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RAPD markers linked to a block of genes conferring rust resistance to the common bean*

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Abstract

Rust, caused by the fungus *Uromyces appendiculatus*, may cause a significant loss to common bean (*Phaseolus vulgaris* L.) yield. RAPD markers tightly linked to the resistance genes may be used in breeding programs to aid the development of rust-resistant bean cultivars. In this sense, the objective of the present work was to identify RAPD markers linked to a rust resistance gene block present in the cultivar Ouro Negro. Two hundred and fourteen F_2 individuals from a cross between the resistant cultivar Ouro Negro and the susceptible cultivar US Pinto 111 were inoculated with a mixture of eight races of *U. appendiculatus*. The segregation ratio obtained suggested that resistance is monogenic and dominant. Bulked segregant analysis was used in conjunction with the RAPD technique to search for markers linked to rust resistance genes. Two molecular markers flanking the rust resistance gene block were identified, one at 5.8 ± 1.6 cM ($OX11_{630}$) and the other at 7.7 ± 1.7 cM ($OF10_{1,050}$) of the gene. Simulated indirect selection efficiency in the F_2 population using the two markers was 100%. The molecular markers identified in this work are currently being used for the selection of disease-resistant plants in the commom bean breeding program of the Federal University of Viçosa.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is susceptible to be attacked by a large number of pathogens which may qualitatively and quantitatively affect plant growth and yield (Vieira, 1983; Stavely and Pastor-Corrales, 1994). In particular, rust caused by the fungus *Uromyces appendiculatus* may cause a significant loss of yield (Lindgren *et al*., 1995).

Recommended measures for controlling rust include crop rotation and the elimination of contaminated material (Hall and Nasser, 1996). Fungicides and resistant cultivars are also useful for limiting the progress of this disease (Stavely and Pastor-Corrales, 1994).

The chemical control of rust is rarely employed by small farmers who do not use irrigation and advanced cultivation technologies, mainly because of the need for specialized knowledge in handling these compounds and because of the cost involved. In addition, chemicals contaminate the environment and affect human health. For these reasons, the use of resistant cultivars is preferred by most plant breeders and researchers because they provide an effective, safe and inexpensive method of rust control.

Conventional breeding methods have been widely used to develop new cultivars resistant to *U. appendiculatus* (Coyne and Schuster, 1975). However, resistance is easily overcome by the extensive pathogenic variability of the fungus (Faleiro, 1997). New strategies to bypass this problem, such as the pyramiding of resistance genes, have been proposed. According to Kelly *et al.* (1994), the pyramiding of three rust resistance genes (*Ur*-4, *Ur-5* and *Ur*-3) would be enough to confer resistance to 63 of the 65 races of *U. appendiculatus* identified in the United States. However, the use of this strategy in conventional breeding procedures is extremely difficult, mainly because of the need for multiple inoculations which may lead to erroneous evaluation of the symptoms (Vinhadelli *et al*., 1997).

Random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990) markers have been used in breeding programs to help in the production of rust-resistant bean cultivars (Haley *et al.*, 1993; Miklas *et al.*, 1993; Kelly *et al.*, 1994). Markers tightly linked to the resistance genes may be used for the indirect selection of resistant plants in segregating populations, without the need for multiple inoculations.

The objective of the present work was to identify RAPD markers linked to a rust resistance gene block present in the cultivar Ouro Negro, one of the major rust resistance sources used in the bean breeding program of the Federal University of Viçosa, MG, Brazil.

MATERIAL AND METHODS

An F_2 population of 214 individuals derived from a cross between the cultivars Ouro Negro and US Pinto 111 was used for the experiments. Cultivar Ouro Negro, which was introduced in Brazil as Honduras 35 and released as a variety in 1991 (Araújo *et al*., 1991), is resistant to several

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races of *U. appendiculatus* identified in Brazil (Faleiro *et al.*, 1996), whereas cultivar US Pinto 111 is considered to be universally susceptible to rust.

F_2 seeds were sown in a greenhouse in 2.5-liter pots containing substrate. Ten days after sowing, when the primary leaves had reached approximately two-thirds of their full development, the plants were inoculated with a mixture containing equal amounts of spores from *U. appendiculatus* races 1, 2, 6, 7, 9, 10, 11 and 13 identified by Faleiro *et al*. (1999b). The spores were suspended in distilled water containing 0.05% Tween 20 and the suspension was diluted to give approximately 2 x 10^4 spores/ml. The inoculum was sprayed onto both leaf surfaces with the aid of a De Vilbiss No. 15 atomizer powered by an electric compressor (Carrijo *et al.*, 1980). After a brief drying period, the plants were transferred to a mist chamber (20 ± 1°C and >95% relative humidity) where they were kept for 48 h, under a 12-h photoperiod. The plants were then returned to the greenhouse where they were evaluated for disease symptoms 15 days after inoculation, using a scale reported by Stavely *et al.* (1983).

Table I - Segregation analyses between rust resistance and the molecular markers $OX11_{630}$ and $OF10_{1,050}$ in the F_2 population derived from a cross between cultivars Ouro Negro and US Pinto 111.

Locus tested	Observed ratio	Expected ratio	χ^2	Probability
Resistance:susceptibility	151:63	3:1	1.12	0.32
Presence:absence $OX11_{630}$	149:65	3:1	3.29	0.08
Presence:absence $OF10_{1,050}$	150:64	3:1	2.75	0.16

After symptom evaluation, leaf DNA was extracted from each F_2 plant (Doyle and Doyle, 1990). To identify the RAPD markers linked to the resistance gene, DNA bulks from contrasting plants were prepared as described by Michelmore *et al.* (1991). DNA amplification was done according to Vasconcelos *et al.* (1996). A total of 1,280 decamer primers were tested (Operon Technologies, Alameda, CA, USA). Amplification products were separated in 1.2% agarose gels containing 10 µg ethidium bromide/ml, visualized under UV light and photographed with an Eagle Eye II photodocumentation system (Stratagene, La Jolla, CA, USA).

To confirm the segregation between the marker and the resistance phenotype, primers which produced polymorphic bands between the two bulks were tested in the progenitors and 214 F_2 plants. The data were analyzed by

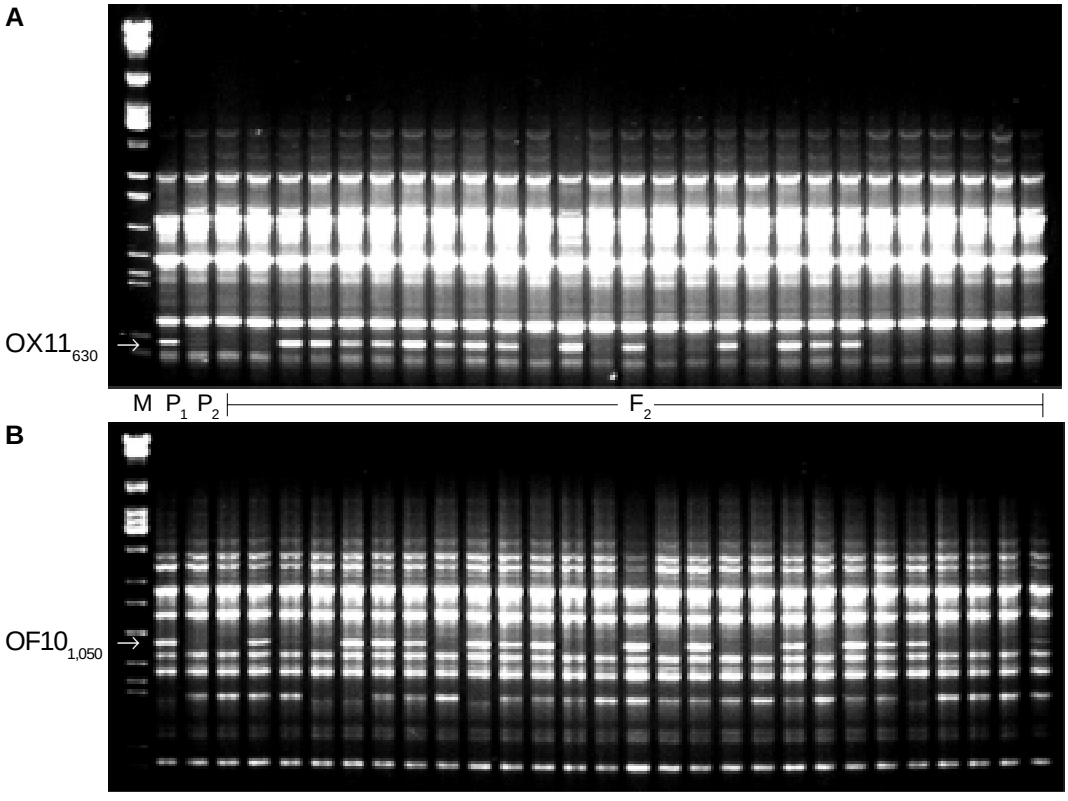

Figure 1 - Electrophoretic analyses of DNA amplification products obtained with primers OX11 (A) and OF10 (B). In both gels, P_1 corresponds to cultivar Ouro Negro, P_2 to cultivar US Pinto 111 and F_2 to 27 individuals from the segregating population. Lane M contains lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers). The arrows indicate markers $OX11_{630}$ (A) and $OF10_{1,050}$ (B).

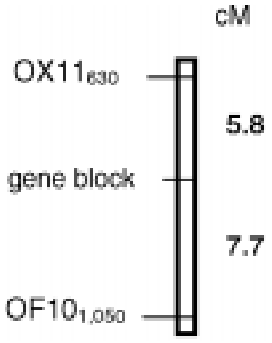

Figure 2 - The positions of markers $OX11_{630}$ and $OF10_{1,050}$, expressed as genetic distances (cM), relative to the rust resistance gene block.

Table II - Indirect selection efficiency (ISE) of rust resistant plants based on the presence/absence of single and associated molecular markers.

Molecular markers	Selected plants			ISE (%)*
	Resistant	Susceptible	Total	
$OX11_{630}$	144	5	149	96.6
$OF10_{1,050}$	143	7	150	95.3
$OX11_{630}$ and $OF10_{1,050}$	137	0	137	100.0

* ISE (%) = (No. of resistant plants x 100) / No. of selected plants.

the chi-square test to verify the segregation ratios. Recombination frequencies and genetic distances between the markers and the resistance gene block were estimated using the software MAPMAKER.EXP 3.0 (Lander *et al*., 1987; Lincoln *et al.*, 1992).

RESULTS AND DISCUSSION

The segregation analyses suggested that a major dominant gene confers rust resistance in cultivar Ouro Negro (Table I). Preliminary studies on resistance inheritance in this cultivar using one *U. appendiculatus* race indicated that major genes are indeed the main determinants of bean rust resistance (Faleiro, 1997).

Two RAPD markers linked in coupling-phase to a rust resistance gene block were identified: one with 630 bp ($OX11_{630}$) and the other with 1,050 bp ($OF10_{1,050}$). The amplification patterns with the two primers generating these markers were highly reproducible (Figure 1). Segregation analyses of the markers in the F_2 population confirmed their dominant nature (Table I). The number of recombinants showed that markers $OX11_{630}$ and $OF10_{1,050}$ are located at 5.8 ± 1.6 cM and 7.7 ± 1.7 cM of the gene block, respectively (Figure 2).

That the two markers flank the resistance gene block (Figure 2) is extremely important for the indirect selection of resistant plants. Two cross-over events would be necessary to give rise to a resistant plant bearing no marker. This is particularly evident when the indirect selection efficiency (ISE) is estimated using the markers separately and simultaneously. Preliminary estimates of ISE in 214 individuals of the F_2 population were 96.6 and 95.3% using the markers $OX11_{630}$ and $OF10_{1,050}$, respectively. The simultaneous use of the two markers gave an ISE of 100% (Table II).

RAPD markers linked to a resistance gene block have been reported by others. Miklas *et al*. (1993) identified a marker ($OA14_{1,100}$) tightly linked to the resistance gene *Ur-4* (no recombinants in 84 BC_6F_2 individuals). Haley *et al.* (1993) identified a marker ($OF10_{970}$) linked at 2.15 cM of the rust resistance gene block present in line B-190 and a marker $OI19_{460}$ tightly linked to the same gene block (no recombinants in 97 BC_6F_2 individuals). Haley *et al.* (1994) identified a marker ($OK14_{620}$) linked at 2.23 cM of the resistance gene *Ur*-3. All these markers were used in the pyramiding of these resistance genes in the same genetic background (Stavely *et al.*, 1994, 1997).

These markers were also tested in our segregating population, but none were polymorphic and, in some cases, the corresponding DNA bands were not detected (data not shown). However, as already discussed, primer OF10 produced a 1,050-bp fragment linked to the rust resistance gene block present in cultivar Ouro Negro. This primer also produced a DNA fragment (970 bp) linked to a rust resistance gene block in line B-190 (Haley *et al.*, 1993). The small difference between the two bands is apparently related to the way their sizes were estimated. The resistance gene blocks present in line B-190 and in cultivar Ouro Negro are very similar and probably map to the same location. Both confer the same type of resistance, with immunity to certain *U. appendiculatus* races and small pustules (< 0.3 mm in diameter) to other races (Haley *et al.*, 1993; Faleiro *et al*., 1999a). Interestingly both Ouro Negro and B-190 are highly productive black seed cultivars.

The molecular markers identified in this work are currently being used in the selection of disease-resistant plants in the common bean breeding program of the Federal University of Viçosa.

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RESUMO

A ferrugem, causada pelo fungo *Uromyces appendiculatus*, pode ocasionar perdas significativas na produtividade do feijoeiro-comum (*Phaseolus vulgaris* L.). Marcadores RAPD fortemente ligados a genes de resistência à ferrugem podem ser usados em programas de melhoramento para auxiliar no desenvolvimento de cultivares resistentes. Nesse sentido, o objetivo do presente trabalho foi o de identificar marcadores RAPD ligados a um bloco de genes de resistência à ferrugem presente no cultivar Ouro Negro. Para isso, foram utilizados 214 indivíduos F_2 originados do cruzamento Ouro Negro (resistente) x US Pinto 111 (suscetível

universal). As plantas foram inoculadas com uma mistura de 8 raças de *U. appendiculatus* e a análise da segregação revelou uma herança monogênica dominante. Foi empregada a metodologia de *bulk segregant analysis*. Os *bulks* foram amplificados pela técnica de RAPD com 1280 primers. Foram encontrados dois marcadores flanqueando o bloco gênico que confere resistência à ferrugem: $OX11_{630}$ (5,8 ± 1,6 cM) e $OF10_{1050}$ (7,7 ± 1,7 cM). Considerando os indivíduos F_2, a seleção indireta de plantas resistentes com base no uso dos dois marcadores simultaneamente leva a 100% de eficiência. Os marcadores identificados nesse trabalho estão sendo extremamente úteis no programa de melhoramento da Universidade Federal de Viçosa.

REFERENCES

Araújo, G.A.A., Vieira, C. and **Souza Filho, B.F.** (1991). *'Ouro Negro' Nova Variedade de Feijão-Preto para os Estados de Minas Gerais e Rio de Janeiro*. EPAMIG (Boletim Técnico No. 1), Viçosa, MG.

Carrijo, I.V., Chaves, G.M. and **Pereira, A.A.** (1980). Reação de vinte e cinco variedades de *Phaseolus vulgaris* a trinta e nove raças fisiológicas de *Uromyces phaseoli* var. *typica* Arth., em condições de casa-de-vegetação. *Fitopatol. Bras. 5*: 245-255.

Coyne, D.P. and **Schuster, M.L.** (1975). Genetic and breeding strategy for resistance to rust (*Uromyces phaseoli* (Reben) Wint.) in bean (*Phaseolus vulgaris* L.). *Euphytica 24*: 795-803.

Doyle, J.J. and **Doyle, J.L.** (1990). Isolation of plant DNA from fresh tissue. *Focus 12*: 13-15.

Faleiro, F.G. (1997). Identificação de raças, diversidade genética de *Uromyces appendiculatus* var. *appendiculatus* e herança da resistência no feijoeiro. Master's thesis, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Faleiro, F.G., Paula Jr., T.J., Barros, E.G., Freitas, M.A. and **Moreira, M.A.** (1996). Resistência de cultivares de feijoeiro comum a *Uromyces appendiculatus* da Zona da Mata de Minas Gerais. *Fitopatol. Bras. 21*: 123-125.

Faleiro, F.G., Vinhadelli, W.S., Ragagnin, V.A., Paula Jr., T.J., Moreira, M.A. and **Barros, E.G.** (1999a). Resistência de variedades de feijoeiro-comum a quatro raças de *Uromyces appendiculatus*. *Rev. Ceres 46*: 19-27.

Faleiro, F.G., Vinhadelli, W.S., Ragagnin, V.A., Zambolim, L., Paula Jr., T.J., Moreira, M.A. and **Barros, E.G.** (1999b). Identificação de raças fisiológicas de *Uromyces appendiculatus* no estado de Minas Gerais, Brasil. *Fitopatol. Bras. 24*: 166-169.

Haley, S.D., Miklas, P.N., Stavely, J.R., Byrum, J. and **Kelly, J.D.** (1993). Identification of RAPD markers linked to a major rust resistance gene block in common bean. *Theor. Appl. Genet. 86*: 505-512.

Haley, S.D., Afanador, L., Miklas, P.N., Stavely, J.R. and **Kelly, J.D.** (1994). Heterogeneous inbred populations are useful as sources of near-isogenic lines from RAPD marker localization. *Theor. Appl. Genet. 88*: 337-342.

Hall, R. and **Nasser, L.C.B.** (1996). Practice and precept in cultural management of bean diseases. *Can. J. Plant Pathol. 18*: 176-185.

Kelly, J.D., Haley, S.D., Afanador, L., Miklas, P.N. and **Stavely, J.R.** (1994). Application of RAPD markers for disease resistance breeding in beans. *Ann. Rep. Bean Impr. Coop. 37*: 15-16.

Lander, E., Green, P., Abrahamson, J., Barlon, A., Daley, M., Lincoln, S. and **Newburg, L.** (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics 1*: 174-181.

Lincoln, S., Daly, M. and **Lander, E.** (1992). *Constructing Genetic Maps with MAPMAKER/EXP 3.0*. 3rd edn. Technical Report: Whitehead Institute, Cambridge.

Lindgren, D.T., Escridge, K.M., Steadman, J.R. and **Schaaf, D.M.** (1995). A model for dry bean yield loss due to rust. *Hort. Technol. 5*: 35-37.

Michelmore, R.W., Paran, I. and **Kesselli, R.V.** (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA 88*: 9828-9832.

Miklas, P.N., Stavely, J.R. and **Kelly, J.D.** (1993). Identification and potential use of a molecular marker for rust resistance in common bean. *Theor. Appl. Genet. 85*: 745-749.

Stavely, J.R. and **Pastor-Corrales, M.A.** (1994). Roya. In: *Problemas de Producción del Frijol en los Tropicos* (Pastor-Corrales, M.A. and Schwartz, H.F., eds.). 2nd edn. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, pp. 185-225.

Stavely, J.R., Freytag, G.F., Steadman, J.R. and **Schwartz, H.F.** (1983). The 1983 Bean Rust Workshop. *Ann. Rep. Bean Impr. Coop. 26*: iv-vi.

Stavely, J.R., Kelly, J.D., Grafton, K.F., Steinke, J., Steadman, J.R., Coyne, D.P., Lindgren, D.T. and **Silbernagel, M.J.** (1994). Recent rust resistant bean germplasm releases. *Ann. Rep. Bean Impr. Coop. 37*: 247-248.

Stavely, J.R., Kelly, J.D., Grafton, K.F., Mullins, C.A., Straw, A., McMillan Jr., R.T., Beaver, J.S., Miklas, P.N., Steinke, J., Steadman, J.R., Coyne, D.P., Lindgren, D.T. and **Silbernagel, M.J.** (1997). Rust resistant bean germplasm releases, 1994-1996. *Ann. Rep. Bean Impr. Coop. 40*: 120-121.

Vasconcelos, M.J.V., Barros, E.G., Moreira, M.A. and **Vieira, C.** (1996). Genetic diversity of *Phaseolus vulgaris* L. as determined by DNA-based molecular markers. *Braz. J. Genet. 19*: 447-451.

Vieira, C. (1983). *Doenças e Pragas do Feijoeiro*. Imprensa Universitária, Viçosa, MG.

Vinhadelli, W.S., Faleiro, F.G., Mesquita, A.G.G., Paula Jr., T.J., Carvalho, G.A., Barros, E.G. and **Moreira, M.A.** (1997). Inoculação simultânea dos fungos causadores da ferrugem e da antracnose do feijoeiro. *Fitopatol. Bras. 22* (Suppl.): 319-320 (Abstract).

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and **Tingey, S.V.** (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res. 18*: 6531-6535.

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