025, umc1030, umc1102, umc1300* and *umc1351*, from the centromere region of chromosome 3. These SSR markers were included in the genetic map (Fig. 4).

Identification of AFLP markers putatively linked to SCMV resistance genes

About 12% of the 456 AFLP primer combinations did not amplify any band in one of the two individuals analyzed in Step 1 because of technical problems such as primer labeling. These 56 AFLP primer combinations were not repeated. On average, 36 AFLP bands were reliably scored for each of the other 400 AFLP primer combinations. In total, 5,600 AFLP bands were polymorphic between the resistant parent FAP1360A and the susceptible parent F7. A total of 3,008 polymorphic bands

Table 1 Different backcross (BCi) and self-fertilized backcross (BCi-S1) families of cross F7 × FAP1360A evaluated for sugarcane mosaic virus (SCMV) disease symptoms during the summer seasons 1997 to 2000

Year	Generation	Number of symptomless ancestor individuals	Number of planted families	Number of segregating families	Proportion of segregating families (%)	Total number symptomless of individuals	Mean proportion of symptomless individuals within a segregating family (%)
1997	BC5	7 BC3	75[a]	20	26.6	40	8.0
1998	BC7	13 BC5	130[a]	15	11.5	26	6.9
1999	BC6	13 BC5	37[b]	26	70.2	79	12.1
	BC8	4 BC7	13[b]	11	84.0	26	9.5
	BC9	4 BC7	99[a]	27	27.2	104	15.4
					43.5[c]		10.4[d]
1999	BC7-S1	13 BC5	17[b,e]	17	100.0	174	40.0
2000	BC6-S1	13 BC5	37[b]	34	91.9	252	29.6
	BC8-S1	4 BC7	8[b]	6	75.0	100	66.0
	BC9-S1	4 BC7	35[b]	32	91.4	231	28.9
					89.6[c]		41.1[d]

[a] Families of unselected BC individuals
[b] Families of symptomless BC individuals
[c] Weighted average of segregating families
[d] Weighted average of symptomless individuals within a segregating family
[e] No of seedlings available for nine BC7-S1 families tracing back to a symptomless BC7 individual

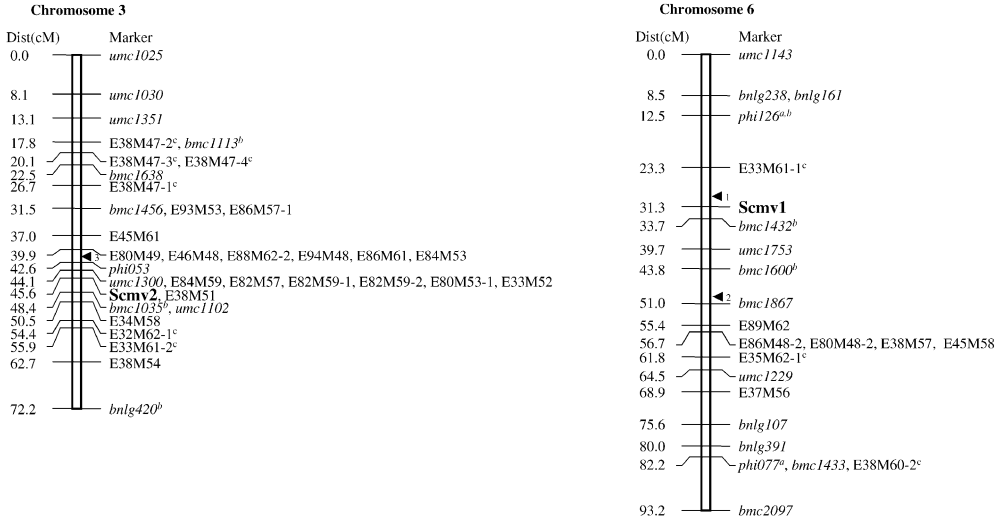

Fig. 4 High-resolution maps of chromosome regions harboring the SCMV resistance genes *Scmv1* and *Scmv2*. *Left*: centomere region of chromosome 3 harboring *Scmv2*. *Right*: short arm of chromosome 6 harboring *Scmv1*. The maps were generated by joint linkage analyses of the BC1, BC5, BC7, and BC9 populations of cross FAP1360A × F7 with 25 SSR and 34 AFLP markers. SSR markers are indicated by *lowercase italics*. a = SSR markers used for preselection of symptomless BC7 individuals, b = SSR markers used for preselection of symptomless BC9 *individuals*, c = AFLP markers previously mapped with 27 symptomless BC5 individuals (Xu et al. 1999). *1, 2, 3* = *Scmv1a, Scmv1b* and *Scmv2* identified by QTL analysis (Yuan et al. 2002)

originated from the susceptible parent F7 and 2,592 bands originated from the resistant parent FAP1360A.

In Step 1 of the tBSA, 49 of 400 AFLP primer combinations identified 56 marker bands present in the symptomless BC7 plant 7R-7, but were absent in the susceptible parent F7. Fifteen of the 56 polymorphic AFLP bands, revealed bands in both BC1 individuals BC1-1 and BC1-2 for Step 2 of the tBSA. Out of the remaining 41 AFLP bands, polymorphic between BC1-1 and BC1-2, 15 were present only in BC1-1 (donor region on chromosome 6) and 26 only in BC1-2 (donor region on chromosome 3). For chromosome 3, 18 AFLP bands were identified in pool 3A as well as in pool 3B, whereas eight AFLP bands were present in only one of the two pools (Table 2). For chromosome 6, six AFLP markers were present in both pools 6A and 6B. Nine AFLP bands were identified in either pool 6A or 6B. In total, 24 AFLP bands and 13 SSR markers were found to be located within the flanking SSR marker intervals employed by tBSA (Fig. 4).

Fine-scale mapping of *Scmv1* and *Scmv2* with SSR and AFLP markers

All markers identified with the BCi individuals of cross F7 × FAP1360A for the chromosome-6 region mapped

Table 2 AFLP primer combinations mapped in step 4 of the tBSA, identifying marker bands tightly linked to either *Scmv1* (chromosome 6) or *Scmv2* (chromosome 3)

Chromosome 3			Chromosome 6		
Marker	*Eco*RI[a]	*Mse*I[a]	Marker	*Eco*RI[a]	*Mse*I[a]
E38M51	ACT	CCA	E45M58	ATG	CGT
E33M52	AAG	CCC	E38M57	ACT	CGG
E94M53	TTG	CCG	E80M48	TAC	CAC
E82M57	TAT	CGG	E86M48	TCT	CAC
E84M59	TCC	CTA	E89M62	TGG	CTT
E82M59	TAT	CTA	E37M56	ACG	CGC
E34M58	AAT	CGT			
E80M49	TAC	CAG			
E94M48	TTT	CAC			
E46M48	ATT	CAC			
E45M61	ATG	CTG			
E84M53	TCC	CCG			
E86M61	TCT	CTG			
E88M62	TGC	CTT			
E86M57	TCT	CGG			
E38M54	ACT	CCT			
E80M53	TAC	CCG			

[a] Selective bases of the respective *Eco*RI and *Mse*I AFLP primers

in an interval of 93.2 cM flanked by the SSR markers *umc2097* and *umc1143* on the short arm of chromosome 6. All newly identified AFLP markers were located within the interval of the markers, *phi077* and *phi126*, employed for pre-selection of symptomless BC7 individuals (Fig. 4). SSR markers *bnlg238* and *umc1229* restricted the donor region on chromosome 6 of BC7 individual 7R-7. Between both SSR markers, all SSR markers revealed the marker allele of the resistant parent. Markers assigned for the chromosome-3 region mapped in a 72.2-cM window between SSR markers *umc1025* and *bnlg420* near the centromere region of chromosome 3. The AFLP markers identified for the chromosome-3 region mapped into a window of 31.2 cM between the preselected markers *bnlg420* and *bmc1113*.

Discussion

Selection of AFLP markers by tBSA

About 3% of the 2,592 polymorphic bands originating from FAP1360A were expected to map to the target regions, given a total genetic map length of the maize genome of about 1,600 cM (Helentjaris et al. 1988; Gardiner et al. 1993) and about 30 cM between SSR markers *umc1030* and *umc1102* (chromosome 3) and 15 cM between *umc1143* and *umc1229* (chromosome 6) for the donor regions, according to the intermated B73 × Mo17 (IBM) map (http://www.cafnr.missouri.edu/mmp/ibm-maps.htm). However, only 41 AFLP markers (1.6%) were located at either of both target regions in Step 2 of tBSA. One explanation is an inflated ratio of genetic versus physical map distance due to an increased level of recombination in both genome regions, which would result in the identification of a lower number of physically equidistant distributed AFLP markers. Gill et al. (1996) and Künzel et al. (2000), by detailed physical and genetic mapping studies of grass genomes supported the hypothesis that much of the meiotic recombination occurs in genes and most recombination events are restricted to few chromosome regions containing gene clusters. Putative resistance gene clusters harboring *Scmv1* and *Scmv2* might be gene-rich regions with increased recombination rates in the direct neighborhood of the centromere.

Another explanation is an unequal distribution of *Eco*RI/*Mse*I AFLP markers across the maize genome (Castiglioni et al. 1999; Vuylsteke et al. 1999). Only a small number of *Eco*RI/*Mse*I AFLP markers mapped to the respective regions above *umc102* on chromosome 3 and above *phi077* on chromosome 6 in two maize populations (Vuylsteke et al. 1999). This suggests an underrepresentation of *Eco*RI/*Mse*I AFLP markers in the SCMV target regions. For comparison, out of 1,753 SSRs mapped in total (http://www.agron.missouri.edu/ssr.html), 131 SSRs mapped to Bin regions 3.04/05 and 6.00/01, harboring *Scmv2* and *Scmv1*, respectively. Therefore, about 7% of all SSR markers mapped to about 5% of the total genetic map (http://www.agron.missouri.edu/maps.html). Consequently, the AFLP markers analyzed in the present study seem to be underrepresented in the *Scmv1* and *Scmv2* regions compared to the SSR markers.

Fifteen out of 2,600 polymorphic AFLP markers (0.6%) were present in both BC1 individuals of Step 2. Five of these bands were identified in the resistant bulk but not in the susceptible bulk and are, therefore, associated with SCMV resistance. These markers might be linked to a third locus apart from *Scmv1* or *Scmv2*, not detected by Xu et al. (1999) because of the lower number of markers screened in their BSA study. Alternatively, these bands might be located on donor segments close to *Scmv1* or *Scmv2* shared by both Step-2 individuals. However, this is unlikely, because both BC1-1 and BC1-2 have been genotyped with 31 AFLP and SSR markers in the *Scmv1* and the *Scmv2* region prior to their selection as diagnostic genotypes. The other ten bands seem not to be involved in SCMV resistance. Since, on average, 0.4% of the genome derive from the donor in BC7 10, out of 2,600 might be attributable to residual heterozygosity.

By tBSA, fewer markers linked to the target genes were identified in Step 1 compared to conventional BSA (Xu et al. 1999). This was expected due to the short donor regions of the BC7 individual 7R-7 employed for marker identification in Step 1. Xu et al. (1999) identified 23 AFLP markers linked with either *Scmv1* or *Scmv2* analyzing 54 AFLP primer combinations. In contrast, only 24 markers with 400 primer combinations were uncovered in the present study. Whereas the bulks employed by Xu et al. (1999) spanned the whole region between distant SSR markers, the individual 7R-7 covered only about 43% of these donor regions. As shown in Fig. 3, the segments of pools 6A and 6B were overlap-

ping between markers E33M62 and *umc1753*, which spanned only 16.4 cM (17%) of the whole donor region analyzed by Xu et al. (1999). Pools 3A and B overlapped in a region of 19 cM (data not shown) and, therefore, only 26% of the donor region was analyzed by Xu et al. (1999). Consequently, only 1/5 of the number of markers identified by Xu et al. (1999) were expected in the present study. However, the number of 24 AFLP markers identified in the present study is 25% lower than expected. Compared to the study of Xu et al. (1999), who found up to four bands linked to the SCMV resistance genes for 7 of 11 primer combinations, only one primer combination revealed more than two specific bands in the present study. For BSA only those primer combinations were selected amplifying high quality results (Dr. Xu, personal communication), as recommended by the manufacturer (http://www.lifetech.com/Content/TechOnline/molecular_biology/manuals.pps). The primers of the present study were randomly chosen, which might explain the differences in the quantity of amplified bands with a single primer combination.

Comparing the number of 56 AFLP markers identified in Step 1 of tBSA to the number of 24 AFLP markers assigned to a genetic window around the target genes in Step 3, the number of markers, mapped individually in Step 4, were reduced by 60%. Since all AFLP markers identified by tBSA were mapped within the pre-selected SSR marker intervals, tBSA seems to enrich markers in the target regions while substantially reducing the subsequent mapping effort.

Comparison of linkage maps

A comparison of SSR marker distances in the present study and the inter-mated B73 × Mo17 map (IBM map, http://www.cafnr.missouri.edu/mmp/ibmmaps.htm) revealed a good fit of the SSR marker orders on chromosome 3. Map distances between SSR markers *umc1030* and *umc1102* were 39 cM (IBM) and 40.3 cM (Fig. 4). The order and map distances of SSR markers employed in tBSA were consistent with results of previous studies including an independent population of cross F7 × FAP1360A used for QTL mapping (Xu et al. 1999; Yuan et al. 2002). Therefore, the estimates of map positions and the distances of AFLPs in addition to SSR markers seem to be reliable for the chromosome-3 region.

In contrast, map distances between SSR markers *bnlg161* and *umc1229* on chromosome 6 spanned 13 cM (IBM) versus 56 cM in the present study, and 31 cM in the study of Yuan et al. (2002). Moreover, the genetic distance between SSR markers *bmc1432* and *umc1229* was three-times larger in the present study compared to the QTL study conducted for the same cross (Yuan et al. 2002). In addition, the distance between SSR markers *umc1143* and *bnlg161* spanned 2 cM in the IBM map, 8.5 cM in the present study and 24.5 cM in the QTL analysis of Yuan et al. (2002). Although marker orders were consistent for the *Scmv1* region, map distances seem to be overestimated in the present study. Alternatively, because the QTL (Yuan et al. 2002) and the tBSA-map spanned a similar map size, marker distances of the IBM map might be underestimated.

A possibility for the larger distances, at least in some subregions of chromosome 6, could be the presence of more than one SCMV resistance gene in the *Scmv1* region. This would also explain the lack of clustering of AFLP markers identified in Steps 3 and 4 close to *Scmv1*. Yuan et al. (2002), in a companion QTL analysis of cross F7 × FAP1360A, identified two QTLs in the *Scmv1* region, *Scmv1a* and *Scmv1b*, at four out of seven scoring dates. Assuming two QTLs were located in the target region on chromosome 6, both QTLs could: (1) interact complementary, i.e. both QTLs are simultaneously required to confer SCMV resistance, or (2) act redundantly, and each QTL confers a sufficient degree of resistance. In the first case, selection for symptomless individuals would act against single recombinations (but favor double cross-overs) between both resistance genes over BC generations. As a consequence, map distances would tend to be underestimated due to the lack of recombinants between resistance genes.

In the case of two linked QTLs, where one QTL is sufficient for resistance expression, symptomless BCi individuals applied in Step 3 of tBSA would carry only one of both flanking SSR markers, and either one or both linked QTLs. Hence, the donor regions of some bulk 6A and 6B individuals would not overlap and, therefore, complicate the mapping of the hypothetical *Scmv1* gene in Step 4. Because Cri-Map is not able to dissect two linked QTLs, instead of a single postulated *Scmv1* gene, markers being recombinant between both linked QTLs would be mapped incorrectly and most-likely map distances would be inflated.

QTL analysis uncovered overdominant gene action for *Scmv1a*, whereas *Scmv1b* showed additive gene action (Yuan et al. 2002). Therefore, *Scmv1a* confers a higher degree of resistance in BCi individuals than *Scmv1b*. The resistance allele of *Scvm1a* should be present in Step-3 individuals with a higher likelihood than *Scmv1b*. In contrast, *Scmv1b* should only be present in some of the individuals. As shown in Fig. 3 all individuals of pools 6A and 6B harbored the small overlapping region around *Scmv1a* between AFLP marker E33M61-1 and SSR marker *bmc1432*, explaining the low number of markers found in the *Scmv1a* region. Additionally, all 317 symptomless BC5 to BC9 individuals carried the short overlapping region for *Scmv1a*. Therefore, the presence of only one SCMV resistance gene on chromosome 6 and selection of the *Scmv1b* region by chance cannot be ruled out entirely. In contrast, only individuals of pool 6B should harbor the resistance marker alleles of the *Scmv1b* region. However, three pool-A individuals, which should not carry the *Scmv1b* region based on the selection of SSR markers, harbored the donor allele for AFLP marker E37M56 distal to *Scmv1b* due to a double-crossover. This might explain the clustering of AFLP markers in this region. Two of these three individuals

trace back to the same BC5 individual. Clear evidence of whether the *Scmv1b* region is present by selection or by chance is not possible because of the small number of individuals applied for tBSA. For 25% of 317 symptomless BC7 to BC9 individuals analyzed with the flanking SSR markers, the donor region below SSR marker *bmc1600* was missing. Although *Scmv1b* seems to be redundant for SCMV resistance, individuals carrying this donor region are likely to have favored expression of resistance to SCMV compared to individuals lacking this donor region. Consequently, clustering of at least two SCMV resistance genes on chromosome 6 seems to be very likely.

Assessment of the gene model chosen for tBSA

Based on earlier studies (Melchinger et al. 1998; Xu et al. 1999; Dußle et al. 2000), the gene model underlying the SCMV resistance in cross F7 × FAP1360A was assumed to involve two dominant, independently inherited, complementary acting resistance genes, *Scmv1* and *Scmv2*. According to this gene model a proportion of the 25% segregating families, as well as symptomless individuals within the segregating families, was expected for all BCi generations. The proportion of segregating families in generations BC7 and BC8 fitted well with the expected values. In contrast, in the case of two linked redundant genes on chromosome 6 and one complementary resistance gene on chromosome 3, the proportion of resistant individuals within a segregating BCi family is expected to be 37%. However, proportions of symptomless plants within and among segregating families in all other generations was generally below 25%. These results could be due to the threshold character of the SCMV resistance, incomplete penetrance, environmental effects or due to selection of an incorrect genetic model. Melchinger et al. (1998) found a varying proportion of susceptible individuals in the heterozygous F1 generation of cross F7 × FAP1360A under field and greenhouse conditions. In the present study, six BC6 families with no symptomless individuals in 1998 showed a mean proportion of 22% symptomless individuals in 1999. Furthermore, no symptomless individuals were found in the BC8 families tracing back to symptomless BC7 individuals 7R-15 and 7R-16. In contrast, self-fertilization of 7R-15 and 7R-16 individuals revealed segregating BC7-S1 families with 40% symptomless individuals. These results indicated that incomplete penetrance affects heterozygous more than homozygous individuals. Therefore, evaluation of the progenies of the symptomless individuals is essential to assure the presence of the target regions and exclude false-positive individuals from further analysis. However, misclassification of symptomless BCi individuals in years with a high infection level seems to be very unlikely, because heterozygous individuals are much more affected by incomplete penetrance than homozygous ones. In conclusion, incomplete penetrance or environmental effects explain well the small proportion of symptomless individuals within the BCi and BCi-S1 generations and obscures the derivation of a genetic model for inheritance of SCMV resistance from segregation data alone.

The question of whether (1) the *Scmv1* region contains only one or more resistance genes against SCMV, and (2) the *Scmv1* and the *Scmv2* regions each harbor only a single locus or clusters of resistance loci against different viruses and other pathogens, can only be solved by cloning of these genes. Cloning of the *Scmv1* region has been complicated because of the putative presence of two resistance genes in this region and the resulting difficulties in mapping the markers closely linked to one of the two resistance genes in that target region. Identification of recombinants between both QTLs is necessary to analyze them independently. In contrast, markers identified for the *Scmv2* region seem to be suitable for MAS and map-based cloning.

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