Crop Breeding and Applied Biotechnology 7:11-15, 2007
Brazilian Society of Plant Breeding. Printed in Brazil

ARTICLE

Inheritance study and validation of SCAR molecular marker for rust resistance in common bean

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Received 01 February 2006

Accepted 05 June 2006

ABSTRACT - *Common bean cultivar Mexico 309 presents a wide resistance spectrum to several pathotypes of the fungus Uromyces appendiculatus that occurs in the states of Minas Gerais and Goiás, in Central Brazil. This pathogen is the causal agent of common bean rust. The present study had the following objectives: (i) to determine the inheritance of rust resistance in segregating populations obtained from crosses between cultivars Mexico 309 and Rudá (commercial cultivar susceptible to rust), and (ii) to validate the SCAR marker* $SI19_{460}$ *as linked to 'Mexico 309' resistance gene. Results confirmed that 'Mexico 309' has a single dominant rust resistance gene (Ur-5) and that marker* $SI19_{460}$ *is linked in coupling phase at 3.31 cM of this gene. This marker can be used for indirect selection of gene Ur-5 during the development of common bean lines resistant to rust using 'Rudá' as one of the progenitors.*

Key words: marker-assisted selection, *Phaseolus vulgaris*, resistance genes, *Uromyces appendiculatus*.

INTRODUCTION

In Brazil, common bean (*Phaseolus vulgaris* L.) is grown in distinct regions and different seasons of the year. It is grown by small farmers with low technological input and by rural entrepreneurs who apply modern technology. In this context, investment on research aiming at an increased production of this crop is justified. In spite of the increasing yield, the national average productivity is still very low compared to the yield potential of the new common bean varieties (Vieira et al. 2005). One factor that partly explains this situation is the high number of diseases that affect the crop. Among these diseases is rust, incited by the fungus *Uromyces appendiculatus* (Pers.: Pers.) Unger [sin. *U. phaseoli* (Reben) Wint.], which can cause great yield losses. According to Lindgren et al. (1995) a 1% increase in rust severity leads to an yield loss of approximately 19 kg/ha. Moderate temperatures (17-27 °C) and high relative air humidity (> 95%) over long periods of time provide the most favorable conditions for *U. appendiculatus* incidence (Paula Júnior and Zambolim 1998).

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The fungus *U. appendiculatus* presents high variability. Many pathotypes have already been identified in Brazil (Faleiro et al. 1999, Rios et al. 2001, Souza et al. 2005). Such variability is an obstacle for the development of rust-resistant commercial cultivars. There are, however, genotypes that are incompatible with several isolates of the pathogen, although in many cases these genotypes do not have the desirable agronomic and commercial traits (Souza et al. 2005).

The individual or simultaneous introgression (pyramiding) of resistance genes (R) in common bean elite lines is a strategy that has been used for the development of commercial cultivars with broad and durable resistance. Molecular markers, mainly DNA markers, have been used as a tool for the indirect selection of R genes during the breeding process (Corrêa et al. 2000, Miklas et al. 1993).

Faleiro et al. (1999) demonstrated that cultivar Mexico 309 is immune to nine and moderately resistant to two of 13 *U. appendiculatus* pathotypes that are frequently found in the state of Minas Gerais. Souza et al. (2005) confirmed the resistance stability of 'Mexico 309' to the most virulent pathotypes identified by Faleiro et al. (1999). This cultivar further proved to be resistant to 11 pathotypes from the state of Goiás (Santos and Rios 2000, Souza et al. 2005). In addition, 'Mexico 309' was resistant to pathotypes 1 and 3 collected on an experimental station of Embrapa Arroz e Feijão, Santo Antônio de Goiás, Goiás (Rios et al. 2001). These data demonstrate the importance of using 'Mexico 309' as a rust resistance source in Central Brazil.

Haley et al. (1993) identified the RAPD (Random Amplified Polymorphic DNA) molecular marker $OPI19_{460}$ as linked in coupling phase and without recombinants to the rust resistance gene or gene cluster found in common bean line B-190 (gene *Ur-5*). This line has 'Mexico 309' as the only resistance donor parent. This RAPD marker was later converted to the SCAR (Sequence Characterized Amplified Region) marker $SI19_{460}$ by Melotto and Kelly (1998).

In our breeding program, 'Mexico 309' as well as cultivar Ouro Negro (gene *Ur-ON*) and the line Belmidak RR-3 (gene *Ur-11*) have been used as resistance-donor parents in backcrossings (BC) with cv. Rudá, aiming at the development of commercial rust-resistant "carioca-type" lines. Cultivar Rudá has many desirable agronomic traits and "carioca-type" grains, which Brazilian consumers prefer, but it is susceptible to *U. appendiculatus*. The main goals of the present study were: (i) to define the inheritance of rust resistance in cv. Mexico 309 by the phenotypic analysis of F_1, F_2, BC_1F_1, BC_2F_1, BC_3F_1 and BC_3F_2 populations derived from the cross Rudá x Mexico 309; and (ii) to validate SCAR marker $SI19_{460}$ in the BC_3F_2 population derived from the cross Rudá x Mexico 309.

MATERIAL AND METHODS

Seeds of the parental cultivars Rudá and Mexico 309 and controls 'Ouro Negro', 'Belmidak RR-3' (both resistant) and 'US Pinto 111' (susceptible) were provided by the Active Germplasm Bank of the Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO) of the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. The plants were artificially crossed under greenhouse conditions. The F_1 plants were phenotypically identified (flower color) and used to generate the F_2 population. Backcrossings (BC) were performed up to the BC_3F_1 generation using cv. Rudá as recurrent parent. In each BC cycle, the resistant individuals were selected by artificial inoculations and used to generate the subsequent BC populations.

The *U. appendiculatus* pathotypes 6 and 10 were used in inoculation procedures. They are among the most virulent pathotypes of the fungus collected in the state of Minas Gerais (Faleiro et al. 1999). These pathotypes are compatible with 'Rudá' and 'US Pinto 111', and incompatible with 'Mexico 309', 'Ouro Negro', and 'Belmidak RR-3'. Before the inoculations, the uredospores were multiplied in 'US Pinto 111' through successive inoculations, in order to increase the inoculum virulence.

Ten days after sowing, 19 F_1 plants, 170 F_2 plants and 10 plants from each parent and the resistant and susceptible controls were inoculated with *U. appendiculatus* pathotype 10, according to a methodology described by Faleiro et al. (2001). The inoculum concentration was 2.0×10^4 uredospores/ml distilled water containing 0.05% Tween 20. The solution was applied on both sides of the primary leaves. After inoculation, the plants were placed in a mist chamber (at 20 ± 1 °C and relative air humidity > 95%), where they remained for 48 h under a 12-h light/12-h dark light regime. Then they were transferred to a greenhouse (20 ± 5 °C) and kept there until the pustules were completely formed. The disease was evaluated around 15 days after inoculation.

The plants were visually evaluated based on a scale of six reaction degrees (Stavely et al. 1983). Plants with degrees 1 to 3 (pustule absence, necrotic spots without

sporulation or sporulating pustules with diameters under 300 µM) were considered resistant, whereas those with degrees 4 or higher (sporulating pustules with diameters equal to 300 µM or higher) were considered susceptible.

In addition, around ten days after sowing 41 BC_1F_1 plants, 23 BC_2F_1 plants, 17 BC_3F_1, and 61 BC_3F_2 plants (Rudá x Mexico 309) as well as 10 plants of each parental cultivar and control were inoculated with *U. appendiculatus* pathotype 6. Incubation and disease evaluation procedures were similar to those mentioned above.

The DNA samples were extracted from all 61 BC_3F_2 individuals (Rudá x Mexico 309), from the parents and the rust resistance sources 'Ouro Negro' (gene *Ur-ON*) and 'BelmidaK RR-3' (gene *Ur-11*). The DNA was extracted according to Doyle and Doyle (1990).

Each amplification cycle of the DNA fragments with marker $SI19_{460}$ consisted of: one initial denaturation step at 94 °C for 3 min; 34 cycles at 94 °C for 1 min, 50 °C for 1 min and 30 s, 72 °C for 1 min and 30 s; and a final step at 72 °C for 7 min. Each amplification reaction with 25 mL contained: 30 ng DNA, 0.1 mM of each dNTP, 2.8 mM $MgCl_2$, 10 mM/50 mM Tris/KCl (pH 8.3), 0.2 mM of each primer $SI19_{460}$ (F: 5' - AAT GCG GGA GTT CAA TAG AAA AAC C - 3' and R: 5' - AAT GCG GGA GAT ATT AAA AGG AAA G - 3'), and one unit of *Taq* DNA polymerase. The amplification products were analyzed in a 1.2% agarose gel containing ethidium bromide (0.2 mg/mL), immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0). The DNA bands were visualized under ultraviolet light and digital images were recorded by an Eagle Eye II image system (Stratagene, La Jolla, CA, USA).

The Chi-square test was used to define the segregation pattern of rust resistance in the F_2, BC_1F_1, BC_2F_1, BC_3F_1 and BC_3F_2 populations. Haldane's Mapping Function was used to estimate the genetic distance between the $SI19_{460}$ marker and the rust resistance locus based on the data of the 61 BC_3F_2 individuals.

RESULTS AND DISCUSSION

All F_1 (Rudá x Mexico 309) plants showed the same resistance pattern as cv. Mexico 309 when inoculated with *U. appendiculatus* pathotype10. The phenotypic segregation for resistance to pathotypes 6 and 10 in the BC_3F_2 and F_2 populations, respectively, was 3:1 (3R_:1rr). A 1:1 segregation ratio of resistant to susceptible plants (1R_:1rr) was observed in the BC_1F_1, BC_2F_1 and BC_3F_1 populations when inoculated with *U. appendiculatus* pathotype 6 (Table 1). These results confirm that rust resistance in 'Mexico 309' is monogenic and dominant.

The molecular analysis of the BC_3F_2 population showed that SCAR marker $SI19_{460}$ is polymorphic between cultivars Mexico 309 and Rudá, as well as in resistant and susceptible BC_3F_2 plants (Figure 1). The co-segregation analysis revealed that $SI19_{460}$ is linked

Table 1. Inheritance of rust resistance in the common bean cultivar Mexico 309 (gene *Ur-5*) and its co-segregation with SCAR marker $SI19_{460}$ in populations obtained from crosses with cv. Rudá (susceptible)

Locus tested	Population[1]	Nº of plants	Expected ratio[2]	Observed ratio	χ^2	P(%)[3]	cM[4]
Ur-5	F_1	19	1(R):0(S)	19(R):0(S)	-	-	-
Ur-5	F_2	170	3(R):1(S)	131(R):39(S)	0.3843	53.53	-
Ur-5	BC_1F_1	41	1(R):1(S)	20(R):21(S)	0.0243	87.59	-
Ur-5	BC_2F_1	23	1(R):1(S)	9(R):14(S)	1.0869	29.71	-
Ur-5	BC_3F_1	17	1(R):1(S)	8(R):9(S)	0.0588	80.84	-
Ur-5	BC_3F_2	61	3(R):1(S)	44(R):17(S)	0.2677	60.48	-
$SI19_{460}$	BC_3F_2	61	3(R):1(S)	46(+):15(-)	0.0054	94.11	-
Ur-5/$SI19_{460}$	BC_3F_2	61	9(R/+):3(R/–):3(S/+):1(S/–)	44(R/+):0(R/–):2(S/+):15(S/–)	54.5155	0.00	3.31

[1]The F_1 and F_2 populations were inoculated with *U. appendiculatus* pathotype 10 and the other populations with pathotype 6; both pathotypes were identified by Faleiro et al. (1999)
[2]Resistant plants (R), susceptible plants (S), presence of marker (+), absence of marker (–)
[3]Percent probability; 1 (one) degree of freedom was established in all Chi-square tests (χ^2)
[4]Genetic distance in centimorgans (cM) between molecular marker $SI19_{460}$ and rust resistance gene *Ur-5*; the calculated LOD score was 11.55

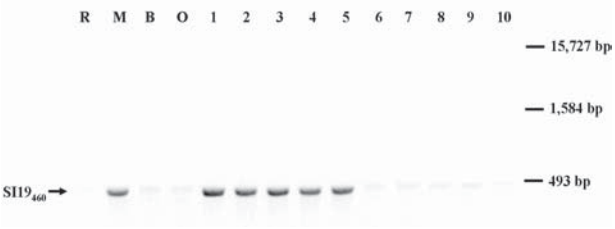

Figure 1. Electrophoretic analysis of DNA amplification products of common bean rust resistance sources with the SCAR marker $SI19_{460}$. Lanes are as follows: R, 'Rudá' (susceptible); M, 'Mexico 309' (gene *Ur-5*); B, 'Belmidak RR-3' (gene *Ur-11*); O, 'Ouro Negro' (gene *Ur-ON*); 1-5, BC_3F_2 (Rudá x Mexico 309) resistant plants; and 6-10, BC_3F_2 susceptible plants to *U. appendiculatus* pathotype 10. The arrow indicates the marker $SI19_{460}$, a DNA band with 460 base pairs (bp) linked in coupling phase at 3.31 centimorgans (cM) to rust resistance gene *Ur-5*

in coupling phase at 3.31 cM of the resistance locus with a LOD score of 11.55 (Table 1).

It was observed that SCAR marker $SI19_{460}$, developed from a RAPD marker associated to the resistance gene or gene cluster present in the rust-resistant bean line B-190 (gene *Ur-5*) (Haley et al. 1993), is also linked to the locus that confers resistance in cv. Mexico, the resistant donor parent of B-190. These molecular evidences give support to the idea that rust resistance in Mexico 309 is also governed by gene *Ur-5* (Haley et al. 1993). The same statement based on phenotypic data is found in the literature (Basset 2004) and was corroborated in the present study.

All BC_3F_2 (Rudá x Mexico 309) plants with the resistant phenotype, as well as two plants of this population that were considered susceptible presented the SCAR marker $SI19_{460}$ (Table 1). The selection efficiency estimate based on the presence of the dominant marker was therefore 91.32%.

No amplification products were observed when DNA samples of the rust resistance sources 'Ouro Negro' (gene *Ur-ON*) and 'Belmidak RR-3' (*Ur-11*), both resistant to the *U. apendiculatus* pathotypes 6 and 10, were amplified with marker $SI19_{460}$. In other words, this marker was able to discriminate the source carrying gene *Ur-5* in relation to those carrying the genes *Ur-ON* and *Ur-11* (Figure 1).

The data obtained in the present study showed that the use of marker $SI19_{460}$ is suitable for monitoring gene *Ur-5* during its introgression in cv. Rudá. In addition, this marker can be used for assisted selection of *Ur-5* during pyramiding with the genes *Ur-ON* and *Ur-11*. This strategy is currently used in our breeding program, which aims at developing commercial cultivars with a durable and wide resistance spectrum, adapted to Central Brazil.

Gene *Ur-ON* used to be the only *U. apendiculatus* resistance source in our breeding program. The RAPD marker $OPX11_{630}$ (Faleiro et al. 2000) and the SCAR markers $SF10_{1,072}$ and $SBA08_{530}$ were used for the indirect selection of *Ur-ON* (Corrêa et al. 2000). More recently, another rust resistance gene, *Ur-11*, was characterized and introgressed in the Rudá background (Souza et al. 2003). To assist the introgression of *Ur-11*, RAPD marker $OPAE19_{890}$ was validated in an F_2 population derived from the cross Rudá x Belmidak RR-3 (Alzate-Marin et al. 2004). Later on, $OPAE19_{890}$ was converted into a SCAR marker ($SAE19_{890}$) by Queiroz et al. (2004).

In the present study, it was confirmed that gene *Ur-5*, which has intra-allelic interaction of complete dominance, governs the rust resistance expressed by 'Mexico 309'. It was demonstrated that marker $SI19_{460}$ can be used for indirect selection of gene *Ur-5* during its individual or simultaneous introgression with the genes *Ur-ON* and *Ur-11* in the 'Rudá' genetic background.

ACKNOWLEDGEMENTS

The present study was financed by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Brazilian government. Thiago Lívio P. O. de Souza received an M.S. scholarship from CNPq.

Estudo de herança e validação de marcador molecular SCAR para resistência à ferrugem do feijoeiro comum

RESUMO - *A cultivar Mexico 309 apresenta um amplo espectro de resistência a vários patótipos do fungo Uromyces appendiculatus, agente causal da ferrugem do feijoeiro comum, provenientes dos estados de Minas Gerais e Goiás, Brasil Central. Assim, este trabalho teve como objetivos: (i) determinar a herança da resistência à ferrugem de Mexico 309 em*

populações segregantes obtidas do seu cruzamento com a cv. Rudá (cultivar comercial, suscetível à ferrugem); e (ii) validar o marcador molecular SCAR $SI19_{460}$ como ligado ao gene de resistência presente em Mexico 309. Os resultados mostraram que Mexico 309 possui um único gene dominante conferindo resistência ao U. appendiculatus. A análise com o marcador $SI19_{460}$ demonstrou que o mesmo está ligado, em aproximação, a 3,31 cM do gene de resistência de México 309 (gene Ur-5). Este marcador pode ser utilizado para a seleção indireta de Ur-5 durante o desenvolvimento de linhagens com background genético Rudá resistentes à ferrugem.

Palavras-chave: genes de resistência, *Phaseolus vulgaris*, seleção assistida por marcdores, *Uromyces appendiculatus*.

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