IDENTIFICATION OF POTENTIAL CANDIDATE GENETIC MARKERS IN PHASEOLUS VULGARIS FOR RESISTANCE TO PHAKOPSORA PACHYRHIZI

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Introduction: *Phakopsora pachyrhizi*, the fungus that causes the Asian soybean rust (ASR) disease, is a very aggressive pathogen that can significantly reduce soybean yield (up to 80%). The host range of *P. pachyrhizi* is broad, with more than 90 plant species including common beans (*Phaseolus vulgaris* L.) (Miles et al., 2007). *P. pachyrhizi* was first observed in Louisiana, USA, in 2004, and within two years, it was found in 274 counties in 15 states (http://www.sbrusa.net; USDA, 2007). ASR poses a major threat to North American soybean production since none of the U.S. soybean commercial cultivars are resistant to *P. pachyrhizi* (Miles et al., 2003). Recently, Miles et al. (2007) identified *P. vulgaris* cultivars [Compuesto Negro Chimaltenango (CNC), Aurora, PI 181996 and Pinto 114] that were resistant to six isolates of *P. pachyrhizi* from Asia, Africa and Latin America. These cultivars had lower disease severity, less sporulation and consistent reddish-brown (RB) lesions, which is associated with resistance in soybean. These findings suggest that these four cultivars have genes for resistance to *P. pachyrhizi* that might be a source of protection for common bean and soybean against ASR. The current study was undertaken to identify molecular markers in these common bean cultivars that are associated or linked to ASR resistance. Here, we report preliminary findings on potential molecular markers using an F_2 population derived from a cross between the susceptible and resistant *P. vulgaris* cultivars, Mexico309 and CNC, respectively (Miles et al., 2007).

Material and Methods: Cultivars Mexico 309 (susceptible) and CNC (resistant) were crossed to produce the F_2 population used in this study. Young leaf tissue was collected from both parental cultivars and 117 F_2 plants, prior inoculation with the *P. pachyrhizi* isolate BZ01-1. Tissue samples were frozen in liquid nitrogen and stored at -80°C. Each sample was ground to a fine powder in the presence of liquid nitrogen, and genomic DNA was extracted from 100 mg using a DNeasy Plant Mini kit (Qiagen). Primers were based on SSR, plant defense-related genes, and conserved regions associated with resistance (R) genes. Selected sequences were blasted against *P. vulgaris* ESTs, *Glycine max* and other leguminous plants for primer design. Their length ranged from 17 to 26 nucleotides, and the forward primer had a WellRed-D4 dye label coupled at its 5' end. Thirty oligonucleotide primer sets (herein designated Pri1 through Pri30) were initially tested with Mexico 309 and CNC. Those that produced signals representing polymorphic fragments between the parents were subsequently tested with the F_2 population. PCR was conducted using 3 ng DNA in 12 µL, which contained 0.2 m dNTPs, 0.5 mM $MgCl_2$, 500 nM primer, 1 x Qiagen *Taq* buffer and 0.6 U of HotStar*Taq* DNA polymerase (Qiagen). The annealing temperature, depending on the primer set tested, varied from 48 to 58°C. Amplicons were analyzed in the CEQ™ 8000 Genetic Analysis System / Beckman Coulter (Fullerton, CA). Disease symptoms on Mexico309, CNC, the F_2 population and soybean cultivar Williams 82, a positive control, were rated in the USDA-ARS FDWSRU Biosafety Level 3 Containment

Greenhouse at Fort Detrick, MD according to Miles et al. (2007). Chi-square (χ^2) was used to test for goodness-of-fit of observed to the expected 3:1 and 9:2 ratios in the F_2 population.

Results and Discussion: Signals representing polymorphic DNA fragments were obtained with primers #13 (CNC had a 664-bp fragment not found in Mexico309); # 14 (CNC had a 694-bp fragment not present in Mexico309) and #27 (Mexico 309 had a 272-bp fragment not found in CNC). The phenotypic data for the F_2 population (117 plants) were 69 resistant and 48 susceptible plants, suggesting that resistance was controlled by a putative two-gene segregation ($\chi^2 = 0.35$). The genotypic results for the F_2 plants, based on these primers, and associated with the phenotypic data are as follow:

Primer 13

Genotype of the F2 plants	Phenotype	# Plants	Total	Ratio	χ^2
with 664 bp	Resistant	51	86	3:1	0.01712
with 664 bp	Susceptible	35			
without 664 bp	Resistant	18	31		
without 664 bp	Susceptible	13			

Primer 14

Genotype of the F2 plants	Phenotype	# Plants	Total	Ratio	χ^2
with 694 bp	Resistant	50	82	3:1	0.93103
with 694 bp	Susceptible	32			
without 694 bp	Resistant	19	34		
without 694 bp	Susceptible	15			

Primer 27

Genotype of the F2 plants	Phenotype	# Plants	Total	Ratio	χ^2
with 272 bp	Resistant	30	44	9:7	1.59606
with 272 bp	Susceptible	14			
without 272 bp	Resistant	38	72		
without 272 bp	Susceptible	34			

The 3:1 ratios suggest that a putative gene is involved, while the 9:7 ratio suggests a putative two-gene segregation. Although none of the markers showed a cosegregation that completely matched the resistance/susceptible phenotypes, these preliminary findings are encouraging for tagging resistance to ASR in *P. vulgaris*. About 150 additional F_2 plants will be tested to confirm these findings. Other primers based on known genomic regions are being evaluated.

References

Miles, MR, Frederick, RD and Hartman, GL (2003). Soybean Rust: Is the U.S. Soybean Crop At Risk? http://www.apsnet.org/online/feature/rust/top.asp

Miles, MR; Pastor-Corrales, MA; Hartman, GL and Frederick, RD (2007). Differential response of common bean cultivars to *Phakopsora pachyrhizi*. Plant Disease, *in press*.