Development of a SNP Assay to Detect an Asian Soybean Rust Resistance Gene from 'Hyuuga' Soybean

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I. INTRODUCTION

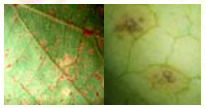
- Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Syd., is a widespread disease of soybean [*Glycine max* (L.) Merr.] with the potential to cause serious economic losses in soybean production.
- Four unique sources of ASR resistance have been reported: PI200492 ('Komata') has *Rpp1* (McLean and Byth, 1980; Bromfield and Hartwig, 1980), PI230970 ('Ankur') is the source of *Rpp2* (Singh and Thapliyal, 1977), PI462312 has the *Rpp3* gene (Hartwig and Bromfield, 1983), and PI459025 'Bing Nan' possesses *Rpp4* (Hartwig, 1986). Three of these sources of resistance have been associated with a red-brown (RB) lesion type.
- A novel rust resistance gene, *Rpp?*(Hyuuga), from the Japanese cultivar 'Hyuuga' producing a RB or resistant lesion type (LT) was identified and mapped to a 3.5 cM region on linkage group (LG) C2 (Monteros et al., 2007; Fig. 1; Fig 2).
- A combination of 32 soybean ancestors and first progeny contribute at least 95% of the alleles found in public cultivars released in North America from 1947 to 1988 (Gizlice et al., 1994; Table 1).
- Several SNP genotyping techniques are available for use in marker-assisted applications (MAS) and include hybridization and PCR-based methods (Syvänen, 2001).
- The objective of this study was to identify single nucleotide polymorphisms (SNPs) linked to the *Rpp?*(Hyuuga) ASR resistance gene for use in the development of "breeder friendly" SNP marker assays for MAS.

II. MATERIALS AND METHODS

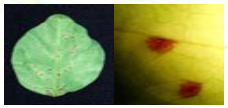
- A recombinant inbred (RIL) population consisting of 100 individuals from the cross of Dillon (tan LT or susceptible) × Hyuuga and the four previously reported sources of ASR resistance genes were screened in the field in Attapulgus, GA and in the greenhouse in Griffin, GA for their reaction to ASR and genotyped with simple sequence repeat (SSR) markers as described in Monteros et al. (2007).

Figure 1. Types of lesion produced by *P. pachyrhizi* in soybean.

a. Tan (S) lesion type



b. RB (R) lesion type



- Twenty-five seeds of each genotype were planted in the greenhouse 20 Oct 2006. Trifoliolate leaves were harvested 1 Nov 2006 and processed for DNA extraction as previously described (Monteros et al., 2007).
- The Dillon x Hyuuga RIL population, the 32 ancestral genotypes, the four sources of previously reported ASR resistance genes, and the Brazilian cultivar FT-2 were genotyped with unlabeled oligonucleotide probes targeting SNP sites near *Rpp*(?)Hyuuga and a saturation DNA dye.
- All PCR reactions were performed in 384-well plates with a total volume of 3 µL per well and conducted as asymmetry. The PCR reaction mixture consisted of 20 - 30 ng of genomic DNA, 3 m*M* MgCl2, 1 µ*M* of excess primer, 0.2 µ*M* of limiting primer, 0.5× of LightCycler 480 Genotyping Master mix (Roche Diagnostics, Indianapolis, IN), and 0.6× LCGreen Plus (Idaho Technology, Salt Lake City, UT).
- PCR was performed in the LightCycler® 480 (Roche Applied Science, Indianapolis, IN) for 50 cycles with 10 s of denaturation at 95 °C, 15 s of annealing at 55 °C, and 20 s extension at 72 °C.
- After amplification, 0.5 µ*M* of unlabeled oligonucleotide probe was added and a final melting cycle was performed by raising the temperature to 95°C for 2 min, lowering the temperature to 40°C for 5 min and increasing the temperature to 90°C with continuous fluorescent acquisition followed by a cool down to 40°C.
- The fluorescence signal (*F*) was plotted in real time against temperature (*T*) to produce melting curves for each sample. Melting curves were then converted to negative derivative curves of fluorescence with respect to temperature (-d*F*/d*T*) by the LightCycler® Data Analysis software (Roche Diagnostics, Indianapolis, IN).
- The same software was used to group similar melting curves and automatically call genotypes based on melting standards for known genotypes in the experiment or software-defined melting standards.
- A genetic linkage map of the LG-C2 was constructed with Joinmap 4.0 linkage analysis software (Van Ooijen, 2006). Linkage groups were determined by a log-likelihood (LOD) threshold of 5.0 using the Kosambi mapping function.

III. RESULTS

- The order of the SSR markers on LG-C2 (Fig. 2) corresponds with the USDA-ARS consensus soybean genetic linkage map (Song et al., 2004).

Figure 2. Soybean linkage group C2. a. Consensus soybean map (Song et al., 2004), b. Location of *Rpp?*(Hyuuga) (Monteros et al., 2007), c. Mapping additional SNP markers.

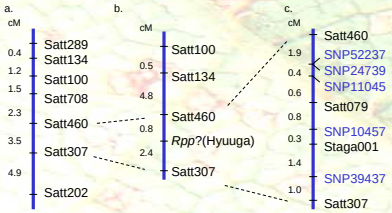


Figure 3. Derivative melting curves of unlabeled probes for genotyping.

a. SNP11045, b. SNP24739, c. SNP522237, and d. SNP39437

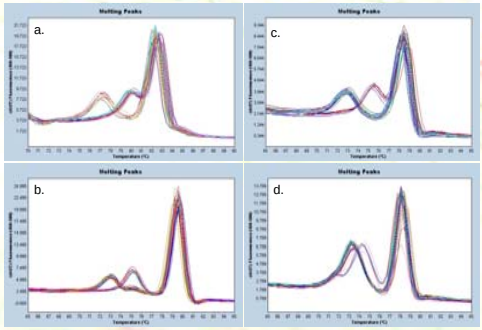


Figure 4. Graphical genotype and phenotype of selected Dillon × Hyuuga RILs.

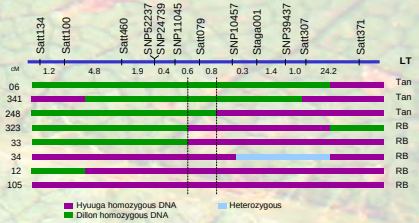


Table 1. SNP haplotypes near *Rpp?*(Hyuuga) of ancestral soybean genotypes and sources of ASR resistance.

Genotype	PI No.	52237	24739	11045	10457	39437
Rpp1	PI2004982	A	G	C	A	G
Rpp2	PI230970	A	G	C	A	A
Rpp4	PI459025A	A	G	C	A	G
Rpp3	PI462312	A	C	A	A	G
Rpp?	FT-2	A	C	A	A	G
FiskebyIII	PI438471	A	G	C	A	A
Fiskeby840-7-3	PI438477	A	G	C	A	A
Capital	PI548311	A	G	C	A	G
Dunfield	PI548318	A	G	C	A	G
Lincoln	PI548362	A	G	C	A	G
StrainNo.18	PI180501	A	G	C	A	G
BilomiNo.3	PI240664	A	G	C	A	G
Flambeau	PI548325	A	G	C	T	G
Haberlandt	PI548456	A	G	C	T	G
Mandarin (Ottawa)	PI548379	A	G	C	T	G
Mukden	PI548391	A	G	C	T	G
Perry	PI548603	A	G	C	T	G
Richland	PI548406	A	G	C	T	G
Anderson	FC33243	A	G	C	T	G
Bansei	PI548302	A	G	C	T	G
CNS	PI548445	A	G	C	T	G
Jackson	PI548657	A	G	C	T	G
Jogun	PI548352	A	G	C	T	G
Kanro	PI548356	A	G	C	T	G
Manitoba Brown	PI548382	A	G	C	T	G
Ogden	PI548477	A	G	C	T	G
Roanoke	PI548485	A	G	C	T	G
FC31745		A	G	C	T	G
Dillon	PI592756	A	G	C	T	G
Hyuuga	PI506764	G	C	A	A	A
AK(Harrow)	PI548298	G	C	A	A	G
Illini	PI548348	G	C	A	A	G
S-100	PI548488	G	C	A	A	G
Peking	PI548402	G	C	A	A	G
Arksoy	PI548438	G	C	A	T	A
Ralsoy	PI548484	G	C	A	T	A
Mejiro	PI80837	G	C	A	T	A
Korean	PI548360	G	C	A	T	G
Improved Pelican	PI548461	G	G	A	A	G

IV. DISCUSSION

- Seven additional markers (5 SNPs and 2 SSRs) have been mapped in the 3.5 cM interval between Satt460 and Satt307 in which *Rpp?*(Hyuuga) was previously located (Monteros et al., 2007; Fig. 2).
- The identification of SNP markers near *Rpp?*(Hyuuga) combined with the lesion type of selected RILs indicates that the location of this ASR resistance gene is located in a 1-cM region on LG-C2 flanked by SNP11045 and SNP10457 (Fig. 4). The use of these SNPs would enable breeders to select for this ASR resistance gene while minimizing the Hyuuga background.
- Three SNPs (SNP52237, SNP10457, and SNP39437) closely linked to *Rpp*(?)Hyuuga can be used to distinguish the rust resistant cultivar Hyuuga from the soybean ancestral genotypes and the previously reported sources of ASR resistance (*Rpp1-4;* Table 1), indicating that these SNPs would be useful in a wide range of soybean breeding programs for selecting the *Rpp*(?)Hyuuga gene.
- Both the *Rpp1* and the *Rpp4* loci have been mapped to LG-G and the *Rpp2* loci has been mapped to LG-J (Hyten et al., 2007; Abdelnoor et al., 2007) indicating that *Rpp?*(Hyuuga) is different from *Rpp1*, *Rpp2*, and *Rpp4*.
- Although the ASR resistance from the Brazilian cultivar FT-2 and from PI462312 (*Rpp3)* have been mapped to LG-C2 (Brögin et al., 2004; Perry Cregan, personal communication), in greenhouse evaluations of FT-2, PI462312, and Hyuuga using Brazilian isolates of ASR, both FT-2 and PI462312 had a tan LT (susceptible), while Hyuuga produced the RB LT (unpublished data).
- The different haplotype among entries representing the different sources of ASR resistance genes and the lesion type data suggests that *Rpp?*(Hyuuga) likely represents a novel source of resistance (Table 1; unpublished data).
- The identification of SNPs closely linked to *Rpp?*(Hyuuga) will allow development of "breeder-friendly" SNP assays for use in MAS for *Rpp?*(Hyuuga) and allow breeders to pyramid *Rpp?*(Hyuuga) with other ASR resistance genes. These strategies should accelerate the development of ASR resistant soybean cultivars adapted to the various production regions of the USA.

V. REFERENCES

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