

Genome-Wide Approaches for Identification of Genomic Regions Associated with Halo Blight Resistance in the USDA Core Collection of Common Bean



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Abstract

Halo blight, an important common bean (*Phaseolus vulgaris* L.) bacterial disease, is caused by an important seed-borne bacterium *Pseudomonas syringae* pv. *phaseolicola* (*Psp*). Prevalent in most dry bean growing areas in the world, race 6 of *Psp* causes significant losses in yield (about 45%) and quality, especially on susceptible cultivars. A chlorotic area of yellow-green tissue resembles a halo that may appear around necrotic lesions, a characteristic symptom of the disease (Fig. 1). Severe infection may develop systemic chlorosis, and extend to stems and pods (Fig. 2). To identify genomic regions linked to the resistance to race 6 of *Psp*, we used a genome-wide association study (GWAS) approach on plant accessions (PIs) collected from the United States Department of Agriculture-National Plant Germplasm System (USDA-NPGS) bean core collection. In this study, we identified two potential genomic regions associated with resistance to race 6 of *Psp*.



Figure 1. Necrotic lesions on the leaves surrounded by a characteristic chlorotic halo.



Figure 2. Reddish brown water-soaked lesions on pods exhibiting bacterial ooze on surface.



Figure 3. Inoculation using the multiple-needle florist pin frog method. Inoculum in the moistened sponge is drawn into the leaves via the wounds.

Materials and Methods

Plant material:

❖ A total of 383 PIs from the USDA-NPGS bean core collection and 10 standard cultivars as checks were included for an experiment.

❖ Under greenhouse conditions, the experiment was arranged in a randomized complete block design (RCBD) with four replicates.

Phenotypic Evaluation:

❖ Second trifoliolate from single plant of each accessions and checks were inoculated at the V-2 growth stage i.e. 21 d after sowing, with a 48 h old *Psp* race 6 culture at a density of 1×10^8 cfu/mL using the multiple-needled florist pin frog method^b (Fig. 3).

❖ The inoculated plants were maintained at 100% RH and 19°C \pm 1°C for 48 h in a humidity chamber.

❖ Inoculated trifoliolates were rated 10 d post inoculation using a disease severity scale of 1 – 5 [1 = resistant (R), 2-5 = susceptible (S)] (Fig. 4)^{a,e}

GWAS

❖ Using a 6K SNP chip, a DNA sample of each genotype extracted in Dr. Paul Gepts lab was genotyped in Dr. Perry Cregan's lab^d.

❖ A total of 5398 SNPs were obtained from 383 accessions.

❖ Data imputation was performed using FastPHASE v. 1.2 software^c.

❖ Markers were selected based on the loci with a minor allele frequency (MAF) >5%.

❖ Population structure was controlled by Principal Component Analysis (PCA).

❖ QQ-plot (Quantile-Quantile) identified PCA3 as best model for genome-wide marker-trait associations using GAPIT (Genome Association and Prediction Integrated Tool) in R software^f.

Result and Discussions

❖ Ten of 383 accessions had significantly higher levels of resistance to race 6 of *Psp* when compared to susceptible accessions and some of the standard checks (Fig. 5). These accessions are potential sources of resistance.

❖ Potential regions associated with resistance to race 6 of *Psp* were identified on chromosome Pv05 (39.7 Mbp) and Pv07 (95 kbp) following the GWAS analysis (Fig. 6).

❖ NBS-LRR (nucleotide-binding site leucine-rich repeat) genes located in the identified regions of each respective chromosome may have a regulatory function under stress.

❖ Stepwise regression revealed three markers explaining 19.9% of the genetic variation, where 12.8% of variation was accounted by the most significant marker.

❖ These genomic regions and markers associated may be useful for marker assisted selection (MAS) in common bean breeding program to develop cultivars resistant to race 6 of *Psp*.



Figure 4. Halo blight disease symptoms on the second trifoliolate evaluated 10 days post inoculation representing the disease severity scale from 1-5.

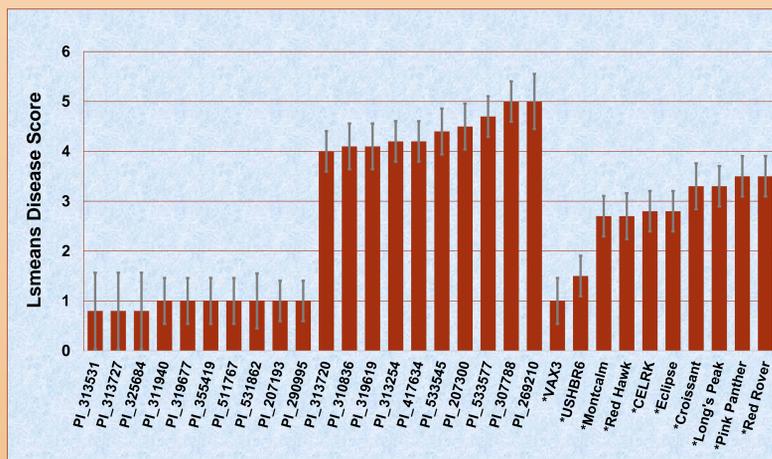


Figure 5. Lsmeans disease score of 10 resistant accessions, 10 susceptible accessions and 10 standard checks to race 6 of *Psp*.

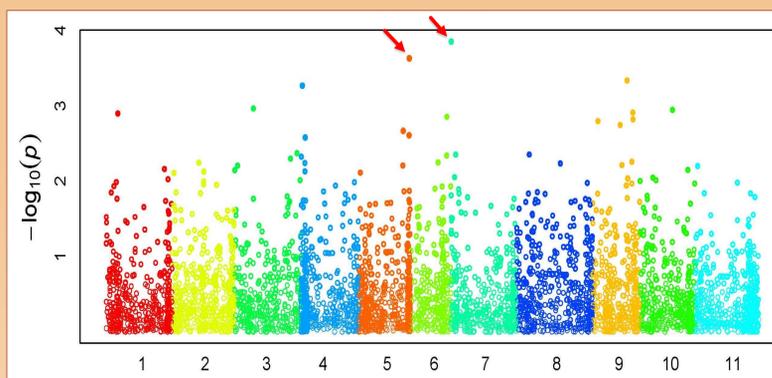
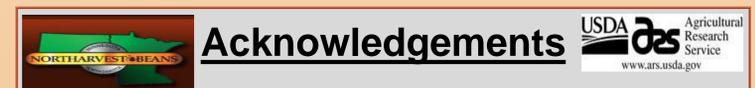


Figure 6. Manhattan plot representing markers associated with halo blight resistance caused by race 6 of *Psp*. Red arrows point out the most significant markers after stepwise regression.



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