Preliminary Mapping of Soybean Dominant Locus Hrcs7 Confering Resistance to Cercospora sojina Race 7

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Abstract
Molecular marker-assisted selection can significantly improved resistance breeding efficiency. The soybean frogeye leaf spot (FLS) is caused by the fungus Cercospora sojina Hara. In order to find the resistant markers linked to C. sojina race 7 in soybean, we carried out the resistant genetic ananlysis and the resistant locus mapping. The F2 population derived from the cross of 'Gang 95144-1' and 'Gongjiao 9723-6' was employed to analyze the resistance genetics. it was sure that the resistance conferrin g to FLS was controlled by one major dominant gene. . Bulk Segregant Analysis (BSA) and Simp le Sequence Repeat (SSR) method were used to map the resistant locus. Among the 600 selected SSR primers, there were four primer pairs exhibiting the polymorphisms between DNA bulk of resistant and susceptible as well as both parents. Segregation distribution of the SSR markers, satt384, were conformed significantly to be as 1:2:1.of Mendel Segregation. The marker, satt411 and satt384 were assigned to link with the resistant locus based on estimation of MAPMAKER/EXP 3.0 software. The linkage order and distance would be Satt384 - 5.7cM - Satt411 - 7.9cM - Hrcs7. The resistant locus was integrated to soybean linkage group E based on the Cregen’s genetic linkage map of soybean.

Keywords Soybean (Glycine max); Cercospora leaf spot race 7; Resistant locus; Hrcs7; Genetic mapping

Background
Frogeye leaf spot (FLS) is an important disease of soybean in the world for several years (Yorinori, 1997). It is caused by Cercospora sojina K. Hara. There are several races of this fungal pathogen, which are distinguished by their ability to infect soybean varieties. There were 12 races in America, 20 races in Brazil, 11 races in China. But only China race 4 and America race 1, and China race 3 and Brazil race 2 exist in similar pathopoiesia (Hu et al., 1995). Among 11 races of the fungal pathogen in China, races 1 and 7 were identified as the dominant ones, whose incidence rate were 50% and 22% respectively.

The main symptoms of FLS are small, spots like frogeye on the leaves. The infection lesions can also develop on stems, pods and seeds (Sinclair and Backman, 1989). The disease incidence favored by hot and humid environment and it brought about the reduction of the photosynthetic area, premature defoliation. Susceptible cultivars were 12%~15% in less at average case, 30% at severe case (Akem and Dashiel, 1994). Germination ratio of susceptible seeds were lower, and it’s quality fall off, 1.2% in protein, 2.9% in fat, 2g in thousand seed weight.

The utilizing natural genetic resistance and breeding resistant cultivar are the most economical and efficient means to control FLS, because the chemical control with fungicides, in addition to being expensive, hasn't been shown to be effective. In the process of the traditional resistance breeding, it was aimless to select resistant parents and segregation offspring in terms of resistance phenotype identification. Otherwise, resistance identification was expertise-required, time-consuming, laboursome and environmentally sensitive. In order to solve these problems well, DNA markers linked to resistant genes can be used. Among 11 races of C. sojina recognized in China, only the three markers Satt565, SOYGPATR, Satt396 (Zhang et al., 2004) linked with resistance gene physiological
race 1 and one RAPD marker OPS036 with resistance gene against physiological race 7 (Zhou et al., 1998) were identified. These DNA markers were insufficient for assisted-select resistant breeding against FLS. The main purpose of this research was to find new molecular markers linked with resistance gene by analyzing resistance genetic and mapping the resistance locus against FLS.

1 Results

1.1 Inheritance of the resistance

After inoculation with C. sojina race 7, the FLS reaction of the two parents, ‘Gang 95144-1’ and ‘Gongjiao 9723-6’ are significantly different. The reaction of ‘Gongjiao 9723-6’ was susceptible and the reaction of ‘Gang 95144-1’ was highly resistant. Among the 184 F2 individuals inoculated, 138 individuals were resistant and 46 individuals were susceptible. A good fit to a 3:1 resistant:susceptible ratio ($\chi^2 = 1.15$, $P = 0.868$) was showed. It was indicated that the resistance in ‘Gang 95144-1’ was completely dominant and controlled by a single gene.

1.2 Identification of SSR markers

A total of 600 SSR primers were selected to survey the polymorphisms between ‘Gang 95144-1’ and ‘Gongjiao 9723-6’. 24 primer pairs couldn’t obtain amplified DNA bands, the remaining 363 primer pairs couldn’t obtain polymorphism between the two parents, and 213 primer pairs obtained polymorphic bands that ranged from 100 bp to 300 bp in size. These polymorphic primer pairs between parents were used to detect the polymorphisms between the two DNA bulks (Resistant and susceptible). Only 4 primer pairs (Satt207, Satt411, Satt384 and Satt491) exhibited polymorphisms between the two DNA bulks. The results suggest that these markers were relevant possibly to resistant locus to Cerocospara sojina race 7 in soybean.

1.3 Linkage analysis

The polymorphism markers between the two DNA bulks were screened in the entire F2 population to analyze the resistance segregation. The $\chi^2$ test was used to analyze the genetic segregation of the 4 polymorphic primer pairs in F2 population. The $\chi^2$ value of the Satt411 and Satt384 marker were respectively 1.570 and 2.562, and the P value were respectively 0.473 and 0.317, indicating that their maternal type, heterozygous type and paternal type conformed significantly to 1:2:1 (Table 1, Figure 1 and Figure 2). The $\chi^2$ value of the Satt207 and Satt491 marker were respectively 66.687 and 334.607, and the P value were respectively $5.32 \times 10^{-15}$ and $2.19 \times 10^{-73}$, indicating that their maternal type, heterozygous type and paternal type did not conform to 1:2:1 (Table 1).

Table1 Genetic Segregation of Polymorphic SSR primer pairs between the two DNA bulk in F2 population

| Marker   | Maternal type | Heterozygous type | Paternal type | No band | Total (plant) | $\chi^2 (1:2:1)$ | $P$       |
|----------|---------------|-------------------|---------------|---------|---------------|----------------|-----------|
| Satt207  | 62            | 24                | 61            | 37      | 184           | 66.687         | $5.32 \times 10^{-15}$ |
| Satt411  | 52            | 85                | 42            | 5       | 184           | 1.570          | 0.473     |
| Satt384  | 50            | 75                | 44            | 15      | 184           | 2.562          | 0.317     |
| Satt491  | 19            | 5                 | 144           | 16      | 184           | 334.607        | $2.19 \times 10^{-73}$ |
Figure 2 DNA amplified products for the SSR marker Satt384
Note: P1: ‘Gang95144-1’; P2: ‘Gongjiao9723-6’; R: resistant DNA bulk; S: susceptible DNA bulk; 1~38 some plants among F2 population derived from the cross of ‘Gang 95144-1’ × ‘Gongjiao 9723-6’

Analyzed based on the Mapmaker3.0 software, it was found that Satt411 and Satt384 were linked with the resistant locus. The linkage order and distance was satt384 - 5.7 cM - satt411 - 7.9 cM - Hrcs7. (Figure 3).

Based on the Cregen’s genetic linkage map of soybean (Cregen, 1999), the resistant locus was located in E linkage group.

Figure 3 The position of resistant locus to soybean Cercospora leaf spot Race 7 integrated into the Cregen’s genetic linkage map of soybean

2 Discussions

Genetics of plant-pathogen interactions specify that plants often contain single dominant resistance genes that specifically recognize pathogens that contain complementary avirulence genes (Flor, 1971). Our results showed that one major dominant gene in ‘Gang 95144-1’ confer resistance to C. sojina race 7, which was consistent with those reported on the resistance to C. sojina race 7 and race 1 in China (Yang et al., 1995; Zhou et al., 1998; Zhang et al., 2004), as well as other races in America (Ahow and Probst, 1952; Philips and Boerma, 1982).

The genetic mapping result show the resistant locus was located in E linkage group and linked with satt411 and satt384, Which was different from the MLG J of American Rcs3 (Mian et al., 1999) and MLG C1 of China race 1(Zhan g et al., 2004). It can be speculated that the resistant genes to the different races of C. sojina possibly distributed in different genetics linkage groups. Together with resistance controlled by one major dominant gene, we conclude molecular markers assisted-polymerizing muti races was relatively easy and was significant for the improvement of resistance to frogeye leaf spot.

MLG E was also the cluster distribution of resistant locus to soybean cyst nematode and Sclerotinia sclerotiorum. There were respectively 3 and 2 QTL distributed in MLG E (Guo et al., 2006; Guo et al., 2008). And near the resistant locus to C. sojina race 7, there was 1 QTL of the resistance locus to soybean cyst nematode and Sclerotinia sclerotiorum. QTL of the former were located between Satt411 and Satt384 (Guo et al., 2006) and that of the later were located between Satt411 and Satt212 (Guo et al., 2008). It can be speculated that the region around Satt411 was a new cluster of resistant locus and MLG E was possibly a main resistance linkage group to fungi.

3 Materials and Methods

3.1 Genetic materials and phenotypic assay
A mapping population of soybean was gained from a cross between the resistant ‘Gang 95144-1’ and the susceptible ‘Gongjiao 9723-6’ to C. sojina race 7. The F1 seeds were grown and selfed, and the leaf of F2 plants were used for disease evaluation and DNA extraction. The parents (‘Gang 95144-1’ and ‘Gongjiao 9723-6’) and the F2 population including 184 individuals were scored for the inoculation with race 7 of C. sojina. The inoculation of the pathogen and the evaluation of symptoms were fulfilled as described by Dong et al. (2007).

3.2 DNA bulks and PCR amplifications
Two DNA bulks (resistant and susceptible) were produced by respectively equally pooling the DNA of 15 resistant and 15 susceptible F2 plants. The DNA extraction was carried out as described by Rogers (1998).
PCR reactions were composed of 1×PCR Buffer, 2 mmol/L MgCl₂, 100 μmol/L of each dNTP, 0.4 μmol/L of each primer, 20 ng–25 ng template DNA, and 1 U of Taq DNA polymerase in a total volume of 20 μL. PCR was carried out with the conditions 94°C for 30s, 47°C for 30s, 72°C for 30s, return to step 1 35 times, 72°C for 3 min. PCR products were separated on 6% SDS-polyacrylamide gel electrophoresis and visualized by silver staining (Tiler et al., 1997).

3.3 SSR and linkage analysis

A total of 600 SSR markers were selected out of 20 genetic linkage groups (http://bldg6-arsusda.gov/~pooley/soy/cregen/soymap.htm) every 5 cM–10 cM and tested for DNA amplification. The polymorphism markers between the ‘Gang 95144-1’ and ‘Gongjiao 9723-6’ were screened against two DNA bulks (resistant and susceptible). The polymorphism markers between the two DNA bulks were screened against the 184 F₂ individuals.

The resistance trait and genetic segregation distribution of 184 F₂ individuals were both detected by the Chi-square (χ²) test for goodness of fit. The data obtained from the F₂ population of ‘Gang 95144-1’ × ‘Gongjiao 9723-6’ were analyzed using Mapmaker/Exp v 3.0 (Lander et al., 1987). The Kosambi map function of Mapmaker/Exp v 3.0 was used to develop a linkage map and obtain centimorgan (cM) values. The markers were assigned to linkage groups by the “group” command with a 3.0 minimum LOD and 50 cM maximum distance and then arranged by the “order” command. Basing on the published map the linkage groups were anchored to soybean chromosomes (Cregen, 1999).

Authors’ contributions

DZM and WSM analyzed the trait phenotype and the genotype data and drafted the manuscript. LZJ worked on the trait phenotype identification. LZ had the experiment on identification of SSR markers. LZG performed the field experiment management. All authors read and approved the final manuscript.

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