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SSR Analysis of Resistance Gene to Head Smut Physiological Strain No.3 on Sorghum

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Abstract: The objectives of the research were molecular markers screening of sorghum resistance gene to head smut physiological strain No.3 by using SSR. Restorer segregation population (2381R/Aisi) were used in the research. Once the molecular markers were found, it would become true to select resistant lines in the indoor laboratory and provided reliable foundation of agricultural production for molecular markers assisted selecting and breeding resistant varieties. The results showed that 150 pairs of primers were used to screen Restorer segregation population and 102 pairs of primers have clear and stable bands, with the rate of 68%. One pair of primers, IS10 264, has consistent polymorphism bands between parents and bulks. IS10 264 located in linkage group I. DNA bands of 114 F_2 resistant individuals and 60 F2 susceptible individuals were amplified with primer IS10 264. The recombination percentage between marker and resistant gene are 9.8% and the genetic distances to resistant gene are 9.9cM.

Key words: Sorghum · Head smut · SSR

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench is the fifth rank of the crop in the world, has the resistance to drought, flooding, barren and salinization. Sorghum is one of the oldest crops and is widely spreading among 89 countries in five continents including arid, semi-arid and Waterlogged Lowland. However, Sorghum is facing the threat of pest and disease in the process of planting [1]. Head Smut is the severe disease worldwide and the incidence of disease is between 15%-20%, even more than 80%, which results in the fewer production [2]. Usually people control the disease by means of crop rotation patterns for field with continuous cropping, chemicals, selection of resistant cultivars. The resistant cultivars selection is the most effective way till now. How to choose persistently resistant cultivars has become the great program for china, even for the whole world.

Resistant cultivar selection is a complicated work. The traditional way is based on the crop phenotype to select the resistant parents, with low breeding efficiency. With the development of DNA molecular markers technology, it comes to a new world for the study of plant resistance to disease and pest. Nowadays, some institutions study the major agronomic traits of rice [3], wheat [4-5], corn [6], soybean [7], sorghum [8-12] and so on and make a big progress in marking, locating and genetic mapping construction by RFLP, RAPD, AFLP and SSR. However, in the research of molecular marker of sorghum smut head, there is no report about it in China and fewer reports in the world.

The effective way of the prevention of sorghum smut head is to apply the resistance cultivar. Sorghum smut head resistant cultivar breeding combines hybridization with back cross by means of inoculation and identification selection which restricts the identification in breeding process. Furthermore, with the effect of environmental condition such as temperature, it is not ideal to broadcasting. Molecular marker technology assists the identification of the resistance to sorghum smut head in the lab, which improves the efficiency and accuracy, saves time and fund and breaks the season restriction. This experiment is based on the genetics mechanism of the resistance to smut head in sorghum in Liaoning Agricultural Science institution: the resistance to Head Smut Physiological Strain No. 3 on Sorghum is qualitative trait [13, 14]. With the help of high polymorphism of SSR marker, dominance and codominance, good stability and less technological difficulties, the SSR markers closely linked to the resistant

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gene to Head Smut Physiological Strain No.3 on Sorghum can be discovered avoiding the inoculation for identification of smut head in the field. The selection of Smut head can be realized by the analysis of seedlings.

MATERIALS AND METHODS

Plant Materials: The mapping population, consisting of Aisi, 2381R and 174 RIL lines (F_2) was derived from a cross between the two sorghum lines, Aisi and 2381R. Aisi is susceptible and 2381R resistant to head smut, the parent Aisi and 2381R come from Liaoning Academy of Agricultural Sciences. 114 $F_2$was resistant to head smut, 60 F_2 is susceptible to head smut.

DNA Extraction: NA extraction was done using the CTAB method (Doyle J J,1990) [15]. Genomic DNA from 174 F_2 plants and both parents was extracted from fresh leaves of one week old seedlings and the DNA was further purified by extraction with phenol/choroform/iso-amylalcohol (25:24:1) and ethanol precipitation. Completely dried pellets were re-suspended in 100 to 150 μl of TE buffer and kept at room temperature to dissolve completely. The extracted DNA was stored at 4°C. Agarose gel (0.8%) was used to check the quality and concentration of the DNA samples.

Bulk Segregant Analysis: Based on phenotypic data, two sets of bulks (one for Head Smut resistance and one for Head Smut susceptible) were constructed to detect Head Smut resistance-related markers. For each component assessed, 114 $F_2$was resistant to head smut and 60 F_2 is susceptible to head smut were constructed the bulks by mixing equal amounts (1 μl) of DNA from each line. A total of 150 SSR primer pairs were first screened on the parents and two sets of bulks. Primer pairs showing specific bands to both 2381R and the resistant bulks, or Aisi and the susceptible bulks, were used to genotype the F2 population. Polymorphic markers tested with all 174 F2 individuals were scored and used to construct linkage maps.

SSR Primer Pairs: 150 pairs of SSR primers is provided by International Crops Research Institute Semi-Arid Tropics (ICRISAT). 26 primer pairs is on A linkage group, 26 on B linkage group, 16 on C linkage group, 14 on D linkage group, 9 on E linkage group, 6 on F linkage group, 7 on G linkage group, 11 on H linkage group, 13 on I linkage group, 10 on J linkage group, 12 linkage groups are unknown.

Reaction System: 10×Buffer 2.0 μl 25mmol/L $MgCl_2$ 2.0 μl 10mmol/L dNTPs 1.5 μl 1U/ μl Taq, 2.5 μl 2μmol/ μl primer 1.5 μl DNA template 100ng (2 μl) the total volume is 20 μl by ddwatwer.

Reaction Condition: 94°C 5 min →(94°C denaturation for 20s →57°C annealing for 30 s →72°C extension for 40 s) 35 cycles →72°C extension for 10 min →4°C in store PCR products were separated in 6% non-denaturing polyacrylamide gels and silver stained using the procedure.

Percentage of Recombination and Linkage Map Distance: Map distance (in centimorgans) was calculated according to the Kosambi mapping function (Kosambi 1944) [16].

Percentage of recombination(r) =
exchange- segregation individual/(resistant-segregation individual + susceptible-segregation individual)×100%

Linkage map distance (M) = 1/4×ln(1+2r)/(1-2r)

RESULTS

DNA Check: Clear and even bands are presented (Fig. 1), which means that DNA didn't happen to degrade and it can be used in SSR-PCR reaction.

Polymoprphic SSR Products Linked to Head Smut Resistance Gene in Sorghum: SSR screening analysis of head smut resistant gene was carried out among restoring line parent and maintainer line parent. Among 150 primer pairs, 102 primer pairs have the amplification product and clear, stable bands and amplication rate is 68% (Fig. 2, 3).

The co-segregation analysis of SSR polymorphic fragment about resistant gene to Sorghum Head Smut: The differentiate bands are the same among parents, resistant and susceptible pool and partial F_2 individuals in primer IS10264 (Fig. 4). The resistant parent has the band with the size of 170bp using primer IS10264 and have a weak band at 190bp. The susceptible parent has the 190bp band but has nothing at 170bp. Meanwhile, the resistant pool has the band with the size of 170bp and has a weak band at 190bp. The susceptible pool has the 190bp band but has nothing at 170bp.

The test result of the 114 resistant plants and 60 susceptible plants of F_2 is listed in Table 1. It showed that the recombination rate between primer IS10 264 and resistant gene is 9.8% and genetic distance is 9.9cM. This DNA fragment is closely linked to resistant gene

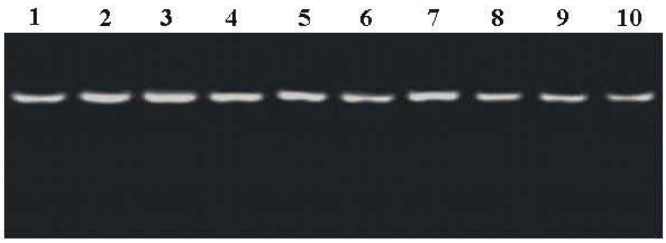

Fig. 1: The result of DNA extraction

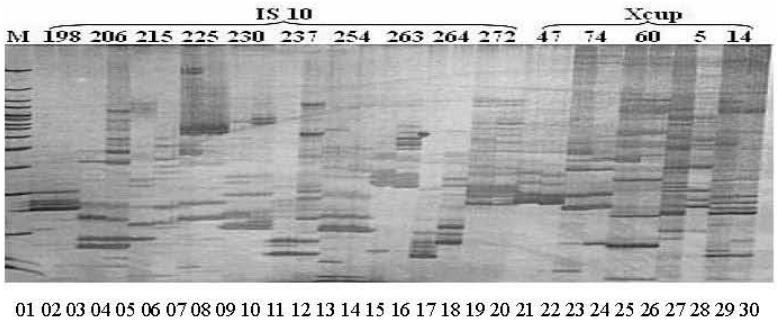

Fig. 2: PCR results of partial IS10 primer and partial Xcup primers resistant parent (01, 03, 05, 07, 09, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29) susceptible parent (02, 04, 06, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30)

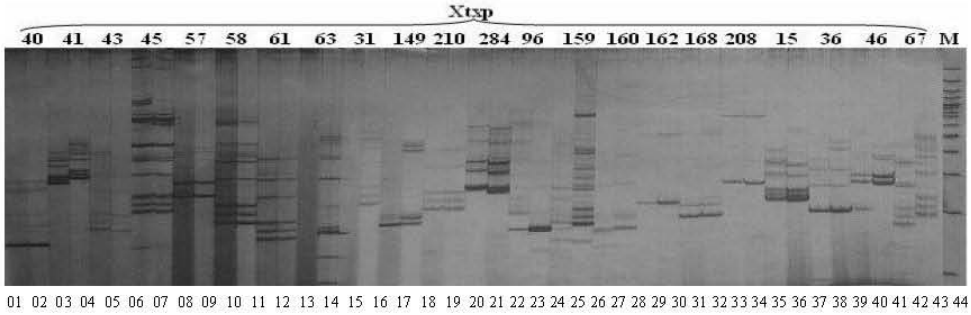

Fig. 3: PCR results of partial Xtxp primersresistant parent(01,03,05,07,09,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39,41,43) susceptible parent (02,04,06,08,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40,42,44)

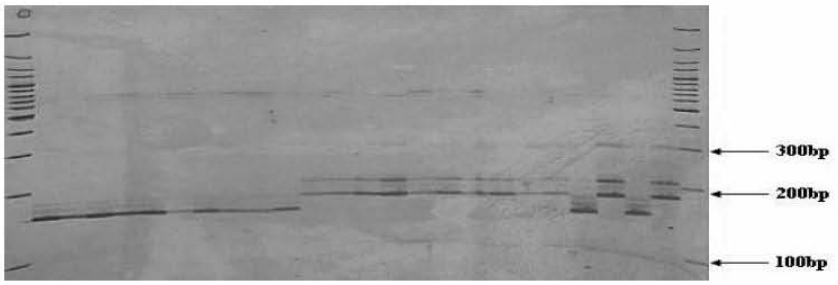

Fig. 4: Analysis of amplified fragment in F_2 individuals using primer IS10 264

Table 1: Co-segregation analysis of amplified fragment in F_2 using primer IS10 264

		Polymorphism fragment			
F_2	Plant number	Present	Absent	No Clear	Percentage of recombination (%)
Resistant to head smut	114	98	12	4	9.8%
Susceptible to head smut	60	51	7	2	

and can be used as the markers of resistance Gene to Head Smut Physiological Strain No.3 on Sorghum.

DISCUSSION

For a long time, there is no obvious conclusion for genetic way of sorghum resistance to head smut. The majority scholoar suppose that there are two quality trait and quantity trait. Xiaoguang Yang, *et al.,* study the resistant heredity of resistance Gene to Head Smut physiological strain No.3 on sorghum. The result shows that the resistant heredity of resistance Gene to Head Smut physiological strain No.2 on sorghum was controlled by 2 pairs of non-allelic major genes and immune material has the difference in gene function. The resistance of the first hybrid is affected by resistance of parents. But the resistance of either parents can not guarantee the resistance of the first generation of hybrid; the resistance of first generation of hybrid is reliable when the resistant source controlled by pure dominant gene. the resistant heredity of resistance Gene to Head Smut physiological strain No.3 on sorghum was controlled by 2 pairs of non-allelic major genes, with the interactive effects and modification of the genes. Before the experimental combination in the resistant heredity study are mostly the hybrid fo maintainer line and maintainer line, or sterility line and restoring line, moreover, we have little understanding of the segregation situation of resistance of the hybrid of restoring line and restoring line. By the analysis of resistance to sorghum head smut of parent-offspring of maintainer lines, restoring lines, sterility lines and restoring lines the result shows that res istance gene to Head Smut physiological strain No.3 on Sorghum is the quality trait heredity. The resistance is dominant and F1 is resistant when one of the parents is resistant. The resistance Gene to Head Smut Physiological Strain No.3 on Sorghum was affected by two independently allelic interactive genes.

The amplification products of different SSR primer has obvious difference. There are many polymorphism of amplification fragments between resistant and susceptible parent, but there are fewer polymorphism between resistant pool and sudceptible pool.

CONCLUSION

The amplification products had obvious difference of different SSR primers. There are amplification polymorphisms between resistant and susceptible parents, but there are fewer amplification polymorphisms in F_2. Even though there are differences, the majority don't have the agreement with parents. By screening and testing, a stable SSR marker, IS10 264, was discovered to apply in resistance Gene to Head Smut physiological strain No.3 on sorghum. Primer IS10 264 is on I chromosome, has the recombination with resistant gene is 9.8%, the relative genetic distance is 9.9cM.

The gene is primarily located with the known molecular map. Based on this marker, more primers are added in the map, therefore we can decrease the region of markers selection and lay the foundation of fine-mapping. In this research, SSR primers have already been located on the linkage group, specify the linkage group while looking for the markers, which are the bases of primer screening and resistant gene location and create the condition for the resistant cultivars selection by molecular marker assisted selection. In future, more SSR markers are needed in order to get more effective markers that are more close to the resistant gene. In this way, we can test the heredity mechanism of sorghum resistance to head smut and lay the foundation of head smut resistance breeding of sorghum Transgenic.

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