ALLELIC RELATIONSHIPS OF ANTHRACNOSE RESISTANCE GENE CLUSTER B4 IN COMMON BEAN.

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Anthracnose is one of the most widespread and economically important diseases of common bean (*Phaseolus vulgaris* L). It is especially harmful in tropical and subtropical areas but it also causes considerable yield losses in temperate areas such as Northern Spain where valuable dry bean landraces are grown. Andecha is a very large white seeded cultivar (proceeding from a selection of Asturian landraces) susceptible to races 6 and 38 and with moderate resistance to races 3, 102 and 787 of *Colletotrichum lindemuthianum*. These five races are the most commonly found in Northern Spain (Ferreira et al. 1998). Two parallel backcross breeding programs were developed using germplasm lines A321 and A493 as resistance donors and Andecha as the recurrent parent. After 6 backcross generations, the lines A1231 and A1220 were obtained, each one carrying one dominant resistance gene proceeding from A321 and A493, respectively. The introgressed gene(s) conferred resistance to races 6 and 38 and enhanced the resistance to races 3, 102 and 787 in both breeding lines. Genetic resistance has shown to be the most effective strategy for protecting crops. To date, up to ten genes that confer dominant resistance (except *co-8*) to different pathogenic races of anthracnose have been described. Genes *Co-1*, *Co-2*, *Co-4*, *Co-6* and *Co-9* are known to be located on the linkage groups of *Phaseolus vulgaris* L. map B1, B11, B8, B7 and B4, respectively. Molecular markers linked to these genes and to *Co-5* have also been identified (Kelly et al. 2003). Little is known about the resistance genes conditioning resistance in the differential cultivar Mexico 222.

In this work, we describe the identification of anthracnose resistance genes present in two breeding lines A1220 and A1231 derived from cultivar Andecha by means of allelism tests and molecular marker analysis.

The segregation ratios observed in the selection for resistance to anthracnose conducted in two breeding programs indicated that both lines A1220 and A1231 each carry a single dominant gene for resistance to race 38. No segregation was observed in the F2 population derived from the cross A1220 x A1231, suggesting that the dominant gene in both lines is located at the same locus. No segregation was observed in F2 populations derived from the crosses A1220 x Mexico 222, A1220 x PI 207262, A1220 x BAT 93, A1231 x Mexico 222, A1231 x PI 207262 and A1231 x BAT 93, indicating that the dominant resistance gene present in lines A1220 and A1231 is located at the same locus as the resistance genes in Mexico 222, PI 207262 and BAT 93. The 3:1 ratio of F2 families derived from the crosses (S x R) Andecha x Mexico 222, Andecha x PI 207262 and Andecha x BAT 93 and the lack of segregation in the cross PI 207262 x Mexico 222 support the theory that the same locus is responsible for resistance to race 38 in all these genotypes (Table 1).

Table 1. Allelism tests for genetic characterization of the resistance to race 38 in lines A1220 and 1231

	Observed frequencies		Expected frequencies				
F_2 Population	Resistant	Susceptible	ratio	Resistant	Susceptible	χ^2	Probability
A1220 x A1231	171	0	15:1	160.3	10.7	11.40(	0.001
A1220 x Mexico 222	115	0	15:1	107.8	7.2	7.66	0.006
A1220 x PI 207262	108	0	15:1	101.3	6.8	7.20	0.007
A1220 x BAT 93	213	0	15:1	199.7	13.3	14.20(	0.000
A1220 x Cornell 49242	98	6	15:1	97.5	6.5	0.04	0.839
A1220 x AB 136	105	7	15:1	105.0	7.0	0.00	1.000
A1220 x TU	116	16	15:1	123.8	8.3	7.76	0.005
A1231 x Mexico 222	246	0	15:1	230.6	15.4	16.40(	0.000
A1231 x PI 207262	222	0	15:1	208.1	13.9	14.80(	0.000
A1231 x BAT 93	95	0	15:1	89.1	5.9	6.33	0.012
A1231 x Cornell 49242	208	9	15:1	203.4	13.6	1.63	0.201
A1231 x TO	102	11	15:1	105.9	7.1	2.34	0.126
A1231 x AB 136	236	13	15:1	233.4	15.6	0.45	0.502
A1231 x TU	102	14	15:1	108.8	7.3	6.70	0.010
Andecha x Mexico 222	76	20	3:1	72.0	24.0	0.88	0.346
Andecha x PI 207262	197	54	3:1	188.3	62.8	1.62	0.202
Andecha x BAT 93	80	22	3:1	76.5	25.5	0.64	0.424
PI 207262 x Mexico 222	159	0	15:1	149.1	9.9	10.60(	0.001

To find molecular markers linked to the resistance gene present in breeding lines A1220 and A1231 the amplification patterns of 374 decamer primers along with twenty different SCAR markers were compared in lines Andecha, A493, A321, A1220 and A1231. Both breeding lines conserved from their corresponding donor parents RAPD markers $OB12_{350}$, $OAH18_{1100}$, $OI19_{460}$ and $OY17_{1100}$ and SCAR markers SI19 (Melotto and Kelly, 1998) and SW12 (Miklas et al. 2000). The SCAR SB12, coming from RAPD $OB12_{350}$ (Méndez de Vigo et al. 2002) amplified a single band only in the two breeding lines, in the resistance donors, in BAT 93 and in PI 207262. The $OY17_{1100}$ RAPD band was also present in genotypes A321, A493, A1231, A1220, BAT 93 and PI 207262 although a weaker amplification was observed in G 2333 and SEL 1308. The SAH18 amplification band (coming from the RAPD $OAH18_{1100}$,) was present in a larger number of genotypes. The SW12 SCAR primers produced 6 different amplification phenotypes in the 22 genotypes analyzed: no amplification (Michelite, MDRK, Widusa, AB136, Catrachita and Jalo EEP558); one single band of 700 bp (Cornell 49242, Kaboon, TO, SEL 1308, A321 and A1231); two bands of 700 and 600 bp (Perry Marrow, TU and Andecha); two bands of 700 and 575 bp (G 2333 and SEL 1360); two bands of 700 and 475 bp (PI 207262, BAT 93, A493 and A1220); and two bands of 700 and 450 bp (Mexico 222).

The F2 population derived from the cross Andecha x A493 was used to test the linkage of these markers and the resistance gene (Table 2). Markers SI19 and $OY17_{1100}$, previously described linked to the rust gene *Ur-5* located on B4 linkage group (Melotto and Kelly, 1998; Miklas et al. 2002) were present. The SCAR marker SW12 linked to a major QTL conferring resistance to bean golden mosaic virus (BGMV) located on B4 (Miklas et al. 2000) was confirmed.

Table 2. Chi-square (χ^2) and linkage analysis of markers $OB12_{350}$, $OAH18_{1100}$, $SI19_{460}$, $OY17_{1100}$ and $SW12_{475}$ and the resistance to race 38 of *C. lindemuthianum.*

Marker	Expected ratio*	Observed frequency	χ^2	p	cM **
$OB12_{350}$	3:6:3:1:2:1	20:27:0:0:4:18	52.96	0.00	3.4
$OAH18_{1100}$	3:6:3:1:2:1	20:29:1:0:3:16	50.66	0.00	4.7
$SI19_{460}$	3:6:3:1:2:1	4:30:17:16:3:1	37.04	0.00	12.6
$OY17_{1100}$	3:6:3:1:2:1	20:29:0:0:2:18	59.91	0.00	1.6
$SW12_{475/575}$	1:2:1:2:4:2:1:2:1	17:1:0:2:30:0:0:1:17	114.13	0.00	2.2

*The expected ratios are based on 1:2:1 genotypic segregation ratio for the resistance gene and marker $SW12_{475/575}$ (codominant inheritance), and 3:1 ratio for the remaining markers (dominant inheritance)

** Distances (cM) between each marker and the resistance gene were calculated using MAPMAKER software (Kosambi function)

Geffroy et al (1999) proposed the symbol *Co-9* for the anthracnose resistance gene present in the breeding line BAT 93 and the names Co-y/Co-z for the resistance present in Jalo EEP558 that also mapped to B4. Due to the origin of the resistant donor A493 (proceeding from BAT 93, derived from a 4-way cross that included the differential cultivar PI 207262) and to the information provided by molecular markers in this work, we conclude that the resistance gene present in line A1220 is in fact the *Co-9* gene. The lack of segregation for resistance to race 38 in the F2 derived from crosses with lines A1220, A1231, BAT 93, PI 207262 and Mexico 222 (Table 1), suggests the presence in all five genotypes of the same locus on the B4 linkage group, conferring resistance to race 38.

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