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INTROGRESSION OF A POWDERY MILDEW RESISTANCE GENE INTO MARKET CLASS FABADA

A. Campa

E. Murube

J. J. Ferreira

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Campa A, E. Murube, JJ Ferreira
Área de cultivos Hortofrutícolas y Forestales, SERIDA, Villaviciosa, Spain

Powdery mildew (PM) is being one of the most serious diseases affecting bean crops in the north of Spain. Two independent resistance genes were reported in the cultivar Porrillo Sintetico, one of them located on linkage group (LG) Pv11, conferring partial resistance, and other located on LG Pv04 conferring a total resistance response (Campa and Ferreira 2017). The objective of this work was the introgression of the gene conferring total resistance from Porrillo Sintetico into the line X2776. Genomic regions introgressed after the breeding program were studied using 'genotyping by sequencing' (GBS).

MATERIAL AND METHODS

X2776 is a breeding line developed at the SERIDA having fabada seed phenotype (white and very large seeds), determinate growth habit (*finfin*) and two introgressed resistance locus against virus (*I* gene) and anthracnose (*Co-2* cluster) (Ferreira et al. 2016). The X2776 line shows a partial resistance response against PM.

Total resistance in X2776 was introgressed using a backcrossing method including six backcross generations followed by four self-pollinated generations with individual plant selection using resistance tests. Seed and plant phenotype were also considered in the selection process of each generation. Resistance tests were carried out according to Trabanco et al. (2012) using a local isolate of PM maintained on plants of susceptible bean cv. Xana in spore-proof chambers.

The parental lines X2776, Porrillo Sintetico and four resistant plants derived from F_4BC_6 families were genotyped through GBS using the *ApeKI* restriction enzyme. Physical positions of the SNP markers were established using the *Phaseolus vulgaris* genome v1 (GenBank Assembly accession GCF_000499855.1).

RESULTS AND DISCUSSION

Four homozygous resistant plants against PM (4562-1, 4562-2, 4562-3, and 4562-4) having white and large seeds (fabada seed phenotype) were obtained in the breeding process. GBS analysis revealed a total of 8763 polymorphic SNPs between the parental lines, which were distributed along the eleven bean chromosomes. The average number of SNPs per chromosome was 796.2 with a minimum of 290 SNPs in chromosome Pv10 and a maximum of 1214 SNPs in chromosome Pv02. The four resistant plants showed the genotype of the parental line X2776 for all the SNPs except for a maximum of 36 SNPs on chromosome Pv04 that showed the Porrillo Sintetico allele (Table 1). These 36 SNPs tag a region between 91,481 and 2,338,026 bp of chromosome Pv04. Individuals 4562-3 and 4532-4 showed the most extensive introgressed region. Individual 4562-1 had a high level of heterozygosity, from 235,860 to 2,338,026 bp, suggesting that this chromosome region is not involved in the resistance. The four individuals showed a common introgressed region between 91,481- 226,281 bp which should contain the gene or genes responsible of the total resistance to PM. Molecular markers that tag this introgressed genomic region can be useful for marker assisted selection.

This result agrees with the genetic analysis conducted in the $F_{2:3}$ population X2776 x Porrillo Sintetico in which a single gene conferring total resistance was identified in Porrillo Sintetico, in a physical position between 84,202 bp and 218,664 bp of chromosome Pv04 (Campa and Ferreira 2017).

Table 1. Genomic profile of four F_4BC_6 homozygous resistant individuals and the parental lines X2776 (recurrent) and Porrillo Sintetico (donor) for 36 SNPs located on chromosome Pv04. The tag sequence and the physical position of each SNP are indicated. -: missing value. R, Y, S, W, K, and M: heterozygous

SNP physical position (bp)	Tag sequence	X2776	Porrillo S	F_4BC_6 individuals 4562-1	4562-2	4562-3	4562-4
91481	CAGCAGGCTAGGGTGAATATT(A/C)ACTGTTCCTGAGGTATTTGCAAATGAAAAGGTGTCACAGAAG	C	A	A	A	A	A
91507	CAGCAGGCTAGGGTGAATATTAACTGTTCCTGAGGTATTT(G/A)CAAATGGAAAGGTGTCACAGAGG	G	A	A	A	A	A
91522	CAGCAGGCTAGGGTGAATATTAACTGTTCCTGAGGTATTTGCAAATGAAAAGGTGTCACAG(A/G)AG	G	A	A	A	A	A
95873	CTGCAAAACGTGTTTGAGCTTTGGTACGGCGGAATAGTTCGTATTA(C/T)TGTTCTGCGTCGGAGAA	T	C	C	C	C	C
100292	CTGCCCTAACTATTA(C/T)GAGATCATTATTTGTAATGTGATTTATTCTTTAACTCGTGTATTTGTA	T	C	C	C	C	C
226281	CAGCCTTAACACTACT(A/T)CCTTGCACTACTTCGTTCCTTATGCTGTTGGAGCTTCAGCAAGGTTT	T	A	A	A	A	A
235860	CTGC(A/T)GTCACGGCTTCTAGCTTGGATTTCGCTCTCAACACCTTGTAACTCAAGTTTCTAACCTT	T	A	W	T	A	A
235905	CTGCAGTCACGGCTTCTAGCTTGGATTTCGCTCTCAACACCTTGTAAC(T/C)CAAGTTTCTAACCTT	T	C	Y	T	C	C
262296	CTGCATGACGTTGT(G/C)AATGACTTCAGTTCTTGACCAAAACTTTGTTTCCTCTTACCATGCAGAA	G	C	S	G	C	C
262491	CAGCGAATTTGAACCATAATAAAAAACTGTCTTATTTTGTTACATAGAAACTAAAATAAGAAG(C/G)	G	C	S	G	C	C
365640	CTGCACAGAAAACAAATCCACTCCCAAGTTAATT(C/T)ATTGCTCAAATACCTTACATGCATCACAG	T	C	Y	T	C	C
514500	CAGCCGGACTGCATCAAACCGTC(G/A)TGCTTCACGCCGCGCTTTTTATCCGGAAAATCCAAGAAAG	G	A	R	G	A	A
564897	CTGCTTTCGCATGCTAGTTGGCTGGCAAAGGATATTATTTGG(C/T)TTGTGGCTGATTCACAATATG	C	T	C	C	T	T
565024	CAGCACTTTTG(G/A)TGGACTTGGACAGGGTCTTTATTTGGATGGAAAGTTAAATGGTGATTTTAGA	G	A	R	G	A	A
592704	CTGCAGTTGTCTTCAGAAAACATCAACAACACCAAAC(A/T)ATGTCGAGCAAAGACTCAAAGGTGAA	A	T	W	A	T	T
592710	CTGCAGTTGTCTTCAGAAAACATCAACAAC(A/G)CCAAACAATGTCGAGCAAAGACTCAAAGGTGAA	A	G	R	A	G	G
1058766	CTGCAA(C/G)TAGCTGGGGACGCATTGGTGGATGATGATGAATTACAGGACCTTTCAGAGAAGGAAG	G	C	S	G	C	C
1104710	CAGCACGCGCCGCCTTC(A/G)AGCGACTTCCCGTTCTCAATCCCATCGTCGAAATGGACGGTAACTT	G	A	R	G	A	A
1143859	CAGCTGAG(G/C)TCGATGGTCTCTCATACTGGGTGACATTGGTCTCGGGATTCCAATAGTAAAGATA	C	G	S	C	G	G
1143891	CAGCTGAGGTCGATGGTCTCTCATACTGGGTGACATTGGT(C/A)TCGGGATTCCAATAGTAAAGATA	A	C	M	A	C	C
1216747	CAGCAACTGAA(C/T)TTGTATGTGATGCTTTAACTTTTCTGGATACTTCAAGGGATTCTGCTTCAAT	C	T	Y	C	T	T
1216789	CTGCGGTTCTGCAATATCCTCCAGTTTATATGGG(T/G)AGGCTTTTTGACAGCTCAAATTACCAAAC	T	G	K	T	-	G
1216801	(C/T)AGCTCAAATTACCAAACCGAGGAAGATGATGGGAAAAAGGTTATTGATCCAAAGAAAATCCAG	C	T	Y	C	-	T
1485452	CAGCACCA(G/A)ATACAAGATTTATCAACACAAAACAAAAAATAAAAGACATTTTTAGATGGTACAA	G	A	R	G	A	A
1485455	CAGCA(C/T)CAGATACAAGATTTATCAACACAAAACAAAAAATAAAAGACATTTTTAGATGGTACAA	C	T	Y	C	T	T
1826541	CTGCCAATGAAATCAAAAAATCCAATAAATCA(T/C)CCTCGGGAGAACAAAGAGATGGGAAATTCTC	T	C	Y	T	C	C
1838570	CTGCGG(A/C)GGCGGCGGCGCGTTGAGGTTGCGTTCGCCGCCGGTAGAATCGGAGAGCTCCATGATC	C	A	M	N	A	A
1854518	CTGCATCAAAGAT(A/G)TCAGAGCAGTTGATCCATACTGTTCCAACTCGCAATGCCCGCATCAAGGT	G	A	-	G	A	A
1919879	CAGC(A/G)TAGTTGGAGCCATAATAGTATCAATTGGCCTTTATGTTGTATTGTGGGGCAAGGCAACA	A	G	R	A	G	G
1919890	CAGCATAGTTGGAGC(C/T)ATAATAGTATCAATTGGCCTTTATGTTGTATTGTGGGGCAAGGCAACA	C	T	Y	C	T	T
2121101	CTGCGTTGTCCAGAAAGTGTTTGTGGAAATGCTCACTCACTAAAT(C/T)CTGAAATTCCGAGTTTTT	C	T	Y	C	T	T
2138790	CTGC(C/T)TTAACAGTCGGTCAAATCAGTAATACAATCAATATAAAACTGATAGTGGATCTTTGAAT	T	C	Y	T	C	C
2138801	CTGCCTTAACAGTCG(G/A)TCAAATCAGTAATACAATCAATATAAAACTGATAGTGGATCTTTGAAT	A	G	R	A	G	G
2199473	CTGCATTAAAAT(A/G)TCGCTTCCCTGTAATCATTTGAAGGACCATGTTGAATATCAGATGAGAAAA	A	G	R	A	G	G
2338021	CTGCCTTTTAATGATTTTTTCTTTTTCCGTTTTTGCAGATTACTGTTATATTACAA(T/C)ATCATGG	T	C	Y	T	C	C
2338026	CTGCCTTTTAATGATTTTTTCTTTTTCCGTTTTTGCAGATTACTGTTATAT(T/C)ACAATATCATGG	C	T	Y	C	T	T

The new breeding line was obtained from individual 4562-2 after two more self-pollinated generations (F_6Bc_6). This new line (X4562) has fabada seed phenotype, determinate growth habit (*finfin*), resistance against virus (*I* gene) and anthracnose (*Co-2* cluster) like the recurrent parental line X2776, and it has incorporated a gene conferring total resistance against PM derived from the donor line Porrillo Sintetico.

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