MARKER ASSISTED SELECTION OF *Rpp5* GENE FOR RUST SOYBEAN (*Phakopsora pachyrhizi*) RESISTANCE IN HL203, AN ELITE SOYBEAN GENOTYPE

Truong Quoc Anh, Ly Hau Giang, Nguyen Van Chuong, Nguyen thi Lang, Bui Chi Buu

INTRODUCTION

Soybean rust (SBR) caused by *Phakopsora pachyrhizi* Syd. & P.Syd is considered to be the most destructive foliar disease in soybean (*Glycine max* (L) Merr.) (Miles et al, 2003). The disease is disseminated through urediniospores carried by the wind and can develop rapidly, causing loss of foliar area and a severe reduction in grain yield.

Chemical spray containing fungicides is the only effective method to control the disease. This strategy increases production costs and exposes the environment to higher levels of fungicides. Introduction of resistant varieties is the most effective measure to control this disease. Presently, five different loci carrying dominant alleles have been reported: *Rpp1* identified in PI 200492 (McLean and Byth 1980), *Rpp2* from PI 230970 (Bromfield and Hartwig 1980), *Rpp3* (PI 230970 (Bromfield and Melching 1982), *Rpp4* (PI 459025) (Hartwig 1986) and *Rpp5* (Gacia et al, 2008). Other recent research has identified recessive genes controlling SBR resistance (Calvo et al. 2008).

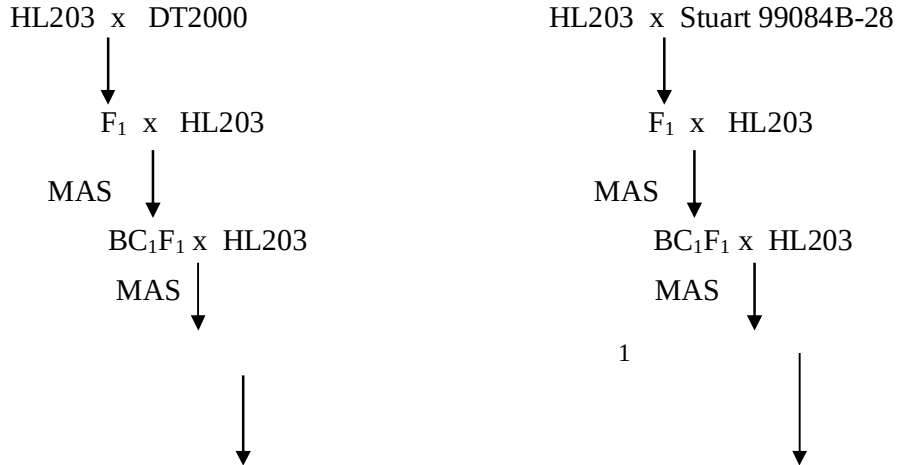
The use of molecular markers is an effective tool for gene identification and transfers (Tanskley 1983; Tanskley and McCouch 1997), and can speed up the development of soybean cultivars carrying single or multiple resistance genes. Soybean has a reasonably dense molecular-marker linkage map (Son et al, 2004), and the association of marker to known genes has been pursued by many group. Molecular mapping of SBR-resistance genes in soybean has previously been reported. Brogin et al. (2004) identified single sequence repeat (SSR) markers linked to rust resistance present on the cultivar FT-2 in the linkage group (LG)-C2 of the previous soybean consensus map reported by Cregan et al. (1999). However, the locus could not be identified in the study. An SBR resistance gene from the cultivar Hyuuga was mapped at 3 cM interval on LG-C2 between Satt134 and Satt460 (Monteros et al. 2007). Hyten (2007) recently mapped the Rpp3 locus at the same interval that Monteros et al. (2007). The Rpp1 locus has been mapped to a 1 cM interval on LG-G between Sct_187 and Sat-064 LG-G (Hyten et al. 2007).

The newly developed lines that were obtained in this study exhibited SBR resistance and retain the yield and grain quality traits of HL203. This study represents a successful example of the use of molecular markers, in foreground and background selection, for introgression of genes of interest into a premium soybean variety.

MATERIALS AND METHODS

Plant materials

Resistant analysis was performed in two backcrossing populations obtained from two donors, DT2000 and Stuart 99084B-28. The recurrent parent was a SBR susceptible cultivar, HL203, which is an elite soybean variety in South Vietnam Fig.1.



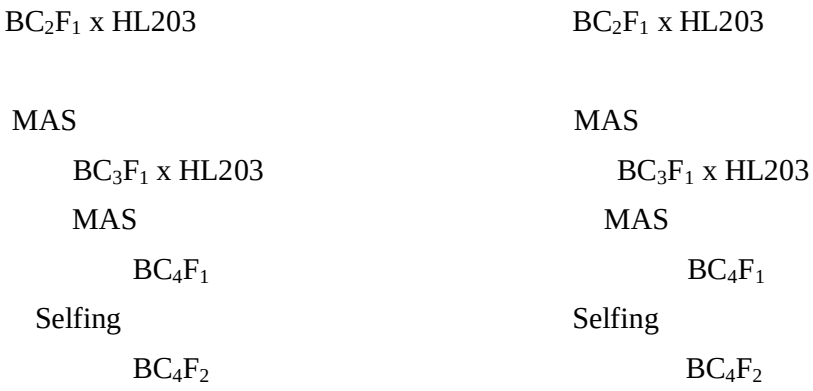


Fig. 1 Breeding schemes were produced for developing Backcrossing population containing SBR resistant gene with genetic background of HL203

***Phakopsora pachyrhizi* inoculation and phenotype**

The isolate used in this study was obtained by collecting spore from naturally infected greenhouse plants of the susceptible cultivar cultured in M_1 medium with a total volume of 1L including 10g glucose, 1g K_2HPO_4, 5g peptone, 0.5 $MgSO_4.7H_20$, 20g agar and adjust sterile distilled water of 1L. Fungal colonies were transferred from a master plate to two pre-prepared M_1 medium plate and incubated at room temperature for 36 – 48 h. The experiment soybean lines were designed by randomly completely block design (RCBD) with three replication; two leaves of each plant per replication were infected. After 8-10 days, evaluation of affected level was recorded following by standard protocol of IRRI. Performance of rust disease on leaves was grouped using NTSYS pc software version.

DNA isolation and molecular markers

Healthy leaf tissue was collected from the parents and backcrossing plants. Tissue was frozen in liquid nitrogen, freeze-dried, and ground to a fine powder using a modified CTAB protocol (Keim et al. 1988). The DNA was precipitated with isopropanol and treated with Rnase A. DNA concentration and integrity was estimated by spectrophotometer analysis and gel electrophoresis, respectively.

Simple sequence repeat (SSR) molecular markers were selected based on the reported genomic location of the known Rpp genes. SSR primer sequences were obtained from soyBase (http://soybase.org/resources/ssr.php).

For SSR analysis, 30ng of DNA was used as template in a 10µl reaction containing buffer (100mM Tris-HCL, 500mM KCL), 1.5mM $MgCL_2$, 32.5µM of each dNTP, 0.2µM of each primer, and 1U of Taq DNA polymerase. The cycling consisted of 5min at 94^0C; 35 cycles of 1min at 94^0C, 1min at 50^0C, 1min at 72^0C; followed by 7min at 72^0C. The amplified fragments were separated by electrophoresis in 3% agarose, stained with ethidium bromide, and visualized under UV light.

RESULTS

Introduction of SBR resistance gene into HL203 background

Rpp5 locus located in N linkage group between flanking markers Sat_275 and Sat_280 (Gacia et al, 2008) in Fig. 2

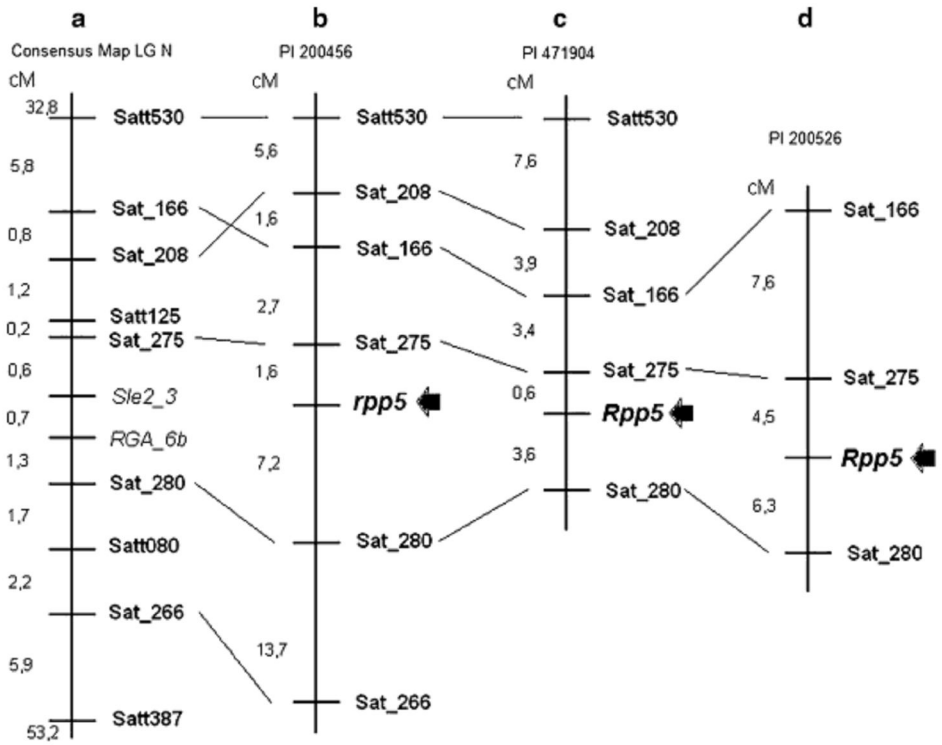


Fig. 2 Genetic linkage map of *Rpp5* locus

The DT2000 and Stuart 99084B-28 soybean varieties (donor of the SBR resistance *Rpp5* gene) were crossed to HL203 with the former as the male parent. The F_1 plants were confirmed for their heterozygosis for the "R" gene linked markers and were backcrossed using HL203 as a female parent. The resulting BC_1F_1 lines were first checked for presence of the marker linked to *Rpp5* resistance allele in a heterozygous condition. All of the plants that were heterozygous for *Rpp5* were backcrossed to HL203 to generate BC_2F_1 plants (HL203 as female parent) and the process was continued up to the BC_4F_1 stage. A representative example of genotyping for background selection is provided in Fig.1. At the BC_4F_1 generation, the plant having maximum contribution from the recurrent parent and containing "R" gene of SBR resistance was selfed to obtain BC_4F_2 lines that were screened using the "R" gene linked marker to identify plants that were homozygous for "R" gene of Rpp5 resistance Fig. 3 and Fig. 4.

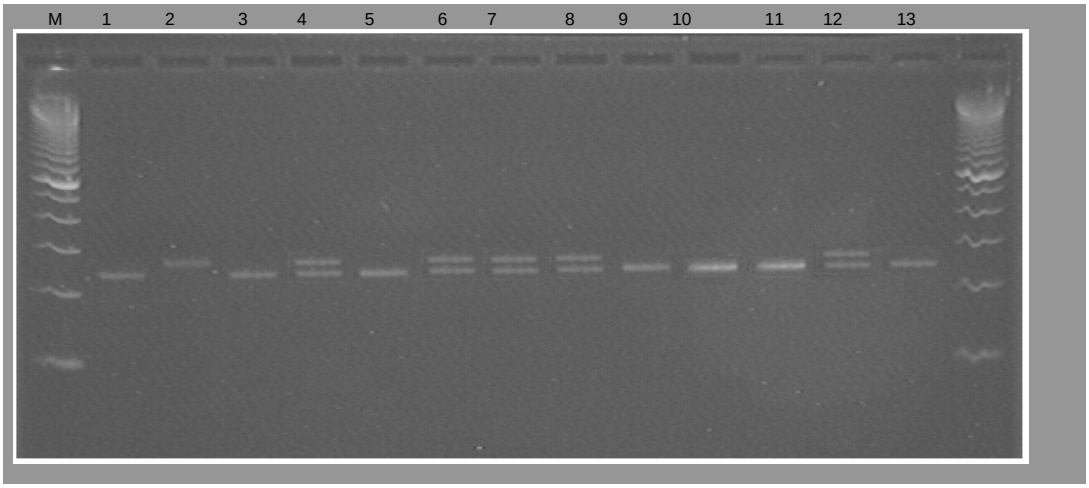


Fig.3. PCR detection of *Rpp5* gene in representative in BC_4F_1 plant. Marker used was Sat_275 (M: 100bp ladder, 1: HL203 recurrent parent, 2: DT2000 donor, 3-13:BC_4F_1 plants

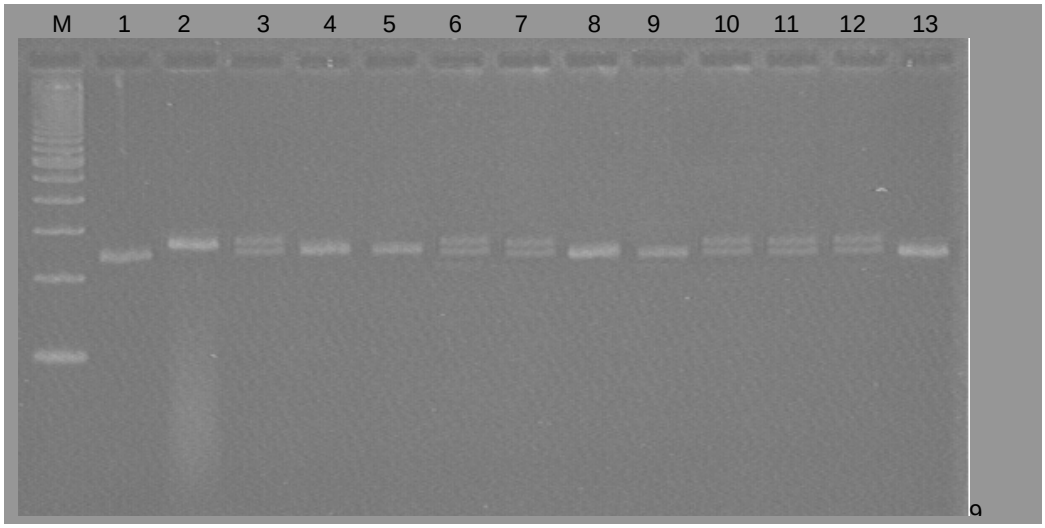


Fig.4. Genotyping for detection of *Rpp5* gene in representative in BC_4F_1 plant using marker sat_275 (M: 100 bp ladder, 1: HL203 recurrent parent, 2: Stuart 99084B-28 donor, 3-13:BC_4F_1 plants

Evaluation of SBR resistance to backcrossing generation

The backcrossing lines were evaluation for their resistance to SBR under glass house condition using several different isolates of SBR. As compared to HL203, the leaves of the backcrossing lines containing *Rpp5* gene exhibited very small lesion lengths indicating the high level of resistance to SBR (data not shown).

DICUSSION

HL203 is a regional soybean variety whose popularity lies in its high yield, grain quality and high adaptability in soybean cultivation region of South Vietnam. In the present study, *Rpp5* resistance gene was introgressed into HL203 with the objective of developing SBR resistant lines, high yield and high quality properties of HL203. The lines containing *Rpp5* exhibited good level of resistance.

This work demonstrates that marker-assisted backcrossing can be gainfully employed for adding new genes into popular and elite soybean genotypes that have been grown by farmers over the years on account of their unique agronomical characters. It can be expected that the availability of the soybean genome sequence will facilitate the development of many more marker for transfer of traits of agronomic value, with greater precision, into commercially important soybean cultivars.

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