Aus dem Institut
für Pflanzenzüchtung, Saatgutforschung und Populationsgenetik
der Universität Hohenheim
Fachgebiet: Angewandte Genetik und Pflanzenzüchtung
Prof. Dr. A.E. Melchinger

DEVELOPMENT AND FINE MAPPING OF MARKERS CLOSELY LINKED TO THE SCMV RESISTANCE LOCI *SCMV1* AND *SCMV2* IN EUROPEAN MAIZE (*Zea mays* L.)

Dissertation
zur Erlangung des Grades eines Doktors
der Agrarwissenschaften
der Fakultät III – Agrarwissenschaften I –
Pflanzenproduktion und Landschaftsökologie
der Universität Hohenheim

von
Diplom-Agrarbiologin
Christina M. Dußle
aus Cleebronn

2002

Die vorliegende Arbeit wurde am 12. Juni 2002 von der Fakultät III - Agrarwissenschaften I - (Pflanzenproduktion und Landschaftsökologie) der Universität Hohenheim als „Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften (Dr. sc. agr.)“ angenommen.

Tag der mündlichen Prüfung:	22. Juli 2002
Dekan:	Prof. Dr. K. Hurle (i.V.)
Berichterstatter, 1. Prüfer:	Prof. Dr. A.E. Melchinger
Mitberichterstatter, 2. Prüfer:	Prof. Dr. A.J.P. Pfitzner
3. Prüfer:	Prof. Dr. R. Blaich

Contents

[1]Dußle CM, Melchinger AE, Kuntze L, Stork A, and Lübberstedt T (2000) Molecular mapping and gene action of *Scm1* and *Scm2*, two major QTL contributing to SCMV resistance in maize. Plant Breeding 119: 299-303

[2]Yuan L, Dußle CM, Melchinger AE, Utz, HF, and Lübberstedt T (2003) Clustering of QTL conferring SCMV resistance in maize. Maydica: In press

[3]Dußle CM, Quint M, Xu ML, Melchinger AE, and Lübberstedt T (2003) Saturation of two chromosome regions conferring resistance to SCMV with SSR and AFLP markers by targeted BSA. Theor Appl Genet 106: 485-493

[4]Dußle CM, Quint M, Xu ML, Melchinger AE, and Lübberstedt T (2002) Conversion of AFLP fragments tightly linked to *Scmv1* and *Scmv2* into simple PCR-based markers. Theor Appl Genet 105: 1190-1195

[5]M Quint, Mihaljevic R, Dußle CM, Xu ML, Melchinger AE, and Lübberstedt T (2002) Development of RGA-CAPS markers and genetic mapping of candidate genes for SCMV resistance in maize. Theor Appl Genet 105: 355-363

ABBREVIATIONS

AFLP	amplified fragment length polymorphism
ANOVA	analysis of variance
BC	backcross
bp	base pair
BSA	bulked segregant analysis
CAPS	cleaved amplified polymorphic sequence
cDNA	complementary DNA
CIM	composite interval mapping
cM	centiMorgan
CTAB	Hexadecyltrimethylammonium bromide
DNA	deoxyribonucleic acid
HPV	high plains virus
indel	insertion/deletion
JGMV	Johnson grass mosaic virus
LOD	$\log_{10}$ odds ratio
LRR	leucine-rich repeat
M	mole
MAS	marker-assisted selection
MDMV	maize dwarf mosaic virus
min	minute
MMV	maize mosaic virus
MSV	maize streak virus
NBS	nucleotide-binding site
PCR	polymerase chain reaction
QTL	quantitative trait locus (*or loci depending on the context*)
QTL E	QTL environment
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RGA	resistance gene analogue(s)
SCAR	sequence characterized amplified region
SCMV	sugarcane mosaic virus
SNP	single nucleotide polymorphisms
SSR	simple sequence repeat
STS	sequenced tagged site
tBSA	targeted bulked segregant analysis
TPIB	tissue print immunoblotting
WSMV	wheat streak mosaic virus

1. General Introduction

Resistance to sugarcane mosaic virus

Sugarcane mosaic virus (SCMV) is one of the most important virus diseases in European maize. It can cause serious yield losses in susceptible cultivars (Fuchs et al. 1995). SCMV and the closely related maize dwarf mosaic virus (MDMV) have been found in some regions of Germany since the 1980s (Fuchs et al. 1984). Whereas MDMV is a widespread viral disease in the southern U.S. Corn Belt (Louie et al. 1991), SCMV is more prevalent than MDMV in Germany (Fuchs et al. 1996). Diagnostic symptoms for both SCMV and MDMV include stunting (Fig. 1.1.), chlorosis (Fig. 1.2.), reduction in plant weight, and therefore, a reduction in yield (Fuchs et al. 1995).

Fig. 1.1. SCMV infection in European maize. The susceptible plants in the foreground show reduction of plant height, compared to the resistant plants in the background.

Fig. 1.2. SCMV infected maize leafs with different levels of mosaic symptoms. Infection level increases from left to right.

SCMV was formerly denoted as a strain of MDMV, MDMV-B (Shukla et al. 1989). SCMV as well as MDMV belong together with wheat streak mosaic virus (WSMV), Johnson grass mosaic virus (JGMV) and sorghum mosaic virus (SrMV) to a group of related pathogenic potyviruses in maize (Kuntze et al. 1995). Chemical control of SCMV is not possible because of the non-persistent mode of virus transmission by aphids. Hence, the most efficient method to control SCMV infections is the cultivation of resistant maize varieties.

Resistance to SCMV, MDMV and WSMV with U.S. germplasm was investigated in a number of studies. Inbred Pa405 showed complete resistance to MDMV and SCMV inoculation under both field and greenhouse conditions (Louie R. et al. 1991). Roane et al. (1977) concluded that resistance to MDMV in maize inbred Oh7B is controlled by a single dominant gene. Rosenkrantz et al. (1987) reported five genes in Pa405 causing resistance to MDMV. Mikel et al. (1984) identified three genes in Pa405, where one gene is essential with either of the other two for complete resistance to a mixture of MDMV and SCMV. One to three major genes in Pa405 causing resistance to MDMV strains A, B, D, E, and F were reported by Findley et al. (1984). Louie et al. (1991) confirmed a single dominant gene conferring resistance to all five strains of MDMV.

Restriction fragment length polymorphism (RFLP) analysis mapped a major gene, *Mdm1*, near the centromere of chromosome 6 that causes resistance to MDMV in Pa405 (McMullen et al. 1989). *Mdm1* is perfectly cosegregating with the nucleolus organizer region (*nor*) and closely linked to the RFLP marker *csu70* (0.2 cM) in maize (Simcox et al. 1995).

Kuntze et al. (1997) screened 122 early-maturing European maize inbreds under both greenhouse and field conditions. Three lines (D21, D32 and FAP1360A) displayed complete resistance and four lines (D06, D09, R2306 and FAP1396A) displayed partial resistance to SCMV and MDMV. Field trials and segregation analysis uncovered one to three genes involved in resistance to SCMV in different crosses (Melchinger et al. 1998). Two major genes, *Scmv1* on the short arm of chromosome 6 and *Scmv2* near the centromere of chromosome, were mapped by Melchinger et al. (1998). QTL analysis confirmed the two major resistance genes (*Scmv1, Scmv2*) and uncovered three minor QTL affecting SCMV resistance on chromosomes 1, 5, and 10 in cross D32 (resistant) D145 (susceptible) (Xia et al. 1999). Both major resistance genes are essential for expression of complete resistance to SCMV. Whereas *Scmv1* suppresses the expression of SCMV symptoms during all stages of infection, *Scmv2* was expressed in later stages of plant development (Xia et al. 1999). Due to the relatively small numbers of genes, SCMV resistance is employed as a model for oligogenic inherited disease resistances. In the present study, the gene action of *Scmv1* and *Scmv2* was investigated in a different population than employed by the previous authors.

Resistance gene analogues

One strategy towards the identification of resistance genes is the application of conserved homologous sequences within resistance genes. Genes conferring resistance to the major classes of plant pathogens, including bacteria, fungi, nematodes and virus, have been isolated from different plant species. Sequence comparisons among these genes have revealed significant similarities in general structure, and conservation of specific domains that participate in protein-protein interactions and signal transduction. The majority of plant disease resistance genes contain nucleotide-binding sites (NBS) and a leucine-rich repeat (LRR) domain (Seah et al. 1998).

A sensitive and efficient method to identify and characterise resistance gene analogues (RGAs) is the polymerase chain reaction (PCR) amplification using degenerate primers based on conserved motifs of NBS-LRR genes (Yu et al. 1996). This approach has been successfully used in the monocotyledon species maize, wheat, barley and rice (Collins et al. 1998, Seah et al. 1998, Leister et al. 1999, Mago et al. 1999) and in dicotyledonous species such as potato, lettuce and *Brassica napus* (Leister et al. 1996, Meyers et al. 1998, Joyeux et al. 1999). In several studies, mapped RGAs showed close linkage to disease resistance loci indicating that this approach can contribute to the identification of candidate resistance genes in different species and can provide starting points for cloning strategies. Eleven classes of non-cross-hybridising sequences with amino acid identity to known NBS-LRR resistance proteins were amplified in maize by Collins et al. (1998). Four of them mapped to the potential resistance gene clusters on chromosomes 6S and 3L, containing as well SCMV resistance genes *Scmv1* and *Scmv2.*

Bulked segregant analysis

A major obstacle in elucidating the biochemical basis of disease resistance in plants is the lack of cloned resistance genes (Simcox et al. 1995). This deficiency was overcome with the cloning of plant disease resistance genes by the use of methods like transposon tagging and chromosome walking (Johal et al. 1992, Martin et al. 1993). Cloning a gene by chromosome walking for example requires a high-resolution map in the region around the gene of interest (Martin et al. 1993). Furthermore, recovery of individuals with crossovers between the target locus and flanking molecular markers is a prerequisite for determining the direction and physical distance to be crossed during a chromosome walk (Simcox et al. 1995). Genetic studies of traits with complex inheritance require for their mapping the determination of genotypes at several hundred polymorphic loci in several hundred individuals. Because only a minority of markers are expected to show linkage and association for a particular trait, a simple screen of genetic markers to identify those showing linkage in pooled DNA samples can greatly facilitate gene identification.

To obviate the analysis of thousands of individuals for mapping, Arnheim et al. (1985) first suggested the idea of using pooled DNA in the context of case-control studies in human genetics. These authors argued that alleles in linkage desequilibrum with a disease would be enriched or deficient in a pooled sample of affected individuals in comparison with a pooled control sample. Thus, beyond testing association for specific alleles, this principle was used to search for associated alleles at specific genes, as they successfully did for *HLA* class II DR and DQ alleles in insulin dependent diabetes mellitus (IDDM) (Arnheim et al. 1985). In plant genetics, the first method using samples of pooled DNA for identification of markers in trait associated genome regions was invented by Michelmore et al. (1991) and named bulked segregant analysis (BSA). This method involves comparing two pooled DNA samples of individuals from a segregating population originating from a single cross. The individuals within each pool were identical for the trait of interest, but arbitrary for all other genes. For example, one pool contains resistant plants, whereas the other pool contains plants susceptible to a particular disease. Employing molecular markers with these two pools reveals markers that are polymorphic between both pools and, therefore, linked to the target region harboring the gene of interest (Michelmore et al. 1991).

BSA was initially proposed for screening qualitative traits known to express variation at a single locus of large effect (Giovannoni et al. 1991, Michelmore et al. 1991). However, the simplicity and low cost of BSA have led to its use for more complex traits (Mackay et al. 2000, Chagué et al. 1997). This method depends on the marker type and the segregating population employed. For a dominant trait like SCMV, a BC population combined with amplified restriction length polymorphism (AFLP) markers seems to be the most efficient way to find markers tightly linked to the resistance genes (Mackay et al. 2000). AFLP markers generate in a single reaction many more polymorphic bands than any other PCR-based markers such as simple sequence repeat (SSR) or random amplified polymorphic DNA (RAPD) markers.

The availability of molecular markers flanking the SCMV resistance genes *Scmv1* and *Scmv2* provides a very efficient tool for rapid introgression into maize breeding germplasm by marker-assisted selection (MAS). Therefore, Xu et al. (1999) constructed a genetic linkage map for the SCMV resistance regions on chromosomes 6S and 3L harbouring the resistance genes *Scmv1* and *Scmv2* by means of BSA with 27

symptomless BC individuals of cross (F7 FAP1360A) F7. The authors mapped 12 AFLP markers to the *Scmv1* and 11 AFLP markers to the *Scmv2* region. Based on these results, the regions on chromosomes 3 and 6 were enriched in this study with further AFLP and SSR markers to construct a high-resolution map for these resistance gene regions. As a tool for marker enrichment in the target regions, a modified targeted BSA (tBSA) was developed (Lübberstedt et al. 2002). This method reduces the experimental effort in analysing large numbers of putatively linked markers and enables the selection of closely linked markers without analysing all individuals of the mapping population.

Marker conversion

The use of AFLP markers for genetic map construction in plants has accelerated genome analysis and genetic improvement. However, it is difficult to employ the AFLP technique directly in MAS and map-based gene cloning due to its high cost and complicated procedure (Xu et al. 2001). Therefore, conversion of AFLP markers into sequence-specific PCR-based primers is essential to expand the usefulness of this technique.

Different molecular marker types have been successfully converted into sequence-specific PCR based sequenced tagged site (STS) markers such as sequence characterized amplified regions (SCARs) and cleaved amplified polymorphic sequences (CAPS) (Adam-Blondon et al. 1994, McDermott et al. 1994, Horvath et al. 1995, Barret et al. 1998, Bradshaw Jr et al. 1994, Cheung et al. 1997, Jung et al. 1999). The converted CAPS and SCAR markers are very reliable, less expensive, easy to apply, and, therefore a helpful tool in large-scale and locus-specific applications like MAS and map-based cloning (Bradeen et al. 1998, Shan et al. 1999). Conversion of AFLP markers has also been attempted (Shan et al. 1999, Bradeen et al. 1998, Qu et al. 1998, Schwarz et al. 1999, Xu et al. 2001). Polymorphisms revealed by AFLPs include single nucleotide polymorphisms (SNPs), insertion and deletions (indels), and microsatellites (Bradeen et al. 1998, Wei et al. 1999). However, AFLP marker conversion turned out to be more difficult than conversion of any other marker type. Many converted AFLP markers lost their sequence-specificity or even their ability in amplification of genomic DNA (Shan et al. 1999). The reasons regarding the difficulties of conversion remain so

far unknown. In this study two dominant AFLP markers, closely linked to resistance genes *Scmv1* and *Scmv2*, were converted into an indel and a CAPS marker.

For map-based cloning and MAS thousands of individuals have to be screened with molecular markers. Hence, easy manageable molecular markers converted of AFLP markers can accelerate both procedures. Cloning of the SCMV resistance genes implies detailed information about the gene action and the localization of the target genes. Therefore, molecular markers linked with the target genes and a comprehensive linkage map are essential tools towards the identification of the genes conferring resistance to SCMV.

The objectives of this study were to

(1) determine precisely the location of *Scmv1* and *Scmv2* on chromosomes 3 and 6 in cross F7 FAP1360A,
(2) determine the gene action of the alleles present at these loci,
(3) enrich the SCMV resistance regions covering *Scmv1* (chromosome 6) and *Scmv2* (chromosome 3) with AFLP and SSR markers employing the tBSA,
(4) evaluate RGAs as potential candidate genes for *Scmv1* and *Scmv2*, and
(5) convert AFLP markers into codominant, simple PCR-based markers as a tool for marker-assisted selection and for map-based cloning of *Scmv1* and *Scmv2*.

References

Adam-Blondon AF, Sévignac M, Bannerot H, Dron M (1994) SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theor Appl Genet 88: 865-870

Arnheim N, Strange C, Erlich H (1985) Use of pooled DNA samples to detect linkage desequilibrum of polymorphic restriction fragments and human disease: Studies of *HLA* clas II loci. Proc Natl Acad Sci USA 82: 6970-6974

Barret P, Delourme R, Foisset N, Renard M (1998) Development of a SCAR (sequence characterised amplified region) marker for molecular tagging of the dwarf BREIZH (*Bzh*) gene in *Brassica napus* L. Theor Appl Genet 97: 828-833

Bradeen JM, Simon PW (1998) Conversion of an AFLP fragment linked to the carrot Y_2 locus to a simple, codominant PCR-based marker form. Theor Appl Genet 97:

960-967

Bradshaw Jr HD, Villar M, Watson BD, Otto KG, Stewart S, Stettler RF (1994) Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. Theor Appl Genet 89: 167-178

Chagué V, Mercier JC, Guénard M, de Courcel A, Vedel F (1997) Identification of RAPD markers linked to a locus involved in quantitative resistance to TYLCV in tomato by bulked segregant analysis. Theor Appl Genet 95: 671-677

Cheung WY, Champagne G, Hubert N, Landry BS (1997) Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. Theor Appl Genet 94: 569-582

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor A (1998) The isolation and mapping of disease resistance gene analogs in maize. Mol Plant-Microb Interact 11: 968-978

Findley WR, Louie R, Knoke JK (1984) Breeding corn for resistance to corn viruses in Ohio. *In* Wilkinson DJ (ed) Proc. Annual Corn Sorghum Res. Conf. Am. Seed Trade Assoc., Washington D.C. 52-67

Fuchs E, Grüntzig M (1995) Influence of sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) on the growth and yield of two maize varieties. J Plant Dis Protect 102: 44-50

Fuchs E, Grüntzig M, Kuntze L, Oertel U, Hohmann F (1996) Zur Epidemologie der Potyviren des Maises in Deutschland. In Bericht über die 46. Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter im Rahmen der "Vereinigung österreichischer Pflanzenzüchter" 46: 43-49

Fuchs E, Kozelska S (1984) Zum Auftreten und zur Schadwirkung des Europäischen Mais Mosaik Virus (EMMV) in der DDR, sowie Ergebnisse der Prüfung der Anfälligkeit von Inzuchtlinien und Hybriden. Nachrichtenbl Pflanzenschutz DDR 38: 162-164

Giovannoni JJ, Wing RA, Ganal MW, Tanksley SD (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res 19: 6553-6558

Horvath DP, Dahleen LS, Stebbing J-A, Penner G (1995) A co-dominant PCR-based marker for assisted selection of durable stem rust resistance in barley. Crop Sci 35:

1445-1450

Johal GS, Briggs SP (1992) Reductase activity encoded by the HM1 disease resistance gene in maize. Science 258: 985-987

Joyeux A, Fortin MG, Mayerhofer R, Good AG (1999) Genetic mapping of plant disease resistance gene homologues using a minimal *Brassica napus* L. population. Genome 42: 735-743

Jung G, Skroch PW, Nienhuis J, Coyne DP, Arnaud-Santana E, Ariyarathne HM, Marita JM (1999) Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. Crop Sci 39: 1448-1455

Kuntze L, Fuchs E, Grüntzig M, Schulz B, Hennig U, Hohmann F, Melchinger AE (1995) Evaluation of maize inbred lines for resistance to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Agronomie 15: 463-467

Kuntze L, Fuchs E, Grüntzig M, Schulz B, Klein D, Melchinger AE (1997) Resistance of early-maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breeding 116: 499-501

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat Genet 14: 421-429

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Graner A, Schulze-Lefert P (1999) RFLP- and physical mapping of resistance gene homologues in rice (*O.sativa*) and barley (*H. vulgare*). Theor Appl Genet 98: 509-520

Louie R, Findley WR, Knoke JK, McMullen MD (1991) Genetic basis of resistance in maize to five maize dwarf mosaic virus strains. Crop Sci 31: 14-18

Lübberstedt T, Dußle CM, Xu ML, Melchinger AE (2002) Targeted bulked segregant analysis (tBSA) - a strategy for identification of markers closely linked to a resistance gene. Plant Breeding: to be submitted

Mackay IJ, Caligari PDS (2000) Efficiencies of F2 and backcross generations for bulked segregant analysis using dominant markers. Crop Sci 40: 626-630

Mago R, Nair S, Mohan M (1999) Resistance gene analogues from rice: cloning, sequencing and mapping. Theor Appl Genet 99: 50-57

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu

T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:

McDermott JM, Brändle U, Dutly F, Haemmerli UA, Keller S, Müller KE, Wolfe MS (1994) Genetic variation in powdery mildew of barley: Development of Rapid, SCAR, and VNTR Markers. Phythopathology 84: 1316-1321

McMullen MD, Louie R (1989) The linkage of molecular markers to a gene controlling the symptom response in maize dwarf mosaic virus strains. Mol Plant-Microb Interact. 6: 309-314

Melchinger AE, Kuntze L, Gumber RK, Lübberstedt T, Fuchs E (1998) Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor Appl Genet 96: 1151-1161

Meyers BC, Shen KA, Rohani P, Gaut BS, Michelmore RW (1998) Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell 11: 1833-1846

Michelmore R, Paran I, Kesseli R (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828-9832

Mikel MA, D´Arcy CJ, Rhodes AM, Ford RE (1984) Genetics of resistance of two dent corn inbreds to maize dwarf mosaic virus and transfer of resistance into sweet corn. Phytopathology 74: 467-473

Qu L-J, Foote TN, Roberts MA, Money TA, Aragón-Alcaide L, Snape JW, Moore G (1998) A simple PCR-based method for scoring the ph1b deletion in wheat. Theor Appl Genet 96: 371-375

Roane CW, Genter CF, Tolin SA (1977) Inheritance of resistance to maize dwarf mosaic virus in maize (Abstr.). Proc Am Phytopathol Soc 4: 140

Rosenkrantz E, Scott GE (1987) Type of gene action in the resistance to maize chlorotic dwarf virus in corn. Phytopathology 77: 1293-1296

Schwarz G, Michalek W, Mohler V, Wenzel G, Jahoor A (1999) Chromosome landing at the *Mla* locus in barley (*Hordeum vulgare* L.) by means of high-resolution mapping with AFLP markers. Theor Appl Genet 98: 521-530

Seah S, Sivasithamparam K, Karakousis A, Lagudah ES (1998) Cloning and

characterisation of a family of disease resistance gene analogs from wheat and barley. Theor Appl Genet 97: 937-945

Shan X, Blake TK, Talbert LE (1999) Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. Theor Appl Genet 98: 1072-1078

Shukla DD, Tosic M, Jilka J, Ford RE, Toler RW, Langham MAC (1989) Taxonomy of potyviruses infecting maize, sorghum, and sugarcane in Australia and the United States as determined by reactivitites of polyclonal antibodies directed towards virus-specific N-termini of coat proteins. Phytopathology 79: 223-229

Simcox KD, McMullen MD, Louie R (1995) Co-segregation of the maize dwarf mosaic virus resistance gene, *Mdm1*, with the nucleolus organizer region in maize. Theor Appl Genet 90: 341-346

Xia XC, Melchinger AE, Kuntze L, Lübberstedt T (1999) QTL mapping of resistance to sugarcane mosaic virus in maize. Phythopathology 89: 660-667

Xu M, Huaracha E, Korban SS (2001) Development of sequence-characterized amplified regions (SCARs) from amplified fragment length polymorphism (AFLP) markers tightly linked to the *Vf* gene in apple. Genome 63-70

Xu ML, Melchinger AE, Xia XC, Lübberstedt T (1999) High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Mol Gen Genet 261: 574-581

Yu YG, Buss GR, Maroof MAS (1996) Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93: 11751-11756

Plant Breeding 119, 299—303 (2000)

ISSN 0179-9541

Molecular mapping and gene action of *Scm1* and *Scm2*, two major QTL contributing to SCMV resistance in maize

C. M. DUBLE[1], A. E. MELCHINGER[1], L. KUNTZE[1], A. STORK[1] and T. LÜBBERSTEDT[1,2]

[1] Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, D-70593 Stuttgart, Germany; [2] Corresponding author

With 1 figure and 2 tables

Received November 25, 1999/Accepted February 21, 2000
Communicated by C. Jung

Abstract

Sugarcane mosaic virus (SCMV) is one of the most important virus diseases of maize in Europe. In this study, the gene action at two major quantitative trait loci (QTL) affecting resistance to SCMV in maize was mapped and characterized. A total of 121 F_3 lines from cross F7 (susceptible) × FAP1360A (resistant) were evaluated for SCMV resistance in replicated field trials across two environments under artificial inoculation at seven scoring dates. The genotypic variance was always highly significant and heritability increased up to 0.92 for later scoring dates. The method of composite interval mapping was employed for QTL mapping using four simple sequence repeat (SSR) markers flanking two regions identified in a previous study with cross D145 × D32. The presence of two QTL for SCMV resistance, one on chromosome 6 (*Scm*1 region) and one on chromosome 3 (*Scm*2 region), was confirmed. These two QTL together explained between 15% (first score) and 62% (final score) of the phenotypic variance at various stages of plant development. Gene action was additive for the *Scm*2 region but completely dominant for the *Scm*1 region. Comparison of results of this study with those obtained for cross D145 × D32 suggested that the resistance alleles in the two populations are identical for the *Scm*1 region but different for the *Scm*2 region.

Key words: *Zea mays* — QTL mapping — sugarcane mosaic virus — SCMV resistance — SSR markers

Sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) are important potyviruses causing significant losses in grain and forage yield in susceptible genotypes of maize and related crops (Fuchs and Grüntzig 1995). Owing to the non-persistent transmission of both viruses, control of aphid vectors by chemical means is not effective. Hence, cultivation of resistant maize varieties is the most efficient method of virus control. Inheritance of resistance to different strains of SCMV and MDMV, as well as wheat streak mosaic potyvirus (WSMV), has been intensively studied for US maize inbred line Pa405 (Louie et al. 1991, McMullen et al. 1994, Simcox et al. 1995). Segregation analyses indicated the presence of one to five genes conferring resistance to SCMV, MDMV and WSMV in Pa405. One dominant major gene (*Mdm*1) was mapped with restriction fragment length polymorphism (RFLP) markers on chromosome arm 6S (McMullen and Louie 1991) and was shown to cosegregate with the nucleolus organizer region (*nor*) (Simcox et al. 1995). In addition, McMullen et al. (1994) described three genes involved in resistance to WSMV in Pa405 located on chromosomes 6 (*Wsm*1), 3 (*Wsm*2), and 10 (*Wsm*3).

Kuntze et al. (1997) screened early-maturing European germplasm for resistance to SCMV and MDMV and identified three dent inbreds (D21, D32 and FAP1360A) with complete resistance under both field and greenhouse conditions. Segregation analyses suggested the presence of one to three SCMV resistance genes strongly affected by different environmental conditions (Melchinger et al. 1998a). Two major genes (*Scm*1 and *Scm*2) conferring resistance to SCMV were mapped to chromosome arms 6S and 3L, respectively, in cross D145 × D32 by QTL analysis (Xia et al. 1999) and in cross F7 × FAP1360A by bulked segregant analysis (BSA) (Xu et al. 1999). Except for a preliminary study based on high-density fingerprinting of a greater number of maize inbred lines (Xu et al. 2000), no information exists on whether the resistance genes found in these populations in the *Scm*1 and *Scm*2 regions are identical or whether they represent different alleles at the same loci or members of gene clusters present in these regions. While the chromosomal location is known for both populations, gene action was determined only for D145 × D32 (Xia et al. 1999). In the present study, a QTL approach was applied to map the SCMV resistance genes segregating in the cross F7 × FAP1360A and analysed their gene action. Our objectives were to (1) confirm the location of *Scm*1 and *Scm*2 on chromosomes 6 and 3 in this cross and (2) determine the gene action of the alleles present at these loci.

Materials and Methods

Plant materials: The two early-maturing European inbred lines F7 (flint) and FAP1360A (dent) of *Zea mays* L. were crossed and the F_1 and F_2 generations were subsequently selfed to produce a random set of 121 F_3 lines. Line FAP1360A is completely resistant to SCMV, whereas line F7 is highly susceptible (Kuntze et al. 1997).

Artificial inoculation: Virus inoculum for testing resistance against the SCMV isolate 'Seehausen' was prepared as described by Fuchs and Grüntzig (1995). Young leaves with typical mosaic symptoms of the SCMV-infected maize variety 'Bermasil' were homogenized using five volumes of a 0.01 M phosphate buffer at pH 7.0. Carborundum was added to the sap. During sap preparation and mechanical inoculation, the inoculum was kept at 4°C. Plants at the three- to four-leaf stage were mechanically inoculated twice at weekly intervals by an air-brush technique using a tractor-mounted air-compressor at a constant pressure of 800 kPa (Fuchs et al. 1996).

Agronomic trials: The parental F_2 plants of the 121 F_3 lines were evaluated in 1995 for resistance to SCMV at Hohenheim, Germany. The 121 F_3 lines from cross F7 × FAP1360A plus two entries of the F_1 hybrid, three entries of the F_2 generation, two entries of the BC_1 generation to the susceptible parent F7 and one entry for each parent were evaluated

in 1996 for resistance to SCMV at Eckartsweier and Hohenheim. The 130 entries were tested in a 13 × 10 α-lattice design (Patterson and Williams 1976).

Plots consisted of single rows, 0.7 m apart and 3 m long, which were overplanted and later thinned to a final density of 9 plants/m² with a total of 20 plants per row. Resistance to SCMV was visually scored 2 weeks after initial inoculation for the first time. The number of plants with virus symptoms was recorded at weekly intervals on seven dates (VIR1 to VIR7) at each location. The number of infected plants was converted into a percentage of plants with disease symptoms.

Leaf collection and DNA extraction: Leaves were harvested from 16 plants of each F_3 line grown in the greenhouse. Equal amounts of leaf material from each plant per line were bulked for DNA extraction to determine the marker genotype of the parental F_2 plant. Harvested leaves were freeze-dried and ground to powder. DNA extraction was performed according to the cetyltrimethylammonium bromide (CTAB) method (Hoisington et al. 1994).

SSR analyses: Four microsatellite (SSR) markers were employed to genotype a random subset of 118 parental F_2 plants of the 121 F_3 lines: *phi*029 and *phi*053 located on chromosome 3, and *phi*126 and *phi*077 located on chromosome 6S, flanking the *Scm*1 and *Scm*2 regions, respectively. The distance between the flanking markers determined for cross F7 × FAP1360A was 11.6 cM for chromosome 6 and 28.7 cM for chromosome 3 (Xu et al. 1999). Sequences of these primers were obtained from the maize data base (http://teosinte.agron.missouri.edu/Coop/SSR-Probes/SSR1-htm) and synthesized by Amersham Pharmacia Biotech (Freiburg, Germany). Polymerase chain reaction (PCR) amplification and MetaPhor gel (FMC, Rochland, USA) electrophoresis were performed according to Lübberstedt et al. (1998).

Segregation and linkage analyses: Segregation at each marker locus was checked for deviations from Mendelian segregation ratios (1 : 2 : 1) and allele frequency 0.5 by χ^2 tests (Weir 1990). A linkage analysis of the SSR markers was conducted with software package MAPMAKER 3.0b (Lander et al. 1987) using Haldane's mapping function. The threshold used for declaring linkage was set to a LOD value of 3.0 and a maximum distance of 50 cM.

Statistical analyses: The biometric analyses of phenotypic data (VIR1 to VIR7) and QTL mapping were performed as described in detail by Xia et al. (1999) without further data transformation. Briefly, adjusted entry means and effective error mean squares were used to compute combined analyses of variance (ANOVA) across environments. The sums of squares for entries were subdivided into orthogonal contrasts (1) between both parent lines F7 and FAP1360A, (2) between the means of both parents ($\bar{P}$) and the mean of the 121 F_3 lines ($\bar{F}_3$), (3) among the means of generations F_1, F_2 and BC_1, and (4) the variation among the 121 F_3 lines. A corresponding subdivision was conducted on the entry × environment interaction sums of squares.

Estimates of variance components σ^2_g (genotypic variance), σ^2_{ge} (genotype × environment interaction variance), and σ^2 (error variance) of F_3 lines and their standard errors were calculated as described by Searle (1971, p. 415). Heritabilities (h^2), on an entry-mean basis, were estimated as described by Hallauer and Miranda (1981, p. 90):

$$\hat{h}^2 = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \hat{\sigma}^2_{ge}/e + \hat{\sigma}^2/re}$$

where r = number of replications and e = number of environments. Exact 90% confidence intervals of $\hat{h}^2$ were calculated according to Knapp et al. (1985). Phenotypic correlations among scoring dates were calculated by applying standard procedures.

Composite interval mapping (CIM) (Zeng 1994) was employed for mapping of QTL and estimation of their effects as described by Bohn et al. (1997). A LOD threshold of 3.0 was chosen for declaring a putative QTL significant. For the analysis of data from each environment and also for the joint analyses across environments, cofactors were selected by stepwise regression. Final selection was for the model that minimized Akaike's information criterion with penalty = 3.0 (Jansen 1993). QTL positions were determined at the local maxima of the LOD ($\log_{10}$ odds ratio) plot curve in the regions under consideration. The proportion of the phenotypic variance ($\hat{\sigma}^2_p$) explained by a single QTL was obtained from the square of the partial correlation coefficient (R^2) (Melchinger et al. 1998b). Estimates of R^2 values and gene effects at QTL as well as tests for QTL × environment (QTL × E) and digenic epistatic interactions among QTL were obtained by fitting a model that included all QTL for the respective trait simultaneously (Melchinger et al. 1998b). All computations were performed with software package PLABQTL (Utz and Melchinger 1996).

Results

Phenotypic data

Both parents differed significantly ($P < 0.01$) in their SCMV resistance at both environments; no infected plant was found for FAP1360A while the percentage of infected plants of parent F7 at the seventh scoring reached 100% at Eckartsweier and 99% at Hohenheim. The mean proportion of infected plants across all 121 F_3 lines averaged across both environments increased from 3.4% at the first scoring date (VIR1) to 32.9% at the final scoring date (VIR7) (Table 1). As reflected by the mean proportion of ($\bar{x}$) infected plants, the disease onset (VIR1) started at a higher level at Hohenheim than at Eckartsweier ($\bar{x} = 0.1\%$) This difference prevailed until the final scoring date (VIR7) with $\bar{x} = 37.1\%$ at Hohenheim and $\bar{x} = 28.55\%$ at Eckartsweier. The F_2 and F_3 generation did not differ significantly in the mean proportion of infected plants. No infected F_2 plant could be identified from VIR1 to VIR3, but their mean proportion increased to 5.2% until VIR7. The phenotypic correlation of F_3 line means between both environments ranged from 0.4 (VIR1) to 0.8 (VIR7), demonstrating a similar ranking of infected F_3 lines at both environments for the final scoring date. No significant ($P < 0.05$) difference existed between the means of both parents ($\bar{P}$) and the mean of all 121 F_3 lines ($\bar{F}_3$) for VIR7.

Genotypic variances $\hat{\sigma}^2_g$ for SCMV resistance were highly significant ($P < 0.01$) for scoring dates VIR2 to VIR7 (Table 1). Estimates of $\hat{\sigma}^2_{ge}$ were significant only for VIR1, VIR2 and VIR7 and for later scoring dates (≥ VIR3) considerably smaller than $\hat{\sigma}^2_g$. Heritabilities for VIR2 to VIR7 ranged from 0.42 to 0.92.

Segregation of SSRs

All four SSR markers showed a codominant segregation in agreement with the expected ratio, 1 : 2 : 1. Allele frequencies did not deviate significantly ($P < 0.05$) from 0.5 at any marker locus. The estimated map distance determined from genotypes of parental F_2 plants of the 118 F_3 lines was 18.4 cM for markers *phi*029 and *phi*053 on chromosome 3 and 30.7 cM for markers *phi*126 and *phi*077 on chromosome 6.

QTL analyses

With the four codominant SSR markers, the presence of two QTL on chromosomes 6 and 3 for SCMV resistance in the population F7 × FAP1360A was confirmed. The largest QTL on chromosome 6S was detected at all scoring dates; it explained between 16 and 54% of $\hat{\sigma}^2_g$ for VIR1 to VIR7. The QTL on chromosome 6 showed significant additive (a) and dominance (d) effects, with a decreasing degree of dominance (ratio d : a) from VIR1 (1.7) to VIR7 (0.94). The QTL on chromosome 3 was identified only at the final scoring date (VIR7); it explained

Table 1: Estimates of means, variance components, and heritabilities for resistance to sugarcane mosaic virus (SCMV) in 121 F_3 lines from maize population F7 × FAP1360A measured in two environments

Parameter	Trait[1] VIR1	VIR2	VIR3	VIR5	VIR7
Means					
F_7	5.0 ± 3.2[2]	17.5 ± 6.3	55.3 ± 11.5	78.0 ± 9.6	99.5 ± 3.2
FAP1360A	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
F_1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 1.3	5.2 ± 2.9
F_2	2.2 ± 1.3	7.0 ± 2.0	10.0 ± 1.7	20.3 ± 2.1	28.0 ± 3.1
F_3	3.4 ± 0.3	5.6 ± 0.5	11.8 ± 0.9	22.5 ± 1.6	32.9 ± 2.2
BC_1	1.7 ± 1.1	7.4 ± 2.4	20.7 ± 4.3	41.4 ± 5.7	49.6 ± 5.3
Variance components					
$\hat{\sigma}^2_g$	−0.4 ± 2.7	12.4 ± 4.4**	81.4 ± 14.1**	294.1 ± 42.0**	533.5 ± 74.4**
$\hat{\sigma}^2_{gy}$	9.9 ± 3.5**	14.2 ± 4.9**	12.5 ± 7.8	7.3 ± 10.5	29.2 ± 13.1**
$\hat{\sigma}^2$	15.0 ± 1.5	20.7 ± 2.0	39.9 ± 3.9	58.5 ± 5.7	61.6 ± 6.0
Heritability (F_3 lines)					
$\hat{h}^2$	n.c.	0.42	0.76	0.89	0.92
90% C.I. on $\hat{h}^2$[3]	n.c.	(0.16, 0.59)	(0.65, 0.83)	(0.86, 0.93)	(0.88, 0.95)

** Significant at P = 0.01.
[1] Scoring of SCMV symptoms at the first, second, third, fifth and seventh date are denoted VIR1, VIR2, VIR3, VIR5 and VIR7, respectively.
[2] Standard errors are attached.
[3] Confidence intervals of $\hat{h}^2$ were calculated by the method of Knapp et al. (1985).
n.c. = not calculated, because $\hat{\sigma}^2_g$ was negative.

Table 2: Parameters associated with putative quantitative trait loci (QTL) significantly affecting resistance to SCMV in 118 F_3 lines of maize population F7 × FAP1360A

Chromosome	Position	Parameter	Trait (%) VIR1	VIR2	VIR3	VIR5	VIR7
3	8–10[2]	a					−11.2**
		d					−1.6
		R^2					15.0
6	14–18	a	−2.4**	−4.8**	−11.9**	−23.4**	−33.6**
		d	−4.1**	−7.8**	−15.9**	−24.2**	−31.1**
		R^2	16.1	28.5	43.6	54.5	55.5
Total[1]		R^2	16.1	28.5	43.6	54.5	64.3

** Significant at P = 0.01.
[1] Estimates of total R^2 obtained by a simultaneous fit of the two QTL affecting the respective trait using a model with additive and dominance effects.
[2] Chromosomal position of the QTL calculated for VIR1 to VIR7.

15% of $\hat{\sigma}^2_g$ and showed only a significant additive effect. At both loci the QTL alleles conferring resistance to SCMV were contributed by the resistant parent FAP1360A. Neither significant (P < 0.05) epistatic nor QTL × E interactions were detected for both QTL on chromosomes 3 and 6 for all scoring dates.

Discussion

Comparison of the *Scm1* and *Scm2* region in different populations

In a companion study with 220 F_3 lines derived from cross D32 (completely resistant) × D145 (highly susceptible), Xia et al. (1999) found major effects on SCMV resistance for the *Scm*1 region on chromosome 6S and the *Scm*2 region on chromosome 3L. Furthermore, the additive gene effects were four times larger for the *Scm*1 region than for the *Scm*2 region at VIR7. Xu et al. (1999) identified two major genes (*Scm*1 and *Scm*2) in a BC_5 population of (F7 × FAP1360A) backcrossed five times to the susceptible parent, F7. With regard to resistance breeding as well as cloning of genes, there are two important questions: (1) Is the same number of resistance genes segregating in both crosses? (2) Are the alleles segregating at the QTL in the *Scm*1 and *Scm*2 region identical?

A major difference between our results of cross F7 × FAP1360A and those of Xia et al. (1999) for cross D145 × D32 is the twofold higher mean infection level of F_3 lines at VIR7 in the latter study, which was even more pronounced at earlier scoring dates (VIR4: 7% vs. 49%) (Fig. 1). Furthermore no F_3 line showed complete resistance to SCMV at VIR7 in D145 × D32 compared with 5% in the present study. These different infection levels could be due to environmental effects, because only one environment, Eckartsweier 1996, was common in both studies. However, a comparison of results from the common environment confirmed the different infection levels for both populations presented in Fig. 1. Hence, it seems safe to conclude that the lower infection level in cross F7 × FAP1360A compared with cross D145 × D32 is attributable to genetic differences.

A possible explanation for these findings is a smaller number of segregating resistance genes in population F7 × FAP1360A due to the presence of fixed resistance genes in the susceptible parent F7. Surprisingly, it was possible to detect *Scm*2 by bulked segregant analysis, although the effect of the *Scm*2 region was much smaller compared with the *Scm*1 region (Xu et al. 1999). However, from the ratio (R^2/h^2), which yields an

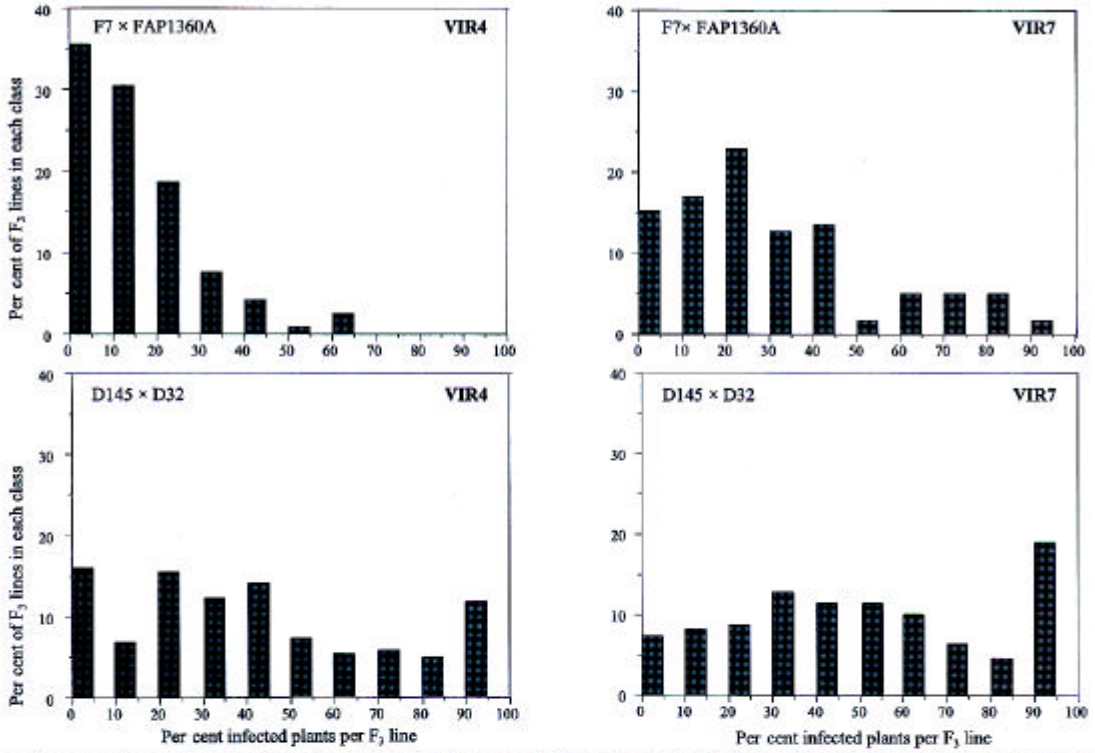

Fig. 1: Histogram for resistance to SCMV of F_3 lines of cross F7 × FAP1360A measured 1996 at Hohenheim and Eckartsweier, and cross D145 × D32, measured 1996 at Eckartsweier and 1997 at Hohenheim (Xia et al. 1999)

estimate of the total genetic variance explained by the QTL in the model (Melchinger 1997), one can conclude that the two QTL found in the present study explain only about 70% of the total genetic variance for VIR7. Therefore, in addition to the two QTL in the *Scm*1 and *Scm*2 region, it is likely that further QTL with small effects segregate in cross F7 × FAP1360A. This is in close agreement with the corresponding estimates reported by Xia et al. (1999), who identified three minor QTL on chromosomes 1, 5 and 10 in addition to the two major QTL in the *Scm*1 and *Scm*2 region.

Comparison of the *Scm1* region in both crosses

The *Scm*1 region had a much larger effect on virus resistance at all scoring dates than the *Scm*2 region in cross F7 × FAP1360A, as well as in cross D145 × D32. However, gene effect of the *Scm*1 region was partial dominance in cross D145 × D32 and complete dominance in cross F7 × FAP1360A. This is most likely attributable to the influence of the different alleles in the susceptible parents F7 and D145, because D32 and FAP1360A probably carry the same resistance alleles according to the results of the high-density fingerprinting approach in this region (Xu et al. 2000). A further explanation could be epistatic effects between *Scm*1 and other genomic regions influencing virus resistance. In conclusion, the general mode of inheritance of genes at the *Scm*1 region appears to be similar for both populations and an indication of similar identity.

Comparison of the *Scm2* region in both crosses

The other major QTL (*Scm*2) was detected for all scoring dates except VIR1 by Xia et al. (1999) and displayed increasing R^2 values and partial dominance for resistance to SCMV. The effect of the *Scm*2 region was much smaller than for the *Scm*1 region in both populations D32 × D145 (Xia et al. 1999) and F7 × FAP1360A. Xu et al. (2000) suggested the presence of different resistant loci in the *Scm*2 region of the three European inbred lines FAP1360A, D21 and D32. The fact that it could be detected at a later stage of plant development for cross F7 × FAP1360A corroborates that the resistance genes of the *Scm*2 region are not identical for the populations D145 × D32 and F7 × FAP1360A. A clustering of resistance genes in the *Scm*2 region has also been reported by Xu et al. (2000). However, identity of *Scm*2 in both populations cannot be ruled out completely.

Joint action of *Scm1* and *Scm2*

The discrepancies between the distances of the flanking SSR markers in the analysis of Xu et al. (1999) and our results could be due to the different sizes of the mapping populations. Whereas Xu et al. (1999) mapped 40 resistant BC_5 plants, the mapping population in the present study included 118 F_3 lines. Furthermore, the higher degree of homozygosity in the BC_5 plants could influence the recombination frequency in meiosis, so that the marker distances in the F_3 lines seemed to be larger than in the BC5 population.

Xu et al. (1999) suggested that *Scm*1 is not sufficient for

resistance without additional resistance genes. Also, Quint et al. (1999) suggested in a comparison of the recombination frequencies of cross F7 × FAP1360A and D21 × D408 that further resistance genes are required for complete resistance to SCMV. In the present study, no epistatic effects were found between the two major QTL detected. In contrast, significant epistatic effects were identified between the major QTL *Scm*1 and *Scm*2 for all scoring dates except VIR1 in population D145 × D32 (Xia et al. 1999). The results of the present study support the hypothesis that the genes in the *Scm*1 region are sufficient for resistance in earlier stages of plant development, whereas the gene in the *Scm*2 region is expressed only at a later stage of plant development and contributed a higher degree of resistance in population D145 × D32 and F7 × FAP1360A. A similar kind of resistance to MDMV was described by Mikel et al. (1984). The segregation of resistance genes to MDMV fitted a two-gene model at an earlier and a three-complementary-gene model at a later stage of plant development.

Based on a literature survey, resistance genes to different pathogens are often clustered in the same chromosome regions of the maize genome (McMullen and Simcox 1995). It is unknown whether resistance genes cluster due to linkage or whether some of them are identical and display pleiotropy. In support of closely linked but different major resistance genes, some susceptible plants were found in a test of allelism between the three European dent inbreds D21, D32 and FAP1360A (Lübberstedt et al. 1999). Only cloning of these genes can resolve the question of whether the *Scm*1 and the *Scm*2 regions each harbour only a single locus or clusters of resistance loci and whether the genes present at these loci in the resistant inbreds D32 and FAP1360A are identical or not. Further research is underway to accomplish this goal.

Acknowledgements

The present study was supported by a grant from the Deutsche Forschungsgemeinschaft, Grant No. LU601/2. The authors gratefully acknowledge the skilled technical assistance of F. Mauch and B. Devezi-Savula at Hohenheim, as well as the staff of the Plant Breeding Research Station at Eckartsweier in conducting the field trials.

References

Bohn, M., M. M. Khairallah, D. Gonzales-de-Leon, H. F. Utz, J. A. Deutsch, D. Jewell, J. A. Mihm, and A. E. Melchinger, 1997: QTL mapping in tropical maize: II. Comparison of genomic regions for resistance to *Diatrea spp.* Crop Sci. **37,** 1892—1902.

Fuchs, E., and M. Grüntzig, 1995: Influence of sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) on the growth and yield of two maize varieties. J. Plant Dis. Protect. **102,** 44—50.

Fuchs, E., M. Grüntzig, L. Kuntze, U. Oertel, and F. Hohmann, 1996: Zur Epidemologie der Potyviren des Maises in Deutschland. Bericht Arbeitstagung Arbeitsgem. Saatzuchtleiter Gumpenstein, Österreich, **46,** 43—49.

Hallauer, A. R., and F. J. B. Miranda, 1981: Quantitative Genetics in Maize Breeding. Iowa State Univ. Press, Ames.

Hoisington, D. A., M. M. Khairallah, and D. Gonzales-de-Leon, 1994: Laboratory Protocols. CIMMYT Applied Molecular Genetics Laboratory, Mexico, DF.

Jansen, R. C., 1993: Interval mapping of multiple quantitative trait loci. Genetics **135,** 205—211.

Knapp, S. J., W. W. Stroup, and W. M. Ross, 1985: Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. **25,** 192—194.

Kuntze, L., E. Fuchs, M. Grüntzig, B. Schulz, D. Klein, and A. E. Melchinger, 1997: Resistance of early-maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breeding **116,** 499—501.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg, 1987: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1,** 174—181.

Louie, R., W. R. Findley, J. K. Knoke, and M. D. McMullen, 1991: Genetic basis of resistance in maize to five maize dwarf mosaic virus strains. Crop Sci. **31,** 14—18.

Lübberstedt, T., C. M. Dußle, and A. E. Melchinger, 1998: Application of microsatellites from maize to teosinte and other relatives of maize. Plant Breeding **117,** 447—450.

Lübberstedt, T., X. C. Xia, M. L. Xu, L. Kuntze, and A. E. Melchinger, 1999: Inheritance of resistance to SCMV and MDMV in European maize. In: G. T. S. Mugnozza (ed.), Proc. XVth EUCARPIA 1998 General Congr.: Genetics and Breeding for Crop Quality and Resistance, 241—250. Kluwer Acad. Publ., Dordrecht.

McMullen, M. D., and R. Louie, 1991: Identification of a gene for resistance to wheat streak mosaic virus in maize. Phytopathology **81,** 624—627.

McMullen, M. D., and K. D. Simcox, 1995: Genomic organization of disease and insect resistance genes in maize. Mol. Plant-Microb. Interact. **8,** 811—815.

McMullen, M. D., M. W. Jones, K. D. Simcox, and R. Louie, 1994: Three genetic loci control resistance to wheat streak mosaic virus in the maize inbred Pa405. Mol. Plant-Microb. Interact. **7,** 708—712.

Melchinger, A. E., 1997: Advances in the analysis of data on quantitative trait loci. Proc. 2nd Int. Crop Sci. Congr., 773—791.

Melchinger, A. E., L. Kuntze, R. K. Gumber, T. Lübberstedt, and E. Fuchs, 1998a: Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor. Appl. Genet. **96,** 1151—1161.

Melchinger, A. E., H. F. Utz, and C. C. Schön, 1998b: Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics **149,** 383—403.

Mikel, M. A., C. J. D'Arcy, A. M. Rhodes, and R. E. Ford, 1984: Genetics of resistance of two dent corn inbreds to maize dwarf mosaic virus and transfer of resistance into sweet corn. Phytopathology **74,** 467—473.

Patterson, H. D., and E. R. Williams, 1976: A new class of resolvable incomplete block designs. Biometrika **63,** 83—92.

Quint, M., C. M. Dußle, S. Dreisigacker, A. E. Melchinger, and T. Lübberstedt, 1999: Clusterung von SCMV-Resistenzgenen bei Mais? Vortr. Pflanzenzüchtg. **46,** 228—234.

Searle, S. R., 1971: Linear Models. Wiley and Sons, New York.

Simcox, K. D., M. D. McMullen, and R. Louie, 1995: Co-segregation of the maize dwarf mosaic virus resistance gene with the nucleolus organizer region in maize. Theor. Appl. Genet. **90,** 341—346.

Utz, H. F., and A. E. Melchinger, 1996: PLABQTL: a program for composite interval mapping of QTL. J. Quant. Trait. Loci. http://probe.nalsuda.gov:8000/otherdocs/jqtl.

Weir, B. S., 1990: Genetic Data Analysis. Sinauer, Sunderland.

Xia, X. C., A. E. Melchinger, L. Kuntze, and T. Lübberstedt, 1999: QTL mapping of resistance to sugarcane mosaic virus in maize. Phytopathology **89,** 660—667.

Xu, M. L., A. E. Melchinger, X. C. Xia, and T. Lübberstedt, 1999: High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Mol. Gen. Genet. **261,** 574—581.

Xu, M. L., A. E. Melchinger, and T. Lübberstedt, 2000: Origin of *Scm*1 and *Scm*2 – two loci conferring resistance to sugarcane mosaic virus (SCMV) in maize. Theor. Appl. Genet. **100,** 934—941.

Zeng, Z. B., 1994: Precision mapping of quantitative trait loci. Genetics **136,** 1457—1468.

Clustering of QTL conferring SCMV resistance in maize[2]

Abstract

Sugarcane mosaic virus (SCMV) is an important virus disease of maize (*Zea mays* L.) in Europe. In this study, we mapped and characterized two major quantitative trait locus (QTL) regions conferring resistance to SCMV in a maize population consisting of 121 F_3 lines from cross F7 (susceptible) FAP1360 (resistant). This population was evaluated for SCMV resistance in replicated field trials across two environments under artificial inoculation. Two previously identified chromosome regions (chromosome 6S and 3L) containing QTL conferring resistance were mapped with 24 SSR markers by composite interval mapping. Both regions together explained between 33.0% and 71.1% of the phenotypic variance for SCMV resistance at various stages of plant development. The *Scmv1* region harbored two QTL rather than one QTL as identified in previous studies. Clustering of QTL for SCMV resistance was confirmed for at least one chromosome region.

Key words: QTL mapping, SSR markers, SCMV resistance, *Zea mays*

[2] Yuan L, Dußle CM, Melchinger AE, Utz, HF, and Lübberstedt T (2003) Clustering of QTL conferring SCMV resistance in maize, Maydica. In press

Introduction

Sugarcane mosaic virus (SCMV) and maize mosaic virus (MDMV) are two important diseases in maize (*Zea mays* L.) and related crops, causing serious yield losses in susceptible cultivars (Fuchs and Grüntzig 1995). Direct chemical control of SCMV and MDMV is impossible due to non-persistent transmission of both viruses. Therefore, the most efficient way to control virus infections is by cultivation of resistant maize varieties.

Effective natural resistance against SCMV and MDMV has been identified in U.S. maize germplasm such as inbred line Pa405 (Louie et al. 1990). The studies in Pa405 indicated that there were one to five genes conferring resistance to SCMV, MDMV, and wheat streak mosaic virus (WSMV) (Louie et al. 1991, McMullen et al. 1994, Simcox et al. 1995). Likewise, in a study with 122 early maturing European maize inbred lines, three lines (D21, D32 and FAP1360A) were identified to be completely resistant to SCMV and MDMV under both field and greenhouse conditions (Kuntze et al. 1997). Melchinger et al. (1998a) proposed that one to three genes confer resistance to SCMV in different crosses, and two resistance genes, *Scmv1* and *Scmv2*, mapped to the short arm of chromosome 6 and the centromere region of chromosome 3.

In order to develop tools for marker-assisted selection (MAS) as well as cloning of the major genes for SCMV resistance, both regions were analyzed in cross F7 FAP1360A by bulked segregant analyses (BSA) (Xu et al. 1999) and in cross D145 (susceptible) D32 (resistance) by QTL analyses (Xia et al. 1999). Dußle et al. (2000) employed a QTL approach to confirm the location and gene action of both regions in a population of 121 F_3 lines from cross F7 FAP1360A using four SSR markers flanking both regions. In the present study, 24 polymorphic SSR markers were applied to QTL analyses of the same population. Our objectives were to (1) address the question of whether single or clustered QTL within both regions are responsible for SCMV resistance, and (2) determine the exact position and gene action of the respective loci.

Materials and Methods

Plant materials

The two early-maturing European maize inbred lines FAP1360A (dent) and F7 (flint) were crossed and the F_1 and F_2 generations were subsequently selfed to produce a random set of 121 F_3 lines as described in detail by Dußle et al. (2000). Line FAP1360A is completely resistant to SCMV, whereas line F7 is highly susceptible (Kuntze et al. 1997). For the present study, only a subset of 118 F_3 lines could be analyzed due to lack of seed for three F_3 lines.

Artificial inoculation

Virus inoculum for testing resistance against SCMV isolates "Seehausen" was prepared as described by Fuchs and Grüntzig (1995). Young leaves with typical mosaic symptoms of the SCMV- infected maize variety 'Bermasil' were homogenized using 5 volumes of a 0.01 M phosphate buffer at pH 7.0. Carborundum was added to the sap. During sap preparation and mechanical inoculation, the inoculum was kept at + 4° C. Plants at the three- to four-leaf stage were mechanically inoculated twice at a weekly interval by an air brush technique with a tractor-mounted air compressor at a constant pressure of 800 kPa (Fuchs et al. 1996).

Agronomic trials

The parental F_2 plants of the 121 F_3 lines were evaluated in 1995 for resistance to SCMV at Hohenheim. The 121 F_3 lines from cross F7 × FAP1360A plus two entries of the F_1 hybrid, three entries of the F_2 generation, two entries of the BC1 generation to the susceptible parent F7 and one entry for each parent were evaluated in 1996 for resistance to SCMV at Eckartsweier and Hohenheim. The 130 entries were tested in a 13 × 10 alpha lattice design (Patterson and Williams 1976). Plots consisted of single rows, 0.7 m apart and 3 m long, which were overplanted and later thinned to a final density of 9 plants/m^2 with a total of 20 plants per row. Resistance to SCMV was

visually scored two weeks after initial inoculation for the first time. The number of plants with virus symptoms was recorded at weekly intervals for seven dates (VIR1 to VIR7) at each location. The number of infected plants was converted into percentage of plants with disease symptoms.

Leaf collection and DNA extraction

Leaves were harvested from 16 plants of each F_3 line grown in the greenhouse. Equal amounts of leaf material from each plant per line were bulked for DNA extraction to determine the marker genotype of the parental F_2 plant. Harvested leaves were freeze-dried and ground to powder. DNA extraction was performed according to the CTAB method (Hoisington et al. 1994).

SSR analyses

Twenty-four SSR markers were employed to genotype the parental F_2 plants of the 118 F_3 lines. The markers were polymorphic based on the alleles between the parental lines FAP1630A and F7 as previously reported by Xu et al. (1999) and Dußle et al. (2002). Eleven makers were located on chromosome 3 and 13 markers on chromosome 6. Sequences of these primers were obtained from the maize database (http://teosinte.agron.missouri.edu/Coop/SSR-Probes/SSR1-htm) and synthesized by Amersham Pharmacia Biotech (Freiburg, Germany). PCR amplification and MetaPhor® gel electrophoresis were performed according to Lübberstedt et al. (1998). Additionally, pic19, a RGA-CAPS marker, was used for genotyping according to the procedure described in detail by Quint et al. (2002a).

Segregation and linkage analyses

Segregation at each marker locus was checked for deviations from Mendelian segregation ratios (1:2:1 or 3:1) and allele frequency 0.5 by chi-square tests (Weir 1990). A linkage analysis of the SSR markers was conducted with software package Mapmaker 3.0b (Lander et al. 1987) using Haldane's mapping function. The threshold

used for declaring linkage was set to a LOD value of 3.0 and a maximum distance of 50 cM.

Statistical analyses

The biometric analyses of the phenotypic data (VIR1 to VIR7) and QTL mapping were performed as described in detail by Xia et al. (1999) without further data transformation. Combined analysis of variance (ANOVA) for the phenotypic data of resistance to SCMV in the 121 F_3 lines of cross F7 FAP1360A was identical with that reported by Dußle et al. (2000). We employed the method of composite interval mapping (CIM) (Zeng 1994) for mapping of QTL and estimation of their effects as described by Bohn et al. (1996). A LOD threshold of 3.0 was chosen to declare a putative QTL significant. For the analyses of data from each environment and also for the joint analyses across environments, cofactors were selected by stepwise regression. Final selection was for the model that minimized Akaike's information criterion (AIC) with penalty = 3.0 (Jansen 1993). QTL positions were determined at the local maxima of the LOD ($\log_{10}$ odds ratio) plot curve in the regions under consideration. The proportion of the phenotypic variance ($\hat{s}_p^2$) explained by a single QTL was obtained by the square of the partial correlation coefficient (R^2) (Melchinger et al. 1998b). Estimates of R^2 values and gene effects at QTL as well as tests for QTL × environment (QTL × E) and digenic epistatic interactions among QTL were obtained by fitting a model including all QTL for the respective trait simultaneously (Melchinger et al. 1998b). All computations were performed with software package PLABQTL (Utz and Melchinger 1996).

Results

Phenotypic data

Both parents differed significantly ($P<0.01$) in their SCMV resistance; no infected plants were found for resistant parent FAP1360A while 99% of plants for susceptible parent F7 were infected at the final scoring dates. The mean proportion of infected

plants across all F_3 lines increased from 3.4% at VIR1 to 32.9% at VIR7. Genotypic variances for SCMV were highly significant ($P<0.01$) at VIR2 to VIR7. Heritabilities for VIR2 to VIR7 ranged from 0.42 to 0.92. For a detailed report of the phenotypic data, see Dußle et al. (2000).

Segregation of SSR markers

Nineteen SSR markers showed a codominant segregation ratio (1:2:1) and five markers displayed a dominant segregation ratio (1:3). Allele frequencies did not deviate significantly ($P<0.05$) from 0.5 at any marker locus. For marker umc1143, the observed genotype frequency deviated significantly ($P<0.001$) from the Mendelian expectation (1:2:1) as heterozygous genotypes were underrepresented. The map spanned about 126 cM with all 24 SSR markers, covering 57.6 cM (11 markers) on chromosome 3 and 68.7 cM (13 markers) on chromosome 6 (Fig. 1). pic19 was mapped in the middle between markers umc1143 and phi126 on chromosome 6 (Fig. 1C).

QTL analyses

With the linkage maps based on twenty-four SSR markers, both QTL regions on chromosome 3 and 6 were confirmed to confer SCMV resistance in the population of cross F7 FAP1360A (Table 1). All QTL alleles affecting resistance to SCMV were contributed by the resistant parent FAP1360A. One QTL on chromosome 3 (*Scmv2* region) was identified between markers umc1300 and phi053 at all scoring dates except for VIR3 and VIR4 (Fig. 1A and Fig. 2). It explained between 12.4% and 30.1% of the phenotypic variance ($\hat{s}^2_p$), decreasing from VIR1 to VIR2 but increasing from VIR 5 to VIR7. The QTL region on chromosome 6 (*Scmv1* region) was identified at all seven scoring dates. At VIR1 and VIR2, one QTL was identified at position 45 cM between markers bmc1432 and umc1753, and explained 21.2% and 32.4% of $\hat{s}^2_p$, respectively. However, two QTL were identified from VIR3 to VIR7. The first one (*Scmv1a*) was located at position 33-36 cM between markers phi126 and bmc1432, and the second one (*Scmv1b*) at position 51 cM between markers bmc1600 and bmc1867 (Fig. 1C and Fig.

2). *Scmv1a* explained between 23.8% and 33.3% of $\hat{s}_p^2$ from VIR3 to VIR7, and *Scmv1b* explained between 22.7% and 33.2% of $\hat{s}_p^2$. All three QTL together explained between 33.0% and 71.1% of $\hat{s}_p^2$ from VIR1 to VIR7 with increasing values in later stages. *Scmv2* showed partial dominance for resistance to SCMV, while gene action was overdominant for *Scmv1a* and additive for *Scmv1b*. No significant (P<0.05) digenic epistatic interactions were found between these QTL. Significant (P<0.05) QTL E interactions were detected for both QTL regions for some dates of SCMV scoring (Table 1).

Discussion

Comparison of the *Scmv1* and *Scmv2* regions in cross F7 × FAP1360A

In a previous study with the same maize population of 121 F_3 lines from cross F7 × FAP1360A reported by Dußle et al. (2000), two major QTL regions (*Scmv1* and *Scmv2*) for SCMV resistance were identified on chromosomes 6 and 3 with the aid of four SSR markers (Fig. 1B and Fig. 1D, phi126 and phi077 on chromosome 6, and phi029 and phi053 on chromosome 3, flanking the *Scmv1* and *Scmv2* regions, respectively). Gene action was additive for *Scmv2*, but completely dominant for *Scmv1*. Meanwhile, further SSR markers were developed with the progress in the maize genome project. In the present study with the same plant materials and field data, the locations of QTL and their gene actions were confirmed based on a much denser linkage map with 24 polymorphic SSR markers covering these two regions. *Scmv2* was identified at the same location, but gene action was partial dominant. Furthermore, the *Scmv1* region was resolved into two closely linked QTL (*Scmv1a* and *Scmv1b*) at scoring dates VIR3 to VIR7. The single QTL in the *Scmv1* region at VIR1 and VIR2 is most likely a 'ghost' QTL derived from *Scmv1a* and *Scmv1b* (Fig. 2) due to the low heritability for the first two scoring dates, which results in a low power of QTL detection and resolution.

Two major genes for SCMV resistance (*Scmv1* and *Scmv2*) were also identified in a BC5 population of cross F7 × FAP1360A with the high resolution maps based on

RFLP, SSR and AFLP markers by BSA analyses (Xu et al. 1999). Furthermore, these two regions were enriched with SSR and AFLP markers in generations BC5, BC7 and BC9 of cross F7 × FAP1360A by applying targeted BSA (tBSA) (Dußle et al. 2002). This permits a direct comparison of BSA and QTL analyses for both regions in the same cross. S*cmv1* was mapped between the SSR markers phi126 and phi077 by Xu et al. (1999), and was mapped 2.4 cM distal to SSR marker bmc1432 by Dußle et al. (2002) (Fig. 1D). One QTL (*Scmv1a*) was located at the position of 33-36 cM adjacent to SSR marker bmc1432 in the present study. It seems safe to conclude that this gene is located at the same position in both analyses. However, the second QTL (*Scmv1b*) was exclusively identified by QTL analyses at the position of 51 cM between markers bmc1600 and bmc1867. In previous studies, a genetic model supposing one major gene in each of both regions, *Scmv1* and *Scmv2*, was applied. Both QTL in the *Scmv1* region were mapped as a single 'ghost' QTL because with the population and mapping program (Crimap) employed, it was not possible to resolve two tightly linked QTL. Furthermore, this 'ghost' QTL was located adjacent to the position of *Scmv1a* due to its overdominant gene action, conferring a higher degree of resistance in BC individuals than the additively acting QTL *Scmv1b*.

The order of SSR markers in the *Scmv1* region agreed well with the results of Dußle et al. (2002), but differences were observed for the map distances. For example, the distance between markers phi126 and phi077 was 37.4 cM compared to 69.7 cM in the latter study. The presence of a second redundant resistance QTL could explain the inflated map distance. As explained by Dußle et al. (2002), instead of estimating the correct map position of a segregating marker relative to one of the real QTL, the markers identified by BSA would be grouped to the 'ghost' QTL, and, therefore, to the same linkage group. In this case, the position of the markers linked to one of the real QTL might be changed and the map distance would be overestimated. Consequently, if there are two QTL in the *Scmv1* region, the QTL approach with unselected F_3 lines seems more appropriate to map this region than BSA with a BC population.

Scmv2 was 1 cM distant from marker phi053 in the present study (Fig. 1) but in about 5 cM distance with BSA (Xu et al. 1999, Dußle et al. 2002). Furthermore, the order of SSR markers and map distance for the *Scmv2* region was largely consistent with the BSA fine mapping study of Dußle et al. (2002). In contrast to the *Scmv1*

region, this is an indication for either one QTL or extremely tightly linked QTL in the *Scmv2* region.

Comparison of the *Scmv1* and *Scmv2* regions in different crosses

In a previous study with a maize population of 220 F_3 lines from cross D145 (susceptible) × D32 (resistant) reported by Xia et al. (1999), two major QTL regions (*Scmv1* and *Scmv2*) for SCMV resistance were identified on chromosomes 6S and 3L, showing the same location as confirmed in the present study. The gene effects were much larger for the *Scmv1* region than for the *Scmv2* region at all scoring dates in both crosses D145 × D32 and F7 × FAP1360A. Gene action of the *Scmv1* region was partially dominant at all scoring dates in cross D145 × D32 similar to the supposed 'ghost' QTL at VIR1 and VIR2 in cross F7 × FAP1360A. It is very likely that the organization of the *Scmv1* region is identical in both crosses, as suggested by identical haplotypes of both resistant lines D32 and FAP1360A in this region (Xu et al. 2000)

The gene action of the other major QTL region (*Scmv2*) was partially dominant in both crosses. Obviously, the resistance attributable to the *Scmv2* region is influenced by the stage of plant development, because this QTL was not detected at VIR1 in cross D145 × D32 and likewise at VIR3 and VIR4 in cross F7 × FAP1360A. The latter also suggests that the resistance alleles in the *Scmv2* region differ in both crosses. This is agreement with Xu et al. (2000) and Quint et al. (2002a), who identified different marker haplotypes and RGA alleles in the three resistant lines FAP1360A, D32, and D21. However, it is unclear for the *Scmv2* region whether there is allelic variation for one single resistance gene or also a cluster of tightly linked genes.

Clustering of resistance genes in the *Scmv1* and *Scmv2* regions

Resistance genes conferring resistance to different pathogens are often clustered in the same chromosome regions of the maize genome (McMullen and Simcox 1995). For instance, chromosome 6S includes resistance genes for SCMV, MDMV, WSMV, high plains virus, rice bacterial streak, sorghum bacterial stripe, and southern corn leaf blight. Likewise, the cluster on chromosome 3L contains genes conferring resistance to SCMV,

WSMV, high plains virus, maize mosaic virus, and maize chlorotic dwarf virus (Quint et al. 2000). Close linkage of different resistance genes seems more likely than pleiotropy for resistance to different pathogens in cross F7 × FAP1360A (Xu et al. 1999). In fact, Lübberstedt et al. (1999) suggested the presence of closely linked major genes for SCMV resistance because some susceptible plants were found in F_2 populations when testing for allelism between the three European resistant inbreds FAP1360A, D32, and D21. Moreover, field experiments, BSA (Xu et al. 1999), and QTL analyses (Xia et al. 1999, Dußle et al. 2000) do not exclude the presence of more than one SCMV resistance gene in the *Scmv1* region. Our results from QTL analyses in cross F7 × FAP1360A support the hypothesis of closely linked but different major resistance genes in the *Scmv1* region. Cloning the SCMV resistance genes is necessary to resolve the question of whether they each harbor a single locus or clusters of resistance loci and whether the genes present at these loci in the resistant inbreds D32 and FAP1360A are identical or not. However, clustered target loci will complicate map-based gene isolation.

Quint et al. (2002a) suggested that pic19 is a candidate for *Scmv1*, although the authors could not distinguish between close linkage and identity of pic19 and *Scmv1*. In the present study, pic19 was mapped between SSR markers umc1143 and phi126, outside the *Scmv1* region (Fig. 1C). Hence, pic19 seems to be no candidate for *Scmv1* based on its map position reported here. However, Quint et al. (2002b) argued that pic19 might have been mapped as a single-copy 'ghost' marker analogous to 'ghost' QTL due to the high sequence similarity of the linked pic19 homologues, which can result in a wrong map position. Therefore, pic19 homologues should not be excluded as candidates for *Scmv1a* or *Scmv1b* without further investigations.

Acknowledgements The present study was supported by a grant from the Deutsche Forschungsgemeinschaft, Grant No. LU601/2. The financial support to Mr. L. Yuan from German Ministry of Agriculture is gratefully acknowledged. We also thank Nadine Burkarth for her support in the SSR analyses.

References

Bohn, M., M. M. Khairallah, D. Gonzales-de-Leon, H. F. Utz, J. A. Deutsch, D. Jewell, J. A. Mihm, and A. E. Melchinger, 1997: QTL mapping in tropical maize: II. Comparison of genomic regions for resistance to *Diatrea spp.* Crop Sci. **37**, 1892-1902.

Dußle, C. M., A. E. Melchinger, L. Kuntze, A. Stork, T. Lübberstedt, 2000: Molecular mapping and gene action of Scm1 and Scm2, two major QTL contributing to SCMV resistance in maize. Plant Breeding **119,** 299-303.

Dußle, C. M., M. Quint, A. E. Melchinger, T. Lübberstedt, 2002: Saturation of two chromosome regions conferring resistance to SCMV by targeted BSA. Submitted to TAG.

Fuchs, E., and M. Grüntzig, 1995: Influence of sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) on the growth and yield of two maize varieties. J. Plant Dis. Protect **102**, 44-50.

Fuchs, E., M. Grüntzig, L. Kuntze, U. Oertel, and F. Hohmann, 1996: Zur Epidemologie der Potyviren des Maises in Deutschland. In: Bericht über die 46. Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter im Rahmen der "Vereinigung Österreichischer Pflanzenzüchter" **46**, 43-49.

Hoisington, D.A., M. M. Khairallah, and D. Gonzales-de-Leon, 1994: Laboratory protocols. CIMMYT Applied Molecular Genetics Laboratory, CIMMYT, Mexico, DF.

Jansen, R. C., 1993: Interval mapping of multiple quantitative trait loci. Genetics **135**, 205-211.

Kuntze, L., E. Fuchs, M. Grüntzig, B. Schulz, D. Klein, and A.E. Melchinger, 1997: Resistance of early-maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breeding **116**, 499-501.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg, 1987: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1**, 174-181.

Louie R., J. K. Knoke, and W. R. Findley, 1990. Elite maize germplasm: Reaction to maize dwarf mosaic and maize chlorotic dwarf viruses. Crop Sci. **30**, 1210-1215.

Louie R., W. R. Findley, J. K. Knoke, and M. D. McMullen, 1991: Genetic basis of resistance in maize to five maize dwarf mosaic virus strains. Crop Sci. **31**, 14-18.

Lübberstedt, T., C. M. Dußle, and A. E. Melchinger, 1998: Application of microsatellites from maize to teosinte and other relatives of maize. Plant Breeding **117**, 447-450.

Lübberstedt T., X. C. Xia, M. L. Xu, L. Kuntze, and A. E. Melchinger, 1999: Inheritance of resistance to SCMV and MDMV in European maize. In: Proc. of the XVth EUCARPIA 1998 General Congress: Genetics and Breeding for Crop Quality and Resistance, G.T.S. Mugnozza (Ed.), Kluwer Academic Publishers, Dordrecht, The Netherlands, 241-250.

McMullen, M. D., M. W. Jones, K. D. Simcox, and R. Louie, 1994: Three genetic loci control resistance to wheat streak mosaic virus in the maize inbred Pa405. Mol. Plant-Microb. Interact. **7**, 708-712.

McMullen, M. D., and K. D. Simcox, 1995: Genomic organization of disease and insect resistance genes in maize. Mol. Plant-Microb. Interact. **8**, 811-815.

Melchinger, A. E., L. Kuntze, R. K. Gumber, T. Lübberstedt, and E. Fuchs, 1998a: Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor. Appl. Genet. **96**,1151-1161.

Melchinger A.E., H. F. Utz, and C. C. Schön, 1998b: Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics **149**, 383-403.

Patterson, H. D., and E. R. Williams, 1976: A new class of resolvable incomplete block designs. Biometrika **63**, 83-92.

Quint, M., A. E. Melchinger, C. M. Dußle, and T. Lübberstedt, 2000: Breeding for virus resistance in maize. Genetika **32**, 529-545.

Quint, M., R. Mihaljevic, C. M. Dußle, M. L. Xu, A. E. Melchinger, T. Lübberstedt, 2002a: Development of RGA-CAPS markers and genetic mapping of candidate genes for sugarcane mosaic virus resistance in maize. Theor. Appl. Genet. In press.

Quint, M., C. M. Dußle, A. E. Melchinger, T. Lübberstedt, 2002b: Identification of genetically linked RGAs by BAC screening in maize and implications for gene cloning, mapping, and MAS. Submitted to MGG.

Simcox, K. D., M. D. McMullen, and R. Louie, 1995: Co-segregation of the maize dwarf mosaic virus resistance gene with the nucleolus organizer region in maize. Theor. Appl. Genet. **90**, 341-346.

Utz, H. F., and A. E. Melchinger, 1996: PLABQTL: A program for composite interval mapping of QTL. J. Quant. Trait. Loci. http://probe.nalsuda.gov:8000/otherdocs/jqtl.

Weir, B. S., 1990: Genetic Data Analysis. Sinauer, Sunderland, MA.

Xia, X. C., A. E. Melchinger, L. Kuntze, and T. Lübberstedt, 1999: QTL mapping of resistance to sugarcane mosaic virus in maize. Phytopathology **89**, 660-667.

Xu, M. L., A. E. Melchinger, X. C. Xia, and T. Lübberstedt, 1999: High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Mol. Gen. Genet. **261**, 574-581.

Xu M. L., A. E. Melchinger, and T. Lübberstedt, 2000: Origin of *Scm1* and *Scm2* - two loci conferring resistance to sugarcane mosaic virus (SCMV) in maize. Theor. Appl. Genet. **100**, 934-941.

Zeng Z. B., 1994: Precision mapping of quantitative trait loci. Genetics **136**, 1457-1468.

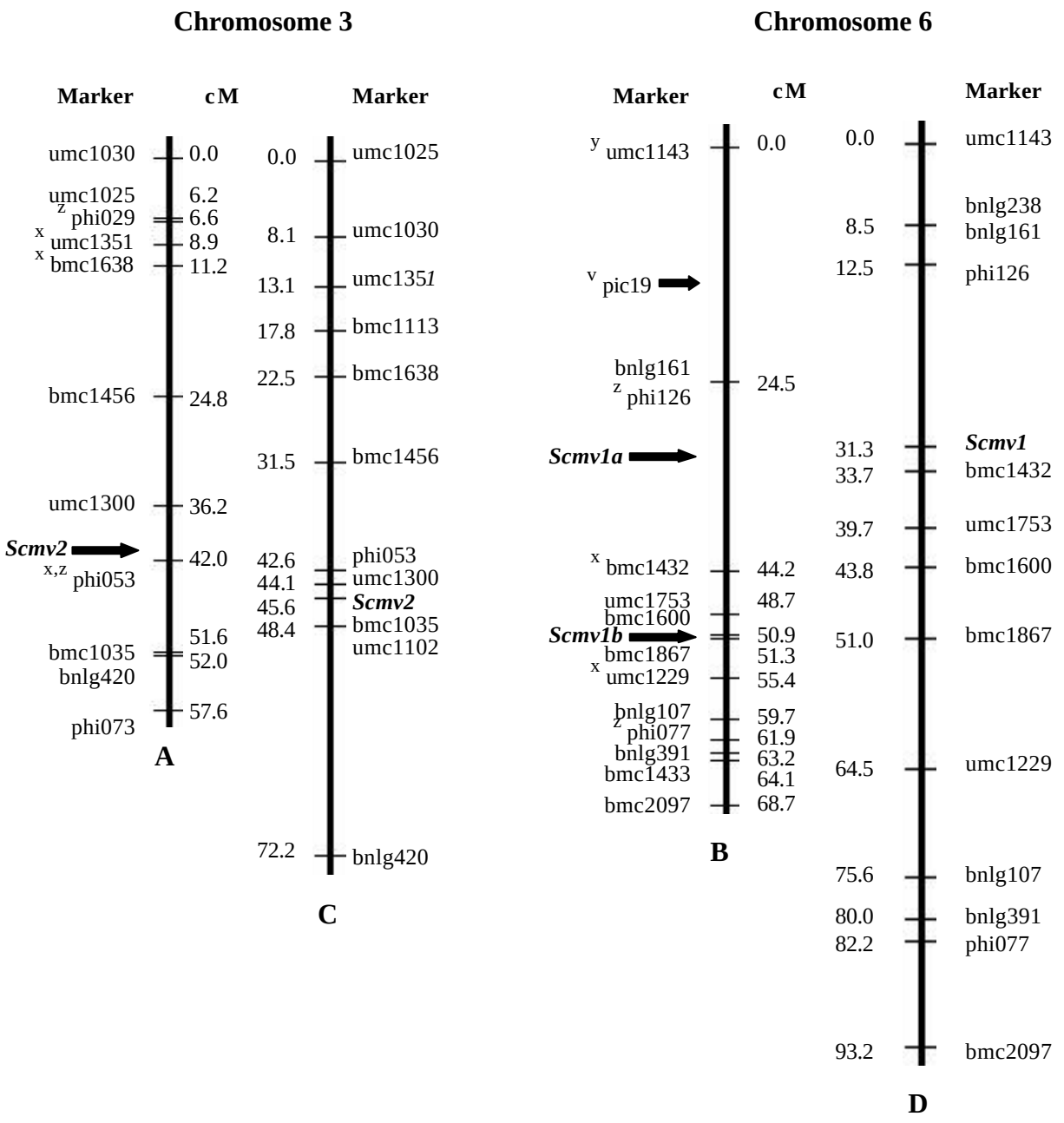

Fig. 1 A, B: The genetic maps obtained by linkage analysis of 118 F_3 lines of cross F7 FAP1360A with 24 SSR markers. **A**: Centomere region of chromosome 3 harboring *Scmv2*. **B**: Short arm of chromosome 6 harboring the *Scmv1* region (*Scmv1a* and *Scmv1b*). ^{v} = RGA-CAPS marker pic19; ^{x} = dominant markers; ^{y} = genotype frequencies deviated significantly from the Mendelian expectation (1:2:1); ^{z} = four markers used in the previous study by Dußle et al. (2000).
C, D: The genetic maps obtained by joint linkage analyses of the BC1, BC5, BC7, and BC9 populations of cross F7 FAP1360A with 25 SSR markers and 34 AFLP markers (AFLP markers not shown) (Dußle et al. 2002). **C**: Centomere region of chromosome 3 harboring *Scmv2*. **D**: Short arm of chromosome 6 harboring the *Scmv1* region.

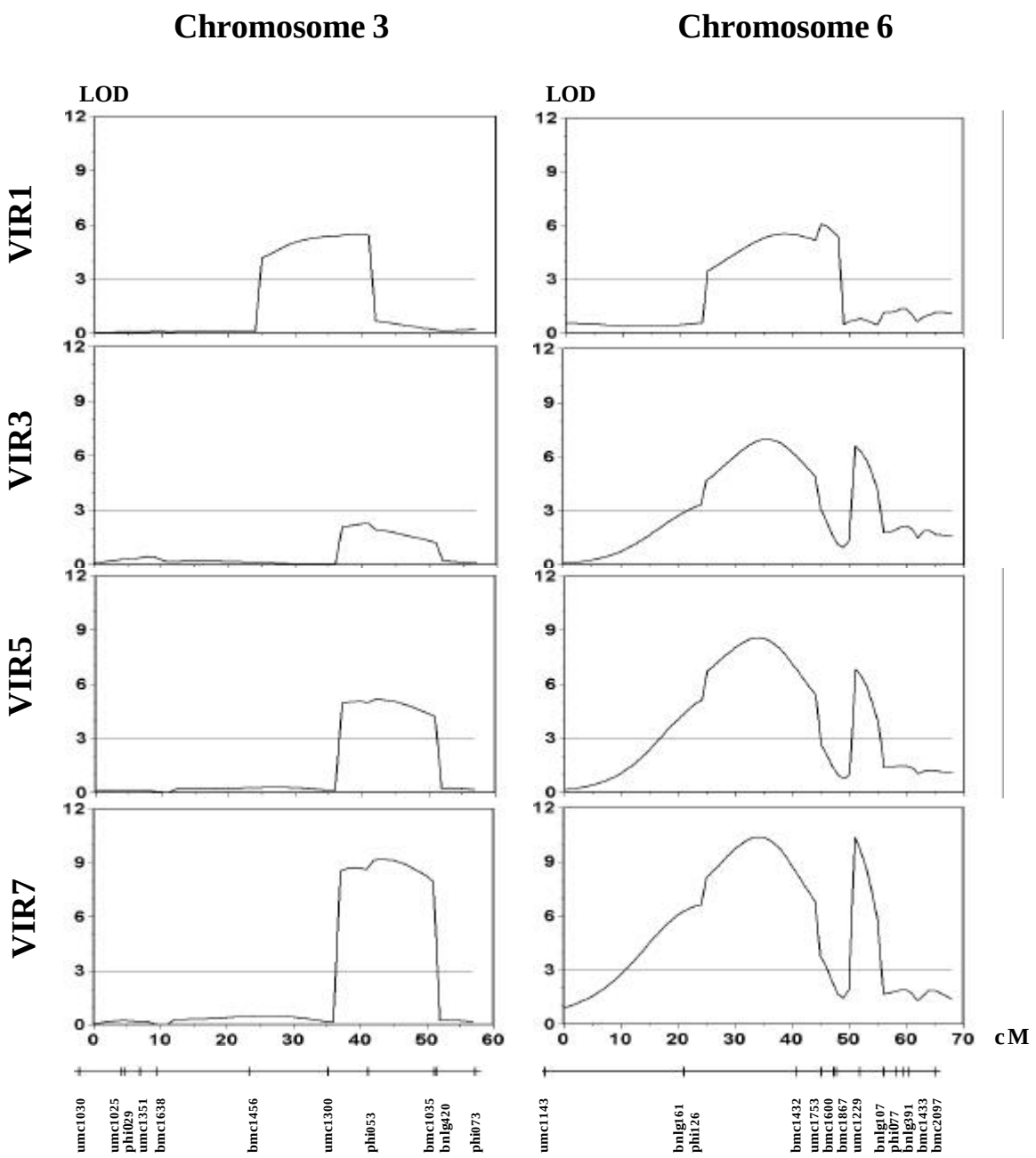

Fig. 2: QTL likelihood maps showing the LOD scores on chromosome 3 (*Scmv2*) and chromosome 6 (*Scmv1*) at various scoring dates (VIR1, VIR3, VIR5, VIR7). A LOD threshold of 3.0 was chosen for declaring a putative QTL significant.

Table 1: Parameters associated with putative quantitative trait loci (QTL) significantly affecting resistance to SCMV in 118 F_3 lines of maize population F7 FAP1360A at various scoring dates (VIR1 to VIR7).

			Trait [3] (%)						
Chromosome	Position[2]	Parameter	VIR1	VIR2	VIR3	VIR4	VIR5	VIR6	VIR7
3	39-43	a	-2.0**	-2.4**			-9.3**	-10.7**	-15.1**
		d	-1.1	-1.4			3.4	0.7	3.2
		R_1^2	19.3	12.4			18.3	23.8	30.1
		a E	**	**			**	ns	*
		d E	ns	ns			ns	ns	ns
6	33-36	a			-10.6**	-12.6**	-18.1**	-23.7**	-23.0**
		d			-18.5**	-19.4**	-22.6**	-31.5**	-23.8*
		R^2			23.8	23.5	28.4	31.9	33.3
		a E			ns	*	ns	ns	ns
		d E			ns	ns	ns	ns	ns
	45	a	-2.7**	-5.3**					
		d	-2.0	-4.3*					
		R^2	21.2	32.4					
		a E	**	**					
		d E	ns	ns					
	51	a			-2.4	-5.1	-6.6*	-7.8*	-12.3*
		d			2.3	0.6	2.9	6.5	1.6
		R^2			22.7	21.4	23.4	29.0	33.2
		a E			ns	*	ns	ns	ns
		d E			ns	ns	ns	ns	ns
Total		R^2	33.0	36.9	48.7	48.7	60.8	67.5	71.1

*, ** = Significant at the 0.05 and 0.01 probability level, respectively. ns = no significant.

[1] QTL position based on the linkage map (Fig. 1)

[2] a = Additive effect and d = dominance effect of QTL. R^2 estimates the phenotypic variance (%) explained by detected QTL. Total R^2 refers to the simultaneous fit of all putative QTL affecting the respective trait using a model with additive and dominance effects. a E and d E = Interaction between QTL and environment for the a and d effect, respectively.

[3] Scoring of SCMV symptoms at scoring dates VIR1 to VIR7.

Theor Appl Genet (2003) 106:485–493
DOI 10.1007/s00122-002-1107-x

C. M. Dußle · M. Quint · A. E. Melchinger
M. L. Xu · T. Lübberstedt

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

Received: 26 March 2002 / Accepted: 21 May 2002 / Published online: 2 November 2002

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

Communicated by G. Wenzel

C.M. Dußle · M. Quint · A.E. Melchinger · M.L. Xu
T. Lübberstedt (✉)
Institute of Plant Breeding, Seed Science,
and Population Genetics, University of Hohenheim,
D-70593 Stuttgart, Germany
e-mail: thomas.luebberstedt@agrsci.dk
Fax: ++45-58-11-33 01

Present address:
M.L. Xu, College of Biosciences and Biotechnology,
Yangzhou University, 225009, Yangzhou,
Peoples Republic of China

Present address:
T. Lübberstedt, Danish Institute of Agricultural Sciences,
Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark

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

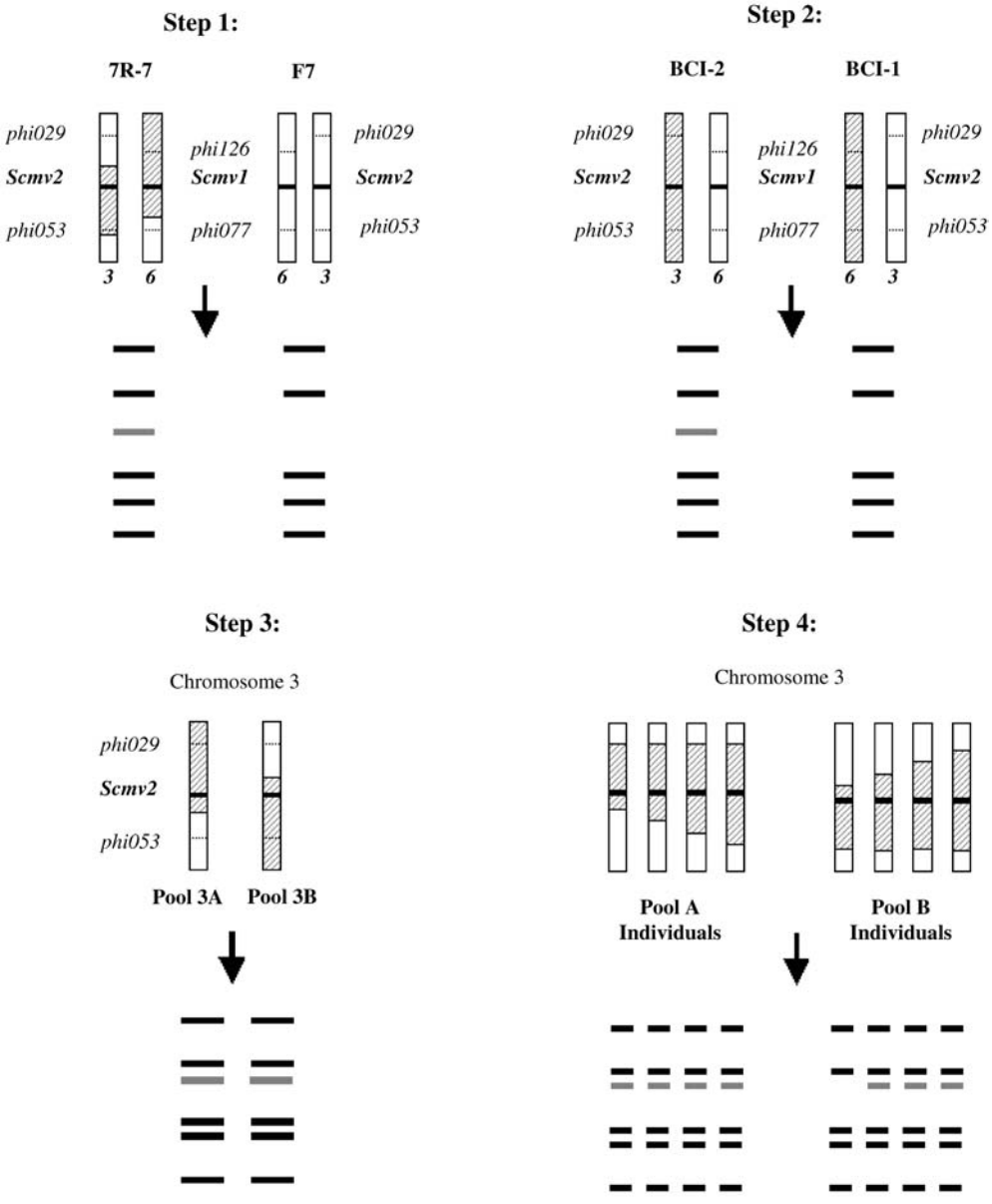

Fig. 1 Steps 1–4 Scheme of the four steps for evaluation and mapping of markers closely linked to *Scmv1* and *Scmv2* applying a modified targeted BSA (t-BSA). **Step 1**: AFLP primer combinations were screened for polymorphism in susceptible parent F7 and the resistant backcross 7 (BC7) individual 7R-7. **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).

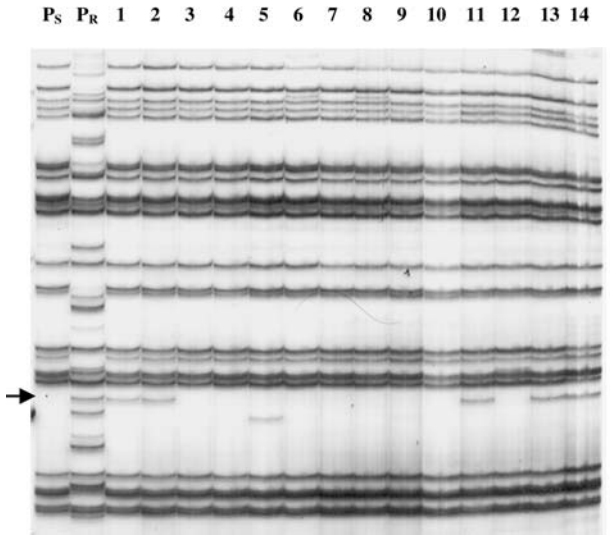

Fig. 2 Analysis of AFLP markers putatively linked to SCMV resistance genes using primer E15M7 (E45M61) with resistant BC5, BC7 and BC9 individuals of pools 3A and 3B. The putatively linked AFLP marker was identified by the first three steps of the tBSA strategy. *Lanes*: P_S susceptible parent FAP1360A,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

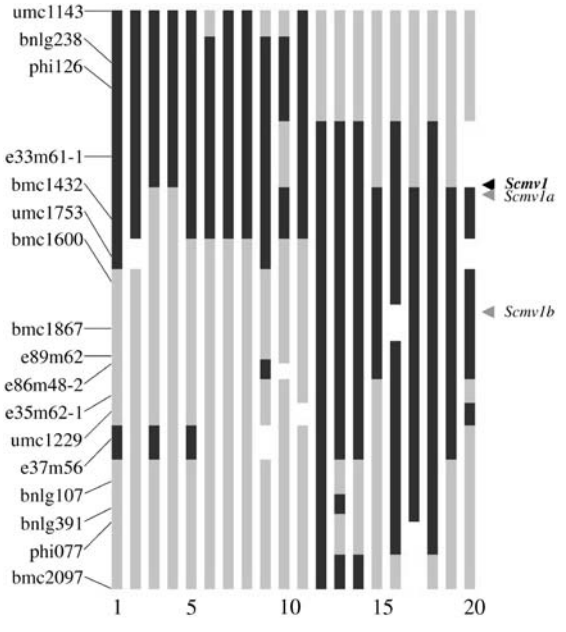

Fig. 3 Graphical genotypes of 11 BC7 to BC9 individuals of Pool 6A (1–11) and 9 BC7 to BC9 individuals of Pool 6B (12–20). *Black* = segments, heterozygous for the resistant parent FAP1360A and the susceptible parent F7, *gray* = segments homozygous for the susceptible parent F7, *white* = unknown segment 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

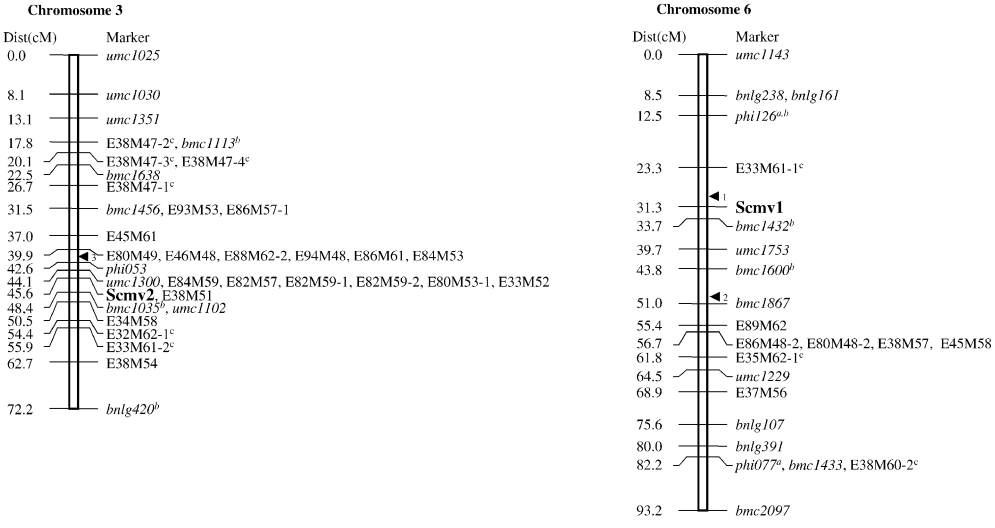

Fig. 4 High-resolution maps of chromosome regions harboring the SCMV resistance genes *Scmv1* and *Scmv2*. *Left*: centomere region of chromosome 3 harboring *Scmv2*. *Right*: short arm of chromosome 6 harboring *Scmv1*. The maps were generated by joint linkage analyses of the BC1, BC5, BC7, and BC9 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/ibmmaps.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.

Acknowledgements The present study was supported by grants from the Deutsche Forschungsgemeinschaft, Grant No. LU601/2-3. We thank Susanne Dreisigacker, Sebastian Haesler and Nadine Burkarth for their support with the SSR analyses. The skilled technical assistance of Franz Mauch and Astrid Karg in conducting the field trials is gratefully acknowledged.

References

Ballvora A, Hesselbach J, Niewöhner J, Leister D, Salamini F, Gebhardt C (1995) Marker enrichment and high-resolution map of the segment of potato chromosome VII harbouring the nematode resistance gene *Gro1*. Mol Gen Genet 249:82–90

Castiglioni P, Ajmone-Marsan P, van Wijk R, Motto M (1999) AFLP markers in a molecular linkage map of maize: codominant scoring and linkage group distribution. Theor Appl Genet 99:425–431

Cervera MT, Gusmao J, Steenackers M, Peleman J, Storme V, Vanden Broeck A, Van Montagu M, Boerjan W (1996) Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. Theor Appl Genet 93:733–737

Chagué V, Mercier JC, Guénard M, de Courcel A, Vedel F (1997) Identification of RAPD markers linked to a locus involved in quantitative resistance to TYLCV in tomato by bulked segregant analysis. Theor Appl Genet 95:671–677

Dußle CM, Melchinger AE, Kuntze L, Stork A, Lübberstedt T (2000) Molecular mapping and gene action of *Scm1* and *Scm2*, two major QTLs contributing to SCMV resistance in maize. Plant Breed 119:299–303

Fuchs E, Grüntzig M (1995) Influence of sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) on the growth and yield of two maize varieties. J Plant Dis Protection 102:44–50

Fuchs E, Grüntzig M, Kuntze L, Oertel U, Hohmann F (1996) Zur Epidemologie der Potyviren des Maises in Deutschland. In: Bericht über die 46. Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter im Rahmen der "Vereinigung österreichischer Pflanzenzüchter" 46:43–49

Gardiner JM, Coe EH, Melia-Hancock S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized F_2 population. Genetics 134:917–930

Gill KS, Gill BS, Endo TR, Taylor T (1996) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891

Green P, Falls K, Crooks S (1990) Cri-Map, version 2.4. http://biobase.dk/Embnetut/Crimap

Helentjaris T, Weber D, Wright S (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction length polymorphisms. Genetics 118:353–363

Hoisington DA, Khairallah MM, Gonzales-de Leon D (1994) Laboratory protocols: CIMMYT Applied Molecular Genetics Laboratory, CIMMYT, Mexico, DF

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation break points. Genetics 154:397–412

Kuntze L, Fuchs E, Grüntzig M, Schulz B, Klein D, Melchinger AE (1997) Resistance of early maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breed 116:499–501

Lübberstedt T, Dußle CM, Melchinger AE (1998) Application of microsatellites from maize to teosinte and other relatives of maize. Plant Breed 117:447–450

Melchinger AE, Kuntze L, Gumber RK, Lübberstedt T, Fuchs E (1998) Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor Appl Genet 96:1151–1161

Michelmore R, Paran I, Kesseli R (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Thomas CM, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8:785–794

Van Berloo R (1999) Computer note. GGT: software for the display of graphical genotypes. J Hered 90:328–329

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lübberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. Theor Appl Genet 99:921–935

Xia XC, Melchinger AE, Kuntze L, Lübberstedt T (1999) QTL mapping of resistance to sugarcane mosaic virus in maize. Phythopathology 89:660–667

Xu ML, Melchinger AE, Xia XC, Lübberstedt T (1999) High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Mol Gen Genet 261:574–581

Yuan L, Dußle CM, Melchinger AE, Utz HF, Lübberstedt T (2002) Clustering of QTL conferring SCMV resistance in maize. Maydica (in press)

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application EP 0534858A1

Theor Appl Genet (2002) 105:1190–1195
DOI 10.1007/s00122-002-0964-7

C. M. Dussle · M. Quint · M. L. Xu · A. E. Melchinger
T. Lübberstedt

Conversion of AFLP fragments tightly linked to SCMV resistance genes *Scmv1* and *Scmv2* into simple PCR-based markers

Received: 31 October 2001 / Accepted: 14 December 2001 / Published online: 22 October 2002

Abstract In a previous study, bulked segregant analysis with amplified fragment length polymorphisms (AFLPs) identified several markers closely linked to the sugarcane mosaic virus resistance genes *Scmv1* on chromosome 6 and *Scmv2* on chromosome 3. Six AFLP markers (E33M61-2, E33M52, E38M51, E82M57, E84M59 and E93M53) were located on chromosome 3 and two markers (E33M61-1 and E35M62-1) on chromosome 6. Our objective in the present study was to sequence the respective AFLP bands in order to convert these dominant markers into more simple and reliable polymerase chain reaction (PCR)-based sequence-tagged site markers. Six AFLP markers resulted either in complete identical sequences between the six inbreds investigated in this study or revealed single nucleotide polymorphisms within the inbred lines and were, therefore, not converted. One dominant AFLP marker (E35M62-1) was converted into an insertion/deletion (indel) marker and a second AFLP marker (E33M61-2) into a cleaved amplified polymorphic sequence marker. Mapping of both converted PCR-based markers confirmed their localization to the same chromosome region (E33M61-2 on chromosome 3; E35M62-1 on chromosome 6) as the original AFLP markers. Thus, these markers will be useful for marker-assisted selection and facilitate map-based cloning of SCMV resistance genes.

Communicated by G. Wenzel

C.M. Dussle · M. Quint · M.L. Xu · A.E. Melchinger
T. Lübberstedt (✉)
Institute of Plant Breeding, Seed Science, and Population Genetics,
University of Hohenheim, 70593 Stuttgart, Germany
e-mail: thomas.luebberstedt@agrsci.dk
Fax: +49-4558-113301

Present address:
M.L. Xu, College of Biosciences and Biotechnology,
Yangzhou University, 225009, Yangzhou, PRC

Present address:
T. Lübberstedt, Danish Institute of Agricultural Sciences,
Research Centre Flakkebjerg, 4200 Slagelse, Denmark

Keywords AFLP · CAPS · Indel marker · Maize · Marker conversion · Sugarcane mosaic virus (SCMV)

Introduction

Sugarcane mosaic virus (SCMV) is one of the most important virus diseases of maize (*Zea mays* L.) and causes serious yield losses in susceptible cultivars (Fuchs and Grüntzig 1995). It is naturally transmitted by aphids in a non-persistent manner, which makes control of SCMV vectors rather inefficient. Therefore, cultivation of resistant varieties is the most promising approach for controlling of SCMV.

Kuntze et al. (1997) screened 122 early-maturing European inbred lines for resistance to SCMV and MDMV (maize dwarf mosaic virus) and identified three dent inbreds (D21, D32 and FAP1360A) displaying complete resistance under both field and greenhouse conditions. Two major genes, *Scmv*1 and *Scmv*2 (previously named *Scm1* and *Scm2*), conferring resistance to SCMV were mapped to chromosome arms 6S and 3L, respectively, in cross D145 × D32 by quantitative trait loci (QTL) analysis (Xia et al. 1999) and in cross F7 × FAP1360A by bulked segregant analysis (BSA) (Xu et al. 1999) and QTL analysis (Dussle et al. 2000). As resistance against SCMV is strongly affected by environmental conditions (Melchinger et al. 1998), molecular markers turned out to be a good tool to determine the resistance genotype.

Identification of molecular markers closely linked to the SCMV resistance genes is an essential step towards both marker-assisted selection (MAS) and map-based cloning of these genes. Xu et al. (1999) identified 23 tightly linked amplified fragment length polymorphism (AFLP) markers for both major resistance genes by BSA: 11 markers linked to *Scmv2* on chromosome 3 and 12 linked to *Scmv1* on chromosome 6, including one AFLP marker cosegregating with *Scmv1*.

Although the AFLP technique is powerful and reliable in identifying markers closely linked to genes of in-

terest, it has some disadvantages for use in MAS and map-based cloning. Limitations to the large-scale, locus-specific application of AFLPs include their dominant type of inheritance, the intensity of labour involved, and the high costs. Hence, conversion of AFLP markers into sequence-specific polymerase chain reaction (PCR) markers is required for screening large breeding populations at low costs.

Sequence-specific PCR markers have been successfully developed by conversion of different marker types such as RFLPs, RAPDs and SSRs, (Bradshaw et al. 1994; Cheung et al. 1997; Jung et al. 1999). The conversion of AFLP markers into PCR-based markers has been accomplished for several species such as carrot (Bradeen and Simon 1998), brassica (Negi et al. 2000), asparagus (Reamon-Büttner et al. 2000), soybean (Meksem et al. 2001), apple (Xu et al. 2001), barley and wheat (Shan et al. 1999). However, the conversion of AFLP markers seems to be more difficult than the conversion of other marker types due to the loss of their sequence specificity after amplification of the AFLP-derived internal primers (Shan et al. 1999). Hence, AFLP polymorphisms related to *Eco*RI or *Mse*I restriction site differences will not be reflected in primers from an internal sequence (Shan et al. 1999).

The objective of the study reported here was to sequence the respective AFLP bands linked to SCMV resistance genes in order to convert these dominant markers into either indel (insertion/deletion) or cleaved amplified polymorphic sequence (CAPS) markers. These AFLP markers were previously identified by BSA to be closely linked with *Scmv1* on chromosome 6 (two markers) or *Scmv2* on chromosome 3 (six markers). Our goal was to obtain codominant, simple PCR-based markers as a tool for marker-assisted selection as well as for map-based cloning of *Scmv1* and *Scmv2*.

Materials and methods

Plant materials

Inbred lines used in this analysis were the SCMV-resistant European inbred lines FAP1360A, D21 and D32 and the highly susceptible lines F7, D408 and D145. The mapping population consisted of (1) a subset 87 $F_{2:3}$ families derived from a cross between D32 and D145 previously used by Vuylsteke et al. (1999) to develop a high-density AFLP map (1,355 markers), and (2) 27 resistant BC_5 individuals from the cross (F7 × FAP1360A) × F7.

Isolation and cloning of tightly linked AFLP markers

AFLP markers flanking *Scmv1* (E35M62-1, E33M61-1) and *Scmv2* (E33M61-2, E33M52, E38M51, E82M57, E84M59, E93M53) were identified in a BSA employing four DNA samples: both parental lines FAP1360A (resistant parent) and F7 (susceptible parent), as well as a resistant and a susceptible bulk (Xu et al. 1999). 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). AFLP primer pairs corresponding to the tightly linked AFLP markers were used to re-amplify the linked AFLP markers from resistant parent FAP1360A. The resulting bands were excised from the dried gel with a sharp, clean razor blade. The sliced DNA-containing gel was transferred into an Eppendorf tube, eluted twice with 200 µl TE (1 h each) and once with 200 µl ddH_2O (2 h). The eluted gel was then mixed with 50 µl ddH_2O and kept on boiling water for 5 min to release the DNA from the gel. After the gel debris was spun down, the DNA-containing supernatant was transferred into a new Eppendorf tube and used as template for the subsequent amplification.

For a given linked AFLP marker, the corresponding primer pair and the same reaction conditions as for the main amplification of AFLPs (Vos et al. 1995) were employed to re-amplify the isolated AFLP marker bands. Re-amplification products were excised from an agarose gel, extracted with Nucleospin Kit (Macherey & Nagel) and blunt-end cloned into the pBluescript vector.

Conversion of AFLP markers

After cloning, five white colonies from each transformation event were selected. Respective inserts were sequenced using the ALF-Express automated sequencer (Amersham Pharmacia, Freiburg). Sequencing reaction conditions were chosen following the manufacturer's (Amersham Pharmacia) suggestions, and the DNA sequences were analysed using the ALIGNPLUS 2.0 software package (http://www.scied.com/ses_alim.htm). If the sequencing of these first five clones showed identity for at least three of the five clones, new primers internal to the AFLP selective primers were designed using the PRIMER1.02 programme (http://www.scied.com/ses_pd4.htm) (Table 1). Otherwise, additional five white clones were sequenced to receive a majority of identical sequences for one genotype. The internal primers synthesized for fragments corresponding to the AFLP markers were employed to amplify fragments from the inbred lines F7 and FAP1360A, which represent the parent lines of the mapping population for BSA. Internal primers of the three AFLP markers E33M61-1, E33M61-2 and E35M62-1 were additionally employed on the four inbred lines D21, D32 (SCMV resistant), and D145, D408 (susceptible) in order to evaluate the relationship between polymorphisms and SCMV resistance. The extension "STS" was added to the names of the AFLP marker after synthesizing the internal primers in order to distinguish AFLP markers and converted markers.

Sequenced tagged site (STS) markers that differed in length after amplification were used immediately as indel (insertion/deletion) markers. In the case of an identical sequence length, enzyme recognition sites were identified using the CLONE manager software package (http://www.scied.com/ses_cm6.htm). Sequence regions displaying single nucleotide differences in restriction enzyme recognition sites between parent lines of mapping populations were used to identify CAPS markers, which were separated on a 3% MetaPhor agarose gel in 0.5× TBE buffer.

Linkage and statistical analyses

Based on the segregation data, the STS markers were mapped to previously constructed genetic linkage maps (Xia et al. 1999; Xu et al. 1999). Marker orders and map distances for population D32 × D145 were calculated with MAPMAKER 3.0B (Lander et al. 1987) using a LOD threshold of 3.0 and the mapping function of Kosambi (1944). Marker orders and genetic distances for population FAP1360A × F7 were calculated with CRIMAP 2.4 (Green et al. 1990) taking into account the meiotic interdependence of progenies and ancestors within a population of BC individuals from different generations.

Table 1 STS marker development in maize: detailed information on eight STS markers converted from AFLPs that are closely linked to resistance genes *Scmv1* (chromosome 6) and *Scmv2* (chromosome 3)

Marker locus	Chromosome	STS forward primer (5′ → 3′) STS reverse primer (5′ → 3′)	Sequence length after amplification with STS primers (bp)	Number of SNPs[a]	Marker type
		Primers analysed with inbred F7 and FAP1360A			
E33M52STS	3	CCATATCGTGTTGAGAAGGC CCACTCAATGCGGTGTCTAT	173	1	–
E38M51STS	3	CACCAAGAAGGTTTGGATCC GCGTACCAATTCACTAACCG	146	–	–
E82M57STS	3	AACCTCCTAGCGTCATGTAG AGTCCTGAGTAACGGATCC	166	–	–
E84M59STS	3	AACAACAGTTACCAGGCCAG CTTCAGATTCTCCCGAACCA	168	2	–
E93M53STS	3	GCTTGCCAATTCTGCATGCA	203	–	–
		Primers analysed with inbred lines F7, FAP1360A D21, D32, D145 and D408			
E35M62-1STS	6	GAGTCCTGAGTAACCGCCTA CTTCATGCCTCTCGTCG	152; 160	7	Indel
E33M61-1STS	6	ACTGCTTAGTCCTCGACAGA CGTACCAATTCAAGAGCGAC	195	–	–
E33M61-2STS	3	TCTTGTGCAACTACGACACC GATGATGGCATTGTCGAGGA	152	8	CAPS

[a] Identified between pairs of inbred lines

Results

The polymorphic markers linked to SCMV resistance, obtained after AFLP analysis, were in the range of 200 bp to 300 bp. All sequenced AFLP fragments contained the *Eco*RI adapter on the one end and the *Mse*I adapter on the other end. Five to ten clones obtained from inbred line FAP1360A were used to design primers for PCR amplification of genomic DNA (Table 1). Following amplification using these PCR primers, no difference in sequence length was identified for any of the markers between inbred lines F7 and FAP1360A, with sequence lengths ranging from 146 bp for marker E38M51STS to 203 bp for marker E93M53STS. For the additionally analysed inbred lines (D21, D32, D145 and D408), markers E33M61-1STS and E33M61-2STS showed identical sequence length for all six inbred lines (195 bp and 152 bp, respectively). A 152-bp sequence was identified with marker E35M62-1STS for all of the inbred lines D32, D21 and D408 except D145 (160 bp). This polymorphism of 8 bp between the parents of cross D32 × D145 could be easily detected on a 3% MetaPhor agarose gel (FMC) (Fig. 1). Genetic mapping was performed using 87 $F_{3:2}$ families of cross D32 × D145, which located E35M62-1STS on maize chromosome 6S between markers phi075 and phi077 within the *Scmv1* QTL region previously identified by Xia et al. (1999).

Six out of the eight markers showed identical sequences among the clones within each inbred line. In contrast, markers E33M61-1STS and E84M59STS resulted in single nucleotide differences between the clones within each inbred line. Single nucleotide polymorphisms (SNPs) between inbred lines F7 and FAP1360A were found for the four markers E33M61-2STS, E35M62-1STS, E33M52STS and E84M59STS. Recognition sites for restriction enzymes could be found only for marker E33M61-2STS, resulting in a different number of recognition sites for the restriction enzyme *Mnl*I (Fig. 2). For marker E33M61-2STS, *Mnl*I cuts the fragments of FAP1360A (Fig. 2) and D408 four times. The fragments of inbred lines F7, D21, D32 and D145 were cut only three times with *Mnl*I. Therefore, E33M61-2STS could be used as a CAPS marker (Fig. 3). Mapping of E33M61-2STS with the BC_5 mapping population (FAP1360A × F7) confirmed the same segregation pattern with its corresponding AFLP marker E33M61-2 and its location 7.3 cM above *Scmv*2.

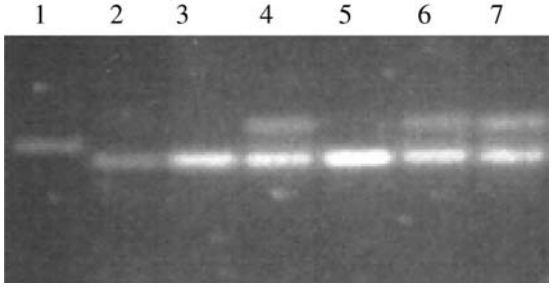

Fig. 1 Polymorphism in population D32 × D145 after conversion of AFLP primer E35M62-1 to the indel marker E35M62-1STS on a 1.5% agarose gel. *Lanes: 1* Susceptible parent D145, *2* resistant parent D32, *3–7* genotypes of the mapping population (*3*, *5* homozygous; *4*, *6*, *7* heterozygous band pattern)

Fig. 2 Recognition sites for restriction enzyme *Mnl*I in parents F7 (susceptible) and FAP1360A (resistant) after amplification with CAPS marker E33M61-2STS

```
F7          (   1)   GATGATGGCATTGTCGAGGATGAAGTCCCTGTGGCTCGATGAAGATGTCG
FAP1360A    (   1)   GATGATGGCATTGTCGAGGATGAAGTCCCTGTGGCTCGATGAAGAgGTCG

F7          (  51)   GAGAAGGATTTCCAGCAACGTGCCCACGAGCTTGTGGGACATGTCCATGG
FAP1360A    (  51)   GAGAAGGATTTCCAGCAACGTGCCCACGAGCTTGTGGGACATGTCCATGG

F7          ( 101)   AGGCAAGTTGTCCTACTATTCCACCCAGGTCGTTCCAGCGAACCAGGTGG
FAP1360A    ( 101)   AGGCAAGTTGTCCTgCTATTCCACCCAGGTCGcTCCAaCGAACCAGGTGG

F7          ( 151)   CCTTGCCGCCAGAAAGTCATGGTGAGGGTGTCGTAGTTGCACAA
FAP1360A    ( 151)   CCTTGCCGCCAGAAAGTCATGGTGAGGGTGTCGTAGTTcCACAA
```

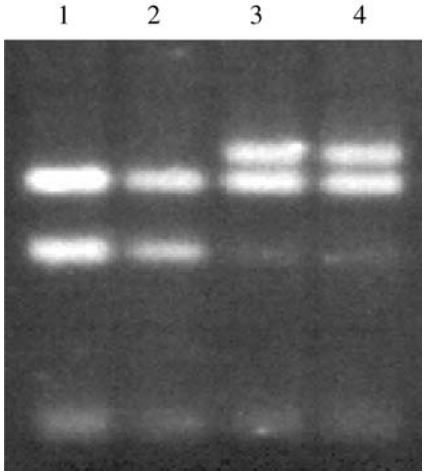

Fig. 3 CAPS marker E33M61-2STS digested with the restriction enzyme *Mnl*I corresponding to AFLP marker E33M61-2. *Lanes 1, 2* Resistant parent FAP1360A, *3*, *4* susceptible parent F7

Four out of the eight investigated markers revealed no SNPs after pairwise comparison of the inbreds. Although only half of the analysed markers showed between one and eight SNPs per inbred pair, 2.1 SNPs were found per inbred pair on average, resulting in one SNP per 71 bp. Analyses for stop codons identified continuous open reading frames for none of the sequences.

Discussion

In combination with BSA, AFLPs proved to be highly efficient for finding tightly linked molecular markers to the SCMV resistance genes *Scmv1* and *Scmv2* (Xu et al. 1999). However, AFLP markers are too costly and laborious for high-throughput monitoring of large numbers of genotypes. Hence, the conversion of AFLP markers closely linked to resistance genes is an important step to implement useful markers for MAS and map-based cloning, both of which require large population sizes of thousands of individuals. Marker conversion requires the characterization of the linked marker sequences and the design of locus-specific primers (Paran and Michelmore 1993). Reports on successful AFLP marker conversion are lacking so far in maize. With a size of 500–1500 bp, RAPD fragments are easier to convert to either sequence-characterised amplified region (SCAR) or CAPS markers than AFLP markers (Barret et al. 1998). Although there are doubts in converting short fragments like AFLPs (Negi et al. 2000), we were able to convert two short AFLP bands (150–300 bp) into PCR-based indel and CAPS markers without using methods like inverse PCR or chromosome walking. In contrast to DeJong et al. (1997) and Negi et al. (2000), who applied either inverse PCR or PCR walking to isolate the flanking regions for conversion of indel markers, we found with E35M62-1STS a polymorphism that could be used directly as an indel marker in populations generated from cross of D32 and D145.

After sequencing five to ten clones for each STS marker, we obtained six markers with identical sequences over most of the clones originating from one inbred. For markers E33M61-1STS and E84M53STS, SNPs were also found within all inbred lines. In total, 8 out of 28 inbreds investigated with the eight markers revealed single nucleotide changes within the inbred lines. The small number of published reports on AFLP marker conversion might be due to similar findings. Because identical sequences for the several clones of one inbred line were found technical problems in sequencing could be ruled out. The probability of residual heterozygosity for the inbred lines is below 0.025% because they were self-fertilized for more than 12 generations. Taking into account that the independent inbred lines revealed the same SNP within these inbreds in every case, it seems very unlikely that these polymorphisms were caused by residual heterozygosity. If we accept, the hypothesis that maize is an ancient tetraploid species (Gaut and Doebley 1997), the whole region harbouring the SCMV resistance gene might be duplicated, even though located at different regions of the genome. Under these conditions the segregation ratio would shift from 1r:3s (1 resistant to 3 susceptible) under a two dominant gene model for BC plants to 1r:7s under a three gene model. Although Xu et al. (1999) found a better fit with a three dominant gene model for the segregation within 20 $BC_{4:5}$ families of population F7 × FAP1360A segregating for SCMV resistance, the presence of additional SCMV resistance genes beside those on chromosomes 3 and 6 could not be confirmed for population F7 × FAP1360A. In contrast, mapping of the original AFLP markers did locate markers E33M61-1STS and E84M59STS exclusively to chromosomes 6 and 3, respectively.

Clusters of resistance genes originating from duplication during the evolution of maize may have led to slight sequence differences of paralogs, which may differ only in single nucleotides but not in total length. In mapping BAC clones of lettuce in order to analyze resistance gene clusters, Meyers et al. (1998) identified duplicates of AFLP markers in the same chromosome region. Genes conferring resistance to different pathogens are often clustered in the same chromosome region in the maize genome (McMullen and Simcox 1995). The fact that the AFLP markers corresponding to the converted STS markers mapped in the same regions previously reported to harbour clusters of resistance genes (McMullen et al. 1995) allows the assumption that the different marker sequences found within one inbred are linked to different resistance genes in the same chromosome region. The closer a marker is linked to a specific resistance gene, the higher might be the probability of being duplicated with the resistance gene during evolution. Hence, the occurrence of different sequences within one inbred line that map to the same chromosome region seems to be possible due to clustering. However, none of the sequenced AFLP fragments revealed any similarity to sequences known to be conserved within resistance genes.

The development of markers that can be easily handled is a prerequisite to the screening of large populations in order to clone the resistance genes *Scmv1* and *Scmv2*. The converted CAPS and indel markers will be useful to identify recombination events close to *Scmv1* and *Scmv2*. So far, it is unknown whether resistance genes cluster due to linkage or whether some of them are identical and display pleiotropy. In support of the existence of closely linked but different major resistance genes, Lübberstedt et al. (1999) found some susceptible plants in an allelism test between the three European dent inbreds D21, D32 and FAP1360A. The converted, closely linked markers identified in the present study could be used as probes for BAC screening in order to solve the question of whether the *Scmv1* and the *Scmv2* regions each harbour only a single locus or clusters of resistance loci.

Rafalski et al. (2001) analysed random cDNA clones in a collection of over 30 maize lines representative for the North American corn germplasm. Their analysis was restricted to coding regions. Sequence alignment revealed one SNP per 70 bp among the 30 lines. The authors emphasized that pairwise comparison between any two lines reveals a lower degree of polymorphism. In similar experiments, Useche et al. (2001) detected one SNP per 49 bp, although in non-coding regions. The low number of one SNP per 71 bp found in our study might be due to the pairwise sequence comparison in contrast to the sequence alignment of a large number of inbreds analysed by the previous authors. Taking into account that only four out of the eight converted AFLP markers showed polymorphism between inbred lines, it seems very likely that extending the fragment size by inverse PCR would increase the number of polymorphic STS-primers.

The CAPS marker E33M61-2STS turned out to be dominant in the mapping population. The polymorphism resulted in the presence of an additional band in the resistant parent FAP1360A that was absent in the susceptible parent F7 (Fig. 3). As in this mapping population the individuals were either homozygous for the susceptible parent allele or heterozygous, mapping with our mapping population of 27 resistant BC_5 individuals was not affected. However, even in this dominant case, CAPS markers are easier to apply than the original AFLP markers. In contrast to the AFLP markers, the converted markers do not require purified, high-molecular-weight DNA. Consequently, the application of simple STS markers enables a faster DNA isolation for a high number of individuals required for fine mapping. Additionally, the polymorphisms revealed by STS markers could be separated by an agarose gel, where no radioactivity is required to visualize the results. Compared to the analyses of AFLP markers in which polyacrylamide gels and radioactivity are used, the application of STS markers can reduce costs to about 20%.

The quality of a marker used for MAS depends on its predictive and/or diagnostic value (Borchardt and Weissleder 2000). Whereas the predictive value of a marker is determined by the inheritance of the marker and the linkage between marker and trait, the diagnostic value can be measured as the frequency of the desired linkage phase between marker and trait. Taking into account that resistant individuals of different populations harbour different resistance alleles of the same gene, cosegregation of these markers with the resistance trait in each population (F7 × FAP1360A, D32 × D145, D21 × D408) is not consequently preconditioned. By analysing inbred lines showing resistance, partial resistance and susceptibility to SCMV, Xu et al. (2000) suggested a single common ancestor for the resistance gene *Scmv1*. In the present study, no marker allele identical for all resistant or susceptible genotypes was identified. Therefore, the diagnostic value of these markers seems to be low. In the case of a low diagnostic value, the allelic phase of a marker has to be checked in each cross before it can be used in MAS (Borchardt and Weissleder 2000). A reason for the lack of resistance-allele-specific, cosegregating markers could be the presence of more than one SCMV resistance gene in the *Scmv1* region. Field experiments, BSA (Xu et al. 1999), and QTL analyses (Xia et al. 1999; Dussle et al. 2000) did not preclude the presence of more than one gene in the *Scmv1* region. Since different ancestors were expected for *Scmv2* (Dussle et al. 2000; Xu et al. 2000) and, therefore, different SCMV resistance genes within the *Scmv2* region, it was not possible to develop one single resistance-allele-specific marker for *Scmv2*.

Acknowledgements The present study was supported by a grant from the Deutsche Forschungsgemeinschaft, Grant No. LU601/2. The authors gratefully acknowledge the skilled technical assistance of Elisabeth Kokai-Kota.

References

Barret P, Delourme R, Foisset N, Renard M (1998) Development of a SCAR (sequence characterized amplified region) marker for molecular tagging of the dwarf BREIZH (*Bzh*) gene in *Brassica napus* L. Theor Appl Genet 97:828–833

Borchardt DC, Weissleder KK (2000) Marker-assisted selection in sugar beet: a breeder's perspective. In: Gallais A, Dillmann C, Goldringer I (eds) EUCARPIA Quantitative genetics and breeding methods: the way ahead. Proc Meet EUCARAPIA Sect Biometrics Plant Breed. INRA, Yessailles, pp 213–220

Bradeen JM, Simon PW (1998) Conversion of an AFLP fragment linked to the carrot Y_2 locus to a simple, codominant PCR-based marker form. Theor Appl Genet 97:960–967

Bradshaw HD Jr, Villar M, Watson BD, Otto KG, Stewart S, Stettler RF (1994) Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. Theor Appl Genet 89:167–178

Cheung WY, Champagne G, Hubert N, Landry BS (1997) Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. Theor Appl Genet 94:569–582

DeJong W, Forsyth A, Leister D, Gebhardt C, Baulcombe DC (1997) A potato hypersensitive resistance against potato virus X maps to a resistance gene cluster on chromosome 5. Theor Appl Genet 95:246–252

Dussle CM, Melchinger AE, Kuntze L, Stork A, Lübberstedt T (2000) Molecular mapping and gene action of *Scm1* and *Scm2*, two major QTL contributing to SCMV resistance in maize. Plant Breed 119:299–303

Fuchs E, Grüntzig M (1995) Influence of sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) on the growth and yield of two maize varieties. J. Plant Dis Protect 102:44–50

Gaut BS, Doebley JF (1997) DNA sequence evidence for the segmental allotetraploid origin of maize. Proc Natl Acad Sci USA 94:6809–6814

Green P, Falls K, Crooks S (1990) CRI-MAP, version 2.4. http://biobase.dk/Embnetut/Crimap

Jung G, Skroch PW, Nienhuis J, Coyne DP, Arnaud-Santana E, Ariyarathne HM, Marita JM (1999) Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. Crop Sci 39:1448–1455

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Kuntze L, Fuchs E, Grüntzig M, Schulz B, Klein D, Melchinger AE (1997) Resistance of early-maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breed 116:499–501

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

Lübberstedt T, Xia XC, Xu ML, Kuntze L, Melchinger AE (1999) Inheritance of resistance to SCMV and MDMV in European maize. In: Mugnozza GTS (ed) Proc 15th EUCARPIA 1998 Gen Congr Genet Breed Crop Quality Resistance. Kluwer, Dordrecht, pp 241–250

McMullen MD, Simcox KD (1995) Genomic organization of disease and insect resistance genes in maize. Mol Plant Microbe Interact 8:811–815

Meksem K, Ruben E, Hyten D, Triwitayakorn K, Lightfoot (2001) Conversion of AFLP bands into high-throughput DNA markers. Mol Gen Genet 265:207–214

Melchinger AE, Kuntze L, Gumber RK, Lübberstedt T, Fuchs E (1998) Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor Appl Genet 96:1151–1161

Meyers BC, Chin DB, Shen KA, Sivaramakrishnan S, Lavelle DO, Zhang Z, Michelmore RW (1998) The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell 10:1817–1832

Negi MS, Devic M, Delseny M, Lakshmikumaran M (2000) Identification of AFLP fragments linked to seed coat color in *Brassica juncea* and conversion to SCAR marker for rapid selection. Theor Appl Genet 101:146–152

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance in lettuce. Theor Appl Genet 85:985–993

Rafalski JA, Ching A, Bhattramakki D, Morgante M, Dolan M, Register JC, Smith OS, Tingey S (2001) SNP markers in maize: discovery and applications. In: Grant D, Lazo G, Carollo V, Marshall K (eds) Plant Anim Genome 9th Conf

Reamon-Büttner SM, Jung C (2000) AFLP-derived STS markers for the identification of sex in *Asparagus officinalis* L. Theor Appl Genet 100:432–438

Shan X, Blake TK, Talbert LE (1999) Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. Theor Appl Genet 98:1072–1078

Useche F, Morgante M, Hanafey M, Tingey S, Martins W, Gao GR, Rafalski A (2001) Computer detection of single nucleotide polymorphisms (SNPs) in maize ESTs. In: Grant D, Lazo G, Carollo V, Marshall K (eds) Plant Anim Genome 9th Conf. San Deigo, p 144

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lübberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. Theor Appl Genet 99:921–935

Xia XC, Melchinger AE, Kuntze L, Lübberstedt T (1999) QTL mapping of resistance to sugarcane mosaic virus in maize. Phythopathology 89:660–667

Xu ML, Melchinger AE, Xia XC, Lübberstedt T (1999) High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Mol Gen Genet 261:574–581

Xu ML, Melchinger AE, Lübberstedt T (2000) Origin of *Scm1* and *Scm2* – two loci conferring resistance to sugarcane mosaic virus (SCMV) in maize. Theor Appl Genet 100:934–941

Xu ML, Huaracha E, Korban SS (2001) Development of sequence-characterized amplified regions (SCARs) from amplified fragment length polymorphism (AFLP) markers tightly linked to the *Vf* gene in apple. Genome 44:63–70

Theor Appl Genet (2002) 105:355–363
DOI 10.1007/s00122-002-0953-x

M. Quint · R. Mihaljevic · C. M. Dussle · M. L. Xu
A. E. Melchinger · T. Lübberstedt

Development of RGA-CAPS markers and genetic mapping of candidate genes for sugarcane mosaic virus resistance in maize

Received: 23 September 2001 / Accepted: 12 December 2001 / Published online: 19 June 2002

Abstract Three previously published resistance gene analogues (RGAs), pic13, pic21 and pic19, were mapped in relation to sugarcane mosaic virus (SCMV) resistance genes (*Scmv1*, *Scmv2*) in maize. We cloned these RGAs from six inbreds including three SCMV-resistant lines (D21, D32, FAP1360A) and three SCMV-susceptible lines (D145, D408, F7). Pairwise sequence alignments among the six inbreds revealed a frequency of one single nucleotide polymorphism (SNP) per 33 bp for the three RGAs, indicating a high degree of polymorphism and a high probability of success in converting RGAs into co-dominant cleaved amplified polymorphic sequence (CAPS) markers compared to other sequences. SNPs were used to develop CAPS markers for mapping of the three RGAs in relation to *Scmv1* (chromosome 6) and *Scmv2* (chromosome 3), and for pedigree analyses of resistant inbred lines. By genetic mapping pic21 was shown to be different from *Scmv2*, whereas pic19 and pic13 are still candidates for *Scmv1* and *Scmv2*, respectively, due to genetic mapping and consistent restriction patterns of ancestral lines.

Keywords RGA · SNP · CAPS · SCMV · Maize

Communicated by C. Möllers

M. Quint · R. Mihaljevic · C.M. Dussle · M.L. Xu
A.E. Melchinger · T. Lübberstedt (✉)
Institute of Plant Breeding, Seed Science,
and Population Genetics, University of Hohenheim,
Fruwirthstraße 21, 70593 Stuttgart, Germany
e-mail: Thomas.Luebberstedt@agrsci.dk
Fax: +45-58-11-33-01

Present addresses:
M.L. Xu, College of Bioscience and Biotechnology,
Yangzhou University, Yangzhou, Jiangsu, 225009, PR China

T. Lübberstedt, Danish Institute of Agricultural Sciences,
4200 Flakkebjerg, Denmark

Introduction

Sugarcane mosaic virus (SCMV) is one of the most important virus diseases of maize and causes serious yield losses in susceptible cultivars (Fuchs and Grüntzig 1995). Owing to the non-persistent transmission, control of aphid vectors by chemical means is not effective and, therefore, cultivation of resistant maize varieties is the most efficient method of virus control. In a study with 122 early maturing European maize inbreds, three lines (FAP1360A, D21 and D32) displayed complete resistance and four lines displayed partial resistance (FAP1396A, D06, D09 and R2306) against SCMV and maize dwarf mosaic virus (MDMV) (Kuntze et al. 1997). In field trials, resistance of all three European lines D21, D32 and FAP1360A seemed to be controlled by one to three genes (Melchinger et al. 1998). Linkage mapping and 'bulked segregant analysis' (BSA) mapped two major genes, *Scmv1* on the short arm of chromosome 6 and *Scmv2* near the centromere of chromosome 3 (Melchinger et al. 1998; Xia et al. 1999; Xu et al. 1999; Dußle et al. 2000). Minor quantitative trait loci (QTLs) affecting SCMV resistance were identified on chromosomes 1, 5, and 10 (Xia et al. 1999). For full resistance to SCMV, the presence of both *Scmv1* and *Scmv2* is essential. *Scmv1* suppresses symptom expression throughout all developmental growth stages at a high level, whereas *Scmv2* was expressed at later stages of infection (Xia et al. 1999; Dußle et al. 2000).

With the cloning of a number of disease resistance genes (R genes) from several plant species, it became obvious that these R genes share homologies in protein domains such as the nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) (reviewed in Bent 1996; reviewed in Hammond-Kosack and Jones 1996). Degenerate primers based on the amino-acid sequence of these domains have meanwhile allowed successful PCR amplification of several RGAs from various plant species with significant homology to known plant disease R genes. Collins et al. (1998) identified 20 RGA loci in maize, which mapped preferentially to chromosomal regions

known to carry R genes (McMullen and Simcox 1995). These RGAs can be further analysed for their potential use in marker-assisted selection (MAS) or even the cloning of target genes. The latter approach has been successfully used in maize to clone the *Rp1-D* gene, a member of the complex locus composed of approximately nine gene homologues, conferring resistance to common rust, *Puccinia sorghi* (Collins et al. 1999).

Most sequence variation is attributable to single nucleotide polymorphisms (SNPs), with the rest attributable to insertions or deletions of one or more bases, repeat length polymorphisms and rearrangements (The International SNP Map Working Group 2001). In maize, one SNP between two randomly sampled sequences occurs approximately every 104 bp (Tenaillon et al. 2001). Therefore, SNPs are present at sufficient density for comprehensive haplotype analysis as applied in this study.

The objectives of the present study were to: (1) clone three RGAs previously mapped to chromosomal regions known to harbour SCMV R genes from six inbred lines resistant or susceptible to SCMV, (2) measure the frequency of SNP and CAPS occurring within RGAs and evaluate their usefulness as a source for marker development, and (3) map RGA-CAPS markers in relation to *Scmv1* and *Scmv2* to examine their role as candidates for SCMV R genes.

Materials and methods

Plant materials

Seventeen maize inbred lines were analysed for CAPS restriction pattern including: (1) three resistant European dent lines D21, D32, and FAP1360A, (2) three highly susceptible European lines D408 (dent), D145 (flint), and F7 (flint), (3) ancestral lines of the three resistant lines, A632, V3, WD, W401, Co158, Co125, FAP493B, and FAP954A, and (4) three partially resistant lines D06, D09, and FAP1396A.

The mapping populations consisted of: (1) 87 $F_{2:3}$ families derived from a cross between D32 and D145 (PopA) (Vuylsteke et al. 1999; Xia et al. 1999), (2) 27 resistant BC5 individuals from the cross (F7 × FAP1360A) × F7 (PopB) (Xu et al. 1999), and (3) 30 resistant BC7 individuals from the cross (D408 × D21) × D408 (PopC). PopB originally consisted of 40 individuals, but 13 were excluded after progeny testing in field trials for resistance to SCMV. In addition, the map of PopB was saturated with SSR markers *phi075*, *umc1002*, *umc1018*, *bmc1600*, *bmc1433*, *bngl107*, *bmc1538*, and *bngl426*. Primer sequences were obtained from the maize database (http://www.agron.missouri.edu/ssr.html). PCR amplification was performed as described by Xu et al. (1999). pic13 was also mapped in oat-maize chromosome addition lines (Ananiev et al. 1997).

Cloning and sequencing of RGAs

Genomic DNA was extracted from 0.1 g of freeze-dried leaf tissue following the CTAB procedure as described by Hoisington et al. (1994). RGAs were isolated and cloned from the parental lines using PCR with specific primers (Table 1) based on the original sequences of pic13 (pic13L2/pic13R2), pic21 (pic21L1/pic21R1), and pic19 (pic19L/pic19R) described by Collins et al. (1998). DNA amplifications were performed in a standard reaction mix containing 100 ng of genomic DNA, 10 mM of Tris–HCl (pH 9), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.3 mM of dNTPs, 0.5 pMol of each primer and 0.625 U of *Taq* DNA polymerase (Amersham Pharmacia, Freiburg). After an initial denaturation step at 94 °C for 2 min, template DNAs were amplified using 35 cycles with the following conditions: 1 min at 94 °C, 2 min at 55 °C, and 2 min at 72 °C. The final extension step was conducted at 72 °C for 2 min. Amplification products were excised from the agarose gel, extracted with the Nucleospin Kit (Macherey and Nagel, Düren), and blunt-end cloned into the pBluescript vector.

Table 1 Primer sequences used for specific amplifications of pic13, pic19 and pic21

RGA	Primer	Sequences 5′–3′
pic13	pic13L2	TTGAAGCCATTGCTGGTGAC
	pic13R2	GCCATGAGCTATCCATTGAG
pic19	pic19L	TAGATGATGTCTGGACGGCT
	pic19R	AGCCAATGGCAAACCATCAC
	pic19X	GCAGTTCCTCTCTGCAACGTG
	ippic19L	CCAGAGTTACATCAGTGTGG
	ippic19R	ACATCAGCCGTCCAGACATC
pic21	pic21L1	GGAAGACCACGCTGCTCAAC
	pic21R1	CTCATCAGGTGGTCGCCAAC

Elongation of pic19 was performed by inverse PCR. Genomic DNA (500 ng) of the resistant parent FAP1360A was digested with *Rsa*I for 1 h at 37 °C, self-ligated overnight at 16 °C, and amplified with inverse PCR primers ippic19L and ippic19R. The elongated pic19 can be recreated after amplification by using primers pic19L and pic19X (Table 1). DNA sequencing was performed using the ALFExpress automated DNA sequencer (Amersham Pharmacia, Freiburg). Sequencing reaction conditions were chosen as suggested by the manufacturer (Amersham Pharmacia, Freiburg). For verification, pic19 was again sequenced by SEQLAB Sequence Laboratories Göttingen GmbH. The DNA sequences and the deduced amino-acid sequences were analysed using the ALIGN Plus 2.0 software package (http://www.scied.com/ses_alim.htm).

Generation and mapping of CAPS markers

Sequence regions displaying single nucleotide changes within restriction enzyme recognition sites between parental lines of mapping populations were used to identify RGA-CAPS using the CLONE Manager 3.11 software package (http://www.scied.com/ses_cm6.htm). PCR products were generated using the primers (Table 1) and the amplification conditions listed above. PCR products were digested with restriction endonucleases *Nla*III (pic19), *Rsa*I (pic13), and *Mbo*II (pic21) at 37 °C for 4 h according to the manufacturers' suggestions. RGAs were separated on 3% MetaPhor agarose gels in 0.5 × TBE buffer except for pic21, which was separated on denaturing polyacrylamide gels (SequaGel6, Biozym, Hessisch Oldendorf). Mapping of pic13, pic21, and pic19 was performed with PopA. In addition, pic13 was mapped in oat-maize addition lines and pic19 in PopB (Xu et al. 1999).

Linkage and statistical analyses

RGAs were mapped using data generated in previous studies (Xia et al. 1999; Xu et al. 1999). Marker orders and genetic distances for PopA were calculated with MAPMAKER 3.0b (Lander et al. 1987) using a LOD threshold of 3.0 and the Kosambi mapping function. Marker orders and genetic distances for PopB were calculated with Cri-Map (http://biobase.dk/Embnetut/Crimap).

Significant differences among the six inbred lines (resistant: D21, D32, FAP1360A; susceptible: D408, D145, F7) for SNP and CAPS frequencies per 100 bp of RGA sequence were tested by ANOVA using the software package PLABSTAT (http://www.uni-

Table 2 Genetic similarities (GS) of RGAs and frequency of SNPs per 100 bp between six maize inbred lines

Inbred lines	F7	FAP1360A	D21	D408	D32	D145	
pic19							
F7		6.38	5.10	6.54	5.58	6.34	GS
FAP1360A	0.34		4.78	6.22	5.90	2.55	
D21	0.50	0.53		2.55	1.91	5.10	
D408	0.34	0.34	0.74		1.59	5.58	
D32	0.45	0.41	0.84	0.86		4.15	
D145	0.38	0.81	0.55	0.47	0.57		
	SNP/100 bp					$\Sigma^a = 58$	
pic13							
F7		0.92	4.94	1.23	3.94	0.56	GS
FAP1360A	0.79		4.80	1.83	4.23	0.89	
D21	0.50	0.43		4.62	3.34	3.30	
D408	0.93	0.86	0.57		3.90	0.84	
D32	0.29	0.36	0.50	0.36		3.62	
D145	0.79	0.86	0.43	0.86	0.36		
	SNP/100 bp					$\Sigma^a = 14$	
pic21							
F7		1.17	0.70	0.70	0.70	3.29	GS
FAP1360A	1.00		0.47	0.94	0.70	3.99	
D21	1.00	1.00		0.47	0.23	3.29	
D408	0.93	0.93	0.93		0.47	3.52	
D32	0.93	0.93	0.93	0.87		3.52	
D145	0.13	0.13	0.13	0.07	0.07		
	SNP/100 bp					$\Sigma^a = 15$	

[a] Total number of SNPs between all six investigated inbred lines

hohenheim.de/~ipspwww/soft.html). Analyses of variance for randomised complete block designs were used to obtain a mean value for each RGA sequence. Least significant differences were calculated to test for differences between individual entry means. To determine SNP frequencies, pairwise comparisons between each two of the six inbred lines were performed. CAPS frequencies were calculated in pairwise comparisons of the three parental pairs of the mapping populations PopA, PopB, and PopC.

Genetic similarity of the RGA sequences between the six investigated inbred lines was measured on the basis of SNPs (Table 2) to test the six inbred lines for clustering into the heterotic flint and dent groups or resistant and susceptible lines using the software package NTSYSpc Version 2.02i (Rohlf 1989). The data set consisted of 58 detected SNPs between all six inbred lines for pic19, 14 for pic13, and 15 for pic21. Graphical genotypes of pic19 were displayed by software package GGT:GraphicalGenoTyping (http://www.spg.wau.nl/pv/pub/ggt).

Results

Isolation of pic13, pic19, and pic21 sequences from six inbred lines

All six RGA alleles of pic19 and pic21 were identical in size and similar to the sequences published by Collins et al. (1998). All pic19 sequences contained continuous open reading frames (ORFs), except those for D145 and D408. Likewise, pic21 sequences of all six inbreds showed continuous ORFs, except F7. The sequence of pic13 was incomplete, i.e. it was sequenced from both ends without identifying overlaps. pic13 showed a continuous ORF for both partial sequences of FAP1360A, D32, and F7. Lines D21, D408, and D145 displayed at least one stop codon.

Frequency of SNPs and probability of conversion into RGA-CAPS

The overall mean for the six genotypes over the three RGAs was 3.05 SNPs per 100 bp (= 1 SNP per 33 bp) of the RGA sequence. SNP frequencies of pic19 (4.70 SNPs per 100 bp) were significantly higher ($P < 0.01$) than those of pic13 (2.86 SNPs per 100 bp) and pic21 (1.61 SNPs per 100 bp). The number of CAPS sites per 100 bp of the RGA sequence was measured for the three parental pairs of the mapping populations FAP1360A/F7, D32/D145, and D21/D408. The number of CAPS varied between 0.00 and 4.15 per 100 bp, with an overall mean of 2.20 CAPS sites per 100 bp. CAPS frequencies of pic19 (3.72) were significantly higher ($P < 0.01$) than those of pic13 (1.69) and pic21 (1.18). PopA revealed the highest degree of polymorphism (3.13), followed by PopC (2.18), and PopB (1.82).

Genetic similarity analyses revealed no clustering into the heterotic flint and dent groups or resistant and susceptible lines (Fig. 1). The graphical haplotype for pic 19 was constructed on the basis of the same SNP data for all six inbred lines, revealing conserved sequence blocks.

Development of CAPS markers

CAPS polymorphisms were identified for all investigated RGAs in at least one of the three mapping populations. Sequence alignment between the cloned pic13 fragments revealed a SNP within a *Rsa*I recognition site resulting in polymorphism between D32 and D145

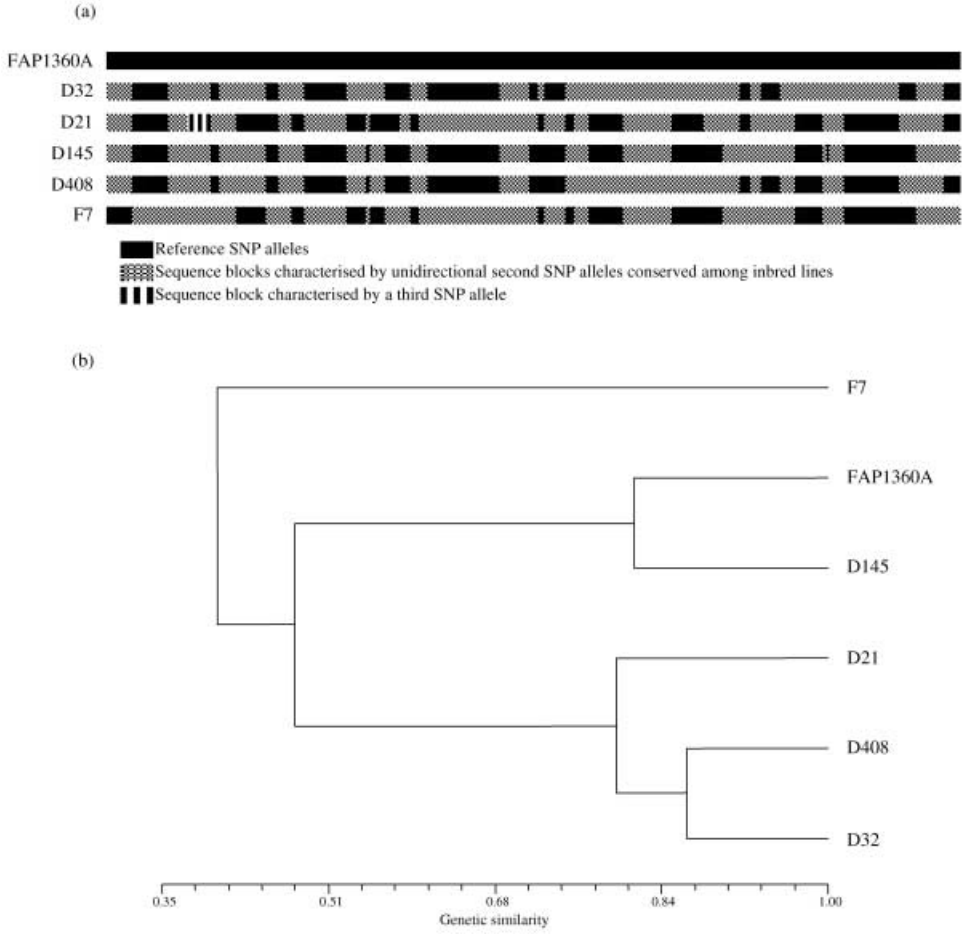

Fig. 1 (**a**) Graphical haplotyping for the example of pic19 (624 bp) in six European inbred lines based on 58 SNPs between the six maize inbred lines FAP1360A, D32, D21, D145, D408 and F7. (**b**) Genetic similarities among the six inbred lines based on pic19 SNPs

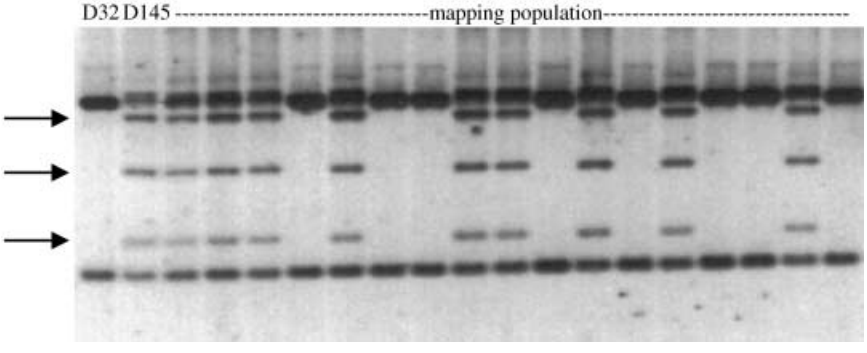

Fig. 2 *Rsa*I restriction pattern of pic13 PCR products from parental inbred lines D32 (SCMV resistant) and D145 (SCMV susceptible) and part of PopA

(PopA) (Fig. 2) and between D21 and D408 (PopC) (data not shown). The sequences of pic21 were polymorphic between D32 and D145 at a *Mbo*II recognition site. Digestion of elongated pic19 amplification products with *Nla*III generated polymorphisms in all three parental pairs (data not shown).

Genetic mapping of pic13, pic21, and pic19 relative to *Scmv1* and *Scmv2*

Amplification of pic13 from oat-maize addition lines showed a PCR product of the expected size only in the oat line carrying an additional maize chromosome 3. Amplification of pic19 and pic21 did not result in differential amplification of the oat line carrying the respective additional maize chromosome. For PopA, pic13 was mapped into the interval of *umc102/csu285b* on chromosome 3L (Fig. 3), which completely overlaps with the *Scmv2*-QTL region previously identified by Xia et al. (1999). However, pic13 could not be mapped in PopB because of identical banding patterns between heterozygotes and the recurrent parent F7 for all tested restriction enzymes.

Using PopA, pic21 was located on chromosome 3L but, contrary to pic13, it was located outside the *Scmv2*

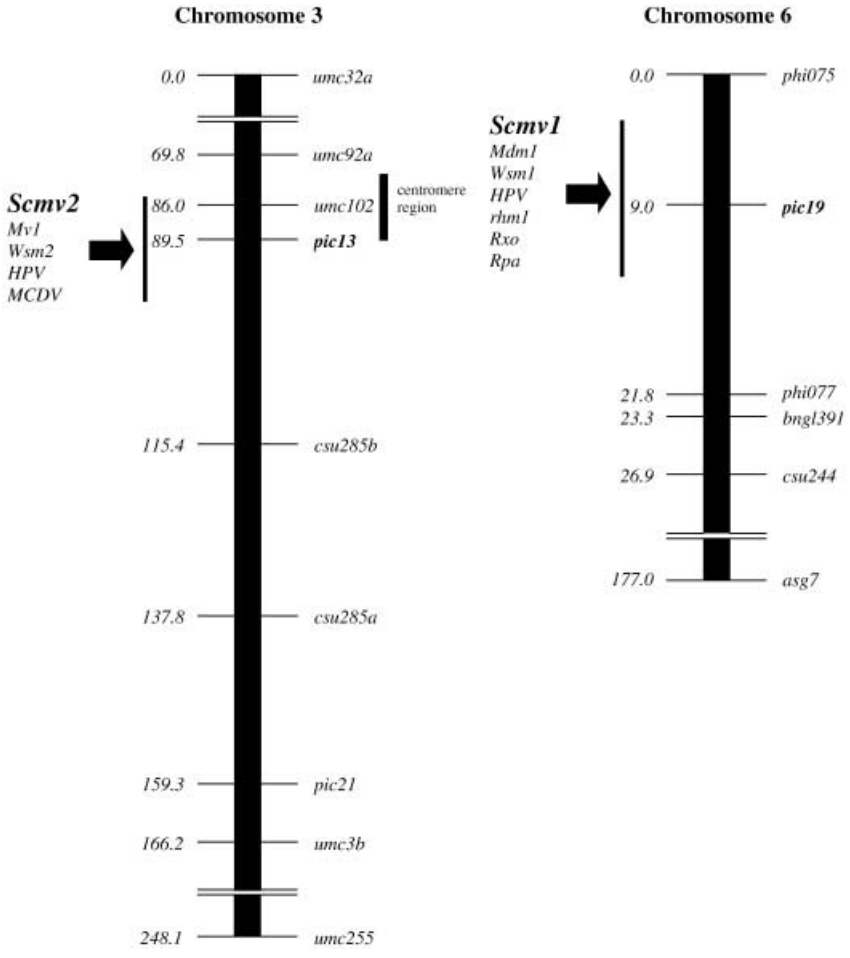

Fig. 3 Genetic map of genome regions conferring resistance to SCMV on maize chromosomes 3 and 6 derived from D32 × D145 $F_{2:3}$ mapping populations (PopA). Chromosome 3 containing RGAs pic13 and pic21, and genes for resistance to sugarcane mosaic virus (*Scmv2*), maize mosaic virus (*Mv1*), wheat streak mosaic virus (*Wsm2*), high plains virus (*HPV*) and maize chlorotic dwarf virus (*MCDV*). Chromosome 6 containing pic19 and genes for resistance to sugarcane mosaic virsu (*Scmv1*), wheat streak mosaic virus (*Wsm1*), high plains virus (HPV), southern corn leaf blight (*rhm1*), rice bacterial streak (*Rxo*) and sorghum bacterial stripe (*Rpa*)

Table 3 Evaluation of restriction pattern of pic19 and pic13 in resistant, susceptible, partially resistant, and ancestral inbred lines

Inbred lines		pic13		pic19		
		Genotype A	Genotype B	Genotype A	Genotype B	Genotype C
Resistant	FAP1360A		X	X		
	D21	X		X		
	D32	X		X		
Susceptible	F7		X		X	
	D408	X				X
	D145	X			X	
Partially resistant	D06	X		X		
	D09	X		X		
	FAP1396A	X		X		
Ancestors	A632	X		X		
	V3	X			X	
	WD	X			X	
	W401	X				X
	Co158	X			X	
	Co125		X		X	
	FAP493B	X			X	
	FAP954A	X		X		

region flanked by markers *csu285a* and *umc3b* (Fig. 3). In PopA, pic19 mapped to maize chromosome 6S between markers *phi075* and *phi077b* (Fig. 3). In PopB, pic19 mapped into the marker interval *umc1023/E2M7-1* with a map distance of 3.5 cM to *Scmv1*.

RGA analyses in ancestral lines and partially resistant inbreds

Two genotypes were observed when pic13 amplification products were digested with *Rsa*I. F7, FAP1360A, and Co125, the direct ancestor and potential donor of *Scmv2*,

showed the same restriction banding pattern (genotype B). The remaining inbred lines displayed a different restriction pattern (genotype A) (Table 3).

Three genotypes were observed for pic19 when digested with *Nla*III. Besides the three resistant lines FAP1360A, D21, and D32, the potential donors of *Scmv1*, A632 and FAP954A, and the three partial resistant inbreds D06, D09, and FAP1396A, as well as FAP1360A, showed the same restriction pattern (genotype A). The second restriction banding pattern (genotype B) was observed for the following six lines: V3, WD, Co158, Co125, FAP493B, and F7. The remaining two lines D408 and W401 lack one *Nla*III restriction site and thus revealed a third restriction pattern (genotype C) (Table 3). In contrast to pic13, the resistant lines revealed the same restriction pattern.

Discussion

One of the long-term aims of molecular marker technology in plant breeding is the selection of superior individuals directly at the level of DNA. RGAs provide an excellent source for the development of molecular markers, especially for resistance traits, because of their high level of polymorphism and their putatively functional character. Thereby, RGAs can be converted to single-copy PCR markers like CAPS (Konieczny and Ausubel 1993), while RGA-RFLPs frequently result in complex banding patterns because of sequence homology to related RGA sequences (Collins et al. 1998). Diagnostic markers like allele-specific RGA-CAPS will have wider applications in MAS strategies in the future.

SNP and CAPS frequency in three maize RGAs

Rafalski et al. (2001) analysed random cDNA clones in a collection of over 30 public and private maize lines representative for the North American corn germplasm. This analysis was restricted to coding regions. Sequence alignment revealed one single nucleotide change per 70 bp among the 30 lines. However, the authors pointed out that pairwise comparison between any two lines – such as in the results presented in this article – reveals a lower degree of polymorphism. Useche et al. (2001) performed similar experiments in maize and reported a SNP frequency of only one SNP per 138 bp in coding regions. Another study revealed that maize has an average of one SNP every 104 bp between two randomly sampled sequences (Tenaillon et al. 2001). Hence, the frequency of one SNP per 33 bp originating from single nucleotide changes in pairwise comparisons is significantly higher in the present study. This result confirms the highly polymorphic character of plant RGAs and possibly R genes, which is an important feature in evolutionary processes at R gene loci and especially R gene clusters (Parniske and Jones 1999).

Among the RGAs, pic19 displayed a significantly higher degree of polymorphism than pic13 and pic21 (Table 2); pic19 might be phylogenetically older than pic13 and pic21 and, therefore, displays a significantly higher degree of polymorphism. A second possibility would be the coding character of pic13 and pic21 in contrast to pic19. Since a SNP in the first two positions within a triplet generates more amino-acid changes, the mutations in coding regions should be mainly in the third position of the triplet. Hence, the significantly higher SNP frequency at the third position of triplets for pic19 suggests expression of the respective sequence, but was not observed in the current study.

The generally high degree of polymorphism of the three RGAs might also be influenced by their map position. Two of the three RGAs map to potential R gene clusters on chromosomes 3L close to the centromere and 6S close to the nucleolus organiser region (nor). In gene clusters the recombination frequency is expected to be very high because of the high density of coding sequences. In many plant species, recombination rates can vary up to an order of magnitude over relatively small intervals (reviewed by Schnable et al. 1998). Detailed physical and genetic mapping of grass genomes revealed the clustering of genes, and supports the hypothesis that much of the meiotic recombination occurs in genes and most recombination events are restricted to few chromosome regions containing gene clusters (Gill et al. 1996; Künzel et al. 2000). The maize genome exhibits a very striking gene distribution with almost all genes present in 10–20% of the genome (Carels et al. 1995). In fact, genes exhibit recombination rates 10 to 100-fold higher than the genome average (reviewed by Lichten and Goldman 1995). In maize, one-fifth of all recombination events in a 140-kb interval between the *anthocyaninless1* (*a1*) and the *shrunken2* (*sh2*) genes were resolved within a 377-bp region of the *a1* gene (Xu et al. 1995). It is not known whether the *Scmv* regions on chromosomes 6 and 3 contain R gene clusters or single pleiotropically acting genes. Zhao et al. (2001) mapped two maize R genes, *Rxo* and *Rpa*, to the same position on the short arm of maize chromosome 6. The authors identified 5–6 RGAs in the direct neighbourhood of *Rxo* and *Rpa*. These findings and the high degree of polymorphism for pic19 support the presence of a R gene cluster on chromosome 6S. However, one member of the putative R gene cluster on chromosome 6S, *Mdm1*, shows 100% linkage with nor (Simcox et al. 1995), whereas *Scmv1* and nor were not absolutely linked (Xu et al. 1999). Very low recombination rates are typically exhibited by regions surrounding the centromeres and the nor. Therefore, the putative R gene cluster on chromosome 6S is located in the direct neighbourhood of regions with suppressed recombination. Interestingly, the situation seems to be similar on chromosome 3 with *Scmv2* in the direct neighbourhood of the centromere.

The structure of plant materials (flint-dent, SCMV resistant-susceptible; relationship by descent of the three resistant lines) was not reflected by the pic19-based genetic similarity analysis (Fig. 1). Explanations for the lack of grouping of the six inbred lines are either a miss-

ing correlation of the RGA allele sequences with these factors or the above mentioned high degree of polymorphism within RGAs. Furthermore, Xu et al. (2000) identified a 7.2 cM interval containing eight molecular markers as well as pic19 flanking *Scmv1* conserved across the three resistant lines D21, D32, and FAP1360A, whereas the susceptible lines displayed a different segregation pattern. Therefore, this 7.2 cM chromosomal segment seemed to be identical in the three resistant lines. However, disagreement of RGA-based genetic similarity analysis with phenotypic, pedigree, and marker data question the utility of SNPs for association or disequilibrium mapping (reviewed in Lazzeroni 2001), at least for this genome region.

Single nucleotide changes in all three RGAs between all six inbred lines generated only two of the possible four SNP alleles except one. The formation of the second SNP alleles was not randomly distributed. Several consecutive SNPs were ordered in sequence blocks (haplotypes) with unidirectional mutations. Most of the sequence blocks contain more than one single SNP (Fig. 1a). Presuming a low number of RGA alleles in European founder materials, the driving force for the development of the high degree of polymorphism and variability within these RGAs seems to be recombination.

In conclusion, RGA-derived markers are especially interesting because of their genomic localisation within R gene clusters which putatively contain numerous genes relevant for plant breeding purposes. The frequencies of SNPs and CAPS are promising prerequisites for conversion of even short RGA sequences into molecular markers. This should further enhance the mapping of RGAs as potential candidates for genes conditioning resistance to pathogens in plant species.

Candidate gene evaluation

The identification of CAPS restriction sites between the parents of three mapping populations facilitated mapping of the three RGAs, pic13, pic19, and pic21, to genomic regions known to be involved in the inheritance of SCMV resistance on chromosomes 3 and 6. To address the question of whether any of the RGAs is a likely candidate for one of the target genes *Scmv1* or *Scmv2*, respectively, we evaluated the following criteria: (1) *Linkage with the target gene*. A potential role as a candidate gene can be ruled out in case of a large map distance to the target gene. Cosegregation and even tight linkage to the R gene indicates a functional role of the RGA because of: (i) incomplete penetrance of SCMV resistance and escapes resulting in mis-scoring, as well as (ii) the potential presence of more than one SCMV R gene within both regions. (2) *Comparison of the R alleles of the six inbred lines with those of the ancestor and partially resistant lines*. Sharing the same allele among resistant, partially resistant inbred lines and putative R gene donors on the one hand and different alleles in the other lines on the other hand, indicates a functional role in the inheritance of SCMV resistance. (3) *Continuous ORFs as a prerequisite for coding regions*. A continuous ORF spanning the complete RGA sequence strongly suggests a coding character on the basis of the statistical probability of the occurrence of stop codons.

Chromosome 6

Mapping in PopA located pic19 within the QTL peak of *Scmv1*. Mapping in PopB showed two recombinant individuals. However, field experiments, BSA (Xu et al. 1999), and QTL analyses (Xia et al. 1999; Dußle et al. 2000) did not exclude the presence of more than one SCMV R gene in the *Scmv1* region. Furthermore, incomplete penetrance of virus resistance and escapes might result in mis-scorings during the phenotypic evaluation of the mapping populations. Therefore, a functional role of pic19 in the expression of SCMV resistance cannot be ruled out by genetic mapping. Moreover, the three resistant, the partially resistant lines, as well as the putative donors of *Scmv1*, A632, and FAP954A, displayed the same restriction patterns for pic19 (Table 3). Xu et al. (2000) investigated the chromosomal regions harbouring *Scmv1* and *Scmv2* by AFLP analyses in the same three resistant European inbreds, their ancestral lines and partially resistant inbred lines. The banding patterns indicated the identity of a 7.2 cM window harbouring the *Scmv1* locus in D21, D32, and FAP1360A. Therefore, it is most likely that these three European inbred lines share the same *Scmv1* allele at this Mega-locus. Absence of stop codons in all three resistant lines strengthens the possible functional involvement in the expression of SCMV resistance. However, a continuous ORF is not restricted to the resistant lines because the susceptible inbred F7 also showed a continuous ORF. In conclusion, pic19 remains a candidate for *Scmv1*, but our findings cannot distinguish between close linkage and identity of pic19 with *Scmv1*.

Chromosome 3

In PopA, pic21 mapped to chromosome 3, and was located approximately 50 cM outside the marker interval flanking *Scmv2*. Therefore, it was excluded as a candidate gene for *Scmv2*.

In contrast, pic13 mapped in PopA within the QTL peak of *Scmv2*. According to Xu et al. (2000), the *Scmv2* region in FAP1360A most likely originated from Co125, while all other ancestral lines showed AFLP patterns in this region different from FAP1360A. The authors proposed the following explanations for the origin of the *Scmv2* region: (1) *Scmv2* in FAP1360A originates from Co125, or (2) Co125 is also the *Scmv2* donor of D32 displaying a very short donor region not detectable by the employed flanking markers or another inbred line. The fact that restriction patterns of the potential donor of *Scmv2*, Co125, and FAP1360A, are identical and show a

different allele at this locus than D21 and D32, confirms the previously published hypothesis of different *Scmv2* genes in FAP1360A and D32 (Xu et al. 2000). The presence of *Scmv1* seems to be sufficient for conferring partial resistance. Hence, *Scmv2* is not necessarily expected to be present in the partially resistant lines, explaining a different genotype in all three partially resistant inbred lines. No continuous ORF was found for pic13 in D21, in agreement with the lack of evidence for *Scmv2* in this inbred line.

The mapping results and analyses of ancestor lines cannot distinguish between identity and close linkage of pic19 and *Scmv1*, and pic13 and *Scmv2*, respectively. The previously mentioned results of Zhao et al. (2001), as well as studies on the *Pto*, *Cf*, and *Dm* clusters in tomato and lettuce (Martin et al. 1994; Thomas et al. 1997; Meyers et al. 1998), revealed the existence of numerous RGAs in the direct physical neighbourhood of the R genes. At least seven apple *Vf* gene analogues have been identified from a BAC contig of 290 kb, encompassing the *Vf* locus (Xu 2001, personal communication). These RGAs showed very similar amino-acid domains except for the LRRs, where deletion of several LRR units and point mutations occurred frequently. Therefore, even in the case of non-identity of pic19 and pic13 with *Scmv1* and *Scmv2*, respectively, they should provide excellent starting points for a map-based approach for cloning of the target genes themselves as well as other members of these clusters, such as MDMV, wheat streak mosaic virus (WSMV), maize mosaic virus (MMV), High Plains virus (HPV), and maize chlorotic dwarf virus (MCDV) R genes. Large and often continuous ORFs of the RGAs investigated in this study indicate that they are part of coding sequences. This is in agreement with large-scale sequencing of the above mentioned tomato and lettuce R gene loci. The majority of RGAs seemed to be functional and expressed, with only a few of them clearly being pseudogenes. Screening of DNA libraries will extend the number of RGAs in these regions and, therefore, broaden the probability of identifying the target genes. In contrast to the simple procedures to clone homologues, establishing potential functions of the RGAs remains challenging. Since the proof of function of a given candidate for a certain resistance is highly dependent on pathogen-specific plant material, exchange of R gene candidates between research groups working on different resistances is essential and can greatly accelerate the cloning of R genes by the RGA approach.

Acknowledgements The present study was supported by a grant from the Deutsche Forschungsgemeinschaft, Grant No. LU601/3. The authors gratefully acknowledge the mapping of SSRs to PopB by Susanne Dreissigacker and the skilled technical assistance of Elisabeth Kokai-Kota.

References

Ananiev EV, Riera-Lizarazu O, Rines HW, Phillips RL (1997) Oat-maize chromosome addition lines: a new system for mapping the maize genome. Proc Natl Acad Sci USA 94:3524–3529

Bent AF (1996) Plant disease resistance genes: function meets structure. Plant Cell 8:1757–1771

Carels N, Barakat A, Bernardi G (1995) The gene distribution of the maize genome. Proc Natl Acad Sci USA 92:11,057–11,060

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor A (1998) The isolation and mapping of disease resistance gene analogues in maize. Mol Plant–Microbe Interact 11:968–78

Collins NC, Drake J, Aycliffe M, Sun Q, Ellis J (1999) Molecular characterisation of the maize *Rp1-D* rust resistance haplotype and its mutants. Plant Cell 11:1365–1376

Dußle CM, Melchinger AE, Kuntze L, Stork A, Lübberstedt T (2000) Molecular mapping and gene action of *Scm1* and *Scm2*, two major QTLs contributing to SCMV resistance in maize. Plant Breed 119:299–303

Fuchs E, Grüntzig M (1995) Influence of sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) on the growth and yield of two maize varieties. J Plant Dis Protection 102:44–50

Gill KS, Gill BS, Endo TR, Taylor T (1996) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891

Hammond-Kosack KE, Jones JDG (1996) Resistance gene-dependent plant defense responses. Plant Cell 8:1773–1791

Hoisington DA, Khairallah MM, Gonzales-de-Leon D (1994) Laboratory protocols. CIMMYT Applied Molecular Genetics Laboratory, Mexico, DF

Konieczny A, Ausubel FA (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Kuntze L, Fuchs E, Grüntzig M, Schulz B, Klein D, Melchinger AE (1997) Resistance of early maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breed 116:499–501

Künzel G, Korzun L, Meister A (2000) Cytogenetically integrated physical restriction fragment length polymorphism maps for barley genome based on translocation breakpoints. Genetics 154:397–412

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

Lazzeroni (2001) A chronology of fine-scale gene mapping by linkage disequilibrium. Stat Methods Med Res 10:57–76

Lichten M, Goldman AS (1995) Meiotic recombination hotspots. Annu Rev Genet 29:423–444

Martin GB, Frary A, Wu T, Brommonschenkel S, Chunwongse J, Earle ED, Tanksley SD (1994) A member of the tomato *Pto* gene family confers sensitivity to fenthion resulting in rapid cell death. Plant Cell 6:1543–1552

McMullen MD, Simcox KD (1995) Genomic organisation of disease and insect resistance genes in maize. Mol Plant–Microbe Interact 8:811–815

Melchinger AE, Kuntze L, Gumber RK, Lübberstedt T, Fuchs E (1998) Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor Appl Genet 96:1151–1161

Meyers BC, Chin, DB, Shen, KA, Sivaramakrishnan S, Lavelle DO, Zhang Z, Michelmore RW (1998) The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell 10:1817–1831

Parniske M, Jones JD (1999) Recombination between diverged clusters of the tomato *Cf-9* plant disease resistance gene family. Proc Natl Acad Sci USA 96:5850–5

Rafalski JA, Ching A, Bhattramakki D, Morgante M, Dolan M, Register JC, Smith OS, Tingey S (2001) SNP markers in maize: discovery and applications. Plant and Animal Genome IXth Conference, p 44

Rohlf FJ (1989) NTSYS-pc numerical taxonomy and multivariate analysis system. Setauket, Exeter Publishing Company Limited

Schnable PS, Hsia AP, Nikolau BJ (1998) Genetic recombination in plants. Curr Opin Plant Biol 1:123–129

Simcox KD, McMullen MD, Louie R (1995) Co-segregation of the maize dwarf mosaic virus resistance gene, *Mdm1*, with the nucleolus organiser in maize. Theor Appl Genet 90:341–346

Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF, Gaut BS (2001) Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). Proc Natl Acad Sci USA 98:9161–9166

The International SNP Map Working Group (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409:928–933

Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones JDG (1997) Characterisation of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in *Cf-4* and *Cf-9*. Plant Cell 9:2209–2224

Useche F, Morgante M, Hanafey M, Tingey S, Gao GR, Rafalski JA (2001) Computer detection of single nucleotide polymorphisms (SNPs) in maize ESTs. Plant and Animal Genome IXth Conference, p 144

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lübberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. Theor Appl Genet 99:921–935

Xia XC, Melchinger AE, Kuntze L, Lübberstedt T (1999) Quantitative trait loci mapping of resistance to sugarcane mosaic virus in maize. Phytopathology 89:660–667

Xu ML, Melchinger AE, Xia XC, Lübberstedt T (1999) High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR, and AFLP markers. Mol Gen Genet 261:574–581

Xu ML, Melchinger AE, Lübberstedt T (2000) Origin of *Scm1* and *Scm2* – two loci conferring resistance to sugarcane mosaic virus (SCMV) in maize. Theor Appl Genet 100:934–941

Xu X, Hsia AP, Zhang L, Nikolau BJ, Schnable PS (1995) Meiotic recombination breakpoints resolve at high rates at the 5′end of a maize coding sequence. Plant Cell 12:2151–2161

Zhao B, Lin X, Leach JE, Hulbert S (2001) Molecular analysis of the maize gene conferring a non-host hypersensitive reaction to rice bacterial streak. Plant and Animal Genome IXth Conference, p 90

General Discussion

SCMV resistance as threshold character

SCMV infected maize plants can be classified as symptomless or susceptible, although SCMV resistance is not inherited as a qualitative trait. The inheritance of SCMV resistance is based on a continuous distribution of disease symptom expression with a threshold, whereas the visible scale, symptomless and susceptible, is discontinuous (Falconer 1961). Hence, individuals whose phenotypic values on the underlying scale exceed the threshold will appear in one visible class while individuals below the threshold will appear in the other. Although the individuals can be divided in two classes concerning SCMV resistance, symptomless individuals can be genetically susceptible (escapes). *Vice versa*, some individuals might show symptoms, although carrying the resistance alleles due to incomplete penetrance. Therefore, the threshold has to be overcome to assign such an individual to the other class. This overcoming of the threshold is caused by genetic, physiological and environmental factors.

After inoculation of the susceptible inbred lines D145, D408, and F7 with SCMV, up to 20 % of the individuals turned out to be escapes (Melchinger et al. 1998). In the same study, 2 to 5% of the F_1 plants of the crosses D21 D145, D32 D408, and FAP1360 F7 in field trials and more than 50% under greenhouse conditions showed incomplete penetrance. Furthermore, resistant maize inbreds can show disease symptoms after mechanical inoculation with MDMV and WSMV (Louie 1995). In addition, a high infection level might lead to a collapse of the resistance threshold. Some susceptible phenotypes were found within the resistant inbred line FAP1360A in 2000, where a high infection level was observed. Overcoming the threshold is more likely for heterozygous individuals than for homozygous. In the present study the gene effect for *Scmv1a* was overdominant, whereas the gene effect for *Scmv1b* and *Scmv2* turned out to be additive. Therefore *Scmv1b* and *Scmv2* might be much more affected by incomplete penetrance than *Scmv1a* with its larger genetic effect on SCMV resistance. Therefore, an order of stability against overcoming the threshold to susceptibility can be stated as follows: absence of resistance alleles in both target regions *Scmv1* and *Scmv2* <

presence of resistance alleles in the *Scmv2* region < presence of resistance alleles in the *Scmv1* region < presence of resistance alleles in both target regions, *Scmv1* and *Scmv2*. Furthermore, epistasis or pleiotropy might cause incomplete penetrance.

Assuming two dominantly acting, complementary resistance genes in F7 FAP1360A, the discrepancy between the observed proportion of BC families segregating for SCMV and the expected proportion of 25%, is most likely due to the phenomenon of incomplete penetrance. Another possibility could be the presence of additional minor resistance genes besides those identified in *Scmv1* and *Scmv2* regions, and therefore, a different gene model. The QTL in the regions on chromosomes 3 and 6 explained only 70% of the total genetic variance according to the QTL analyses in this study. Hence, presence of additional resistance genes with minor effects can not be ruled out. Furthermore, Xia et al. (1999) identified in cross D32 D145 three minor QTL on chromosomes 1, 5 and 10. In contrast, neither BSA (Xu et al. 1999) nor QTL analyses (Xia et al. 1999) identified additional SCMV resistance gene regions in population F7 FAP1360A other than those on chromosomes 3 and 6. Consequently, phenomena like incomplete penetrance or environmental effects might have lead to the small proportion of symptomless individuals within the BC and BC-S1 generations.

Influence of escapes on the identification of closely linked markers

The causes of escapes could be environmental influences or occurrence of additional resistance mechanisms influencing the behavior of the virus transmitting vectors. Misscoring of the SCMV symptoms is very unlikely. A comparison of visual and serological evaluation with tissue print immunoblotting (TPIB) achieved a consistency of 99% (Hohmann et al. 1996).

The occurrence of escapes can impair the mapping of resistance genes. In this study, molecular markers were mapped with selected symptomless BC individuals. In the case of escapes, resistance and marker alleles of the donor would be absent in at least one of resistance gene regions, which might be incorrectly interpreted as recombination between marker and resistance gene. Consequently, the resistance genes would be misplaced in the genetic linkage map and identification of cosegregating markers would

be impossible. With regard to cloning the resistance genes *Scmv1* and *Scmv2*, identification of the exact position of these genes is necessary. However, the occurrence of escapes obscures the evaluation of the resistant genotype by means of the respective phenotype. To avoid misscoring of escapes, progenies of symptomless individuals selected for tBSA and mapping were evaluated for SCMV resistance after inoculation. On average, 15% of the families tracing back to phenotypically symptomless BC individuals were completely susceptible and, therefore, excluded from further analyses.

Influence of incomplete penetrance on the identification of closely linked markers

Incomplete penetrance can change the results of genetic mapping. Phenotypically susceptible individuals would be grouped to the susceptible bulk for BSA although they harbor the SCMV resistance alleles. Consequently, identification of polymorphisms between the resistant and susceptible bulk will be more difficult. In the present study, a modified tBSA with single symptomless individuals instead of pools was employed for identification of markers tightly linked with the SCMV resistance genes. Hence, incomplete penetrance did not influence the mapping results in this case. Furthermore, only those individuals showing a symptomless phenotype were included in further steps of the tBSA.

Genetic analyses of SCMV resistance

The application of molecular markers has improved the ability to obtain reliable estimates of the number of genes underlying a trait and to map these genes on the respective chromosomes. Melchinger et al. (1998) mapped the SCMV resistance gene *Scmv1* on chromosome 6S and *Scmv2* near the centromere region of chromosome 3 using one SSR and four RFLP markers. These two SCMV resistance regions were confirmed by a BSA with 23 putatively linked AFLP markers (Xu et al. 1999) and a QTL analysis with four SSR markers in the present study. With the QTL analysis applying 24 SSR markers, the presence of one gene in *Scmv2* region was confirmed. In contrast, this QTL analysis revealed a cluster of at least two closely linked SCMV resistance genes, *Scmv1a* and *Scmv1b*, in the cross F7 FAP1360A. *Scmv1* mapped as a

single QTL for the first two scoring dates with a similar location as detected by the first QTL analysis, BSA (Xu et al. 1999) and tBSA. However, it was separated for later scoring dates in two QTL, *Scmv1a* with overdominant gene action and *Scmv1b* with additive gene action. Consequently, a higher marker density has increased the power of QTL detection in the present study. Furthermore, Xia et al. (1999) identified three minor QTL on chromosomes 1, 5, and 10 conferring resistance to SCMV in population D32 D145. In contrast, no minor QTL were found for F7 FAP1360 by BSA (Xu et al. 1999) and in the present study. In agreement with these findings, an oligogenic type of inheritance for resistance to SCMV, MDMV and WSMV was reported (Dollinger et al. 1970, McMullen et al. 1994, Xia et al. 1999).

Fine mapping of SCMV resistance genes on chromosomes 3 and 6 with molecular markers

The identification of closely linked or cosgregating molecular markers being polymorphic for the trait of interest is the most time consuming step for fine mapping. BSA with AFLPs identifies rapidly molecular markers tightly linked to the target locus of a monogenic trait (Ballvora et al. 1995), restricting further mapping analyses to a promising subset of linked markers. Three AFLP markers cosegregating with the *Cf*-Locus in tomato were found by Thomas et al. (1995) based on pooled DNA samples. Likewise, three tightly linked markers to the *Mer* locus in *Populus* were identified from a total number of 11500 AFLP bands by Cervera et al. (1996). Xu et al. (1999) demonstrated that AFLP-based BSA can also be successfully applied to the more complex situation of the oligogenic inherited trait of SCMV resistance. The authors identified with BSA 23 AFLP and 11 SSR markers linked to the SCMV resistance regions *Scmv1* on chromosome 6 and *Scmv2* on chromosome 3 using BC5 individuals in population F7 FAP1360A. In the case of an oligogenic trait, the differences with regard to the bands polymorphic between the susceptible and the resistant bulk are no longer qualitative as they are with a dominantly inherited monogenic trait, but quantitative.

Based on the results of Xu et al. (1999) and the utilization of a modified tBSA (Lübberstedt et al. 2002), the SCMV resistance regions were further enriched with 24

AFLP and 19 SSR markers in the present study. Using symptomless BC individuals of advanced BC5 to BC9 generations and an additional step for the tBSA to assign the identified markers to the respective target gene regions *Scmv1* and *Scmv2*, it was possible to reduce the mapping effort by 60% compared to conventional BSA employed by Xu et al. (1999).

Whereas the SSR marker order was identical and marker distances for the chromosome 3 region were comparable between QTL analysis and tBSA, marker distances and the window size between SSR markers *bnlg161* and *umc1229* were substantially different for the target region on chromosome 6. The presence of an additional resistance gene *Scmv1b*, identified by QTL analysis with 24 SSR markers is the most likely explanation for the larger distances in some subregions of chromosome 6. Because of the population type of BC generations and the small population size, it was not possible to dissect the two QTL on chromosome 6 by tBSA and BSA (Xu et al. 1999). *Scmv1b* seems to act independently of *Scmv1a* and increases SCMV resistance. Consequently, markers identified to be tightly linked to *Scmv2* on chromosome 3 region by tBSA can be immediately used for MAS and map-based cloning. However, the question whether there is a cluster of more than one resistance gene on chromosome 6 conferring resistance to SCMV can only be solved after cloning these genes.

Clustering of resistance genes

Clustering of resistance genes against different pathogens in the same chromosomal regions of the maize genome was previously reported by McMullen et al. (1995). Resistance genes against MDMV (McMullen et al. 1989), WSMV (McMullen et al. 1994) and high plains virus (Marçon et al. 1997) were identified in the same region as SCMV resistance genes on chromosome 6. So far, it is unknown whether *Scmv1*, *Mdm1* and *Wsm1* are identical and display pleiotropic effects against different potyviruses or whether they are closely linked genes. Likewise, the *Scmv2* region on chromosome 3 harbors, besides *Scmv2*, the major resistance genes *Rp3* against *Puccina sorghi* (Sans-Alferez et al. 1995), *Mv1* against MMV (Ming et al. 1997), *Wsm2* against WSMV (McMullen et al. 1994) and three QTL for resistance to European corn borer, Fusarium stalk rot (McMullen et al. 1995) and common smut. Supporting the existence of closely

linked but different major resistance genes against SCMV, some susceptible plants were detected in an allelism test between the three resistant European dent inbred lines D21, D32 and FAP1360A (Lübberstedt et al. 1999). Quint et al. (2002) observed linkage of three BAC contigs containing homologues of the RGA pic19 with *Scmv1* for cross D32 D145, indicating a cluster of SCMV resistance genes in this region. In the present study, the identification of two linked QTL on chromosome 6, *Scmv1a* and *Scmv1b*, conferring resistance against SCMV gave further evidence for the clustering of SCMV resistance genes.

Conversion of AFLP markers into simple PCR-based markers

Because AFLP markers are too expensive and laborious for high-throughput monitoring of large numbers of genotypes, conversion of AFLP markers closely linked to the SCMV resistance genes on chromosomes 3 and 6 is an important step to receive easily applicable markers for MAS and map-based cloning. Additionally, the converted STS-markers can reduce the costs of marker analyses to about 20%. In the present study it was possible to convert AFLP markers into STS markers without intermediate steps like inverse PCR or chromosome walking, suggested by DeJong et al. (1997) and Negi et al. (2000) to elongate the short AFLP fragments. Sequencing five to ten clones of two to eight maize inbred lines (D21, D32, D145, D408, F7, FAP1360A) for tightly 6 AFLP markers for SCMV regions *Scmv1* and *Scmv2*, respectively, uncovered one indel marker E35M62-1STS and one CAPS marker E33M61-2STS. However, a sequence comparison between the six maize inbred lines identified SNPs within as well as among these inbred lines for two AFLP markers. Difficulties of AFLP marker conversion based on the loss of their sequence specificity after amplification with the AFLP-derived internal primers were previously reported (Shan et al. 1999). Unfortunately, it was not possible to generate a STS-markers distinguishing all resistant (D21, D32, FAP1360A) from the susceptible (D145, D408, F7) inbred lines. Consequently, the diagnostic value (Borchardt et al. 2000) of the converted markers seems to be low. Hence, markers being polymorphic in the present study between the inbred lines F7 and FAP1360A have to be checked in each new cross before applying them for MAS (Borchardt et al. 2000).

Alternative strategies for resistance gene identification

In addition to marker analyses, an RGA analysis with pic13, pic19 and pic21 was conducted to examine their role as candidates for the SCMV resistance genes on chromosomes 6S and 3L in the present study. A first mapping of the CAPS markers derived from pic19 and pic13 with 27 resistant BC5 individuals from the cross (F7 FAP1360A) F7 identified pic19 and pic13 as candidates for *Scmv1* and *Scmv2*, respectively. However, mapping of pic19 in a population of 118 F_3 lines uncovered a larger distance between pic19 and the SCMV resistance gene. Quint et al. (2002) assumed that three pic19 homologues mapped as a single-copy 'ghost marker', comparable to a 'ghost QTL', because of their high sequence similarity. As a consequence, the position of the 'ghost marker' may change the positions and even the order of other marker loci or QTL in the target region. Likewise, two QTL, *Scmv1a* and *Scmv1b*, were found in the present study, which were probably mapped together as one 'ghost' QTL for the first scoring dates and in the tBSA analyses. Therefore, pic19 homologues can not be excluded as candidates for these resistance genes on chromosome 6 region.

Isolation of resistance genes by RGA sequences is limited because pseudogenes which accumulate more mutations compared with active genes are the source of polymorphisms between genotypes (Kanazin et al. 1996). Therefore, genetic mapping of RGA sequences may be more important in identifying markers close to resistance genes for subsequent map-based cloning, than for detection of resistance genes. RGA are at present mostly applied for MAS or map-based cloning (Collins et al. 1998, Shen et al. 1998). Application of RGA is a reliable tool for both, resistance gene isolation and MAS if there is a correlation between polymorphism and gene expression. However, if the RGA is a sequence of a pseudogene, it can be employed only for MAS and map-based cloning, but not for resistance gene isolation by candidate gene analysis. The genetic and physical clustering of RGA and the abundance of NBS-LRR genes in plant genomes complicate the utilization of RGA as a tool to isolate biologically characterized genes involved in disease resistances. Consequently, the availability of high-resolution maps for a particular resistance gene and of mutant alleles will be crucial complementary tools (Leister et al. 1999). Hence, at the present stage, RGA can

serve as a companion approach for the identification of markers tightly linked to the SCMV resistance genes, but cannot substitute molecular marker analyses for fine mapping.

Conclusion and Prospects

With regard to SCMV resistance as a oligogenic inherited trait it can be concluded that BSA and tBSA are able to detect segregating markers in the target regions on chromosome 3 and 6. For a trait like SCMV resistance, MAS might be superior to conventional phenotypic selection because of (1) the oligogenic inheritance of SCMV resistance, (2) the strong influence of the environment on the expression of the resistance genes, (3) the occurrence of incomplete penetrance and escapes due to the threshold character of SCMV resistance, and (4) the high costs for resistance evaluation. A further benefit of MAS is the selection in off-season nurseries and before flowering.

However, the threshold character of SCMV resistance confuses the generation of bulks for BSA and tBSA. Therefore the choice of the population type is of main importance for the success of identification of closely linked markers for a trait with a threshold character. Because homozygous resistance loci are less affected by incomplete penetrance than heterozygous ones, utilization of segregating BC1-S1 families for marker identification might be superior to BCi individuals. Moreover, the generation of isogenic lines carrying the single resistance genes separately in different genotypes might increase the success to identify closely linked markers for all three QTL *Scmv1a*, *Scmv1b* and *Scmv2* conferring resistance to SCMV. Whereas the tightly linked SSR, AFLP and indel markers for chromosome 3 can be applied immediately for MAS or map-based cloning, generation of isogenic lines will help to identify molecular markers tightly linked to or cosegregating with either *Scmv1a* or *Scmv1b*. Analysis of a large BC1-S1 population with the molecular markers, known to be tightly linked to either one of the target genes on chromosome 6 will help to identify rare recombination events between *Scmv1a* and *Scmv1b*, and might therefore pave the way to clone these genes.

References

Ballvora A, Hesselbach J, Niewöhner J, Leister D, Salamini F, Gebhardt C (1995) Marker enrichment and high-resolution map of the segment of potato chromosome VII harboring the nematode resistance gene *Gro1*. Mol Gen Genet 249: 82-90

Borchardt DC, Weißleder KK (2000) Marker-assisted selection in sugar beet: a breeder's perspective. In: EUCARPIA Quantitative genetics and breeding methods: the way ahead. Paris, France 2000 213-220

Cervera MT, Gusmao J, Steenackers M, Peleman J, Storme V, Vanden Broeck A, Van Montagu M, Boerjan W (1996) Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. Theor Appl Genet 93: 733-737

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor A (1998) The isolation and mapping of disease resistance gene analogs in maize. Mol Plant-Microb Interact 11: 968-978

DeJong W, Forsyth A, Leister D, Gebhardt C, Baulcombe DC (1997) A potato hypersensitive resistance against potato virus X maps to a resistance gene cluster on chromosome 5. Theor Appl Genet 95: 246-252

Dollinger EJ, Findley WR, Williams LE (1970) Inheritance of resistance to maize dwarf mosaic virus in maize (*Zea mays* L.). Crop Sci 10: 412-415

Falconer DS (1961) Introduction to quantitative genetics. Oliver and Boyd, Boston

Hohmann F, Fuchs E, Grüntzig M, Kuntze L (1996) Entwicklung eines Routinetests zum Nachweis des sugarcane mosaic virus (SCMV) und des maize dwarf mosaic virus (MDMV) auf der Grundlage eines tissue print immunoblotting (TPIB). Z Pfl Krank Pfl Schutz 103: 464-471

Kegler H, Friedt W (1993) Resistenz von Kulturpflanzen gegen pflanzenpathogene Viren. Gustav Fischer Verlag Jena

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Graner A, Schulze-Lefert P (1999) RFLP- and physical mapping of resistance gene homologues in rice (*O.sativa*) and barley (*H. vulgare*). Theor Appl Genet 98: 509-520

Louie R (1995) Vascular puncture of maize kernels for the mechanical transmission of maize white line mosaic virus and other viruses of maize. Phythopathology 85: 139-

143

Lübberstedt T, Dußle CM, Xu ML, Melchinger AE (2002) Targeted bulked segregant analysis (tBSA) - a strategy for identification of markers closely linked to a resistance gene. Plant Breeding: to be submitted

Lübberstedt T, Xia XC, Xu ML, Kuntze L, Melchinger AE (1999) Inheritance of resistance to SCMV and MDMV in European maize. In: Proc. of the XVth EUCARPIA 1998 General Congress: Genetics and breeding for crop quality and resistance, G.T.S. Mugnozza (ed.), Kluwer Academic Publishers, Dordrecht, The Netherlands: 241-250

Marçon A, Kaeppler SM, Jensen SG (1997) Resistance to systemic spread of high plains virus and wheat streak mosaic virus cosegregates in two F_2 maize populations inoculated with both pathogens. Crop Sci 37: 1923-1927

McMullen MD, Jones MW, Simcox KD, Louie R (1994) Three genetic loci control resistance to wheat streak mosaic virus in the maize inbred Pa405. Mol Plant-Microb Interact 7: 708-712

McMullen MD, Louie R (1989) The linkage of molecular markers to a gene controlling the symptom response in maize dwarf mosaic virus strains. Mol Plant-Microb Interact 6: 309-314

McMullen MD, Simcox KD (1995) Genomic organization of disease and insect resistance genes in maize. Mol Plant-Microb Interact 8: 811-815

Melchinger AE, Kuntze L, Gumber RK, Lübberstedt T, Fuchs E (1998) Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor Appl Genet 96: 1151-1161

Ming R, Brewbaker RC, Pratt RC, Musket TA, McMullen MD (1997) Molecular mapping of a major gene conferring resistance to maize mosaic virus. Theor Appl Genet 95: 271-275

Negi MS, Devic M, Delseny M, Lakshmikumaran M (2000) Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to SCAR marker for rapid selection. Theor Appl Genet 101: 146-152

Quint M, Dußle CM, Melchinger AE, Lübberstedt T (2002) Identification of genetically linked RGAs by BAC screening in maize and implication for gene cloning, mapping,and MAS. Theor Appl Genet: in review

Sans-Alferez S, Richter TE, Hulbert SH, Bennetzen JL (1995) The *Rp3* disease resistance gene of maize: mapping and characterization of introgressed alleles. Theor Appl Genet 91: 25-32

Shan X, Blake TK, Talbert LE (1999) Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. Theor Appl Genet 98: 1072-1078

Shen KA, Meyers BC, Islam-Faridi MN, Chin DB, Stelly DM, Michelmore RW (1998) Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol Plant-Microb Interact. 11: 815-823

Thomas CM, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8: 785-794

Xia XC, Melchinger AE, Kuntze L, Lübberstedt T (1999) QTL mapping of resistance to sugarcane mosaic virus in maize. Phythopathology 89: 660-667

Xu ML, Melchinger AE, Xia XC, Lübberstedt T (1999) High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Mol Gen Genet 261: 574-581

Summary

Sugarcane mosaic virus (SCMV) is an important disease in European maize cultivars (*Zea mays* L.). Because of its non-persistent transmission by aphid vectors, it is not possible to control SCMV directly. Therefore, cultivation of resistant maize varieties is an efficient way to control SCMV infections.

The overall objectives of this study were the genetic analysis of SCMV resistance in cross F7 FAP1360A and the identification of closely linked markers to the SCMV resistance genes *Scmv1* on chromosome 6 and *Scmv2* on chromosome 3 for map-based cloning and marker-assisted selection (MAS). The technical objectives were to (1) identify in particular the location of *Scmv1* and *Scmv2* on chromosomes 3 and 6 in cross F7 FAP1360A, (2) estimate the gene action of the alleles present at these loci, (3) enrich the SCMV resistance regions surrounding *Scmv1* and *Scmv2* with amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers by applying a modified targeted bulked segregant analysis, tBSA, (4) convert AFLP markers into codominant, simple PCR-based markers as a tool for MAS and map-based cloning of *Scmv1* and *Scmv2* and, (5) assess resistance gene analogues (RGAs) as potential candidate genes for *Scmv1* and *Scmv2*.

Two European inbred lines, FAP1360A and F7, were crossed to produce a population with a random set of 121 F_3 families. Inbred line FAP1360A, a dent line, was completely resistant to SCMV, whereas F7, a flint line, was highly susceptible. Field trials were evaluated for SCMV resistance across two environments under artificial inoculation at seven scoring dates VIR1 to VIR7. Quantitative trait loci (QTL) mapping with four SSR markers confirmed the presence of two QTL on chromosome 6 (*Scmv1*) and chromosome 3 (*Scmv2*) previously identified in cross D32 D145. Both QTL together explained between 15% and 62% of the phenotypic variance for the seven scoring dates. The *Scmv1* region showed complete dominance, whereas the gene action for the *Scmv2* region was additive. A second, more detailed QTL analysis based on 24 SSR markers with the same set of F_3 families of F7 FAP1360A resolved two QTL, denoted as *Scmv1a* and *Scmv1b*, on chromosome 6 for scoring dates VIR3 to VIR7. Similar to the previous study, the QTL on chromosomes 3 and 6 explained between 33% and 71% of the phenotypic variance. Whereas gene action for the QTL on

chromosome 3 was additive, gene action was overdominant for *Scmv1a* and additive for *Scmv1b* on chromosome 6.

Phenotypic evaluation of BC (backcross) field trials after artificial inoculation resulted in proportions of symptomless individuals within families, segregating for SCMV resistance, between 2.6% and 33.3% in BC6 to BC9 generations. The proportion of segregating families in BC6 and BC9 fitted the expected value of 25% segregating families, assuming an underlying model of two complementary acting dominant genes. However, proportions within segregating families in all BCi generations did not exceed an average of 10%. This could be due to the threshold character of SCMV resistance, incomplete penetrance, environmental effects, or the presence of additional resistance genes.

tBSA is a modification of a BSA, minimizing the experimental input for the analysis of large numbers of linked markers and enables the evaluation of closely linked markers without analysing all individuals of a mapping population. After identification of polymorphisms between the susceptible parent F7 and a symptomless BC7 individual known to carry short donor regions for the SCMV resistance genes on chromosomes 3 and 6, markers were assigned to either one of the resistance gene regions *Scmv1* or *Scmv2* in step two. In the third step those markers were identified, which were closer linked to the respective target genes, than the flanking SSR markers employed for preselection of the BCi individuals. Mapping was conducted in the fourth step, employing symptomless BC individuals, which were used in step 3 as pools. tBSA was conducted with symptomless individuals of different advanced backcross generations (BC5 to BC9) of cross F7 FAP1360A. Analyses of 512 AFLP primer combinations and 81 SSR markers identified 24 AFLP and 25 SSR markers adjacent to either *Scmv1* or *Scmv2*. Fourteen SSR and 6 AFLP markers mapped close to *Scmv1*, whereas 11 SSR and 18 AFLPs were located close to *Scmv2*. On chromosome 6, AFLP marker E33M61-1 was closest linked proximal to *Scmv1*, with a distance of 8.0 cM. The closest linked marker distal to *Scmv1* was SSR marker *bmc1432* with a distance of 2.4 cM. One AFLP marker, E38M51, showed prefect cosegregation with *Scmv2* on chromosome 3. Whereas the chromosome 3 map and the results obtained by QTL analysis agreed well, some differences were found for the map distances and the window size on chromosome 6. The reason for the discrepancies between both maps might be the

presence of two genes on chromosome 6, which were detected by QTL analysis but not by tBSA.

AFLP markers are too costly and laborious for high-throughput monitoring of large numbers of genotypes. Therefore, conversion of AFLP markers tightly linked to SCMV resistance genes into simple and reliable PCR-based markers, like insertion/deletion (indel) or cleaved amplified polymorphic sequence (CAPS) markers, provide useful markers for MAS and map-based cloning. Sequencing of eight AFLP markers closely linked to *Scmv1* or *Scmv2* resulted in either completely identical sequences between the investigated inbred lines or revealed single nucleotide polymorphisms (SNPs) within the inbred lines. AFLP marker E35M62-1, closely linked to *Scmv1* on chromosome 6, was successfully converted into an indel marker. For chromosome 3, AFLP marker E33M61-2 was converted into a CAPS marker. Both converted AFLP markers mapped to the same chromosome region as their original AFLP markers.

RGA analysis was conducted with three previously published resistance gene analogues pic13, pic21, and pic19. These RGAs were mapped in relation to the SCMV resistance genes *Scmv1* and *Scmv2*. They were cloned from three SCMV susceptible (D145, D408, F7) and three SCMV resistant (D21, D32, FAP1360A) maize inbred lines. Development of CAPS of the RGAs and mapping in relation to SCMV resistance genes *Scmv1* and *Scmv2* identified pic19 and pic13 as potential candidates for these resistance genes. Genetic mapping identified pic21 to be different from *Scmv2*.

In this study, useful markers were developed for applications in MAS. Because inheritance of SCMV resistance is strongly affected by the environment, MAS enables the selection of resistant individuals independently of field experiments. Furthermore, MAS can assist breeders to identify resistant individuals before flowering and to pyramid resistance genes in elite inbred lines. Another benefit of these closely linked markers is their application for map-based cloning, where thousands of individuals have to be screened with molecular markers cosegregating or at least closely linked with the target genes. Final evidence, whether there are one or more genes clustered on chromosomes 3 and 6, conferring resistance against SCMV, can only be solved after cloning these genes.

Zusammenfassung

Das Zuckerrohrmosaikvirus (sugarcane mosaic virus, SCMV) ist eine wichtige Pflanzenkrankheit im europäischen Maisanbau. Aufgrund der nicht-persistenten Übertragung durch Blattläuse ist es nicht möglich, SCMV direkt zu bekämpfen. Daher stellt der Anbau resistenter Maissorten die einzig wirksame Bekämpfungsmaßnahme dar.

In Rahmen dieser Studie wurde die Resistenz gegenüber SCMV innerhalb der Kreuzung F7 FAP1360A untersucht. Im Vordergrund stand das Auffinden von eng mit den SCMV-Resistenzgenen *Scmv1* und *Scmv2* gekoppelten Markern als Basis für die markergestützte Selektion (marker-assisted selection, MAS) und die kartengestützte Klonierung. Die Ziele der Arbeit waren (1) die genaue Charakterisierung der Kartenposition von *Scmv1* auf Chromosom 6 und *Scmv2* auf Chromosom 3, (2) die Schätzung der Geneffekte und Genwirkungsweise der Chromosomenregionen (quantitative trait loci, QTL) die an der Ausprägung der Resistenz gegenüber SCMV beteiligt sind, (3) das Auffinden von eng mit den Resistenzgenen gekoppelten SSR- (simple sequence repat) und AFLP- (amplified fragment length polymorphism) Markern mittels einer tBSA (targeted bulked segregant analysis), (4) die Konversion von AFLP-Markern zu einfacher handhabbaren PCR-basierten Markern und (5) die Bewertung von Resistenzgenanaloga (RGA) als mögliche Kandidatengene für *Scmv1* und *Scmv2.*

Als Ausgangsmaterial dienten die beiden europäischen Maisinzuchtlinien FAP1360A und F7. Während die Dentlinie FAP1360A vollständig resistent gegen SCMV war, zeigte sich die Flintline F7 hochanfällig gegen SCMV. Für die Kreuzung F7 FAP1360A wurde eine Population mit 121 F_3-Familien hergestellt. Das Resistenzverhalten der Population gegenüber SCMV wurde in zwei Wiederholungen nach mechanischer Inokulation an sieben Terminen (VIR1 bis VIR7) geprüft. Eine QTL-Kartierung mit vier SSR-Markern bestätigte zwei QTL auf den Chromosomen 6 (*Scmv1*) und 3 (*Scmv2*), die zuvor in einer spaltenden Population der Kreuzung D32 D145 identifiziert wurden. Beide QTL erklärten zusammen zwischen 15% und 62% der geschätzten phänotypischen Varianz an den sieben Boniturterminen. Die Genwirkungsweise für *Scmv1* war vollständig dominant, während *Scmv2* eine additive Genwirkungsweise aufwies. Mit einer zweiten, umfassenderen QTL-Analyse mit 24

SSR-Markern und denselben F_3-Familien konnten zwei QTL (bezeichnet als *Scmv1a* und *Scmv1b*) auf Chromosom 6 für die Boniturtermine VIR3 bis VIR7 gefunden werden. Vergleichbar zu der vorhergehenden Studie erklärten die QTL auf den Chromosomen 3 und 6 zwischen 33% und 71% der phänotypischen Varianz. Die Genwirkungsweise der QTL auf Chromosom 6 waren überdominant für *Scmv1a*, sowie additiv für *Scmv1b*. *Scmv2* zeigte ebenfalls eine additive Genwirkungsweise.

Verschiedene Rückkreuzungsgenerationen (backcross, BC) wurden nach mechanischer Inokulation im Feld beurteilt. Dabei wurden zwischen 2,6 und 33,3% symptomlose Individuen innerhalb der für SCMV spaltenden BC6- bis BC9-Familien identifiziert. Der Anteil spaltender Familien in den Generationen BC6 und BC9 stimmten gut mit dem Erwartungswert von 25% spaltender Familien bei zwei komplementär wirkenden, dominanten Genen überein. Tatsächlich konnten jedoch im Durchschnitt kaum mehr als 10% symptomlose Pflanzen innerhalb spaltender Familien für alle anderen Rückkreuzungsgenerationen gefunden werden. Die Gründe für die Abweichungen vom Erwartungswert könnten der Schwellenwertcharakter der SCMV Resistenz, unvollständige Penetranz, Umwelteffekte oder die Anwesenheit weiterer Resistenzgene sein.

Mit der tBSA kann der experimentelle Aufwand für die Untersuchung einer großen Anzahl an Markern deutlich verringert werden. Dadurch können eng mit dem Zielgen gekoppelte Marker identifiziert werden ohne jeden Marker an jedem einzelnen Individuum einer Kartierungspopulation untersuchen zu müssen. Zwischen dem anfälligen Elter F7 und einer symptomlosen BC7-Einzelpflanze mit kurzen Donorregionen im Bereich der SCMV-Resistenzgene auf Chromosom 3 und 6 wurden polymorphe AFLP-Marker identifiziert. Diese wurden in einem zweiten Schritt den jeweiligen Resistenzgenregionen *Scmv1* oder *Scmv2* zugeordnet. Im dritten Schritt wurden diejenigen polymorphen Marker selektiert, die enger mit dem jeweiligen Resistenzgen gekoppelt waren, als jene, die zur Voruntersuchung der symptomlosen BC$_7$-Einzelpflanzen eingesetzt wurden. Die Kartierung der in Schritt drei identifizierten, eng gekoppelten Marker, erfolgte in einem vierten Schritt an symptomlosen BC$_7$-Einzelpflanzen, welche zur Durchführung des dritten Schritts in Pools zusammengefasst waren. Die Analyse wurde mit symptomlosen Einzelpflanzen verschiedener fortgeschrittener Rückkreuzungsgenerationen (BC5 bis BC9) der Kreuzung F7

FAP1360A durchgeführt. Es wurden 512 AFLP-Primerkombinationen und 81 SSR-Marker eingesetzt. Davon waren 24 AFLP- und 25 SSR-Marker eng mit den Zielgenen gekoppelt. Vierzehn SSR- und sechs AFLP-Marker kartierten in die Scmv1-Region, während 11 SSR- und 18 AFLP-Marker für die *Scmv2*-Region identifiziert werden konnten. Der AFLP-Marker E33M61-1 war mit einem Abstand von 8 cM proximal zu *Scmv1* am engsten gekoppelt. Den kürzesten Abstand distal zu *Scmv1* zeigte mit 2,4 cM der SSR-Marker *bmc1432*. Der AFLP-Marker E38M51 kosegregierte vollständig mit *Scmv2*. Die genetischen Karte für Chromosom 3 zeigte eine gute Übereinstimmung mit der bei der QTL-Analyse erhaltenen Karte. Die Karten der tBSA und QTL-Analyse unterschieden sich jedoch in den Markerabständen und der Größe des kartierten Fensters für Chromosom 6. Dies könnte dadurch erklärt werden, dass die *Scmv1*-Region auf Chromosom 6 bei der tBSA als ein Genlocus identifiziert wurde, während mittels der QTL-Analyse zwei eng gekoppelte Loci gefunden wurden.

AFLP Analysen sind sehr teuer und arbeitsaufwändig und deshalb nicht gut geeignet für die Untersuchung einer großen Individuenzahl. Daher ist die Konversion von eng mit den SCMV Resistenzgenen gekoppelten AFLP-Markern zu Indel- (insertion/deletion) oder CAPS- (cleaved amplified polymorphism site) Markern zu einfach anwendbaren PCR-Markern hilfreich für deren weitere Anwendung zur MAS oder kartengestützten Klonierung. Beide Methoden erfordern die Untersuchung von Populationen mit einigen tausend Einzelpflanzen. Die Sequenzierung von acht eng mit *Scmv1* bzw. *Scmv2* gekoppelten Markern führte zu entweder vollständig identischen Sequenzen zwischen den untersuchten Inzuchtlinien oder zu Einzelnukleotidunterschieden (single nucleotide polymorphism, SNP) zwischen den klonierten Fragmenten eines Markers innerhalb einer Inzuchtlinie. Der eng mit *Scmv1* gekoppelte AFLP-Marker E35M62-1 konnte erfolgreich in einen Indel-Marker umgewandelt werden. Ein AFLP-Marker (E33M61-2) für Chromosom 3 wurde zu einem CAPS-Marker konvertiert. Beide konvertierten Marker kartierten in dieselbe Chromosomenregion wie die zur Konversion verwendeten AFLP-Marker.

Die RGA-Analyse wurde mit den Resistenzgenanaloga pic13, pic21 und pic19 durchgeführt. Diese RGA wurden in Bezug auf die SCMV Resistenzgene *Scmv1* und *Scmv2* kartiert. Drei für SCMV anfällige (D145, D408, F7) und drei resistente (D21, D32, FAP1360A) Maisinzuchtlinien wurden zur Klonierung dieser RGA eingesetzt. Die

Entwicklung von CAPS-Markern und deren Kartierung identifizierte pic19 (*Scmv1*) und pic13 (*Scmv2*) als mögliche Kandidaten für diese Resistenzgene, während pic21 sich eindeutig als nicht identisch mit *Scmv2* erwies.

Die in dieser Studie entwickelten und charakterisierten Marker können gut zur MAS eingesetzt werden. Da die Merkmalsausbildung der SCMV-Resistenz stark abhängig von Umweltfaktoren ist, könnten mit Hilfe von MAS resistente Einzelpflanzen unabhängig von Feldversuchen und mechanischer Inokulation noch vor der Blüte selektiert werden. Darüber hinaus können mittels MAS mehrere Resistenzgene in Inzuchtlinien pyramidisiert werden. Ein weiterer Vorteil der in dieser Studie identifizierten eng gekoppelten Marker ist ihre Anwendung zur kartengestützten Klonierung. Wie bei der MAS muss hierbei eine sehr große Zahl von Einzelpflanzen mit molekularen Markern untersucht werden, die mit dem betreffenden Zielgen kosegregieren oder zumindest eng gekoppelt sind. Ein eindeutiger Beweis, wieviele Gene auf den Chromosomen 3 und 6 tatsächlich an der Resistenz gegen SCMV beteiligt sind, kann nur durch die Klonierung dieser Gene erbracht werden.

Acknowledgements

I am deeply indebted to Prof. A. E. Melchinger for his scientific support, advice and creative suggestions in the course of the present investigation.

Many thanks to Dr. Th. Lübberstedt for initiating the experiments and for his continuous advice and scientific support.

I wish to thank Prof. Dr. H.F. Utz for all the valuable advices on statistical problems.

My regards to Dr. M.L. Xu who initiated the marker analyses and supported me at the beginning of my studies.

The skilled technical assistance of Mr. F. Mauch, Ms. A. Karg, Ms. B. Devezi-Savula at Hohenheim as well as the staff at the Plant Breeding Station , Eckartsweier, in conducting the field trials is gratefully acknowledged. Many thanks also to Ms. E. Kokai-Kota who carefully assisted the laboratory experiments. Moreover, I highly appreciate the aid of many students, especially A.Pahlich, S. Dreisigacker, S. Haesler and N. Burkarth who helped me with the marker analyses. Many thanks to L. Yuan who conducted parts of the SSR analyses during his stay at Hohenheim.

I want to thank Ms. B. Boesig for her friendliness and helpfulness in everyday life in the institute.

Finally I want to thank Dr. M. Bohn, Dr. M Frisch, M. Heckenberger, J. Muminovic, Dr. E. Bauer, Dr. M. Wolf, Dr. F. Röber and all the colleagues at Hohenheim for creating a pleasant work environment. I particularly enjoyed the friendship of my laboratory and office mate Marcel Quint.

My personal thanks goes to Markus and last but not least to my parents for their encouragement, great tolerance and patience during the writing up of this treatise.

This research was supported by grants from the Deutsche Forschungsgemeinschaft.

Lebenslauf

Name:	Christina Maria Dußle
Geboren:	06.06.1968 in Bönnigheim
Familienstand:	ledig

Schulausbildung

1974 – 1979, Grundschule in Cleebronn
1979 – 1986, Zabergäugymnasium in Brackenheim
1986 – 1988, Hölderlingymnasium in Lauffen/N
Abschluss: Allgemeine Hochschulreife

Berufsausbildung

August 1988 – Juli 1990, Ausbildung zur Winzergehilfin bei Winzermeister Eberhard Link in Lauffen/N
Berufsabschluss: Winzergehilfin

Studium

WS 1991 – SS 1998, Studium der Agrarbiologie in Stuttgart Hohenheim mit Fachrichtung Pflanzenproduktion
Abschluss: Diplom-Agrarbiologin

1995 – 1998, Wissenschaftliche Hilfskraft im Fachgebiet angewandte Genetik und Pflanzenzüchtung: Einsatz von DNA-Markern (Mais)

Berufl. Tätigkeit

August 1990 – September 1991, Arbeiterin bei der Firma Layher GmbH & Co., KG

Juli 1998 – Januar 2002, Wissenschaftliche Angestellte am Institut für Pflanzenzüchtung, Saatgutforschung und Populationsgenetik, Universität Hohenheim

Seit Februar 2002, Wissenschaftliche Angestellte an der Landessaatzuchtanstalt, Universität Hohenheim