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RESEARCH ARTICLE

Genetic Mapping of Novel Symptom in Response to Soybean Bacterial Leaf Pustule in PI 96188

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Abstract

Soybean bacterial leaf pustule (BLP) is a serious disease caused by *Xanthomonas axonopodis* pv. *glycines*. Typical symptoms of BLP are pustules surrounded by small yellow haloes. Interestingly, PI 96188 only exhibits pustules without chlorotic haloes which suggests a resistant response. The objectives of this study are to understand the inheritance mode of the novel symptom to BLP in PI 96188 and to investigate whether or not a gene controlling BLP resistance in PI 96188 is identical to the *rxp* gene. First, a new BLP resistant genotype, PI 96188 was crossed with the resistant cultivar SS2-2. All F_1 plants showed the same phenotype as SS2-2 and the F_2 population segregated into 75 typical symptoms (haloes presence : 28 novel symptoms (haloes absence) indicating the presence of a single recessive gene. To map the novel symptom to BLP in PI 96188, a population of 88 F_7 recombinant inbred lines was developed from a cross between PI 96188 and the susceptible cultivar Jinjoo1. The BLP resistance gene from PI 96188 was mapped on chromosome (Chr.) 10 (LG O) rather than Chr. 17 (LG D2). This gene was linked with the simple sequence repeat marker, Sat_108 at the distal end of Chr. 10. Thus, the BLP resistance gene from PI 96188 was determined to be a new gene.

Key words: bacterial leaf pustule, novel symptom, PI 96188, *rxp* gene, soybean, *Xanthomonas axonopodis* pv. *glycines*

Introduction

Bacterial leaf pustule (BLP) caused by *Xanthomonas axonopodis* pv. *Glycines* (*Xag*) is a serious bacterial disease in soybean (*Glycine max* (L). Merr.). *X. axonopodis* is a gram-negative bacteria and one of the most prevalent bacterial diseases in the world causing a substantial loss of yield in soybean through premature defoliation (Hartwig and Johnson 1953; Kennedy and Tachibana 1973).

In the earliest study, Hartwig and Lehman (1951) reported that a high degree of resistance in the soybean cultivar CNS (PI 548445) was mediated by a single recessive gene, designated as *rxp* (resistance to *Xanthomonas phaseoli*). Typical symptoms of BLP are pustules surrounded by small yellow haloes. Although these symptoms appeared in both resistant and susceptible cultivars, a number of pustules and haloes in resistant cultivars containing the *rxp* gene were at least six times lower than susceptible cultivars under the same conditions (Groth and Braun 1986). The *rxp* gene confers partial resistance by increasing the number of bacterial cells necessary for infection rather than by restricting pathogen growth within host tissues.

The study of a linkage relationship with the *rxp* locus revealed that *rxp* was linked to the malate dehydrogenase locus

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(Palmer et al. 1992). With the advent of DNA marker technology, a number of molecular genetic maps in soybean have been developed (Cregan et al. 1999). Using simple sequence repeat (SSR) markers, the *rxp* locus was mapped 3.9 cM from Satt372 and 12.4 cM from Satt014 on chromosome (Chr.) 17 (previously linkage group (LG) D2) (Narvel et al. 2001). Satt486 was also identified as the significantly associated marker to BLP resistance (Kim et al. 2004). Recently, three single nucleotide polymorphism markers which are BARC-021191-04000, BARC-0220337-04263, and BARC-040963-07870 were revealed to be located between Satt372 and Satt486 in a soybean transcript map (Choi et al. 2007).

Recently, the BLP resistant source, PI 96188, was found to show a novel response compared to resistant as well as susceptible cultivars after *Xag* inoculation (Han et al. 2007). Without chlorotic haloes which are one of the characteristics of BLP, the novel symptoms which exhibit lesions with only pustules appeared on both sides of the leaves in PI 96188 during bacterial infection. The objectives of the present study are to understand the mode of inheritance of the novel symptom in response to BLP in PI 96188 and to investigate whether a gene controlling BLP resistance in PI 96188 is identical to the *rxp* gene.

Materials and Methods

Plant materials

The five soybean (*G. max* (L). Merr.) genotypes used in this study were PI 96188, Jangyeopkong, Jinjoo1, CNS1, and SS2-2, which differ in response to BLP caused by *Xag* (Fig. 1). Among these cultivars, crosses were made between PI 96188 showing only pustules (BLP-resistant) and SS2-2 showing pustules and haloes (BLP-resistant) responding to *Xag* to construct F_1 and F_2 populations for understanding the inheritance mode of the novel symptom in PI 96188. Additionally, a population of 88 F_7 recombinant inbred lines (RILs) was developed from a cross of PI 96188 × Jinjoo1 (BLP-susceptible and haloes-present) and these RILs were used for linkage mapping.

Bacterial culture and *Xag* inoculation

Experiments were carried out in a greenhouse and a chamber. For the production of inoculum, the *Xag* strain 8ra was cultured on peptone sucrose agar medium at 28°C for 48 h supplemented with 0.1 ppm of the antibiotic rifampicin (Oh et al. 1999). The bacterial culture was diluted with 10 mM $MgCl_2$ to obtain 1×10^8 colony forming units per milliliter at an optical density of 0.3 ~ 0.5 at 600 nm. Four-week-old soybeans were inoculated by spraying the bacterial suspension onto the leaf surface using an atomizer. These plants were grown in a greenhouse at Suwon, Korea during the rainy season, where relative humidity (RH) averaged 80% and average temperature was 25°C. Meanwhile, the inoculated leaves of soybeans were cut to be placed on moisturized kimwipe and incubated in a box made of acrylic in a chamber under the conditions of 28°C, almost 100% RH, and 12hr illumination.

Genomic DNA extraction and SSR marker analysis

Total DNA was extracted from young leaves of soybean using the modified CTAB procedure (Keim et al. 1988). Polymerase chain reaction (PCR) was performed using the genomic DNA with SSR markers as described by Cregan and Quigley (1997) and PCR was run on a Tetrad Thermal Cycler (MJ Research Inc., Watertown, MA, USA). All of the forward SSR primers were labeled with different dyes, 6-FAM (blue), HEX (green), or NED (yellow) (Applied Biosystems, Foster City, CA, USA). A total of 80 SSR markers were used to screen for parental polymorphisms between PI 96188 and Jinjoo1. The PCR products were resolved on an ABI prism 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA). GeneScan 672 fragment analysis software (Applied Biosystems, Foster City, CA, USA) was used for gel image analysis, and Genotyper 3.0 software (Applied Biosystems, Foster City, CA, USA) was used for accurate characterization of the alleles and automated data output.

Data analysis and genetic mapping

The SSR marker data and the BLP phenotypes of the F_7 RILs

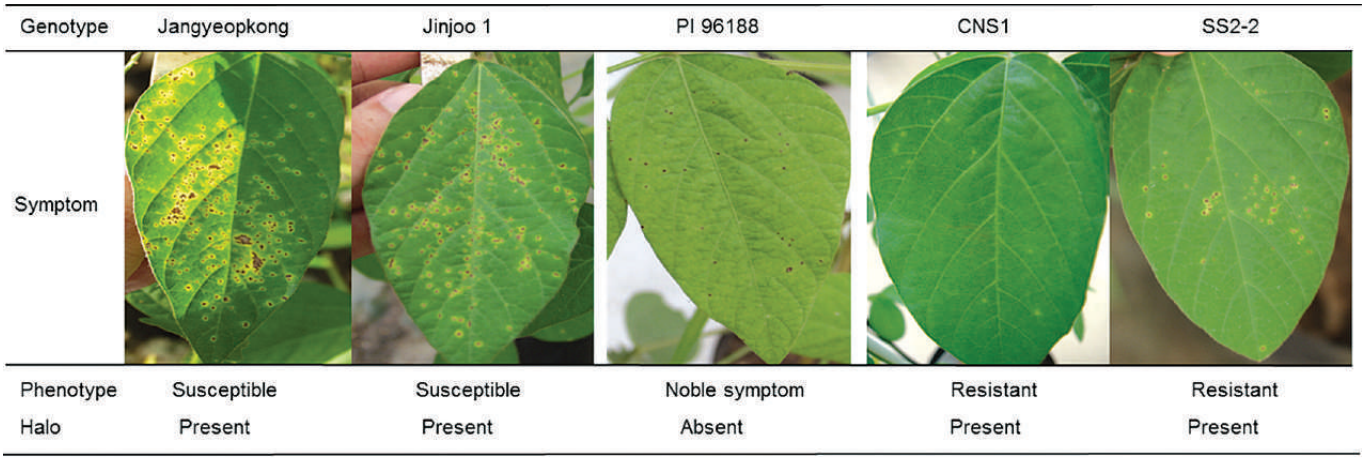

Fig. 1. Comparison of phenotypes among various soybean cultivars after *Xanthomonas axonopodis.* pv. *glycines* (*Xag*) inoculation.

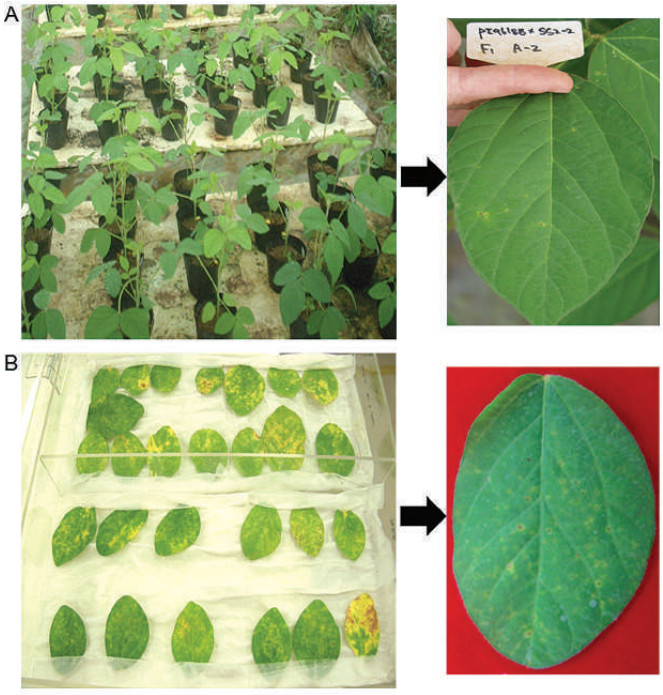

Fig. 2. Lesion development of the F_1 plants of PI 96188 × SS2-2 inoculated by *Xag*. The inoculated plants were grown in a greenhouse (A) and the inoculated leaves were cut to be incubated in a box made by acryl in a chamber (B).

of PI 96188 x Jinjoo1 were analyzed to construct a linkage map with Mapmaker 3.0 (Lander et al. 1987) using the Kosambi mapping function. A logarithm (base 10) of odds score of 3.0 was used to identify linked loci and maximum distance of 50 cM was used. The primary linkage group was determined on the basis of the public USDA map (Cregan et al. 1999).

Results

Novel symptom for BLP resistance in PI 96188

To better characterize a novel symptom of BLP disease in PI 96188, we compared it to four other soybean genotypes. BLP typically generated the pustules edged with haloes in resistant as well as susceptible soybeans (Fig. 1). The susceptible cultivars, Jangyeobkong and Jinjoo1 showed 168 and 151 pustules surrounded by haloes per leaf, respectively. The resistant cultivars CNS1 and SS2-2 showed 12 and 31 spots per leaf, respectively. However, while PI 96188 did not show the chlorotic haloes, one of the typical symptoms of BLP, it did show tiny black pustules as numerous as in the resistant soybeans. Based on the number and intensity of the lesions, PI 96188 appeared to be resistant or partially resistant to BLP disease.

Inheritance mode of BLP resistance in PI 96188

To reduce time and space required in BLP phenotyping following *Xag* inoculation and facilitate the control of BLP induction condition, we first tested BLP virulence by *in vitro* incubation of the inoculated and then cut leaves using two parents, PI 96188 (BLP-resistant and haloes-absent) and SS2-2 (BLP-resistant and haloes-present), as well as their F_1 plants. The incubated leaves showed the symptom similar to that of soybean plants inoculated and grown in a greenhouse. However, the severity of lesions in the incubated leaves increased somewhat because the conditions suitable to disease induction were able to be easily controlled in the growth chamber (Fig. 2). These results allowed for a clear assessment of the degree of BLP susceptibility in soybean genotypes.

Two parents, PI96188 and SS2-2, and their F_1 plants were used to investigate the inheritance mode of BLP resistance in PI96188. All F_1 plants displayed the same phenotype as SS2-2 of haloes presence and did not show any novel BLP symptoms of PI 96188 regardless of whether they were grown in the greenhouse or in the chamber (Fig. 2). The indication of the BLP resistance in PI96188 controlled by a recessive gene was confirmed by polymorphic SSR marker genotyping showing the hybrid nature of F_1 plants (data not shown).

To investigate segregation of the novel BLP symptom in the F_2 population, the leaves of 103 F_2 progenies developed from the F_1 plants of PI 96188 × SS2-2 were *Xag*-inoculated, and they were incubated *in vitro*. Of these, 75 lines showed the typical symptom (haloes present) and 28 showed the novel BLP symptom (haloes absent). This result showed an exact fit to a 3:1 ratio for segregation of a single recessive gene model. Thus, these results indicated that the novel symptom specifying BLP resistance in PI 96188 was controlled by a single recessive gene.

Mapping of BLP resistance in PI 96188

For construction of a genetic map, the population of 88 F_7 RILs was developed from a cross of PI 96188 and Jinjoo1 (BLP-susceptible and haloes-present). Out of 80 SSR markers screened, 32 markers were polymorphic between the mapping parents, and 19 SSR markers were genetically linked. Based on this SSR marker result, a genetic linkage map was constructed including five chromosomes (Chrs. 10 (LG O), 13 (LG F), 16 (LG J), 17 (LG D2), and 20 (LG I)) (data not shown).

Using the genetic linkage map of PI 96188 × Jinjoo1, we performed the mapping of BLP resistance of PI 96188 with segregation data to investigate whether or not a gene controlling BLP resistance in PI 96188 was associated with the reported *rxp*. The presence of haloes was associated with the *rxp* gene but not associated when the absence of haloes. In the 88 RIL population of PI 96188 × Jinjoo1, 73 lines had normal phenotype and 15 showed the pustules without the haloes. Interestingly, the locus conferring BLP resistance of PI 96188 was found to be located on Chr. 10 (LG O) rather than Chr. 17 (LG D2) (Fig. 3). Consequently, the novel symptom gene was linked with Sat_108 marker on Chr. 10. These results indicated that the gene controlling BLP resistance of PI 96188 was different from the *rxp*.

Discussion

In soybean, the *rxp* gene is the only gene known to BLP resistance to *Xag*. In the present study, we found another gene

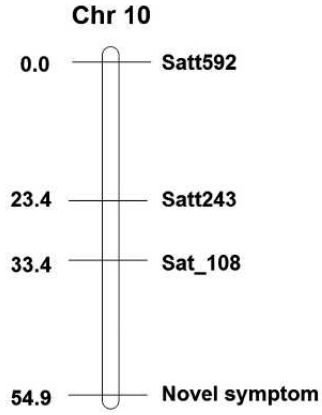

Fig. 3. Linkage map of the novel symptom gene for BLP in PI 96188.

regulating BLP resistance characterized by the novel symptom showing no chlorotic haloes in PI 96188 (Fig. 1). A genetic study using the F_1 and F_2 populations of PI 96188 × SS2-2 revealed that BLP resistance in PI 96188 is controlled by a single recessive gene (Fig. 2). Furthermore, the novel symptom gene was found to be associated with Sat_108 on Chr. 10 (LG O) rather than Chr. 17 (LG D2), where *rxp* is located through mapping analysis with 88 F_7 RILs of PI 96188 × Jinjoo1 (Fig. 3).

Meanwhile, Sharma et al. (1993) previously reported that the soybean genetic stock P-4-2 showed an immune response to *Xag* in spite of a high concentration of inoculums, where no pustule and haloes were observed. BLP resistance in P-4-2 was also determined by new genes which are duplicated recessive genes differing from *rxp* (Manjaya and Pawar 1999). Considering the different response to *Xag* of PI 96188 in this study, there should be four different phenotypes for the BLP disease in soybean. Those phenotypes are no pustule and haloes (P-4-2), resistance showing only pustules (PI96188), resistance showing low pustules and haloes (CNS1 and SS2-2), and susceptibility showing high pustules and haloes (Jangyeobkong and Jinjoo1). Also, the *rxp* gene, its duplicated genes, and several genes including a novel symptom gene in this study appear to be involved in BLP resistance in soybean (Kim et al. 2009). Although very little is known about recessive gene mediating disease resistance in soybean, some clear evidence was reported. Two recessive genes were reported to be relevant to yellow mosaic virus in soybean and mungbean (Shukla et al. 1978; Singh and Malick 1978).

Recently, the study of bacterial growth in PI 96188 was performed to compare the growth pattern difference of *Xag* in PI 96188, the BLP-resistant cultivar CNS1 and the BLP-susceptible cultivar Jinjoo1 (Han et al. 2007). The number of bacteria in PI 96188 showed 10 to 100 fold lower than Jinjoo1, and 10 fold higher than CNS1. This indicated that the novel symptom in PI 96188 may be a defense reaction of plants called hypersensitive reaction (HR) (Goodman and Novacky 1994). HR restricts pathogen growth at the site of the infection and triggers systemic acquired resistance in the non-inoculated site (Durrant and Dong 2004). Although the number of bacteria in PI 96188 was higher than the resistant cultivar, PI 96188 did not show chlorosis around the pustules, suggesting that it is a highly unique source for understanding a complicated plant defense mechanism regulated by multiple recessive genes.

For fine mapping and identification of candidate genes for the novel symptom of BLP, we are utilizing another bigger mapping population instead of the RIL population of PI 96188 × Jinjoo 1. Because this population was consisted of only 88 RILs, it is likely to be caused to the failure of isolation of a target gene owing to low map resolution. Thus, we only revealed which chromosome the locus of the novel BLP symptom was located in this study and we are constructing a highly dense map for the novel BLP symptom to identify candidate genes and compare them to the known *rxp* gene for BLP resistance in the next study.

Acknowledgments

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