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Allelism Tests between the Rust Resistance Gene Present in Common Bean Cultivar Ouro Negro and Genes *Ur-5* and *Ur-11*

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With one figure

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Abstract

The pathogenic variability of the fungus *Uromyces appendiculatus* is an obstacle for the creation of rust-resistant common bean (*Phaseolus vulgaris* L.) varieties. Gene pyramiding is an alternative strategy for the development of varieties with durable resistance. However, to reach this goal it is important to identify different genes with ample resistance spectra. Cultivars Ouro Negro, Mexico 309 and Belmidak RR-3 have been shown to be resistant to several rust races identified in the state of Minas Gerais, Brazil. Ouro Negro is the only rust resistance source being used in the BIOAGRO/Universidade Federal de Viçosa (UFV) breeding programme, which aims at pyramiding resistance genes in the 'carioca-type' cultivar Rudá. It would be also interesting to use Mexico 309 (*Ur-5*) and Belmidak RR-3 (*Ur-11*) in the breeding programme. However, there is no available information on the possible allelic relationships between the Ouro Negro resistance gene and *Ur-5* and *Ur-11*. This work aimed at: (1) determining the allelic relationship between the Ouro Negro resistance gene and *Ur-5* and *Ur-11*; and (2) evaluating a random amplified polymorphic DNA (RAPD) marker previously reported as being linked to *Ur-11*, in populations from crosses between Belmidak RR-3 and Rudá. The allelism tests confirmed that the Ouro Negro rust resistance gene is distinct from *Ur-5* and *Ur-11* and the molecular analyses confirmed that the RAPD marker can be used in our breeding programme to develop 'carioca-type' cultivars with the *Ur-11* gene.

Introduction

Common bean rust, caused by the fungus *Uromyces appendiculatus*, is a worldwide-distributed disease that may cause a significant yield loss in humid tropical and subtropical areas and periodic severe epidemics in

humid temperate regions (Stavelly and Pastor-Corrales, 1989). It is estimated that losses caused by bean rust in Brazil may reach 20–45% of the expected yield (Jesus Junior et al., 2001). In the last few decades a high number of *U. appendiculatus* races has been detected in Central and Southern Brazil (Vieira, 1988; Faleiro et al., 1999; Santos and Rios, 2000).

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

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 this pathogen. Pyramiding of resistance genes assisted by molecular markers has been proposed as an alternative solution for this type of problem. 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 (Kelly, 1995). Random amplified polymorphic DNA (RAPD) (Williams et al., 1990) markers have been identified and used in breeding programmes to aid the production of rust-resistant bean cultivars (Haley et al., 1993; Miklas et al., 1993; Kelly et al., 1994; Johnson et al., 1995; Corrêa et al., 2000; Faleiro et al., 2000). The genes *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6* and *Ur-11* have been used for pyramiding rust-resistant in North American bean germplasm (Stavelly, 2000).

In Central Brazil, cultivar Ouro Negro has been extensively used as a rust resistance source, showing

resistance to 13 races of *U. appendiculatus* collected in the state of Minas Gerais (Faleiro et al., 1999). This cultivar with excellent agronomic characteristics, has been recommended by the National Agricultural Research System (SNPA) for cultivation in several states. In addition, Ouro Negro has been tested with 24 of 94 isolates of *U. appendiculatus* maintained at Beltsville United States Department of Agriculture (USDA), showing resistance reaction to 22 races, a moderate reaction to race 78 and susceptibility only to race 108 (Dr J. R. Stavely, personal communication).

In the BIOAGRO/Universidade Federal de Viçosa (UFV) bean breeding programme that uses Ouro Negro as the only rust resistance source, it was observed that cultivars Mexico 309 (*Ur-5*) and Belmidak RR-3 (*Ur-11*) are also important resistant sources to *U. appendiculatus* races prevalent in the state of Minas Gerais, Brazil (Faleiro et al., 1999, 2000). Information about allelism among cultivars Mexico 309, Belmidak RR-3 and Ouro Negro is essential for effective pyramiding of rust resistance genes from these sources.

In this work the allelic relationship between the rust resistance gene present in Ouro Negro and *Ur-5* and *Ur-11* were determined and a RAPD marker linked to *Ur-11* was tested in segregating populations derived from crosses between Belmidak RR-3 and Rudá.

Materials and Methods

Source of *U. appendiculatus* isolates and culture conditions

Races 6 and 10 of *U. appendiculatus*, used in this work, were collected in the state of Minas Gerais, Brazil (Faleiro et al., 1999). They are part of the collection maintained by the bean-breeding programme of the Biotechnology Institute (BIOAGRO) of the UFV, MG, Brazil. To keep the viability and increase the amount of inoculum the spores were periodically inoculated on leaves of the susceptible cultivar US Pinto 111. Newly generated spores were stored under low humidity, at 4°C, and protected from light.

Genetic material

Seeds from cultivars Mexico 309 and Belmidak RR-3 were provided by the Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. Seeds from 'carioca-type' cultivar Rudá were provided by CNPAF/EMBRAPA (Goiânia, GO, Brazil). Seeds from cultivar Ouro Negro were provided by Dr Clibas Vieira of the Federal University of Viçosa (Minas Gerais, Brazil). Cultivars Mexico 309 and Belmidak RR-3 were used as male progenitors and crossed in a greenhouse with Ouro Negro for allelism tests. Cultivar Belmidak RR-3 was used as male progenitor and crossed with cultivar Rudá for molecular studies. The populations were maintained in the greenhouse.

Analysis of F₁ plants

To determine the effectiveness of the crosses, the F₂ seeds were analysed morphologically. The F₁ plants derived from the crosses between Ouro Negro and

Mexico 309 (both with black seeds and violet flowers) were also analysed with RAPD markers according to Alzate-Marin et al. (1996). As Ouro Negro was the female parent, the presence in the F₁ plant of a DNA band, which was present only in the male parent, confirmed that the plant was indeed a hybrid plant. The true hybrid plants were selfed and the F₂ seeds obtained were used for allelism tests.

Genetic analyses and evaluation of disease symptoms

Allelism tests The F₂ seeds from the crosses Ouro Negro vs. Mexico 309 and Belmidak RR-3 and those of the progenitors and of a susceptible control cultivar (cv. US Pinto 111) were sown in the greenhouse. Fourteen days after sowing the first leaf of each plant was inoculated on the lower and upper leaf surfaces with spore suspensions (2×10^4 spores/ml) of race 10 of *U. appendiculatus*, applied with the aid of a De Vilbiss (Sao Paulo, Brazil) no. 15 atomizer powered by an electric compressor. Preliminary tests showed that the three progenitors were resistant to race 10. The plants were then incubated for 2 days in a mist chamber kept at 20–22°C and 100% relative humidity. The plants were returned to the greenhouse where they were evaluated for disease symptoms 15 days after inoculation, using a scale reported by Stavely et al. (1983).

Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1–3), whereas plants graded 4 or greater were considered to be susceptible (S). The phenotypic class frequencies obtained were tested for goodness-of-fit to theoretical ratios with chi-square tests.

Molecular marker analyses Fifty-three F₂ seeds derived from a cross between Rudá and Belmidak RR-3 were sown in the greenhouse. Primary leaves from all plants were collected and kept at –80°C for DNA extraction. All plants were selfed for generating F₃ seeds. Twelve seeds from each progenitor and from each of the 53 F₂:₃ families were sown in the greenhouse. Fourteen days after sowing the first leaf from each plant was inoculated on the lower and upper leaf surfaces with spore suspensions (2×10^4 spores/ml) of *U. appendiculatus* race 6. Rudá and Belmidak RR-3 present contrasting reactions to *U. appendiculatus* race 6, showing reactions of susceptibility and resistance, respectively. The inoculation conditions and symptom evaluations were as described before.

DNA extraction and amplification

DNA extraction of the primary leaves of the 53 F₂ plants was according to Doyle and Doyle (1990). Amplification reactions were performed in a thermocycler model 9600 (Perkin-Elmer, Norwalk, CT, USA). Each reaction (25 µl) contained: 25 ng DNA, 0.1 mM of each dNTP, 2.0 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.4 µM of primer OPAE19 (Operon Technologies, Alameda, CA, USA) and one unit of *Taq* DNA polymerase. The molecular marker OPAE19₈₉₀ tested was previously reported as linked in

repulsion phase (6.2 cM) to the *Ur-11* gene of cultivar PI 181996 (Johnson et al., 1995), one of the progenitors of cultivar Belmidak RR-3 (USDA, 2003).

Each amplification cycle consisted of one denaturation step at 94°C for 15 s, one annealing step at 35°C for 30 s and one extension step at 72°C for 1 min. After 40 cycles an extra extension step was performed for 7 min at 72°C. Amplification products were analysed on 1.2% agarose gels immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0) and containing ethidium bromide to a final concentration of 0.2 µg/ml. DNA bands were visualized under UV light and photographed with the aid of an Eagle Eye II photosystem (Stratagene, La Jolla, CA, USA).

Linkage analyses

Chi-square analysis was used to test the segregation of rust resistance in the F₂ and F₂:₃ populations derived from crosses between Rudá and Belmidak RR-3 and the segregation between rust resistance and the presence of marker OPAE19₈₉₀ in the F₂ population. The genetic distance between the RAPD marker and the resistance gene was based on the data obtained from the 53 F₂ individuals with the aid of the programme MAP-MAKER III (Lander et al., 1987) using a LOD score minimum of 3.0.

Results

Allelism tests

In the allelism tests the segregation for rust resistance fit a ratio of 15 R to one S plant in the F₂ populations derived from crosses between Ouro Negro and Mexico 309 (*Ur-5*) and Belmidak RR-3 (*Ur-11*) indicating that two independent genes govern resistance in these populations (Table 1). These results indicate that the gene (or complex gene locus) present in Ouro Negro is distinct from *Ur-5* and *Ur-11*.

Validation of RAPD marker OPAE19₈₉₀

In this work we used the molecular marker OPAE19₈₉₀ previously reported by Johnson et al. (1995) as linked (6.2 cM) in repulsion phase to *Ur-11*. The DNA of the F₂ population derived from the cross between Rudá and Belmidak RR-3 (gene *Ur-11*) was amplified with this marker (Table 2). The marker was shown to be polymorphic between Rudá and Belmidak RR-3 and the polymorphism in the F₂ population was clearly observed (Fig. 1). Co-segregation analyses in the F₂ population revealed that marker OPAE19₈₉₀ was located 1.0 cM from the resistance gene *Ur-11* (Table 2). The RAPD marker OPAE19₈₉₀ linked to *Ur-11* was identified based on the correct phenotypic evaluation of the F₂ plants according to the segregation of each F₂:₃ family. Individuals that did not harbour a marker in repulsion phase were homozygous and resistant, whereas resistant individuals with this marker were heterozygous. All but one F₂ homozygous resistant plant did not harbour the OPAE19₈₉₀ marker indicating that selection efficiency based on absence of the marker was 92% (Table 2).

Discussion

The results of this work showed that a rust resistance gene present in Ouro Negro is distinct from genes *Ur-5* and *Ur-11*. The combination of these three genes would provide broad resistance to the rust pathogen worldwide and durable pyramided resistance to specific races that occur in the state of Minas Gerais, Brazil. Ouro Negro is resistant to all 13 races collected in the state of Minas Gerais, *Ur-5* confers resistance to 10 of these races, and *Ur-11* to four of the 13 races (Faleiro et al., 1999). In the state of Goiás (Central Brazil), *Ur-5* confers resistance to 11 identified races of *U. appendiculatus*, but is moderately susceptible or susceptible to 23 races identified in Rio Grande do Sul and Santa

Table 1

Segregation analysis of rust resistance in F₂ populations derived from crosses between Ouro Negro and Mexico 309, and Ouro Negro and Belmidak RR-3 inoculated with *Uromyces appendiculatus* race 10

Cross	Gene	Observed ratio		Expected ratio	χ^2	P-value
		Resistant	Susceptible			
Ouro Negro × Mexico 309	<i>Ur-5</i>	193	15	15 : 1	0.328	56.67
Ouro Negro × Belmidak RR-3	<i>Ur-11</i>	60	4	15 : 1	0.000	100.00

Table 2

Segregation of rust resistance (*Ur-11*) to *Uromyces appendiculatus* race 6, and linkage analysis between molecular marker OPAE19₈₉₀ and the resistance gene in the cross between Rudá and Belmidak RR-3

Cross	Locus	Generation	Expected ratio	Observed ratio	χ^2	P-value	cM ¹
Rudá × Belmidak RR-3	<i>Ur-11</i>	F ₂ : ₃	1 : 2 : 1 ²	12RR : 28Rr : 13S	0.578	74.87	–
Rudá × Belmidak RR-3	OPAE19 ₈₉₀ ³	F ₂	1(–) : 2(+) : 1(+) ⁴	13(–) : 27(+) : 13(+)	0.188	99.06	1.0

¹Distance in centiMorgans in relation to *Ur-11* (resistance gene).

²Resistance/susceptibility segregation in F₂:₃ families.

³Molecular marker linked in repulsion phase to the resistance gene *Ur-11*.

⁴(–), band absent; (+), band present.

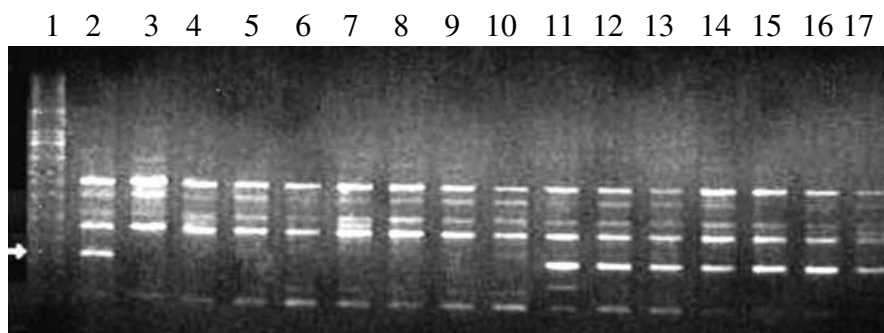


Fig. 1 Electrophoretic analysis of amplification products obtained with primer OPAE19. Lanes are as follows: 1, lambda DNA cut with *EcoRI*, *BamHI* and *HindIII* (size markers); 2, Rudá; 3, Belmidak RR-3; 4–10, homozygous F₂ plants resistant to *Uromyces appendiculatus* race 6; 11–17, homozygous F₂ plants susceptible to *U. appendiculatus* race 6. The arrow indicates a DNA band of 890 bp linked in repulsion phase to the resistance gene, *Ur-11*

Catarina states (Southern Brazil) (Santos and Rios, 2000). Faleiro et al. (2001) demonstrated that cultivar Belmidak RR-3 is resistant to *U. appendiculatus* races 2, 6, 9 and 10 detected in Minas Gerais state. *Ur-11* also confers resistance to 89 of 90 races of *U. appendiculatus* maintained by the USDA (Stavely, 2000).

In this work we also demonstrated that molecular marker OPAE19₈₉₀ linked to *Ur-11* (Johnson et al., 1995) can be used in our breeding programme aimed at pyramiding of rust resistance genes into 'carioca-type' cultivars. At the moment, we use two molecular markers OX11₆₃₀ and SCARF10₁₀₇₂ (Corrêa et al., 2000; Faleiro et al., 2000) flanking the resistance gene of Ouro Negro for the indirect selection of resistant plants. Now we can also use primer OPAE19₈₉₀ to select for the presence of the *Ur-11* gene. As for gene *Ur-5*, at the moment we are in the process of validating in our populations the RAPD marker which was shown to be linked to it (Haley et al., 1993).

The results obtained in this work demonstrate that cultivars Ouro Negro, Mexico 309 and Belmidak RR-3 possess distinct and important rust resistance genes that can be used to develop resistant bean materials adapted to Central Brazil. This diversity is essential for the development of a durable resistance particularly in the context of gene pyramiding. The molecular markers already available will be used to facilitate the development of 'carioca-type' lines containing *Ur-5*, *Ur-11* and the Ouro Negro rust resistance gene in our breeding programme working toward the creation of commercial cultivars with long-lasting resistance to rust.

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