Use of Markers as a Tool to Investigate the Presence of Disease Resistance Genes in Common Bean Cultivars

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ABSTRACT

The molecular marker-assisted selection has a great potential as a breeding tool in common beans. The identification of RAPD markers allows association (pyramiding) of genes for disease resistance or the identification of high potential cultivars with different resistance genes that were not previously identified due to limitations in the screening methodology. In this study previously identified molecular markers were used as a tool to investigate the presence of disease resistant genes as well as to identify pyramided genes that are present in the lines tested in the elite lines trials coordinated by Embrapa Rice and Beans. Fourteen out of 21 elite lines possessed different molecular markers linked to disease resistant genes for anthracnose, angular leaf spot, rust and bean golden mosaic virus. Lines FEB 163 (5) and TB 94-01 (8) presented seven molecular markers linked to different resistance genes. It was not possible to detect the presence of any known sources of disease resistance genes, based in the pedigree of lines LM 93204319 (12), LM 93204328 (13), LM 93203246 (18) and PR 93201472 (21).

KEY WORDS: Colletotrichum lindemuthianum, Phaeoisariopsis griseola, Uromyces appendiculatus , Bean golden mosaic virus, RAPD, Gene resistance.

INTRODUCTION

Marker-assisted selection (MAS) using molecular markers such as random amplified polymorphic DNA (RAPD) has a great potential to be an useful breeding tool in common beans, where monogenic disease resistance genes have been tagged (Haley et al., 1993, 1994; Adam-Blondon et al., 1994; Young and Kelly, 1996a,b; Alzate-Marin et al., 1999a, b, c; Young et al., 1998; Arruda et al., 2000).

The identification of molecular markers linked to resistance genes allows an indirect selection, because the expression of the marker is not masked by epistatic interactions (Kelly, 1995). The identification of a number of RAPD markers allows pyramiding genes for disease resistance, or the identification of potential lines with different resistance genes which could not be previously identified due to difficulties in accomplishing multiple inoculation tests with different pathogens in just one individual. RAPD markers can facilitate the identification of potential lines with pyramided resistance genes especially if they are tightly linked to the locus containing the resistance allele.

The objective of this study was to use previously identified RAPD markers as a tool to investigate the presence of disease resistance genes, as well as to identify pyramided genes from resistance sources to diseases such as anthracnose, angular leaf spot, rust and bean golden mosaic virus, that are present in the pedigree of lines tested in the bean regional elite trials coordinated by Embrapa Rice and Beans.

Table 1- Seed color and pedigree of the 21 elite lines of the Bean Regional Trials coordinated by Embrapa Rice and Beans.

BRT line [1/,2/,3/]	Color [4/]	Pedigree
1. LR 9115398	BL	(((G 2698 x (BRASIL 343 x BRASIL 1096)) x (CAUCA 41)) x (ICA TUI x S 166 AN) x (G 2084 x 51051) x (SB 12)) x (XAN 87)))/HONDURAS 35 = (((S 234 x (BRASIL 343 x BRASIL 1096)) x (CAUCA 41)) x (ICA TUI x S 166 AN) x (GENTRY 21555 x 51051) x (SB 12)) x ((22-G-4 x GENTRY 21439)x (51052 x CORNELL 49-242))))/HONDURAS 35
2. LR 9115453	BR	(DRO 4784) / ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA) x PORRILLO SINTETICO x PI 310878) x NEP BAYO 22))// (AROANA x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))/ (OJO DE LIEBRE x ((PORRILLO SINTETICO x CACAHUATE 73) x (JAMAPA x CACAHUATE 72)))
3. A 774	BR	((51051 x ICA BUNSI) x (51052 x CORNELL 49242))/// (CARIOCA x MEX. 168)/ CARIOCA 80 // (CARIOCA x MEX. 168) / ((ICA TUI x TLALNEPANTLA 64) x (PORRILLO SINTETICO x JULES))
4. PR 9115957	BR	(TLALNEPANTLA 64 x AROANA) / GOIANO PRECOCE
5. FEB 163	RX	(CARIOCA x MEX. 168) / ((AETE 1/38 x (VERANIC 2 x TLALNEPANTLA 64)) x (JAMAPA x TARA))// (CARIOCA x MEX. 168)//(((PORRILLO No. 1 x GENTRY 21439) x (51052 x CORNELL 49242)) x (GENTRY No. 12307 x GARRAPATO))/// CARIOCA x MEX. 168/ ((ICA TUI x TLALNEPANTLA 64) x (PORRILLO SINTETICO x JULES))// ((22-G-4 x GENTRY 21439) x (51052x CORNELL 49-242))/ ((PORRILLO No. 1 x GENTRY 21439) x (51052 x CORNELL 49242) x (GENTRY No. 12307 x GARRAPATO))
6. RAO 33	RD	(((POMPADOUR CHECA) x (G 03645 x G 02045)) x (G 03974 x G 04485))/(51052 x COPAN) =(((POMPADOUR CHECA) x (JAMAPA x GENTRY 21439)) x (JIN 10 x TURRIALBA 1))/(51052 x COPAN)
7. LM 93204217	BL	((PORRILLO SINTÉTICO x TURRIALBA 1) x (ICA PIJAO x NEGRO JAMAPA))// (((VERANIC 2 x TLALNEPANTLA 64)F1 x ((JAMAPA x TARA)F1x AETE 1/37)))/ (((IPA 7419 x (HONDURAS 46 x VENEZUELA 54))///((AROANA x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))) //(G 4326/((S 166 AN x ECUADOR 299) x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))))
8. TB 94-01	BL	(((IPA 7419 x (HONDURAS 46 x VENEZUELA 54))///(((AROANA x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))) //G 4326/((S 166 AN x ECUADOR 299) x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))))/ ((NEP 2 x ICA PIJAO) x F4(RIO TIBAGI*3 x CORNELL 49242))
9. AN 9021334	BL	SAME AS LR 9115398
10. AN 9021336	BL	SAME AS LR 9115398
11.LM 93204303	CA	(CARIOCA/RIO TIBAGI) / (((CARIOCA x ((PI 307824 x PI 310797) x (TURRIALBA 4 x CORNELL 49-242))/// (CARIOCA x MEX. 168)//((AETÉ 1/38 x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA)))/ ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA) x AETÉ 1/37)))
12.LM 93204319	CA	(CARIOCA/RIO TIBAGI)/ ((51052 x CACAHUATE 72) x CARIOCA*2)
13.LM 93204328	CA	SAME AS LM 93204319
14.LM 93204453	CA	(JALO EEP 558, CANARIO 101, BONITAS?)/ (((VERANIC 2xCORNELL 49-242) x (PI 309796 x CACAHUATE 72)) x (PI 310814 x TURRIALBA 1)) x (CRUZAM MULTIPLOS PAIS x (JAMAPA x PI 310878)) x (AETÉ 1/38 x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA)))//(((CARIOCA x ((PI 307824 x PI 310797) x (TURRIALBA 4 x CORNELL 49-242)) /// (CARIOCA x MEX. 168)//(AETÉ 1/38 x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))) /((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA) x AETÉ 1/37)/((51052 x CACAHUATE 72) x CARIOCA*2)
15. AN 9021470	BR	(IPA-7419 x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA)) x (MEX 168))/((IPA 7419 x (HONDURAS 46 x VENEZUELA 54))///(AROANA x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))//G 4326 / ((S 166 AN x ECUADOR 299) x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))))
16. LM 9220225	BR	DRO 4784 / ((((VERANIC 2 x CORNELL 49-242) x (PI 309796 x CACAHUATE 72)) x (PI 310814 x TURRIALBA 1)) x (CRUZAM MULTIPLOS PAIS x (JAMAPA x PI 310878))) x (AETÉ 1/38 x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA)))
17. L 96029	BR	(((VERANIC 2 x TLALNEPANTLA 64)F1 x (JAMAPA x TARA)F1) x AETE 1/37) / (LINEA 32 x TURRIALBA 1)
18.LM 93203246	RS	ADVANCED MATERIAL RECEIVED FROM CIAT 1981/ ROSINHA G2RMC
19.LM 93203304	RS	HI822510?/ (((ICA 10310 x ((VERANIC 2 x TLALNEPANTLA 64) x (TURRIALBA 4 x CORNELL 49-242)) x ((S 166 AN x 51054) x ((VERANIC 2 x TLALNEPANTLA 64) x (JAMAPA x TARA))) // (ADVANCED MATERIAL RECEIVED FROM CIAT 1981/ ROSINHA G2RM)
20. LR 93201684	RX	CF (BEAN COLLECTION) / ((POMPADOUR CHECA) x ((JAMAPA x GENTRY 21439) x (JIN 10 x TURRIALBA 1))) x (51052 x COPAN))
21. PR 93201472	MT	POMPADOUR/IRAI

MATERIAL AND METHODS

The present study included 21 elite bean lines from the regional trials coordinated by Embrapa Rice and Beans (Table 1). Fifteen days after planting, plant material from each line was collected, identified and stored at –80°C. DNA extraction was conducted according to Doyle and Doyle (1990) and DNA amplification reactions were achieved according to Williams et al. (1990). Amplification cycles, product analyses and band visualization were similar to those mentioned by Alzate-Marin et al. (1999b).

To investigate linkage relationships between resistance and molecular markers, the pedigree of all genotypes involved in the development of each elite line was compared (Table 1). Based on this information and through a literature review, a second list of all RAPD molecular markers linked to known resistant genes in each genotype was developed (Table 2). As a result, RAPD molecular markers linked to resistance genes sources present in the 21 elite lines tested in the Bean Regional Trials were validated according to the information showed in Table 2.

RESULTS AND DISCUSSION

A summary of the results obtained in this study is shown in Table 3. In general, previously identified molecular markers were efficient in validating the presence of resistance genes from Cornell 49-242, Honduras 35 (Ouro Negro), Tara, Jules, NEP 2, Pompadour Checa and Garrapato. Marker OPF10912C linked to a resistance gene in the cultivar Tlalnepantla 64 (=PI 207.262) was less specific due to its distance (11.5 cM) from the gene (Castanheira et al., 1999) (Table 3). Different markers, in repulsion (Young and Kelly, 1997) or in coupling phase (Haley et al., 1993; Castanheira et al., 1999), linked to anthracnose and rust resistance genes originated from the same primer OPF10 have already been identified.

Amplifications with the molecular marker OPH20450C (Adam-Blondom et al., 1994), happened to be more efficient in tagging the gene Co-2 from the cultivar Cornell 49-242 than the marker OPQ041440C (Young and Kelly, 1996a). As mentioned by Young and Kelly (1996a), the marker OPQ041440C is more efficient when evaluated in beans with an Andean genetic background which carries the gene Co-2 than in beans with a Middle American background. Although the data from the inoculation tests involving all lines x pathotypes of the pathogen were not obtained, field data provided by EMBRAPA Rice and Beans indicated that elite lines A 774 (3), FEB 163 (5), TB 94-01 (8), AN 9021334 (9) and AN 9021336 (10), were resistant to both 55 and 453 *Colletotrichum lindemuthianum* pathotypes. Elite lines LM 93204303 (11), LM 93204453 (14), LM 9220225 (16) and LM 93203304 (19) were resistant to pathotype 55 and elite lines LR 9115398 (1) and FEB 163 (5) were resistant to a mixture of the pathotypes 95 and 453 of *C. lindemuthianum*. All these lines with a Mesoamerican background (data not shown) showed the OPH20450C marker (Table 3). Previous studies by Rava et al., (1994), showed that the cultivar Cornell 49-242, which presents the molecular marker $OPH20_{450C}$ (Table 3), besides showing resistance to the pathotypes 55 and 453 of *C. lindemuthianum* was also resistant to 18 other pathotypes. These data confirmed that these cultivars carried the resistance gene Co-2 present in cultivar Cornell 49-242 and that the molecular marker $OPH20_{450C}$ was efficient in tagging this gene.

Elite lines A 774 (3), FEB 163 (5), TB 94-01 (8), AN 9021334 (9), AN 9021336 (10), LM 93204453 (14), LM 9220225 (16) and LM 93203304 (19) amplified the band of the $SCARN02_{890C}$ linked, in coupling phase, to the *Phaeoisariopsis griseola* resistance gene in cultivar Cornell 49-242, showing that these lines carry at least one resistance gene for angular leaf spot. Elite lines LR 9115398 (1) and LM 93204303 (11) did not present the $SCARN02_{890C}$ band indicating that the angular leaf spot resistance gene, from Cornell 49-242, was probably lost in these lines during the breeding process.

Amplifications with the co-dominant marker $OPR02_{570C/530R}$, linked in repulsion phase (band of 530 bp) to the recessive bgm-1 gene of BGMV from the resistance source Garrapato, showed that the two bands, linked in coupling and repulsion phase were observed in line FEB 163 (5). The presence of both alleles indicates that the recessive gene locus is heterozygous and susceptible in FEB 163 (+/+=Bgm-1 bgm-1). However, there must be other genes that provide susceptibility or resistance to the bmg-1 originated from the parents of the line FEB 163. According to Urrea et al. (1996), cultivar Porrillo Sintetico exhibits a phenotype +/- (homozygous susceptible: Bgm-1Bgm-1) and the cultivar Garrapato -/+ (homozygous resistance: bgm-1bgm-1) for bgm-1 gene, when amplified with the marker $OPR02_{570C/530R}$. FEB 163 is derived from these two parents, consequently the two markers generated by the primer OPR02 are probably indicating the presence of different genes in FEB 163 (Table 1).

DNA amplified from lines LR 9115398 (1), AN 9021334 (9), and AN 9021336 (10) with the $SCAROPF10_{1050C}$ marker (linked to anthracnose and rust resistance genes), $SCAROPBA08_{530C}$ and $OPX11_{530C}$ (linked only to rust resistance genes) (Corrêa, 1999; Vinhadelli et al., 1997) indicated that line LR 9115398 carries resistance genes for anthracnose and rust that came from the cultivar Ouro Negro (Honduras 35). Elite lines AN 9021334 (9) and AN 9021336 (10) are highly resistant to *Colletotrichum lindemuthianum* pathotypes 55 and 453, and LR 9115398 (1) is resistant to a mixture of 95 and 453 pathotypes, what proves the efficiency of the SCAR marker $OPF10_{1050C}$. Cultivar Ouro Negro is resistant to all pathotypes of *Uromyces appendiculatus* identified in the State of Minas Gerais (Faleiro, 1997) and is also resistant to pathotypes 55, 95 and 453 of *C. lindemuthianum* and other 14 *C. lindemuthianum* pathotypes (data not published).

Concerning to rust resistance, the presence of the markers $OPAA11_{500c}$, $OPAB16_{850C}$ and $OPAD09_{550C}$ linked to the rust resistance gene Ur-7 was observed only in lines A 774 (3), FEB 163 (5), LM 93204303 (11), and LM 93204453 (14) that have Jules or Tara as parents (Table 3). The markers $OPAB16_{850C}$ and $OPAD09_{550C}$ were also observed in the cultivars LR 9115453 (2), LM 93204217 (7), TB 94-01 (8), AN 9021470 (15), LM 9220225 (16), L 96029 (17) and LM 93203304(19) that have Tara as a parent. The molecular markers $OPAD12_{550C}$ and $OPAF17_{900C}$ did not amplify the DNA of the tested cultivars. The results indicate that the eleven cultivars tested carry the rust resistance gene Ur-7 from Jules and Tara.

Amplifications of DNA of LR 9115398 (1), LR 9115453 (2) (data not shown) and TB 94-01 (8) with marker $OPK14_{620C}$ showed that only elite line TB 94-01 (8) possesses the marker linked to the gene Ur-3 (Table 3). This observation shows a high specificity of this marker for the Ur-3 gene and the absence of alleles of this gene among the other two cultivars.

DNA from lines RAO 33 (6) and LR 93201684 (20) that have the cultivar Pompadour Checa as a parent and from line PR 93201472 (21) (Pompadour/Irai), was amplified with primer OPJ13, which tags the rust resistance gene Ur-9. Figure 1 shows that the 1800 bp marker linked to Ur-9 in the cultivar Pompadour Checa is present in lines RAO 33 and LR 93201684 but absent in line PR 93201472. Although cultivar Pompadour Checa was selected from Pompadour (Voysest, 1983; 2000), our results show that there must be some differences between these cultivars and PR 93201472. Field data show that lines RAO 33 and LR 93201684 were resistant to rust while cultivar PR 93201472 was susceptible. Lines RAO 33 and LR 93201684 possess the marker $OPJ13_{1800C}$ while the line PR 93201472 does not (Figure 1). The absence of the marker $OPJ13_{1800C}$ linked to Ur-9 gene and the susceptibility to rust under field conditions indicate that Pompadour, used as parent of PR93201472, is unlikely to have been the parent of Pompadour Checa.

Table 2 - General information on previously identified RAPD markers present in cultivars/lines involved in the breeding program to develop the elite lines of the Bean Regional Trials coordinated by Embrapa Rice and Beans.

LINE/CULTIVAR	ORIGIN	GENES AND MOLECULAR MARKER PREVIOUSLY IDENTIFIED	NOTE
1. CORNELL 49-242	Venezuela (Central America? Voysest, (1983))	Anthracnose: *Co-2* $OPQ04_{1440C}$[1/] (Young and Kelly, 1996a), $OPH20_{450C}$ (Adam-Blondon et al., 1994) Angular leaf spot: $SCARN02_{890C}$ (Nietsche et al., 2000)	*I* and *bc-u* genes[3/] Middle American (Vasconcelos, 1996). Anthracnose and angular spot resistance source and differential cultivar.
2. GARRAPATO	Central America	BGMV : *bgm-1* $OPR02_{530R}$[2/] , $OPR02_{570C}$ (Urrea et al., 1996)	Co-dominant marker
3. HONDURAS 35 (Ouro Negro)	Central America (Honduras)	Anthracnose-Rust $SCARF10_{1050C}$ (Corrêa, 1999) Rust $OPX11_{550C}$ (Vinhadelli et al., 1997) $SCARBA08_{560C}$ (Corrêa, 1999) Angular leaf spot $OPM02_{425C}$ (Corrêa, 1999)	Middle American (Vasconcelos, 1996). Resistant to several pathotypes of *C. lindemuthianum* and resistance to 14 pathotypes of *Uromyces appendiculatus* (Faleiro, 1997; Corrêa, 1999)
4. JULES	USA Nebraska	Rust : *Ur-7* $OPAA11_{500C}$,$OPAD12_{550C}$, $OPAF17_{900C}$, $OPAB16_{850C}$ $OPAD9_{550C}$ (Park et al., 1999)	GN Nebraska #1 sel. 27/GN 1140 (pedigree similar to Tara)[3/]
5. NEP 2	Costa Rica	Rust: *Ur-3* $OPK14_{620C}$ (Haley et al., 1994)	***Rust differential.*** Resistant to Andean isolates[4/]. Small white seed from cultivar San Fernando (S 182 N), selection from Turrialba through mutagenesis; *I* and *bc-u* genes[3/] (Middle American)
6.POMPADOUR CHECA	Dominican Republic	Rust: *Ur-9* $OPJ13_{1800C}$ (Jung et al., 1996)	Andean, rust resistant (Bassett, 1996). Red and pink mottled median size seeds Selected from Pompadour (Voysest, 1983).
7. TARA	USA (Nebraska)	Rust : *Ur-7* $OPAA11_{500C}$,$OPAD12_{550C}$, $OPAF17_{900C}$, $OPAB16_{850C,}$ $OPAD9_{550C}$ (Park et al., 1999)	GN Nebraska #1 Sel. 27/GN 1140 (pedigree similar to Jules)[3/]. Resistant to some pathotypes of *U. appendiculatus*. Tolerant to the bean common bacterial blight. Developed in Nebraska.
8.TLALNEPANTLA 64	Mexico (Tlalnepantla)	Anthracnose $OPF10_{912C}$ (Castanheira et al., 1999)	Collected in 1943[5/]. PI 207,262. Anthracnose differential cultivar. Source of resistance to common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* (Saettler, 1989).

[1/, 2/] Molecular markers linked in coupling (C) and repulsion (R) phases with resistance gene, respectively; [3/] http://probe.nal.usda.gov:8300/cgi-bin/webace?db=beangenes&class=Allele&object=I. *I* and *bc-u* genes for Bean common mosaic virus; [4/] Sandlin et al., 1999; [5/] GRIN-USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: www.ars-grin.gov/cgi-bin/npgs/html/acc_search.pl

Table 3 - Summary of DNA amplification of the Bean Regional Trials elite lines coordinated by EMBRAPA Rice and Beans using previously identified RAPD markers linked to resistance genes.

Molecular marker - Distance	Resistance source	Elite line with resistance source (Table 1)	DNA amplification in elite lines (Table 1)																			Controls[1]			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	R	C	O	P
Anthracnose																											
1.OPQ04$_{1440C}$ 2.0–5.0 cM	Cornell 49-242	1, 3, 5, 8, 9, 10, 11, 14, 16, 19	**A**[2]	-[3]	**A**	-	**A**	-	-	**P**[4]	**A**	**A**	**A**	-	-	**A**	-	**A**	-	-	**A**	-	-	**A**	**P**	-	-
2. OPH20$_{450C}$ 0.5 cM	Cornell 49-242	1, 3, 5, 8, 9, 10, 11, 14, 16, 19	**P**	-	**P**	-	**P**	-	-	**P**	**P**	**P**	**P**	-	-	**P**	-	**P**	-	-	**P**	-	-	**P**	**P**	-	-
3.OPF10$_{912C}$ 11.5 cM	Tlalnepantla 64	2, 3, 4, 5, 7, 8, 11,14,15,16, 17,19	-	**P**	**P**	**P**	**P**	-	**P**	**P**	-	-	**P**	-	-	**P**	**P**	**P**	**P**	-	**P**	-	-	**P**	-	-	**P**
Anthracnose-Rust																											
4.SCARF10$_{1050C}$ 4.3 cM	Honduras 35 (Ouro Negro)	1, 9, 10	**P**	-	-	-	-	-	-	-	**P**	**P**	-	-	-	-	-	-	-	-	-	-	-	**A**	-	**P**	-
BGMV																											
Bgm-1																											
5.OPR02$_{570C/530R}$ 4.2 cM	Garrapato	5	-	-	-	-	**P**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**P**	-	-	-
Angular leaf spot																											
6.SCARN02$_{890C}$ 3.2 cM	Cornell 49-242	1, 3, 5, 8, 9, 10, 11, 14, 16, 19	**A**	-	**P**	-	**P**	-	-	**P**	**P**	**P**	**A**	-	-	**P**	-	**P**	-	-	**P**	-	-	**A**	**P**	-	-
7.OPM02$_{425C}$ 5.6 cM	Honduras 35	1, 9, 10	**P**	-	-	-	-	-	-	-	**A**	**A**	-	-	-	-	-	-	-	-	-	-	-	**A**	-	**P**	-
Rust																											
Ur-?																											
8.OPX11$_{550C}$ 5.3 cM	Honduras 35	1, 9, 10	**P**	-	-	-	-	-	-	-	**P**	**P**	-	-	-	-	-	-	-	-	-	-	-	**A**	-	**P**	-
9.SCARBAO8$_{560C}$ 6.0 cM	Honduras 35	1, 9, 10	**P**	-	-	-	-	-	-	-	**P**	**P**	-	-	-	-	-	-	-	-	-	-	-	**A**	-	**P**	-
Ur-7																											
10.OPAA11$_{500C}$ 0.0 cM	Jules and Tara	Jules: 3, 5	-	**A**	**P**	-	**P**	-	**A**	**A**	-	-	**P**	-	-	**P**	**A**	**A**	**A**	-	**A**	-	-	**A**	-	-	-
11. OPAB16$_{850C}$ 2.2 cM	Jules and Tara	Tara: 2, 5, 7, 8,	-	**P**	**P**	-	**P**	-	**P**	**P**	-	-	**P**	-	-	**P**	**P**	**P**	**P**	-	**P**	-	-	**P**	-	-	-
14.OPAD09$_{550C}$ 2.2 cM	Jules and Tara	11, 14, 15, 16, 17, 19	-	**P**	**P**	-	**P**	-	**P**	**P**	-	-	**P**	-	-	**P**	**P**	**P**	**P**	-	**P**	-	-	-	-	-	-
Ur-3																											
12.OPK14$_{620C}$ 2.2 cM	Nep 2	8	-	-	-	-	-	-	-	**P**	-	-	-	-	-	-	-	-	-	-	-	-	-	**A**	**A**	**A**	-
Ur-9																											
13.OPJ13$_{1800C}$ 5.0 cM	Pompadour Checa	6, 20	-	-	-	-	-	**P**	-	-	-	-	-	-	-	-	-	-	-	-	-	**P**	**A**	**P**	-	-	-
Total number of present bands			5	3	6	1	7	1	3	7	5	5	5	-	-	6	3	5	3	-	5	1	-	-	-	-	-

[1/] R,C,O and P correspond to cultivars Rudá, Cornell 49-242, Ouro Negro and PI 207262 used as a control, respectively; [2/]Absence (A) and [4/]Presence (P) of molecular markers; [3/]No amplified DNA (-).

Most cultivars showed markers linked to anthracnose, angular leaf spot, and rust and bean golden mosaic virus resistance genes (Table 3). Bean elite lines FEB 163 (5) and TB 94-01 (8) were the only lines to show seven bands generated by different molecular markers linked to resistance genes. These data show the importance of these two lines which exhibit great diversity in terms of resistance sources to different bean diseases based on pedigree. Lines LM 93204319 (12), LM 93204328 (13) and PR 93201472 (21) did not show any known disease resistance genes, indicating that they present high vulnerability if commercially released as varieties.

Our work showed a practical application of molecular markers as a tool in the selection process directed toward the commercial release of new cultivars. Our study also indicated that pedigree information has a fundamental importance in the process of validating previously identified disease resistance gene sources.

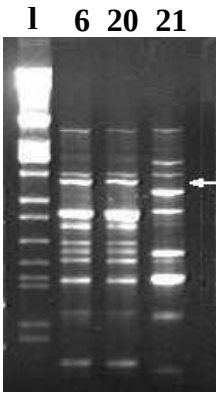

Figure 1- Electrophoretic analysis of amplification products obtained with primer OPJ13. The first lane correspond to lambda DNA cut with EcoRI, BamHI, and HindIII (m.w. marker). Lanes 6, 20 and 21 correspond to the amplification products of elite lines from the Regional Trials coordinated by EMBRAPA Rice and Beans (Table 1). Arrow indicates the 1800 bp band linked in the coupling phase to the rust resistance gene Ur-9 present in cultivar Pompadour Checa.

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RESUMO

Uso de Marcadores Como uma Ferramenta para Verificar a Presença de Genes de Resistência em Cultivares de Feijoeiro Comum

A seleção assistida por marcadores moleculares, apresenta um grande potencial para ser utilizada como ferramenta em programas de melhoramento do feijoeiro comum (Phaseolus vulgaris L.). A identificação de marcadores RAPD permite a transferência de genes através do processo de associação de genes (piramidação) para uma única linhagem e, também, a identificação da presença de genes em outros cultivares os quais não puderam ser previamente detectados devido a dificuldades na metodologia de monitoramento. Neste estudo o DNA das linhagens elite de feijoeiro comum dos Ensaios Regionais coordenados pela Embrapa Arroz e Feijão foi amplificado com marcadores moleculares previamente identificados como ligados a genes de resistência, visando (i) verificar a presença de genes de resistência a doenças e (ii) identificar possíveis associações de genes (piramidações) oriundas de fontes de resistência conhecidas que compõem os pedigrees destas linhagens. Entre os resultados obtidos, pode-se observar que 14 das 21 linhagens apresentaram diferentes bandas ligadas a genes de resistência a antracnose, mancha angular, BGMV e ferrugem. Nas linhagens FEB 163 (5) e TB 94-01 (8) foram observados sete marcadores moleculares ligados a diferentes genes de resistência. Nas genealogias das linhagens LM 93204319 (12), LM 93204328 (13), LM 93203246 (18) e PR 93201472 (21) não foi observada a presença de nenhuma fonte conhecida de genes de resistência.

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