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Tagging downy mildew resistance gene (*Sdm*) and head smut resistance gene (*Shs*) in sorghum using RFLP and RAPD markers

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Sorghum Head Smut

Sorghum head smut, caused by *Sporisorium reilianum* (Kühn) Langdon & Fullerton, is an important disease of sorghum (*Sorghum bicolor* (L.) Moench) in Africa, Asia, Australia, Europe, and North America. Chemical control and effective agronomic practices are not sufficient to efficiently reduce disease incidence. Resistant cultivars are the most effective method to control this disease, but head smut still remains a potentially important disease because of the pathogen's variability (Frederiksen 1986). In the United States, four physiological races have been defined among sorghum isolates of *S. reilianum* on the basis of their reactions on a series of host differentials. An isolate that originated in Taylor, TX, was determined to be race 5 of *S. reilianum*. Resistance to race 5 of *S. reilianum* in several sorghum accessions was expressed as a dominant trait. However, there is no information on linkage of any genes for sorghum head smut resistance.

Since restriction fragment length polymorphisms (RFLPs) were recognized as a useful tool for genome mapping (Botstein et al 1980), RFLP markers have been introduced into general plant breeding programs and used to tag disease resistance genes. Disease resistance genes linked to RFLP markers have been identified in several important crops, including maize, potato, rice, and tomato.

The procedure is developed that employs random DNA primers in a polymerase chain reaction (PCR) to rapidly generate polymorphic markers that can be used to create genetic linkage maps (William et al 1990). Since then, random amplified polymorphic DNAs (RAPDs) have been used to produce maps in several plant species and to tag major genes for disease resistance in common bean, lettuce, and tomato. Bulked segregant analysis was proposed for the identification of RFLP and RAPD markers linked to important genes in plants where

near-isogenic lines are not available (Michelmore et al 1991). The combination of RAPDs and bulked segregant analysis serves to enhance the identification of markers that are tightly linked to the gene of interest.

One of the goals in our sorghum hybrid breeding is to improve the ability to select and combine genes for sorghum head smut resistance via their linkage to easily detectable RFLP or RAPD markers. This study reports two RFLP markers and one RAPD marker that are linked to a sorghum head smut resistance gene in accession SC325.

RFLPs were identified between four resistant accessions and four susceptible accessions by using three different enzymes (*Eco*RI, *Eco*RV, and *Hind*III) and 43 maize genomic clones. Among the 16 pairs of resistant and susceptible accessions, SC325 and RTx7078 showed the maximum level of RFLP that could be easily detected. Therefore, a cross was made between SC325 and RTx7078 to map the *Shs* locus. An F2 population consisting of 52 progeny was used for linkage mapping.

F3 lines derived from 52 single F2 plants were used to determine the resistance genotype of the F2 plants. Of the 52 F2 plants, 19 were inferred to be homozygous resistant to *S. reilianum*, eight were homozygous susceptible, 23 were heterozygous resistant, and two were scored as missing data for a 1:2:1 ratio (χ^2 = 5.038; P>0.05).

To identify RFLP markers for *Shs*, 124 sorghum genomic clones were screened after digestion of sorghum genomic DNA with five different restriction enzymes (*Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, and *Xba*I). Of the genomic clones tested, 102 showed polymorphism between SC325 and RTx70768 with at least one restriction enzymes. To determine which of the 102 RFLP markers were linked to *Shs*, the DNA probes were hybridized to filters that contained DNA from the two parents and 52 F2 progenies that had been digested with a restriction enzymes that generated easily distinguished polymorphisms. Evidence of linkage to *Shs* was found with the locus detected by pSbTXS1294 (Xu et al 1994), which maps on the end of linkage group A in the sorghum RFLP map (Fig. 1A). In further mapping analysis, the locus detected by pSbTXS560, which is located near *txs1294* (Fig. 1A), was also found to be linked to *Shs*. Linkage analysis showed that the *Shs* locus was linked to two RFLP loci, *txs560* and *txs1294*, at 8.8 and 19.9 cM, respectively (Fig. 1B).

Approximately 1,467 discrete products, ranging from 0.3 to 2.6 kbp, were amplified per parental accession (average 4.5 products per primer). Although the two parents are not near isogenic lines, the majority of the products were identical in both parents. Nineteen of 326 (5.8%) RAPD primers produced DNA fragments that were polymorphic between the two parents. In the bulked segregant analysis, only primer OPG5 generated one polymorphic product (OPG5-2) that was amplified from the homozygous resistant, but not the susceptible, bulk. OPG5 generated two polymorphic products (OPG5-2 and OPG5-1) that differed between SC325 and

RTx7078, but only the former was linked to *Shs* by F2 segregation (Fig. 2). When each F2 plant was individually tested, linkage analysis showed that the *Shs* locus was linked to RADP locus *opg5-2* at 6.0 cM (Fig.1B).

In theory, combining different genes for head smut resistance may lead to more durable resistance and alleviate the need to continuously search for new sources of disease resistance. However, in order to do so, it will be imperative to easily identify each of the resistance genes that may be present in a particular individual. Current evaluation protocols, including natural infection, hypodermic inoculation, and seedling reaction, have classified sorghum genotypes as R1, R2, R3, and S1 according to factors for resistance to *S. reilianum* (Craig and Frederiksen 1992), but the genes involved have not been identified. According to the reaction to *S. reilianum* based on natural inoculation and hypodermic inoculation, SC325 is classified as an R3 genotype. Since R3 genotypes are resistant under natural inoculation and by hypodermic inoculation, they have factors for resistance in both nonmeristematic and meristematic tissues that lead to an incompatible host-pathogen interaction. The head smut resistance gene (*Shs*) of SC325 is a valuable source of resistance to *S. reilianum*. RFLP marker loci detected by pSbTXS560 and pSbTXS1294 and a RAPD marker locus from primer OPG5 linked to *Shs* in SC325 may help to identify genotypes of sorghum possessing the resistance alleles, to select for other head smut resistance genes, or to breed resistant sorghum hybrids (Oh et al 1994).

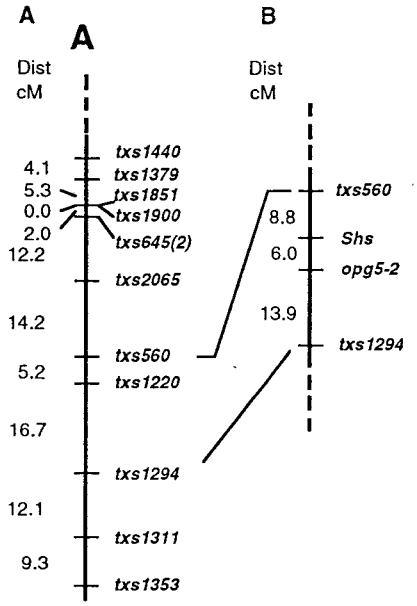

Fig. 1. Position of the *Shs* locus on the restriction fragment length polymorphism (RFLP) linkage map of sorghum. **A,** Linkage group A was derived from the segregating population (IS3620C × BTx623) used for the sorghum RFLP map (31). **B,** A part of the sorghum linkage group showing the linkage maps around the *Shs* locus derived from SC325 (resistant) and RTx7078 (susceptible).

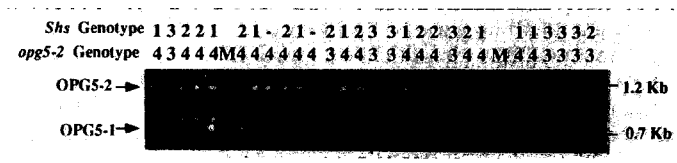

Fig. 2. Segregation analysis by polymerase chain reaction amplification of genomic DNA from 26 F_2 progeny with primer OPG5. A total of 52 F_2 plants derived from SC325 × RTx7078 were scored for their reactions to *Sporisorium reilianum* and for their genotypes with respect to random amplified polymorphic DNA markers selected. Left two lanes are SC325 and RTx7078, respectively. 1 = Homozygous for SC325 alleles (resistant phenotype); 2 = heterozygous (resistant phenotype); 3 = homozygous for RTx7078 alleles (susceptible phenotype); and 4 = not homozygous for RTx7078 alleles (resistant phenotype). M = molecular standard marker (pGEM marker).

Sorghum Downy Mildew

Sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* (Weston & Uppal) C.G. Shaw is one of the most destructive diseases of sorghum. Although chemical control and cultural practices are available for the control of SDM, this disease is mostly controlled by the use of resistant varieties (Frederiksen 1980). However, new races of *P. sorghi* have been continuously developing. In 1979 and 1980, a high incidence of SDM was noted in reputedly resistant sorghum hybrids in Texas. The susceptibility exhibited by these resistant sorghum hybrids was caused by new pathotypes (pathotype 2 and pathotype 3) of *P sorghi* (Craig and Frederiksen 1980). Inheritance of resistance to SDM was recently studied in sorghum accession QL3 and SC414-12 in Texas (Craig and Schertz 1985; Sifuentes and Frederiksen 1988). However, no information on linkage association is available for any SDM resistance gene. Thus, tagging of an SDM resistance gene by RFLP markers could be a valuable resource for the breeding of resistance hybrids in sorghum and will help to identify other genes for resistance to *P. sorghi*. The present work reports the identification of two RFLP markers that are linked to a gene in SC325 that confers resistance to pathotypes (P1) and 3 (P3) of *P. sorghi*.

Segregation analysis for SDM resistance in each F2 plant was first analyzed in F3 progeny inoculated with P1. A second set of progeny from each F2 was inoculated with P1. The segregation patterns following inoculation with P1 and P3 isolates were the same. No isolate of P2 was available for testing. One week after inoculation, susceptible reactions showed sporulation of *P. sorghi* on the abaxial surface of the leaf of inoculated plants. Of 46 F2 plants that were scored based on F3 progeny tests, 12 plants were inferred to be homozygous resistant to P1 and P3, 6 were homozygous susceptible, and 28 were heterozygous resistant. The data support segregation of alleles for a single dominant resistance gene, with equal viability of classes (χ^2 = 3.74; P>0.05)

To identify informative RFLP markers, DNA was digested using five restriction enzymes (*Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, and *Xba*I) and the blots were hybridized to each of the selected clones. Differences between cultivars SC325 and RTx7078 could be identified for 69 of the 93 probes tested. To determine which of the markers that were segregating in SC325 and RTx7078 were linked to *Sdm*, each DNA probes was hybridized to a filter that contained DNA from the two parents and 46 progeny that had been digested with a restriction enzyme that generated easily distinguished polymorphisms.

Linkage analysis showed that the *Sdm* locus was close to loci detected by clones pSbTXS552 and pSbTXS361 at 5.0 cM (LOD 17.2) and 7.9 cM, respectively (Oh et al 1996).

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