

## A3. Molecular Map Construction and Mapping Gene for *rxp* in Soybean

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### Objective

The bacterial pustules (BP) resistance gene, *rxp* is the only locus controlling disease resistance in soybean that has been mapped on LG D<sub>2</sub>. BP resistance soybean mutant, SS2-2, was isolated from M<sub>2</sub> families of BP susceptible Sinpaldalkong2 mutagenized by EMS (Lee et al, 1997). In the present study, we mapped *rxp* locus with simple sequence repeat (SSR) markers which already reported and have identified amplified fragment length polymorphism (AFLP) markers tightly linked to *rxp* using bulked segregant analysis (BSA).

### Material and Method

material - Plant : SS2-2 X JANGYEP F<sub>7</sub> 130 RIL population.

pathogen race : 8ra (*Xanthomonas campestris* pv. *glycines*)

Method - CTAB method, pathogen culture in YDC medium, simple sequence repeat (SSR) analysis, amplified fragment length polymorphism (AFLP) analysis, bulked segregant analysis (BSA), Genescan, Genotyper 3.0, Mapmaker 3.0, SAS V8, QTL cartographer 1.20

### Results and Discussion

A total of 11 SSR markers were screened to construct a genetic map of *rxp* loci. Only 4 SSR markers, *satt* 135, *satt*372, *satt*486, *satt*458, showed polymorphisms between parents. Using these markers, genetic map was constructed covering 26.2 cM, shown in Fig 2. All SSR markers showed male-skewed segregation distortion in chi-square analysis. Among these 4 SSR markers, *Satt* 486 was the most significantly associated with both the resistance ( $P=0.0017$ ,  $R^2=11.48$ ) to BP (Fig 1) and segregation distortion ( $\chi^2:10.47$ ). AFLP marker linked to the resistance gene were identified by BSA. Based on the SSR markers and phenotypic data, resistance and susceptible bulks were made by pooling equal amount of genomic DNAs from each 10 resistance and 10 susceptible plants. A total of 108 primer combination were used to identify specific to the resistance. One putative AFLP marker was selected after screening. This AFLP marker produced the fragment presented in SS2-2 and the resistant bulk, while absent in JANGYEP and the susceptible bulk. A total of 130 F<sub>7</sub> individuals were analyzed to determine the linkages between this AFLP marker and the *rxp*. As the results of linkage analysis, this AFLP marker, *McctEact97* ( $P=0.0009$ ,  $R^2=8.39$ ) was mapped on distal end of LG D<sub>2</sub> 19.2cM apart from *satt*486, which tightly linked to *rxp*. *McctEact97* was accordance to theoretical segregation ratio ( $\chi^2:0.007$ ). We confirmed that AFLP was successfully used for gene tagging in combination with BSA. In further research, we will identify the AFLP markers tightly linked to *rxp*.

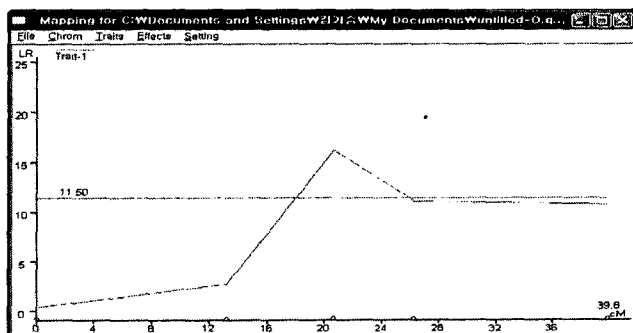


Fig 1. single marker analysis by QTL cartographer

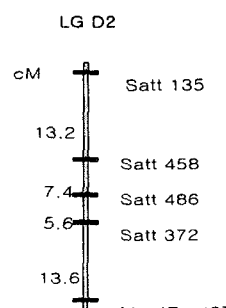


Fig 2. genetic linkage map

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