Molecular analysis of *Sugarcane mosaic virus* resistance in maize

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Summary
The *Potyvirus sugarcane mosaic virus* (SCMV) is an important pathogen of maize (*Zea mays* L.), causing chlorosis, stunting and serious yield loss in susceptible cultivars. Three major resistance genes confer resistance to SCMV, one gene located on chromosomes 3 and two genes closely linked on chromosome 6. However, the molecular mechanisms underlying the establishment and progression of SCMV infection in maize are poorly understood. A near-isogenic line, F7R, carrying the resistance regions from chromosome 3 (*Scmv2*) and 6 (*Scmv1a and 1b*) in a susceptible background was developed. Based on F7R, nine isogenic genotypes segregating in these two regions were developed. Testing the nine genotypes for response to SCMV infection showed that the *Scmv1* and *Scmv2* locus interact epistatically and that one resistance region alone was not sufficient for complete resistance against SCMV. When the resistant allele is fixed either at the *Scmv1* or *Scmv2* loci, the susceptible homozygote at the other locus is easily distinguishable from the genotypes carrying one or two resistance alleles. Based on these findings we are currently in the process of map-based gene isolation using large F_2 populations for each of both genome regions. A BAC library has been constructed from the resistance gene donor of F7R, inbred FAP1360A, for physical mapping of the *Scmv1* and *Scmv2* loci and isolation of the respective genes.

Introduction

SCMV is one of the most important virus pathogens in European maize causing serious yield losses in susceptible cultivars (Fuchs and Grüntzig, 1995). Diagnostic symptoms include stunting, chlorosis, reduction in plant weight, and therefore, a reduction in yield. Since the 1980s, SCMV has been found in Germany (Fuchs and Kozelska, 1984) and SCMV has followed the progression of maize cultivation towards higher latitudes. Chemical control of SCMV is not possible because of the non-persistent mode of virus transmission by aphids. Hence, the only method to control SCMV infection is the cultivation of resistant maize varieties.

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 to SCMV and MDMV. Two major dominant genes, *Scmv1* on the short arm of chromosome 6 and *Scmv2* near the centromere of chromosome 3, were mapped by *Melchinger et al.* (1998). Both major resistance genes are essential for expression of complete resistance to SCMV. More recently, the *Scmv1* region was shown to contain most likely two closely linked resistance genes (Dussle *et al.,* 2003 ; Yuan *et al.,* 2003).

The major limitation so far has been suboptimal high-resolution mapping with traditional mapping populations. In F2 and BC populations incomplete penetrance and escapes obscure a tight relationship between resistance genotype and phenotype, necessary for reliable genetic fine mapping, due to simultaneous segregation of more than two resistance genes and presence of heterozygotes. However, this problem might be overcome by fine mapping of both regions separately using homozygous sub-isogenic lines. While one region is fixed for the resistance allele, a segregating population will be produced for the other region (by selfing a heterozygote), hereby converting the previous quantitative trait locus analysis into separate monogenic analyses. Furthermore, map-based cloning in maize might be impaired by allelic differences between maize inbred lines with respect to frequent insertions and deletions of genes (Brunner *et al*., 2005), stressing the necessity of a BAC library from the resistance donor.

Material and methods

Development of isogenic lines.

The early maturing European maize inbreds, FAP1360A, completely resistant to SCMV, and F7, highly susceptible to SCMV (Kuntze *et al.,* 1997), were crossed to produce F_1 offspring, and backcrossed seven times to F7 with two generations per year from 1995 to 1998 (Dussle *et al.,* 2003). Seed of the homozygous line $F7^{RR/RR}$ (= F7R, a F7 near isogenic line with FAP1360A introgression at the two target regions) was produced by three subsequent selfing steps starting from one SCMV resistant BC7 plant carrying the donor regions from FAP1360A at *Scmv1* and *Scmv2*. In 2001 and 2002, $F7^{RR/RR}$ was confirmed to be completely resistant to SCMV both in the field and greenhouse (Shi *et al.,* 2005).

Starting from the isogenic line pair F7 and F7R, further (sub-) isogenic lines have been developed. The *Scmv2*-flanking SSR markers on chromosome 3, bnlg1035 and bnlg1456 and four SSR markers in the *Scmv1* gene region on chromosome 6 (bnlg161, bnlg1432, bnlg1600 and phi077) (Dussle *et al.*, 2003), were used to screen F_2 populations derived from F7 x $F7^{RR/RR}$. Plants were selfed to produce the sublines $F7^{RR/SS}$ and $F7^{SS/RR}$ with resistance alleles (*Scmv2*, *Scmv1*) fixed in one *Scmv* QTL region, and with F7 alleles (*scmv2*, *scmv1*) fixed in the second region. These two lines together with F7 and $F7^{RR/RR}$ were used to develop all nine genotypes for the two QTL regions (table 1).

Table 1. R and S stand for the alleles of resistant parent FAP1360A and susceptible parent F7, respectively. The letter left of the slash refers to the genotype at *Scmv2* on chromosome 3; the right refers to the genotype of *Scmv1* on chromosome 6.

		Scmv2		
		RR	RS	SS
Scmv1	RR	$F7^{RR/RR}$	$F7^{RS/RR}$	$F7^{SS/RR}$
	RS	$F7^{RR/RS}$	$F7^{RS/RS}$	$F7^{SS/RS}$
	SS	$F7^{RR/SS}$	$F7^{RS/SS}$	$F7^{SS/SS}$

Construction of a BAC library from resistant line FAP1360A.

Inbred FAP1360A is resistant to SCMV, and was used to construct a BAC library. High-molecular-weight maize DNA was partially digested with *Bam*H I or *Hin*d III and ligated into pIndigoBAC-5. The library contains 103,680 clones with an average insert size of approximately 120 kb, giving approximately 5 times haploid genome coverage.

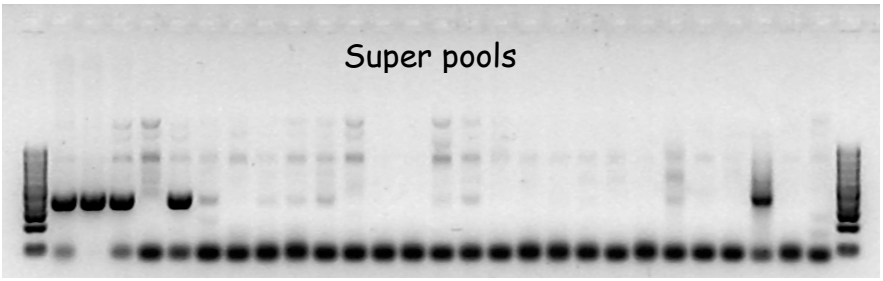
In order to be able to screen the BAC library by PCR, DNA pools were made (http://www.genome.washington.edu/UWGC/mapping/Protocols/Wet_Bench/Clone_Library_Pooling_Protocol.doc). Each of the 270 384-well microtiter plates were replicated in 96-well plates, thus pooling the clones four by four. Using a Hamilton pipetting robot, plate pool DNAs (P1, P2, ...) each containing 384 clones was made. 10 by 10 plates were grouped in turn (P1-10; P11-20; ...) for preparation of 8 row (R1A, R1B, ..., R1H; R2A, ...) and 12 column (C101, C102, ..., C112; C201, ...) pool DNAs. Each row and column pool contains 320 and 480 BAC clones, respectively. Superpools were made by hand by pooling plate pools 10 by 10, giving a total of 27 superpools, 270 plate pools, 324 column pools and 216 row pools.

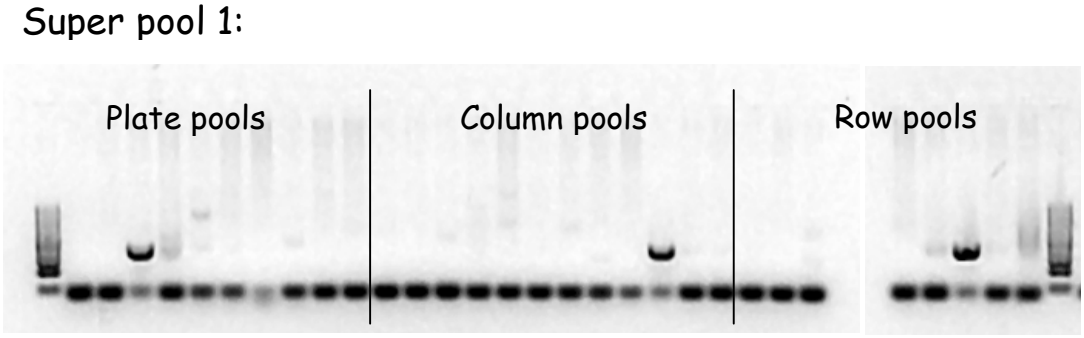
Results and discussion

Genetic fine mapping of both regions has been conducted using targeted bulked segregant analysis (Dussle *et al.,* 2003) with about 1000 AFLP primer combinations (Yuan *et al.*, 2004). Previous work (Xu *et al.*, 1999 ; Dussle *et al.*, 2002 ; Yuan *et al.,* 2003) substantially increased the density of markers in the *Scmv1* and *Scmv2* genome regions to more than two markers per cM. This usually is a good starting point for map based gene isolation. The major limitation so far was the suboptimal high-resolution mapping with traditional mapping populations. Fine

mapping and map-based gene isolation in simple types of genetic populations such as F_2 or BC_1, has been impaired by presence of duplicated sequences (Quint *et al.*, 2003, Frisch *et al.,* 2004) and by use of mapping populations (backcross, F_2) including heterozygous genotypes. The latter problem arises from the high level of phenotypic plasticity of heterozygous genotypes in contrast to homozygous genotypes. Depending on environmental conditions, heterozygotes might show similar infection levels as either the homozygous resistant or susceptible inbred lines, complicating establishment of close association between marker genotype and resistance phenotype as required for high resolution mapping.

Figure 1. Screening of the BAC library with primers for the Scmv resistance candidate gene pic19 (Quint *et al*., 2003). Screening the super pools gave 5 hits in super pools 1, 2, 3, 5 and 25. Screening super pool 1 showed that the clone of interest can be found in plate 3, column 10, row F.





However, this problem can be overcome by fine mapping of both regions separately using homozygous sub-isogenic lines. The oligogenic inherited SCMV resistance has been broken down into two "monogenic" cases, giving a very reliable association between phenotype and genotype classes, which is an important prerequisite for successful map-based gene isolation. When, for example, the *Scmv2* resistance allele is fixed, both homozygote classes at *Scmv1* can be clearly distinguished after virus infection. The material can further be employed as a model system to better understand properties related to quantitative traits such as epistasis, partial dominance, incomplete penetrance (threshold character) of resistance, pleiotropy/clustering of genes with related function.

It has been found that the genome of maize inbreds contains a lot of nonhomologous DNA (Brunner *et al*., 2005). This have an effect on the recombination rate, giving big local differences in the genetic-to-physical distance rations between crosses. Although the majority of the nonhomologous DNA is found outside expressed regions, nonshared genes are also found. This could have a major impact on map-based cloning, as nonshared genes cannot be cloned from certain BAC libraries. Furthermore, the nonshared genes violate the maize colinearity with rice, impairing synteny studies (Brunner *et al*, 2005). It seems that nonshared genes or the homologues are often found somewhere else in the maize genome, also affecting map-based cloning in some populations. We have overcome these putative problems by constructing a BAC library based on the SCMV resistant line FAP1360A, the resistance donor of F7R. After construction of super pools as well as plate pools, row pools and column pools, the library was screened with *Scmv* resistance candidate genes (figure 1).

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References

Brunner, S., Fengler, K., Morgante, M., Tingey, S. & Rafalski, A., 2005. Evolution of DNA sequence nonhomologies among maize inbreds. *Plant Cell* **17**:343-360.

Dussle, C., Quint, M., Melchinger, A.E., Xu, M.L. & Lübberstedt, T., 2002. Saturation of two chromosome regions conferring resistance to SCMV by targeted BSA. *Theor. Appl. Genet.* **105**:1190-1195.

Dussle, C.M., Quint, M., Melchinger, A.E., Xu, M.L. & 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.

Frisch, M., Quint, M., Lübberstedt, T. & Melchinger, A.E., 2004. Duplicate marker loci can result in incorrect locus orders on linkage maps. *Theor. Appl. Genet.* **109**:305-316.

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. & 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.

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

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

Quint, M., Dußle, C., Melchinger, A.E. & Lübberstedt, T., 2003. Identification of genetically linked RGAs by BAC screening in maize and implication for mapping, MAS, and gene cloning. *Theor. Appl. Genet.* **106**:1171-1177.

Shi, C., Ingvardsen, C., Thümmler, F., Wenzel, G., Melchinger, A.E. & Lübberstedt, T., 2005. Identification of genes differentially expressed in association with SCMV resistance in maize by combining SSH and cDNA array techniques. *Mol. Gen. Genom.* **273**:450-461.

Xu, M.L., Melchinger, A.E., Xia, X.C. & 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., Dusle, C.M., Melchinger, A.E., Utz, H.F. & Lübberstedt, T., 2003. Clustering of QTL conferring SCMV resistance in maize. *Maydica* **48**:55-62.

Yuan, L., Dußle, C.M., Melchinger, A.E., Muminovic, J. & Lübberstedt, T., 2004. Application of targeted BSA using *Pst*I/*Mse*I and *Eco*RI/*Mse*I AFLP primer combinations for SCMV resistance genes in maize. Plant Breeding **123**:434-437.