



Full Length Article

Development of Head Smut Resistance-linked Sequence Characterized Amplified Regions Markers in Sorghum

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ABSTRACT

Head smut caused by *Sporisorium reilianum* (Kühn) Clinton is a major limiting factor to sorghum yield. To effectively screen or evaluate resistant varieties, we first developed sequence characterized amplified regions (SCAR) markers tightly linked to head smut physiological race 3 resistant trait in sorghum. Two primers (S18 & S336) of random amplified polymorphic DNA (RAPD) were obtained from a total of 400 RAPD primers tested and used to amplify 144 sorghum F₂ progenies (96 resistant & 48 susceptible) from a cross between accessions Tx622B (susceptible) and 7050B (resistant), and polymorphic fragments of 799 bp and 1419 bp were produced, respectively. The linkage analyses between head smut resistance and the two RAPD markers showed that the recombinant rates for marker S18 and S336 were 8.33% and 10.42%, and corresponding linkage map distances were 8.4 cM and 10.6 cM, respectively. Based on the DNA sequences of these two RAPD markers, two SCAR markers were developed and successfully tagged the resistant trait of sorghum head smut physiological race 3. This would provide a useful tool for the genetic improvement of sorghum resistance to head smut. © 2012 Friends Science Publishers

Key Words: Sorghum; Head smut; Molecular marker; Random amplified polymorphic DNA; Sequence characterized amplified regions (SCAR)

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal grain used as food and animal fodder, and mainly grown in arid and semi-arid regions in the world. However, the sorghum plant is affected by several diseases. Among which the most prevalent diseases of sorghum are caused by fungal pathogens, such as leaf blight (*Helminthosporium turcicum*), downy mildew (*Sclerospora sorghi*), rust (*Puccinia purpurea*), head smut (*Sporisorium reilianum*), and anthracnose (*Colletotrichum graminicolum*) (Maqbool *et al.*, 2001). Head smut is a fatal disease that causes extensive damage and economic loss in sorghum production (Oh *et al.*, 1994; Zou *et al.*, 2010). Therefore, breeding new varieties of sorghum with resistance to head smut is a major target in sorghum breeding strategies.

DNA molecular marker technology has been widely applied in crop genetic improvement, and greatly enhancing the genetic analysis of crop plants (Agarwal *et al.*, 2008; Gupta & Varshney, 2000; Varshney *et al.*, 2005). There have been many researches on the sorghum genome by developing restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and other molecular markers covering all 10 chromosomes (Bhatramakki *et al.*, 2000;

Maqbool *et al.*, 2001; Haussmann *et al.*, 2002; Menz *et al.*, 2002; Wu & Huang, 2007). Oh *et al.* (1994) developed RFLP markers (pFBT, xS 560 & xS 1294) and RAPD marker (OPG5) linked to head smut resistance genes in sorghum. We previously developed two SSR markers (Xtxp13 & Xtxp145) tightly linked with the resistance to sorghum head smut physiological race 3, with the Xtxp13 located in sorghum chromosomes 2, and Xtxp145 located in chromosomes 6, and the recombination percentage between markers and resistant gene are 9.6% and 10.4%, corresponding linkage map distances 9.6 cM and 10.4 cM, respectively (Zou *et al.*, 2010). However, the converted SCARs from above-mentioned markers are highly reliable and repeatable, relatively inexpensive, and can be easily manipulated. Thus, it is valuable in large-scale and locus-specific applications such as marker-assisted selection (MAS) and map-based gene cloning (Bradeen & Simon, 1998; Shan *et al.*, 1999; Negi *et al.*, 2000; Xu *et al.*, 2001; Zhang *et al.*, 2008). To our knowledge, very little information has been available on SCAR markers linked to head smut in sorghum so far. In this study, we used the technologies of RAPD and SCAR to identify the molecular markers linked to resistant traits of sorghum head smut physiological race 3, and obtained two SCAR markers. This would provide an effective method for MAS and map-based breeding in sorghum head smut resistance.

MATERIALS AND METHODS

The sorghum seeds of “Tx622B” (susceptible to head smut) and “7050B” (resistant to head smut), as the parents in this study, were obtained from Liaoning Academy of Agricultural Science, and the F₂ populations were made by cross between the parents. The pathogen *Sporisorium reilianum* (Kühn) Clinton physiological race 3 was provided by Liaoning Academy of Agricultural Science, and used to infect sorghum plants by artificial inoculation. Briefly, the inoculum was made by well-mixing the thick-walled teliospore powder of *S. reilianum* and fine-sieved soil at a percentage of 0.6% (w/w), then wrapped with plastic cloth and incubated at room temperature for 4 d. A 100-g portion of inoculum was added to each bunch planting at sowing time just above the seeds. The resistance or susceptibility was assessed according to the previous method (Zou et al. 2010).

Genomic DNA was extracted from fresh leaves of 7-d-old seedlings of the F₂ plants and both parents using the cetyltrimethylammonium bromide (CTAB) method (Doyle & Koyle, 1990). A DNA concentration of 10 ng μL^{-1} was made in TE buffer and used as the template for PCR. Bulk segregant analysis (BSA) was employed for identifying putative markers associated with the head smut resistance. For the head smut resistant bulk, equal amounts of DNA extract were pooled from 96 resistant F₂ individuals, and for the susceptible bulk, the DNA mixture consisted of equal amounts of DNA from 48 susceptible F₂ individuals. For RAPD amplification, a total of 400 RAPD primers (i.e., S1-S400), bought from the company of Shanghai Shengong, China, were first screened on the parents and two sets of bulks. Then, the primers showing polymorphisms between the resistant parent (7050B) and susceptible parent (Tx622B) or the resistant bulks and susceptible bulks were selected to genotype the F₂ population by PCR. PCR reactions were conducted using the EN 61010-1 DNA Thermal Cycler (USA). The reaction mixtures consisted of 17 μL ddwater, 2.5 μL 10 × reaction buffers, 2.5 mM MgCl₂, 0.2 mM dNTP, 1U Taq enzyme, 0.2 μM primers, and 50 ng DNA in a total volume of 25 μL . The reaction program consisted of an initial denaturation at 94°C for 5 min, and followed by 35 cycles of 20 s at 94°C, 30 s at 38°C, and 40 s at 72°C, last followed by a 10 min extension at 72°C. The PCR products were separated in 1% agarose gels. For sequencing of polymorphic fragments, the corresponding DNA fragments were cut out and extracted from the gels using DNA recovery kit (Sunbitech, Beijing, China), and used to sequence. DNA sequencing was conducted in Takara Biotechnology Co., Ltd (Dalian, China).

Based on the sequence of polymorphic DNA fragments amplified by RAPD, SCAR primers were designed and used to amplify the genome DNA of representative F₂ individuals. Primer S18₇₉₉: 5'-TCCACAGCAGTTGTACATCAG-3' and 5'-

TTCCACAGCAGTACATAATTGCC-3'; Primer S336₁₄₁₉: 5'-TCCCCATCACATCGAATG-3' and 5'-TTCCCCATCACAAGTAGACAAC-3'. The PCR reactions consisted of 15.7 μL ddwater, 2.5 μL 10 × reaction buffers, 2.5 mM MgCl₂, 0.2 mM dNTP, 1U Taq enzyme, 0.2 μM of the forward primer, 0.2 μM of the reverse primer, and 40 ng DNA in a total volume of 25 μL . The reaction program included an initial denaturation at 94°C for 5 min, and then 35 cycles of 20 s at 94°C, 30 s at 58°C for primer S18₇₉₉ or 66°C for primer S336₁₄₁₉, and 1 min at 72°C, followed by an extension at 72°C for 10 min. The PCR products were separated in 1% agarose gels.

Linkage analyses of head smut resistance with the two RAPD markers (S18 & S336) were carried out on the F₂ populations including 96 resistant individuals and 48 susceptible individuals by PCR. The recombinant rates and map distances were calculated according to the Kosambi mapping function (Kosambi, 1944). The percentage of recombination (r) = recombinant individuals/total tested individuals × 100. Linkage map distance (cM) = $1/4 \times \ln(1+2r)/(1-2r)$.

RESULTS

A total of 400 RAPD primers (i.e., S1-S400) were screened in polymorphism between the two parents, and ten primers produced polymorphic fragments (Table I). Two sets of F₂ bulks (one for resistance & another for susceptibility to head smut) were amplified using the 10 primers, and polymorphisms were produced with all the primers (data not shown). However, primer S18 and S336, amplified stable polymorphic fragments with sizes of about 800 bp and 1500 bp, respectively (Fig. 1), were most tightly linked to head smut among the 10 primers. Therefore, they were used to analyze the linkage relationship between molecular markers and head smut resistance in detail.

Linkage analyses between head smut resistance and the two RAPD markers were performed on 96 F₂ resistant individuals and 48 susceptible individuals. Among the resistant plants, 3 individuals did not produce the S18 polymorphic band, and 6 individuals did not have the S336 polymorphic band, whereas among the susceptible plants, 9 individuals produced the S18 polymorphic band, and 9 individuals had the S336 polymorphic band. Therefore, these plants were scored as recombinant individuals. The representative results of amplification of F₂ progenies by primer S18 and S336 were shown in Fig. 2. The recombinant rates for marker S18 and S336 were 8.33% and 10.42%, and corresponding linkage map distances were 8.4 cM and 10.6 cM, respectively (Table II).

The polymorphic DNA fragments amplified by primer S18 and S336 were purified and sequenced. The results showed that the two fragments were 799 bp and 1419 bp, respectively (data not shown). Based on primer design principle avoiding hairpin structure and appropriate G + C contents, two pairs of specific primers to the sequence of

Table I: Amplified polymorphic fragment by RAPD primers

Primers	Sequences (5' → 3')	Polymorphic fragments (bp)
S18	CCACAGCAGT	850
S21	CAGGCCTTC	350
S73	AAGCCTCGTC	350
S127	CCGATATCCC	600
S137	AACCCGGGAA	350
S228	GGACGGCGTT	800
S234	AGATCCCGC	200
S267	CTGGACGTCA	450
S355	TGTAGCAGGG	600
S336	TCCCCATCAC	1500

Table II: Co-segregation of the S18-or S336- amplified Polymorphism fragment in F₂

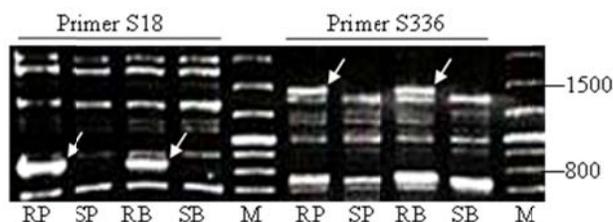
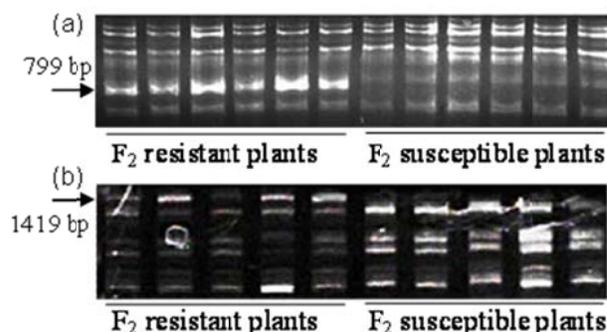
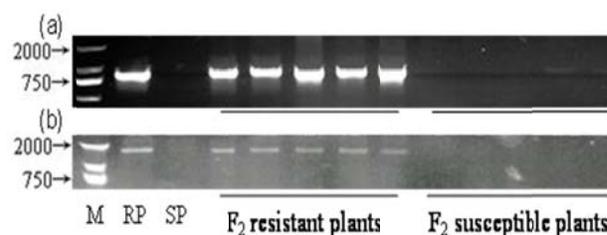
Primers	F ₂ individuals	Total plants tested	Polymorphic fragment		Percentage of recombination (%)
			Present	Absent	
S18	Resistant	96	93	3	8.33
	Susceptible	48	9	39	
S336	Resistant	96	90	6	10.42
	Susceptible	48	9	39	

S18- and S336-amplified fragments were designed, and named as SCAR primer, and used to analyze two parents, as well as representative F₂ individuals. The result indicated that the SCAR markers were agreed well to the RAPD markers in linkage to head smut resistance (Fig. 3).

DISCUSSION

Head smut of sorghum, caused by *Sporisorium reilianum* (Kühn) Clint., has been reported from all parts of the world, where sorghum is grown. Breeding 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 (Oh *et al.*, 1994). Therefore, breeding new varieties of sorghum to resistance head smut is always a major target in sorghum breeding strategies. However, because infected sorghum plants exhibit only slight symptoms until appearance of the inflorescence, cultivar identification by conventional means is time- and labor-consuming task. In this case, molecular tagging technology, combined with BAS, exhibits more powerful advantage in selection of sorghum resistant to head smut, as featured by much more rapid, highly reliable and repeatable, and relatively inexpensive.

Although some sorghum head smut resistance-linked molecular markers have been identified, such as SSR markers (Zou *et al.*, 2010), RFLP and RAPD markers (Oh *et al.*, 1994), it is necessary to develop more markers with more tight linkage to head smut due to the complex manner of genes conferred head smut resistance in sorghum, as well as the variability of head smut pathogens. Furthermore, in theory, combining different genes for head smut resistance in a particular individual may lead to more durable resistance and avoid the need to continuously search for new sources of disease resistance (Oh *et al.*, 1994).

Fig. 1: PCR results of RAPD primer S18 and S336. RP: resistant parent, SP: susceptible parent, RB: resistant bulk, SB: susceptible bulk, M: DNA ladder. The arrows indicated the polymorphic fragments with 799 bp and 1419 bp, respectively**Fig. 2: PCR results on representative F₂ resistant individuals and susceptible individuals. (a) RAPD primer S18, (b) RAPD primer S336****Fig. 3: PCR detection of representative F₂ individuals with SCAR primers. (a) SCAR primer S18₇₉₉, (b) SCAR primer S336₁₄₁₉**

Although much understanding has been made for head smut resistance in sorghum at morphological, cellular, genetic and molecular aspects, the genes involved have not been identified so far. Therefore, our study provides a useful alternative tool to marker-assisted selection or map-based gene cloning in head smut resistance breeding. Especially, we developed two pairs of SCAR molecular markers and successfully tagged the resistant trait of sorghum head smut physiological race 3 in all tested F₂ individuals. To our knowledge, this is the first SCAR markers tagging head smut resistance in sorghum. Compared to other molecular markers, SCAR markers exhibit more stability, reproducibility, easier manipulity, and are less expensive. Thus, it is of great value that RAPD markers associated with head smut resistance were converted to SCAR markers, in

particular for plant breeding applications such as marker-assisted selection and cultivar identification (Paran & Michelmore, 1991).

In conclusion, we obtained two RAPD primers (S18 & S336)-amplified markers, which had 8.33% and 10.4% recombinant rates with the resistant trait of sorghum head smut physiological race 3, and corresponding linkage map distances were 8.40 cM and 10.6 cM, respectively. Based on the sequences of S18- and S336-markers, two SCAR primers were designed and successfully tagged the resistant trait of sorghum head smut physiological race 3.

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