DISSECTION OF THE ANTHRACNOSE RESISTANCE IN THE DIFFERENTIAL CULTIVARS TU AND MDRK

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Anthracnose, caused by fungus *Colletotrichum lindemuthianum* (Sacc.&Magn.) Scrib., is a serious disease in common bean (*Phaseolus vulgaris* L.). This fungus shows numerous pathogenic variants or races (Mahuku & Riascos, 2004). At least nine independent genes involved in the genetic control of the resistance to this pathogen have been described in common bean (see review by Kelly & Vallejos, 2004). The majority of these genes are present in the twelve differential cultivars used for the identification of the pathogenic variants of the fungus. It has been considered that most differential cultivars have only one gene controlling the resistance reaction against different races. Up to now, it has been assumed that differential cultivars MDRK and TU carry only the anthracnose resistance genes Co-1 and Co-5, respectively (Kelly & Vallejos, 2004). In the present work, we show evidences indicating the presence of at least two independent and dominant resistance genes in each one of the bean differential cultivars TU and MDRK. The aim of this work is to consider the possibility of the presence of more than one resistance gene in these two differential cultivars, through the combined analysis of the resistance to different anthracnose races in segregations proceeding from the cross TUxMDRK.

The analysis was carried out in a F_2 population (and the corresponding F_3 families) obtained from the cross between the differential cultivars TU and MDRK. The anthracnose evaluation for races 31, 38, and 1545 was carried out according to standard methods (Pastor Corrales *et al*., 1994) on a total of 86 F_3 families. The resistance to each race was independently evaluated in at least 16 plants per F_3 family.

The segregation for resistance to specific anthracnose races was also analyzed in four F_3 families. In these cases, the genotypes of the F3 plants were determined after the anthracnose evaluation of the corresponding F4 families.

The segregations of different SCAR or RAPD markers linked to anthracnose resistance genes (Kelly & Vallejos, 2004) and SSR markers previously included in the genetic map of common bean (Blair *et al*., 2003), were analyzed in order to know the genes or chromosome regions involved. The different DNA markers were analyzed according to the instructions of the respective authors. The genetic distances between loci were determined with the aid of JOINMAP V3.0 (van Ooijen & Voorrips, 2001).

Table 1. Segregations for the reaction against three different anthracnose races in the F_2 population derived from the cross TUxMDRK. R = F3 families with all plants resistant; R/S = F3 families showing resistant and susceptible plants; S= F3 families with all plants susceptible.

	Parents		F_2 segregation (F_3 families)			Exp. ratio		
Race	Tu	MDRK	R	R/S	S	R : R/S : S	χ^2	p
31	R	S	28	33	8	7 : 8 :1	3.28	0.18
38	R	S	22	38	19	1 : 2 : 1	0.34	0.84
1545	S	R	32	36	2	7 : 8 :1	1.38	0.50

Table 1 shows the observed segregations for the resistance to three anthracnose races in the F_2 population derived from the cross TUxMDRK. For race 38, the observed segregation fitted a 1:2:1 ratio, indicating that a single dominant gene is involved in this resistance. This gene was previously linked to markers SAB3 and Phs, located on B7 linkage group, and identified as Co-5 (Campa *et al*., 2005). However, the observed segregation for resistance to race 31 suggests that two dominant resistance genes are involved in this resistance specificity. In order to confirm this hypothesis, a F3 family (2900.1-9) was analyzed in more detail. In 36 plants of this family, the genotype for resistance to races 31 and 38 was determined through the evaluation of the corresponding F4 families. All plants of this F3 family were

homozygous susceptible to race 38 and had the genotype for markers SAB3 and Phs (linked to Co-5) corresponding to the parental MDRK (susceptible). Concerning race 31, this F3 family showed a segregation of 14 plants homozygous resistant, 17 heterozygous, and 5 homozygous susceptible, ($\chi^2_{1:2:1}$= 4,61; p>0.05). These results indicate that TU carries a gene, conferring resistance to race 31, different from Co-5.

With respect to race 1545, the observed F_2 segregation (table 1) suggests the presence of two independent dominant resistance genes in MDRK. In this case, three F3 families (2900.1-24, 2900.1-29 and 2900.1-74) showing a 3:1 segregation for the resistance were analyzed in more detail. In these families, the genotype for resistance to race 1545 was determined through the evaluation of the corresponding F4 families. In F3 families 2900.1-24 and 2900.1-29, RAPD $OF10_{530}$ (linked to Co-1) was also segregating. The results concerning the joint segregation of the resistance to race 1545 and RAPD $OF10_{530}$ are shown in table 2.

Table 2. Segregation for the reaction against anthracnose race 1545 and RAPD $OF10_{530}$ in F_3 families 2900.1-24 and 2900.1-29 (added). R = F4 families with all plants resistant; R/S = F4 families showing resistant and susceptible plants; S= F4 families with all plants susceptible. += amplification of RAPD $OF10_{530}$ positive; -= amplification of RAPD $OF10_{530}$ negative.

	Segregation of F3 families 2900.1-24 and 2900.1-29 (added)								
	R	R	R/S	R/S	S	S			
Marker	+	-	+	-	+	-	Total	RF	LOD
$OF10_{530}$	4	5	24	0	13	0	46	0.09	3.75

In F3 family 2900.1-74, markers $OF10_{1000}$, SW12, SBA8 and PHVPVPK (all located in linkage group B4) were also segregating. Table 3 shows the results concerning the joint segregation of the resistance to race 1545 and these markers.

Table 3. Segregation for the reaction against anthracnose race 1545 and four molecular markers in F_3 family 2900.1-74. R = F4 families with all plants resistant; R/S = F4 families showing resistant and susceptible plants; S= F4 families with all plants susceptible. += amplification of marker positive; -= amplification of marker negative. PHVPVPK is a microsatellite showing codominant segregation.

	Segregation of F3 family 2900.1-74											
	R	R	R	R/S	R/S	R/S	S	S	S			
Marker	+	+/-	-	+	+/-	-	+	+/-	-	Total	RF	LOD
SW12	4	-	6	9	-	1	6	-	1	27	0.24	0.92
$OF10_{1000}$	4	-	5	9	-	1	6	-	0	25	0.2	1.25
SBA8	6	-	4	9	-	1	7	-	0	27	0.28	0.68
PHVPVPK	0	3	7	0	10	0	7	0	0	27	0.06	7.73

In summary, the results obtained agree with the existence of more than one dominant resistance gene in each of the bean differential cultivars TU and MDRK. The TU cultivar has a resistance gene in B7 linkage group (*Co-5*) involving in the resistance to races 38 and 31. This cultivar has a second gene conferring resistance to race 31 in an unknown location. MDRK carries two dominant and independent resistance genes implicated in the genetic control of the resistance to race 1545. One of these genes is probably Co-1 (linkage group B1), and the other is located in linkage group B4 (it could be allelic of Co-3/Co-9 or Co-10).

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