IDENTIFICATION OF ANTHRACNOSE RESISTANCE LOCI INTROGRESSED FROM *P. coccineus* INTO *P. vulgaris* COMBINING BSA AND WGS

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INTRODUCTION

Bean anthracnose is a race-specific disease for which many pathogenic variants have been described (for example, Padder et al. 2017). *Phaseolus coccineus* L. (Pc) is a species closely related to the common bean (*Phaseolus vulgaris*, Pv) in evolutionary terms (Fig.1). Pc exhibits a high level of resistance to biotic and abiotic stresses that affect Pv. Lines (HYB) derived from the interspecific cross Pv × (Pv × Pc) were developed at SERIDA through six or seven generations of single-seed descendants. The Pv cultivar MDRK, susceptible to the anthracnose race 38, and the Pc accession V215, resistant to this race, were used in this program. Several interspecific lines derived from this program exhibited resistance to anthracnose race 38 in resistance tests.

The goal of this study was to investigate the inheritance of resistance to race 38 identified in the HYB028 line, as well as to identify the genomic regions responsible for this resistance.

Fig 1. P. coccineus accesion V215. Phenotypes of plant (a), inflorescence (b), pods (c), and sedes (d).

MATERIALS AND METHODS

Plant material

Crosses between the MIDAS and HYB028 lines were performed. The progenies were self-crossed, and 85 families F2.3 were obtained. Line Midas is a snap bean susceptible to race 38. Line HYB028 is an interespecific line with resistance to anthracnose race 38 derived from the Pc.

Phenotyping

The response to race 38 was evaluated under controlled conditions using a standardized method (Campa et al. 2017). Al least, sixteen F_3 seedlings descendant of each F_2 plant were evaluated. Seedlings were evaluated after 7 to 9 days using a 1-to-9 scale, where 1 indicated no visible symptoms and 9 indicated very severely diseased or dead

Genetic analysis

The goodness of fit of the observed to expected ratios was tested using chi-square tests ($\chi 2$), considering $\alpha \leq 0.05$.
Bulk Segregant Analysis (BSA) was used to locate the regions involved in resistance. Two bulks with 10 F2 plants each were established: a resistant bulk (R28) and a susceptible bulk (S28).
The two bulks, parents Midas and HYB028 as well as the Pc accession V215 was genotyped. Whole-genome sequencing (WGS) was used for this genotyping (by BMKgene). Sequencing reads were aligned using the bean genome G19833 v2 (NCBI accession GCA_000499845.2). The QTLseqR Package version 0.7.5.2, and the ΔSNP index were used to map the regions involved (Mansfeld and Grumet, 2017).

RESULTS

Phenotypic segregation

The response of the $F_{2:3}$ population showed a continuous variation ranging between absence of symptoms and death (like parent Midas). Families were classified as resistant (all the F_3 plants had a scores <3), susceptible (all the F_3 plants had a scores >5), and heterozygous (presence of resistant as susceptible F_3 plants).
The observed segregation in the $F_{2:3}$ population (Midas x HYB028) was 21 families resistant, 40 heterozygous, and 24 susceptible, which fitted the expected ratio for one major gene ($\chi^2_{1:2:1}$= 0.21, p =0.78). $F_{2:3}$ families with the heterozygous genotype showed segregation that conformed to a 3 resistant:1 susceptible ratio, indicating the dominant nature of this resistance.

Genotyping and Mapping

The WGS of the genotypes V215, Midas, HYB28, bulk R28, and bulk S28 yielded a total of 5,766,560 SNPs. SNPs with many missing values, non-mapped in the 11 bean chromosomes, and physically close positions were removed (5,432,040 SNPs were removed after filtering).
Bulked segregant analysis revealed two statistically significant regions using the ΔSNP index (Fig 12): region 1 at the end of chromosome Pv7 and region 2 at the beginning of chromosome Pv11. The characteristics of these two regions are listed in Table 1.
The involvement of these two regions in the genetic control of resistance in line HYV0B8 was verified using linkage analysis. This also clarifies whether the major gene hypothesis explains the observed segregation.

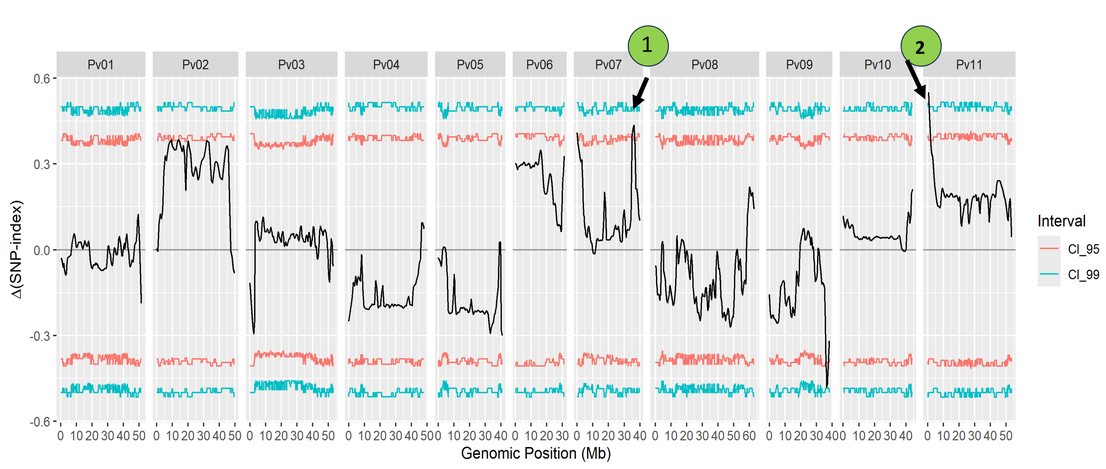

Fig 2. Distribution of the ΔSNP index across of the 11 bean chromosomes considering two confidence intervals (CI). The canditate regions involved in the resistance are indicated.

Table 1. Position in the bean genome of the significant regions associated to resistance to race 38 using the Δ SNP index and the QTLseqR Package.

CHROM	start	end	Num. SNPs	avgSNPs_Mb	peakΔSNI	posPeakΔSNP	avgΔSNP
Pv07	35009891	36838343	6595	3607	0.44	36286114	0.42
Pv11	8452	1988219	4611	2329	0.58	8452	0.48

CONCLUSIONS

- This work is an example of the potential use of the secondary gene pool of the *Phaseolus* spp in the breeding of the common bean.
- The combination of BSA and WGS can be a rapid and cost-effective approach for identifying genomic regions associated with complex traits.

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