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SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKER DISCOVERY AND GENETIC MAPPING ASSOCIATED WITH RESISTANCE TO BEAN GOLDEN YELLOW MOSAIC VIRUS

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INTRODUCTION: Bean Golden Yellow Mosaic Virus (BGYMV) is a severe disease of common bean (*Phaseolus vulgaris* L.) that causes important yield losses. BGYMV is a geminivirus (family Geminiviridae) transmitted by Whitefly in tropical and sub-tropical countries of Latin America and the Caribbean. Breeding for resistance to the virus has been the most effective strategy for controlling the disease. One resistance source, A429, derived from the Durango landrace Garrapato (G2402), and subsequent DOR lines developed at CIAT, were shown to contain a single recessive gene, *bgm-1*, which reduces mosaic and yellowing symptoms. The marker SR2 has been used in marker-assisted selection programs because it is linked to the *bgm-1* gene for resistance to BGYMV. In this study, SNP markers were developed from Genotyping by Sequencing (GBS) data on the region associated with the *bgm-1* gene. These SNPs markers could constitute an important tool for marker-assisted selection programs for improvement of common bean cultivars with resistance to BGYMV.

MATERIALS AND METHODS: Two common bean populations, each 100 recombinant inbred lines (RILs), generated at the International Center for Tropical Agriculture from the cross SEL 1309 x DOR476 and SMC33 x SCR16 were used for this study. The Mesoamerican genotypes DOR 476 and SCR 16 were resistant to BGYMV. The DNA, extracted using the urea protocol from a set of genotypes resistant to BGYMV, was sequenced and genotyped according to Elshire et al. (2014) protocol. Total genomic DNA for each RIL in both populations was isolated from single seed using the extraction method described by Xin et al., 2003 as modified by the Bean Molecular Genetics Laboratory-CIAT. The two populations of 100 RILs were tested in field in El Salvador. Experimental design was a randomized complete block with two replications. Plants were evaluated for chlorosis using a 1–9 scale where 1 was equivalent to very resistant and 9 to very susceptible for each of the symptoms.

Bowtie2 v2.2.3 was used to align reads against the current reference genome of *P. vulgaris*, downloaded from the Phytozome website. NGSEP pipeline was used to discover and genotype SNPs and to perform functional annotation of variants, filtering and conversion from VCF to other formats for further downstream analysis. Primers were designed according to Wang et al. 2005 using the software Primer 3. The markers were amplified by PCR on a fluorescence-detecting thermocycler (CFX384 Real-Time System, Bio-Rad) with EvaGreen© fluorescent dye. Melting point analysis for allele determination of the template DNA was performed using the same equipment.

A linkage map of the target segment around *bgm-1* gene was generated using Mapdisto® with a LOD value 3.0 as the threshold to confirm the order of markers, and the recombination values were converted into Kosambi genetic distances (cM). Additional markers (SSR's, SCAR's, SNP's) from previous studies were incorporated into this map. QTL were identified with QTL IciMapping® v.4.0.6. Map associations were identified with Inclusive composite interval mapping (ICIM) analysis.

RESULTS AND DISCUSSION: In both populations analyzed were found one QTL on chromosome 3 linked to *bgm-1* gene. SEL 1309 x DOR476 was a maximum PVE of 62.28% and SMC 33 x SCR 16 of 89.23%. CB_00352 was the SNP marker with higher correlation with the *bgm-1* gene, between 79 and 84% in these populations. These results suggest that the CB_00352 SNP is better than SR2, which has been used in marker-assisted selection programs for resistance to BGYMV.

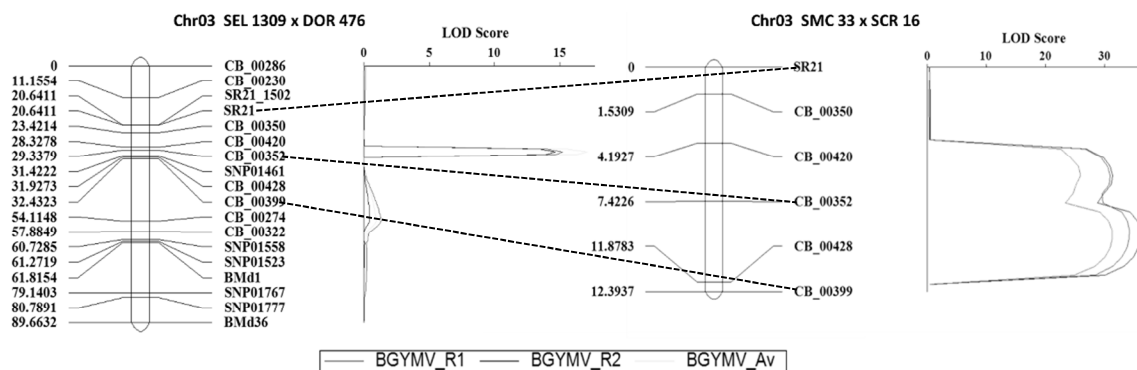


Figure 1. The SEL 1309 x DOR476 and SMC 33 x SCR 16 linkage chr03. One QTL identified on chromosome 3 for resistance to BGYMV. R1: Replication 1, R2: Replication 2 and AV: Average between replications.

Table 1. Quantitative trait loci (QTL) for resistance to BGYMV for the recombinant inbred line (RIL) population from the cross SEL1309 x DOR 476 and SMC 33 x SCR 16. R1: Replication 1, R2: Replication 2 and AV: Average between replications.

Trait	RIL Population	Chrm	Left Marker	Physical Position(bp)	Right Marker	Physical Position(bp)	LOD	PVE(%)	Add
BGYMV_R1	SEL 1309 x DOR 476	3	CB_00352	2,488,398	SNP01461	2,620,505	15.21	58.10	1.63
BGYMV_R2	SEL 1309 x DOR 476	3	CB_00352	2,488,398	SNP01461	2,620,505	14.64	57.47	1.54
BGYMV_Av	SEL 1309 x DOR 476	3	CB_00352	2,488,398	SNP01461	2,620,505	17.06	62.28	1.59
BGYMV_R1	SMC 33x SCR16	3	CB_00420	2,111,735	CB_00352	2,488,398	30.95	86.55	2.01
BGYMV_R1	SMC 33x SCR16	3	CB_00352	2,488,398	CB_00428	2,747,044	34.23	87.06	2.02
BGYMV_R2	SMC 33x SCR16	3	CB_00420	2,111,735	CB_00352	2,488,398	25.97	85.07	1.96
BGYMV_R2	SMC 33x SCR16	3	CB_00352	2,488,398	CB_00428	2,747,044	31.32	87.66	2.00
BGYMV_Av	SMC 33x SCR16	3	CB_00420	2,111,735	CB_00352	2,488,398	31.39	87.64	1.97
BGYMV_Av	SMC 33x SCR16	3	CB_00352	2,488,398	CB_00428	2,747,044	36.13	89.23	2.00

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