Crop Breeding and Applied Biotechnology 5:247-252, 2005
Brazilian Society of Plant Breeding. Printed in Brazil

Phenotypic and molecular characterization of cultivar BRSMG-Talismã regarding the principal common bean pathogens

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Received 17 January 2005

Accepted 21 May 2005

ABSTRACT - *Information about common bean cultivars recommended for commercial use in Brazil regarding the reaction to pathogens is essential for farmers besides furthering future breeding efforts. Our research evaluated the reactions of the common bean cultivar BRSMG-Talismã to several pathotypes evaluated of* Uromyces appendiculatus, Colletotrichum lindemuthianum *and* Phaeoisariopsis griseola. *The cultivar was further characterized through RAPD and SCAR molecular markers used in the marker-assisted selection (MAS) in the bean breeding program of BIOAGRO/UFV which deals with gene pyramiding of rust, anthracnose and angular leaf spot resistance genes. Results showed that BRSMG-Talismã is susceptible to different genotypes of the tested pathogens. All analyzed molecular markers were polymorphic, between BRSMG-Talismã and the resistance sources. Consequently, these markers can be used to monitor a simultaneous introgression of genes with a broad resistance spectrum into the genetic background of BRSMG-Talismã.*

Key words: Pre-breeding, MAS, rust, anthracnose, angular leaf spot.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) cultivars actually released in Brazil have to present a high spectrum of disease resistance, which is nationwide one of the main causes for the low productivity of this crop. This is especially true for small farms with low-technology plantations, but which play an important role; altogether, they account for the greatest fraction of the product for the domestic market supply (Borém and Carneiro 1998). Among the principal diseases of fungal origin that attack common bean and cause serious damage we find bean rust, anthracnose and angular leaf spot, incited by *Uromyces appendiculatus*, *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola*, respectively. In the producing regions, these pathogens occur principally where mild temperatures and dew are observed (Paula Júnior and Zambolim 1998).

The genetic control of these diseases through resistant cultivars is considered effective, safe, cheap and accessible for farmers at any economical level. Most of the common bean cultivars recommended for planting in Brazil have however turned out to be susceptible to the cited pathogens (Faleiro et al. 1996, Lanza et al. 1997, Faleiro et al. 2001).

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Cultivar BRSMG-Talismã is result of a recurrent selection program conducted at the Universidade Federal de Lavras (UFLA). The following parents compose the genealogy: BAT 477, IAPAR 14; FT 84-29, Jalo EEP, A 252; A 77, Ojo of Liebre; ESAL 645, Pintado and Carioca (Ramalho et al. 2002). Besides the "carioca" type grains that meet the market demands (cream-colored with light brown stripes), BRSMG-Talismã presents good culinary features such as a mean cooking time of 28.5 minutes, 9.8% soluble solids and 23.8% proteins (Abreu et al. 2004). The cultivar was recommended for the state of Minas Gerais in 2002 and for the state of Paraná in 2003 (Abreu et al. 2004) and could also be recommended for other regions of the Country. The field evaluations showed that the BRSMG-Talismã yield was in the mean 10.6% superior to the controls Carioca and Pérola (most planted cultivar in Brazil) (Ramalho et al. 2002). In relation to diseases, it presented good resistance to the pathotypes 65 and 89 of *C. lindemuthianum* when inoculated artificially under controlled conditions, besides an intermediary resistance to angular leaf spot and Common bean mosaic virus (CBMV) in field evaluations (Ramalho et al. 2002). In the case of rust, high susceptibility was observed in plantations of the 'Zona da Mata' in Minas Gerais.

Molecular markers are used as tool for assisted selection (MAS) in improvement programs whose objectives include disease resistance since they allow a monitoring of the individual or simultaneous (gene pyramiding) transference of genes of interest to adapted genotypes. Moreover, molecular markers can verify the presence of resistance alleles in commercial cultivars that had not been directly selected during the development of the cultivars due to, principally, the limitations of the conventional methodologies of resistance monitoring (Alzate-Marin et al. 2001).

The objective of this study was therefore the evaluation of the reaction of BRSMG-Talismã to the different pathotypes of *U. appendiculatus*, *C. lindemuthianum* and *P. griseola* in view of the large genetic variability of these fungi (Alzate-Marin et al. 2004a, b, Sartorato and Alzate-Marin 2004). The molecular characterization of this cultivar with RAPD (Random Amplified Polymorphic DNA) and SCAR (Sequence Characterized Amplified Region) markers identified earlier as linked to rust, anthracnose and angular leaf spot resistance genes was also realized. These markers are linked to genes that presents a broad spectrum against the pathotypes from the state of Minas Gerais and other regions of Brazil (Table1). They are already being used for MAS in the ongoing gene pyramiding program of resistance genes of the BIOAGRO/UFV.

MATERIAL AND METHODS

The seeds of BRSMG-Talismã and the cultivars used as rust, anthracnose and angular leaf spot-resistant and susceptible controls (Table2) were taken from the germplasm bank of the common bean improvement program of the BIOAGRO/UFV. The evaluation trials for the reaction of BRSMG-Talismã towards the fungi *U. appendiculatus*, *C. lindemuthianum* and *P. griseola* were realized under greenhouse conditions. Ten plants of each cultivar were inoculated with each of the distinct genotypes of the referred pathogens, which are maintained in the fungus collection of the BIOAGRO/UFV (Table2).

The inoculations with *U. appendiculatus* were realized according to Faleiro et al. (1996). Six grades of disease reaction were considered in the evaluation of the rust symptoms based on the scale proposed by Stavely et al. (1983). We used the methodology described by Lanza et al. (1997) for the *C. lindemuthianum* inoculations and the anthracnose symptoms were evaluated by a nine grade scale of reactions proposed by Rava et al. (1993). For the diagnosis of the reaction incited by the fungus *P. griseola*,

Table 1. Characteristics of the RAPD and SCAR markers linked to rust, anthracnose and angular leaf spot resistance genes

Marker	Cultivar/source	Disease	Gene	Distance cM[1]	TP°C[2]	Reference
OPX-11_{550}	Ouro Negro	Rust	*Ur-ON*	5,8 (A)	36	Faleiro et al. (2000)
SCAR- $F10_{1050}$	Ouro Negro	Rust	*Ur-ON*	6,9 (A)	65	Corrêa et al. (2000)
SCAR-$BA08_{560}$	Ouro Negro	Rust	*Ur-ON*	6,0 (A)	65	Corrêa et al. (2000)
SCAR-$I19_{460}$	México 309	Rust	*Ur-5*	0,0 (A)	53	Melotto and Kelly (1998)
SCAR-$AE19_{890}$	Belmidak RR3	Rust	*Ur-11*	1,0 (R)	64	Queiroz et al. (2004a)
SCAR-$Y20_{830}$	TO	Anthracnose	*Co-4*	0,0 (A)	65	Queiroz et al. (2004b)
OPAZ-20_{940}	AB 136	Anthracnose	*Co-6*	7,1 (A)	36	Alzate-Marin et al. (2000)
SCAR-$H13_{490}$	AND 277	Angular leaf spot	*Phg-1*	5,5 (A)	59	Queiroz et al. (2004c)

[1](A): marker linked in coupling phase to the resistance gene, (R): marker linked in repulsion phase. [2]Temperature of primer pairing

the first trifoliate leaf of the plants to be evaluated was inoculated (Faleiro et al. 2001). Angular leaf spot was evaluated by a nine-level scale based on Van Schoonhoven and Pastor-Corrales (1987).

The determination of the reaction grades to the three diseases was realized by a visual observation of the lesions on the inoculated leaves. All observed grades were recorded, listing the predominant grade first and then those in lower frequency (Table2). In the resistance/susceptibility diagnosis, the plants with grades 1 - 3 were considered resistant in all cases and those with grade four or higher susceptible; based on the three scales in use.

Leaves of the plants of BRSMG-Talismã and of all resistance sources to rust, anthracnose and angular leaf spot (Table1) and of the susceptible controls to the three diseases (Table2) were collected and maintained at -80 ºC for the posterior DNA extraction, which was realized based on the protocol described by Doyle and Doyle (1990). The DNA amplification by the RAPD technique, the electrophoresis in agarose gel and the picture documentation of the amplification products were realized according to the methodology used by Vasconcelos et al. (1996). In the evaluations with the SCAR technique, the procedures were the same as in the trials with the RAPD technique, except that the primer was substituted by 0.2 μM of each specific primer. The temperatures of specific pairing for each SCAR primer are described in Table 1.

RESULTS AND DISCUSSION

Results of the evaluation with *U. appendiculatus* showed that BRSMG-Talismã was susceptible to five of the 11 tested pathotypes. It is important to underline that this cultivar presented high grades of susceptibility to pathotypes 52 and "Coimbra" (Table2). A high incidence of rust in plantations of BRSMG-Talismã in the region 'Zona da Mata', Minas Gerais, has been observed under field conditions, which agrees with our results. The transference of resistance genes to this disease to the BRSMG-Talismã cultivar would therefore be important. To obtain a broad spectrum and durable resistance the simultaneous transference of different resistance genes (gene pyramiding) to the genetic background of interest is recommended. The rust-resistant control used in our study cultivar Ouro Negro (gene *Ur-ON*) was immune to eight (grade 1) and resistant to three (grade 2) of the analyzed pathotypes (Table2). Faleiro et al. (1996) had mentioned its importance as resistance source to rust in improvement programs. The cultivars México 309 (gene *Ur-5*) and Belmidak RR3 (gene *Ur-11*) are also worth mentioning; they performed very well when challenged with the pathotypes of *U. appendiculatus* tested in Minas Gerais and other states of Brazil (Faleiro et al. 1996, Santos and Rios 2000, Faleiro et al. 2001, Alzate-Marin et al. 2004a) as well as in the United States (Pastor-Corrales 2001).

In the evaluation with anthracnose, cultivar BRSMG-Talismã presented susceptibility to pathotypes 85, 1033, 2047, and 453 of *C. lindemuthianu.* A segregating reaction to pathotype 65 (resistant and susceptible plants) and resistance to other eight pathotypes were observed (Table 2). These results confirmed the incompatibility of pathotype 89, one of the most prevailing in Minas Gerais, with BRSMG-Talismã. This reaction had been observed earlier by Ramalho et al. (2002) when working on the development of this cultivar. Due to the susceptibility to the four cited pathotypes, the incorporation of genes that confer resistance on the cultivar would be important. The pathotypes could be multiplied in a large scale in the regions that cultivate BRSMG-Talismã, which would make the quick dissemination of the pathogen, a negative fact from the epidemiological point of view.

In relation to angular leaf spot, BRSMG-Talismã appeared susceptible to three of the seven analyzed *P. griseola* pathotypes: 63-19, 63-31 and 63-39. Segregating reactions (resistance and suscetibility) to the pathotypes 31-23 and 63-23 were observed (Table 2). These results explain the intermediary resistance reactions to angular leaf spot observed in this cultivar under field conditions (Ramalho et al. 2002). In this case as much as for rust and anthracnose, the transference of angular leaf spot-resistance genes to the BRSMG-Talismã gene background is also recommended.

The characteristics of the RAPD and SCAR markers linked to the rust, anthracnose and angular leaf spot resistance genes used in the molecular characterization of resistance of BRSMG-Talismã are resumed in Table 1. All tested markers were polymorphic, between BRSMG-Talismã and the deferent resistance sources to the three diseases, which have genes that have already been characterized and are being used in the ongoing gene pyramiding program of the BIOAGRO/UFV. This result indicates the absence of resistance alleles of these genes in cultivar BRSMG-Talismã (Alzate-Marin et al. 2001). Besides, it indicates that the markers would be useful for a possible monitoring of pyramiding of these genes in the genetic background of the this cultivar.

The results of these evaluations in the phenotypic and molecular range are not only highly important for producers, when choosing the genetic material for cultivation, but also as guidelines for improvement

programs that aim at the development of resistant cultivars to these diseases. The characterization of varieties with a great agronomical potential is useful in the process of choosing parents, a fundamental stage for the success of an improvement program.

Resistance to these three diseases could be transferred to BRSMG-Talismã by means of a program of crossing between this cultivar and isolines developed by the common bean improvement program of the BIOAGRO/UFV; besides having the genetic background of the cultivar Pérola ("carioca" type grains) they are dominant homozygous to the following resistance genes: *Ur-ON* (rust); *Co-04*, *Co-06* and *Co-10* (anthracnose); and *Phg-1* (angular leaf spot). Genetic F_1RC_3 seeds developed by the cited program could also be used as donor parents, once they individually carry the resistance genes to rust *Ur-11* and *Ur-5* and have the genetic background "carioca" of cultivar Rudá.

Table 2. Evaluation of the reaction of common bean cultivar BRSMG-Talismã and controls to the fungi *U. appendiculatus*, *C. lindemuthianum* and *P. griseola*, the causal agents of rust, anthracnose and angular leaf spot, respectively

Pathotypes		Cultivars[1]													
		BRSMG-Talismã		US Pinto 111[2]		Ouro Negro[3]		Rosinha[2]		G 2333[3]		Pérola[2]		AND 277[3]	
U. appendiculatus	32	4	S	6	S	1	R	-		-		-		-	
	45	1	R	6	S	1	R	-		-		-		-	
	46	4	S	6	S	1	R	-		-		-		-	
	47	2.1	R	6	S	2.3	R	-		-		-		-	
	49	4.1	S	6	S	1	R	-		-		-		-	
	50	1	R	6	S	1	R	-		-		-		-	
	52	5	S	6	S	2	R	-		-		-		-	
	54	2	R	6	S	1	R	-		-		-		-	
	58	1	R	6	S	1	R	-		-		-		-	
	59	1	R	6	S	1	R	-		-		-		-	
	"Coimbra"	6	S	6	S	2	R	-		-		-		-	
C. lindemuthianum	65	3.7	R	-		-		9	S	1.2	R	-		-	
	67	1	R	-		-		9	S	1	R	-		-	
	73	1	R	-		-		9	S	1.2	R	-		-	
	75	1	R	-		-		9	S	1.3	R	-		-	
	81	1	R	-		-		9	S	1	R	-		-	
	83	1.3	R	-		-		9.8	S	1	R	-		-	
	85	9	S	-		-		8.9	S	1	R	-		-	
	89	1	R	-		-		9.8	S	1	R	-		-	
	95	1	R	-		-		9	S	1	R	-		-	
	117	1	R	-		-		9.8	S	1	R	-		-	
	453	9	S	-		-		9	S	1	R	-		-	
	1033	9	S	-		-		9	S	1	R	-		-	
	2047	9	S	-		-		9	S	1	R	-		-	
P. griseola	31-17	1.2.3	R	-		-		-		-		9	S	1	R
	31-23	1.3.9	R	-		-		-		-		8.9	S	1	R
	31-39	1.2.3	R	-		-		-		-		9.8	S	1.2	R
	63-19	6.8.4	S	-		-		-		-		9	S	1.3	R
	63-23	1.3.6	R	-		-		-		-		9	S	1	R
	63-31	9.6.5	S	-		-		-		-		8.9	S	1	R
	63-39	9.8.7	S	-		-		-		-		9.8	S	1.2	R

[1]Resistant plants (R): predominance of grades 1 to 3 – absence of symptoms, necrotic spots without sporulation or lesions in a frequency below causing economical damage; susceptible plants (S): predominance of grade 4 or higher – necrotic spots with evident sporulation, lesions in a frequency causing economical damage or plant death; (-): data unavailable. Reaction grades displayed in increasing order of predominance
[2]Susceptible control
[3]Resistant control

Caracterização fenotípica e molecular do cultivar BRSMG-Talismã quanto à reação aos principais patógenos do feijoeiro

RESUMO - *Informações a respeito dos cultivares de feijoeiro comum recomendados para uso comercial no Brasil, no que se refere à reação a patógenos, são de grande importância não só para os produtores, mas também para orientar os futuros trabalhos de melhoramento. Assim, o objetivo deste trabalho foi avaliar a reação do cultivar BRSMG-Talismã frente a vários patótipos de* Uromyces appendiculatus, Colletotrichum lindemuthianum *e* Phaeoisariopsis griseola, *e caracterizá-lo com marcadores moleculares RAPD e SCAR que estão sendo usados para a seleção assistida (SAM) no programa de melhoramento do feijoeiro do BIOAGRO/UFV, que visa a piramidação de genes de resistência à ferrugem, antracnose e mancha angular. Os resultados demonstram que BRSMG-Talismã é suscetível a diferentes genótipos dos patógenos testados. Todos os marcadores analisados foram polimórficos entre BRSMG-Talismã e as fontes de resistência; consequentemente, estes poderão ser usados para monitorar a introgressão simultânea de genes com amplo espectro no background BRSMG-Talismã.*

Palavras-chave: Pré-melhoramento, SAM, ferrugem, antracnose, mancha angular.

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