EVIDENCES FOR ALLELISM AMONG COMMON BEAN DIFFERENTIAL ANTHRACNOSE CULTIVARS BY USING MOLECULAR MARKERS LINKED TO RESISTANCE GENES

Marcia Regina Costa[1], Ana Lilia Alzate-Marin[1*], Everaldo Gonçalves de Barros[1,2] and Maurilio Alves Moreira[1,3]

[1]Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa (UFV), 36571-000 Viçosa, MG, Brazil. [2]Departamento de Biologia Geral, UFV, Viçosa, MG, Brazil. [3]Departamento de Bioquímica e Biologia Molecular, UFV, Viçosa, MG, Brazil.
*Corresponding author: aalzate@alunos.ufv.br

In 1988 occurred the *"Primer Taller de Antracnose del Frijol en América Latina,* in CIAT, Cali/Colômbia, to solve a major problem in the classification of bean anthracnose pathotypes through the use of differential cultivars which, to that date, were not uniform among bean researchers spread over the world. During that meeting it was voted the adoption of a universal set of differentials and a new methodology for classifying the pathotypes of anthracnose causing fungi. This nomenclature is based on a binary system using twelve bean differential cultivars, which are numerically identified from 1 to 12 (1- Michelite, 2 - MDRK, 3 - Perry Marrow, 4 - Cornell 49-242, 5 - Widusa, 6 - Kaboon, 7 - Mexico 222, 8 - PI 207.262, 9 - TO, 10 - TU, 11 - AB 136 and 12 - G 2333). The classification of a given pathotype is accomplished by simple adding the numerical values from each susceptible differential cultivar to this pathotype. Recent evidence has demonstrated the existence of allelism among these differential lines. At present, there are evidences that cultivar G 2333 contains the genes *Co-4*^{2} and *Co-5* (8) and the Andean cultivars MDRK and Kaboon contain alleles of the *Co-1* gene. Therefore, there exist only three Andean differential cultivars, which two carries the same resistance gene (5). Molecular markers highly linked to resistant genes can be used as powerful tools to generate information about allelism among these cultivars. Aiming to identify new potential allelism among resistance genes in the differential cultivars, DNA samples were amplified with RAPD markers linked in coupling to resistant genes (Table 1).

As result from such amplification analyses (Table 1), it was observed that:

1) Differential cultivars PI 207262 and G 2333 possess the band $OPY20_{830C}$, which is tightly linked to *Co-4,* identified in cultivar TO. The presence of this marker in cultivar G 2333 reinforces the finding that this cultivar carries an allele of the *Co-4* gene designated *Co-4*^{2,} which was identified in the derived line "Selection 1308" (8). As PI 207262 affords resistance levels greater than TO (7), two hypotheses can be raised: PI 207262 carries more than one resistance gene for anthracnose or it carries a different allele at the *Co-4* locus. Published data show that PI 207262 carries duplicated dominant genes for resistance to *C. lindemuthianum* (7). On the other hand, if PI 207262 carries an allele at the *Co-4* locus, this allele would be different from *Co-4*^{2}. This is suggested by the different resistance indexes (RI) between Selection 1308 (RI = 97) derived from G 2333 and PI 207262 (RI = 79) (3).

2) Marker $OPC08_{900C}$ (9.7±2.1 cM) was present in most differential cultivars, both Andean and Mesoamerican, suggesting that these cultivars might carry different alleles from gene *Co-4.* Although this marker is relatively distant from the *Co-4* locus, it has been detected in the bean line "P 45", a Brazilian line derived from cultivar TO (4).

3) Marker $OPAS13_{950}$, (tightly linked to gene *Co-4*^{2} from cultivar G 2333) also is present in lines Widusa, PI 207.262 and TO, reinforcing the data obtained with marker $OPY20_{830}$ and suggesting that cultivars Widusa and PI 207.262 carries one allele of Co-*4* gene.

4) Marker $OPAZ20_{940}$ (linked to gene *Co-6* from cultivar AB 136) (1) only amplify in this cultivar, being this marker of great value in the process of pyramiding gene *Co-6* in breeding programs.

The information generated by this work are important to help the understanding the possible interactions and/or allelism among resistant genes in these differential cultivars, which have been extensively used as source of resistance to bean anthracnose in breeding programs.

Allelism studies with Widusa and PI 207.262 will be important to verify allelism information between their resistant genes and gene *Co-4*.

The use of molecular markers tightly linked to known resistant genes could help to identify new potential sources of resistant genes to *C. lindemuthianum*.

Table 1. Resume of DNA amplification of differential cultivars results using RAPD molecular linked to resistance genes

Molecular markers	Linkage in cM /gene (reference)	DIFERENTIAL CULTIVARS[1]											
		1	2 (*Co-1*)	3	4 (*Co-2*)	5	6	7 (*Co-3*)	8	9 (*Co-4*)	10 (*Co-5*)	11 (*Co-6*)	12 (*Co-4*[2]/ *Co-5*)
$OPY20_{830C}$	0.0/*Co-4* (2)	-[2]	-	-	-	-	-	-	+[3]	+	-	-	+
$OPC08_{900C}$	9.7/*Co-4* (2)	+	-	+	+	+	-	+	+	+	-	+	-
$OPAS13_{950C}$	0.0/*Co-4* (8)	-	-	-	-	+	-	-	+	+	-	-	+
$OPAZ20_{C}$	7.1/*Co-6* (1)	-	-	-	-	-	-	-	-	-	-	+	-

[1] 1. Michelite, 2. MDKR, 3. Perry Marrow, 4. Cornell, 5. Widusa, 6. Kaboon, 7. México 222, 8. PI 207.262, 9. TO, 10. TU, 11. AB 136, 12. G2333

[2] band absent (-), [3] band present (+)

Acknowledgement: Maria Regina Costa was supported by undergraduate scholarship from FAPEMIG. Ana Lilia Alzate-Marin was supported by a visitor's fellowship from FAPEMIG.

BIBLIOGRAPHY

1. Alzate-Marin A. L.; Menarim, H.; Chagas J. M.; Barros, E. G. De & Maurilio A. Genetics and Molecular Biology23:633-637. 2000.
2. Arruda, M. C.C.; Alzate-Marin, A. L.; Barros, E. G. De.; Moreira, M. A. Phytopathology 90:758-761. 2000
3. Balardin, R. S. and Kelly, J. D. J. Am. Soc. Hort. Sci. 123: 1038-1047. 1998.
4. Castanheira, A. L. M.; Santos, J. B. Dos; Ferreira, D. F. & Melo, L. C. Genetics and Molecular Biology. 22:565-569.2000.
5. Melotto M.; Awale, H. & Kelly, J.D. BIC 42: 9-10. 1999.
6. Rava, C. A., Purchio, A. F. and Sartorato, A. Fitopatol. Bras. 19: 167-172. 1994.
7. Vidigal, M. C., Cardoso, A, Vieira, C. and Saraiva, L. 1997. Brazilian Journal of Genetics 20: 59-62.
8. Young, R.; Melotto M.; Nodari R. O. & Kelly, J.D. Theor. Appl. Genet. 96: 87-94. 1998.