



# Breeding for common bean (*Phaseolus vulgaris* L.) rust resistance in Brazil

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## ABSTRACT

Common bean is an economically, nutritionally, and socially important crop. It is grown in distinct regions and different seasons around the world by subsistence level farmers with low-technology input as well as by farmers that use high input technologies. One important factor that can limit the bean growing and drastically affect grain yields is the high number of destructive pathogens that attack and cause serious damage to the crop. Among them is bean rust, incited by the fungus *Uromyces appendiculatus*. This disease is distributed throughout the world, but it effectively causes major production problems in humid tropical and subtropical regions. In Brazil, rust causes major losses in south, southeast, and central regions of the country. Bean rust control by resistant cultivars is an easy and economical strategy to be used in association to other rust management practices. Pyramiding of different race-specific resistance genes in association with other genes conferring adult plant resistance, slow rusting, and reduced pustule size can prolong the lifespan of a common bean cultivar by creating a more durable resistance complex against the rust pathogen. This review manuscript presents an overview on bean rust and reports some breeding efforts aiming to develop rust resistant cultivars in Brazil.

**Key words:** *Phaseolus vulgaris*, *Uromyces appendiculatus*, gene pyramiding, host-pathogen interaction, marker-assisted selection, plant resistance

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most important legume directly used for human consumption worldwide. Among the five domesticated and grown species of the genus *Phaseolus*, a genus comprised of some 70 species (Freytag & Debouck, 2002), *P. vulgaris* accounts for more than 90% of the cultivated crop in the world (Singh et al., 1991a, 2001). Dry bean cultivars of the species *P. vulgaris* were grown and consumed on approximately 30 million hectares in about 120 countries during 2011 (faostat.fao.org). The social value of the common bean is extremely high to millions of people in many countries and most especially in developing countries of Latin America, and Eastern and Southern Africa (Pachico, 1989; Wortmann et al., 1998; Broughton et al., 2003). In Brazil, the main producer and consumer country (www.fao.org), dry beans are the main source of vegetable protein, minerals, and vitamins for a major proportion of the population. The set of essential amino acids present in dry bean seeds is complementary to that in grains of cereals such as rice (*Oryza sativa* L.). For this reason beans and rice are regularly consumed together by the Brazilians.

Some bean seed properties have been shown to contribute positively to major health issues, such as the

control of type II diabetes. Regular bean consumption also lowers glycemic and cholesterolemic indices and decreases the incidence of certain types of cancer (Andersen et al., 1984; Hangen & Bennink, 2003).

The attack of pests and pathogens is one of the main causes of yield and quality losses in the common bean crop worldwide (Stavelly & Pastor-Corrales, 1989). This is especially true for small farmers with low-technology inputs, which play an important role, as they account for the greatest fraction of the product for the world market supply. Among the most serious diseases that attack common bean we find bean rust, incited by the highly variable fungus *Uromyces appendiculatus* F. Strauss (syn. *U. phaseoli* G. Winter). This disease is distributed throughout the world, but it effectively causes major production problems in humid tropical and subtropical areas and periodic severe epidemics in humid temperate regions (Souza et al., 2008). Severe bean rust epidemics have been reported in Brazil and other Latin American countries as well as in Australia, China, the United States, and some areas of Europe (Stavelly & Pastor-Corrales, 1989).

According to Lindgren et al. (1995) a 1% increase in bean rust severity leads to a yield loss of approximately 19 kg/ha. The major losses in Brazil occur

in south, southeast and central areas, including the states of Paraná, Rio Grande do Sul, Santa Catarina, Minas Gerais, São Paulo, and Goiás (Souza et al., 2005a). Yield losses higher than 68% have been detected in the state of Minas Gerais located in the southeast region of the country (Vieira et al., 2005).

No single cost-effective or efficient control measure can be recommended to prevent rust infection in all cases or different regions worldwide. Disease management practices for bean rust control include crop rotation, soil incorporation of bean plant debris, planting within recommended dates, growing resistant cultivars, and timely spraying of fungicides (Mmbaga et al., 1996; Souza et al., 2008).

The use of plant resistance not only is harmless to the environment but also an economically sound strategy compared to chemical control. However, the wide variability of *U. appendiculatus* represents an obstacle to breeders aiming at the development of common bean cultivars with durable resistance to rust. The combination of different rust resistance (RR) genes in the same cultivar has been proposed as an important strategy for obtaining effective and durable genetic resistance to rust (Johnson, 1984; Stavely & Pastor-Corrales, 1989; Kelly et al., 1995; Souza et al., 2005b, 2007a). This can be accomplished by gene pyramiding, when distinct genes are combined in the same cultivar, or by using multilines, when different genes are transferred to different lines of the same cultivar. For gene pyramiding, knowledge about the inheritance and organization of the genes and also the use of proper selection tools are of paramount importance (Souza et al., 2008).

Molecular markers have been used to assist different steps of common bean breeding programs aimed at developing cultivars resistant to rust. Isozymes and DNA-based markers have been used to study the genetic diversity of the rust fungus (Lu & Groth, 1988; Linde et al., 1990a, 1990b; McCain et al., 1992; Groth et al., 1995; Maclean et al., 1995; Faleiro et al., 1998) and also for mapping and characterizing resistance genes to *U. appendiculatus* and to other important bean pathogens (Freyre et al., 1998; Miklas et al., 2002; Kelly et al., 2003; Miklas et al., 2006; Souza et al., 2008).

In this review we describe and discuss important aspects about the bean rust. In addition, we present common bean breeding initiatives aiming at the development of rust resistant cultivars in Brazil. We have focused mainly the breeding strategy adopted by the Instituto de Biotecnologia Aplicada à Agropecuária - Bioagro/Universidade Federal de Viçosa Common Bean Breeding Program, which is assisted by molecular markers. This program is the main bean breeding program aiming rust resistance conducted in Brazil. Its objectives include the genetic study of host-pathogen interaction, identification and validation of molecular markers linked to RR genes, and the effective use of these markers to develop commercial lines showing effective and durable resistance to rust.

## CHARACTERIZATION AND MAINTENANCE OF THE RUST PATHOGEN

### Differential cultivars for *U. appendiculatus*

During the "Bean Rust Workshop" (BRW), held in 1983, 35 researchers from different countries proposed a series of 20 cultivars as the international differential standard for *U. appendiculatus* (Stavely et al., 1983) (Table 1). In 1984, cv. 'Mountainer White Half Runner' was eliminated from this series due to its similarity with 'Kentucky Wonder 780' (Stavely, 1984a). Characterization of Brazilian isolates based on those 19 differential cultivars was accomplished by Mora-Nuñez et al. (1992), Santos & Rios (2000) and Souza et al. (2005a). In their work, Mora-Nuñez et al. (1992) concluded that eight out of the 19 cultivars - 'Kentucky Wonder 814', 'Early Gallatin', '51051', 'NEP 2', 'Ecuador 299', 'Olathe', 'Mexico 309' and 'Redlands Pioneer' - were sufficient to discriminate and classify isolates collected in Brazil. Using these eight cultivars, Faleiro et al. (1999a) characterized 13 races of this fungus in the Brazilian state of Minas Gerais.

In the Third BRW held in 2002, a new differential series was proposed for characterization of *U. appendiculatus* isolates (Steadman et al., 2002). This series contains six Andean and six Mesoamerican bean cultivars (Table 2). Cultivars 'Early Gallatin', 'Redlands Pioneer', 'Golden Gate Wax', 'Aurora', 'Mexico 309', 'Mexico 235' and Compuesto Negro Chimaltenango - CNC, which were proposed in the 1983 BRW, were maintained in the present differential series. Cultivars 'Montcalm', 'Pompadour Checa 50 - PC-50', 'PI 260418', 'Great Northern 1140' and 'PI 181996' were added to the series. The wide adoption of this system can contribute to the elaboration of an internationally standardized classification methodology, and facilitate the exchange of information, and the cooperative use of the results obtained by different research groups throughout the world.

### Single-pustule isolates

Obtaining single-pustule isolates from *U. appendiculatus* primary inoculum sampled across

**TABLE 1** - Series of common bean cultivars adopted as differentials for *Uromyces appendiculatus* at the 1983 Bean Rust International Workshop (Stavely et al., 1983)

Common Bean Cultivar	
1. U.S. 3	11. Ecuador 299
2. California Small White 643	12. Mexico 235
3. Pinto 650	13. Mexico 309
4. Kentucky Wonder 765	14. Brown Beauty
5. Kentucky Wonder 780	15. Olathe
6. Kentucky Wonder 814	16. AxS 37
7. Golden Gate Wax	17. NEP 2
8. Early Gallatin	18. Aurora
9. Mountaineer White Half Runner <sup>a</sup>	19. 51051

<sup>a</sup>Deleted of the list because of its similarity to 'Kentucky Wonder 780' (Stavely 1984a).

**TABLE 2** - Differential series and the binary system of nomenclature adopted at the 3<sup>rd</sup> Bean Rust International Workshop as international standard for classification of *Uromyces appendiculatus* physiological races (Steadman et al., 2002)

Binary System Value	Differential Cultivar	Resistance Gene	Gene Pool
1	Early Gallatin	<i>Ur-4</i>	
2	Redlands Pioneer	<i>Ur-13</i>	
4	Montcalm	<i>Ur-?</i> <sup>a</sup>	
8	PC-50	<i>Ur-9, Ur-12</i>	Andean
16	Golden Gate Wax	<i>Ur-6</i>	
32	PI 260418	<i>Ur-?</i>	
1	Great Northern 1140	<i>Ur-7</i>	
2	Aurora	<i>Ur-3</i>	
4	Mexico 309	<i>Ur-5</i>	
8	Mexico 235	<i>Ur-3</i> <sup>+</sup>	Mesoamerican
16	CNC	<i>Ur-?</i>	
32	PI 181996	<i>Ur-11</i>	

<sup>a</sup>*Ur-?* = unnamed gene.

important bean grower areas is an important initial step before the characterization and use of the pathogen for rust resistance screening. Single-pustule isolates are pure lines of the pathogen; specific genotypes of the fungus. They are essential in the study of host-pathogen interaction, host resistance, and pathogen specialization.

*U. appendiculatus* single-pustule isolates can be obtained from inoculum previously collected and maintained in fungal collections. But most often they are isolated from fungal spores collected from plant tissues infected under natural conditions in bean growing areas or experimental stations. For single-pustule isolation, a lower than usual inoculum concentration is used for inoculation in order to increase the chance of getting individual pustules. The spores collected in these single-pustules are multiplied in susceptible varieties for three or more consecutive cycles, using the regular inoculum concentration (Souza et al., 2007a). After this procedure, the uredospores are collected and stored under controlled conditions.

Although the fungus *U. appendiculatus* does not grow in artificial culture as it is an obligate parasite, viable spores can be preserved under laboratory conditions. Dry uredospores in plastic or glass tubes can be successfully maintained under dark conditions at 5±1°C and relative humidity <50% for at least one year. Alternatively, uredospores frozen at -80°C or submersed in liquid nitrogen can be stored for a longer period of time (Souza et al., 2008). Periodical inoculation of the preserved spores on susceptible common bean varieties is highly recommended in order to keep the inoculum virulence.

### Physiological races

Classification of *U. appendiculatus* into physiological races and the consequent knowledge of its virulence diversity is a basic step towards understanding the dynamics of the pathogen distribution and the development of resistant cultivars. In this step it is also possible to identify which pathogen isolates can be used by breeding programs to

monitor the introgression of resistance genes in commercial bean genetic backgrounds (Pastor-Corrales, 2001; Pastor-Corrales & Stavely, 2002; Souza et al., 2007a).

Steadman et al. (2002) proposed a binary nomenclature system for designation of the races in which the evaluation scale was divided in only two reaction degrees: resistant and susceptible. Each race is designated by two numbers separated by a hyphen. The first number is obtained by the sum of the binary values attributed to the susceptible Andean cultivars of the differential series. The second number is obtained by the sum of the binary values of the susceptible Mesoamerican cultivars (Table 2).

Characterization of *U. appendiculatus* isolates collected in the USA, South Africa, Honduras, Argentina and Mozambique has been accomplished based on the procedure proposed in the Third BRW (Steadman et al., 2002; Acevedo et al., 2004; Jochua et al., 2004). Souza et al. (2007a) reported the first work using the standard system for classification of *U. appendiculatus* physiological races in Brazil using *U. appendiculatus* single-pustule isolates obtained from the Bioagro/UFV fungal collection.

## RUST SCREENING

### *U. appendiculatus* inoculation

Inoculations of *U. appendiculatus* are often done when the primary leaves of the bean plants reach approximately 2/3 of their full development, about 10 days after sowing under greenhouse conditions (20±5°C). The standard concentration of inoculum is 2.0×10<sup>4</sup> uredospores/mL of distilled water containing 0.05% Tween-20. The inoculum solution can be applied on both leaf surfaces with a brush or sprayed using a manual atomizer (e.g., atomizer De Vilbiss n° 15) adapted to an electric compressor. After inoculation the plants are transferred to a mist chamber (20±1°C and relative humidity >95%) where they are kept for approximately 48 h under a 12-hour light regime. In order to avoid contamination, plants inoculated with different isolates are kept in separate compartments of

the chamber. After this period the plants are transferred to a greenhouse (20±5°C), where they are kept until symptom evaluation, about 14 days after inoculation (Carrijo et al., 1980; Souza et al., 2005a, 2007a).

The inoculation can also be conducted using bean plant excised leaves, as reported by Souza et al. (2009a). In this alternative method, after inoculation, each leaf is placed in a Petri dish (90×15 mm) on a sterile filter paper previously moistened with 3.0 mL distilled water. The dishes are incubated in a BOD at 20°C, under a 12-hour light regime. Each filter paper is moistened again with 1.5 mL distilled water every three-days until disease symptom evaluation, about 10 days after inoculation.

Both inoculation methods are efficient for evaluating the reaction of the common bean to *U. appendiculatus*. The only differences observed on the results by the two methods is that the number of pustules is higher in the conventional method and disease symptoms appear earlier in the alternative method (Souza et al., 2009a). It is suggested that the conventional inoculation method is more appropriate for spore multiplication and the excised leaf method can be used in the cases where the same plant needs to be assayed several times. Another advantage of the alternative procedure refers to costs and safety; the whole method can be conducted in the laboratory without the need of exposing other plants to the pathogen. In addition, the tested plants are still able to grow and produce seeds.

### Rust reaction evaluation

The fungus *U. appendiculatus* may infect leaves, pods, and, rarely, stems and branches. Symptoms usually appear first on the lower leaf surface as minute, whitish slightly raised spots about five or six days after inoculation. These spots enlarge to form mature reddish brown uredia which rupture the epidermis about two days later. Sporulation begins and the uredia may attain a diameter of 1-2 mm about 8-12 days after inoculation. In some cases, secondary and tertiary uredia develop around the perimeter of these primary uredia. The entire infection cycle occurs within approximately 10-17 days (Stavely & Pastor-Corrales, 1989; Souza et al., 2008).

The reaction degrees to the disease are usually determined based on a scale of six infection degrees: 1 - no pustules (immunity); 2 - necrotic spots without sporulation; 3 - pustules undergoing sporulation with a diameter of <300 µm; 4 - pustules undergoing sporulation with a diameter ranging from 300 µm to 499 µm; 5 - pustules undergoing sporulation with a diameter ranging from 500 µm to 800 µm; and 6 - pustules undergoing sporulation with a diameter of >800 µm (Stavely et al., 1983).

The infection degrees are determined approximately about 10-17 days after inoculation when up to 50% of the pustules are sporulating. The lesions in both surfaces of the primary leaves should be determined visually by at least two evaluators. The cultivars that predominantly presented degrees 3 or lower were classified as resistant,

whereas those with predominant degrees 4 or higher are considered to be susceptible.

### PATHOSYSTEM *P. vulgaris*-*U. appendiculatus*

#### Host-pathogen coevolution

Allozymes studies, variation patterns of the common bean seed protein phaseolin, and morphological evidences revealed the existence of a Mesoamerican and an Andean gene pool in common bean (Gepts et al., 1986; Singh et al., 1991a, 1991b). The Andean cultivars originated in the Andean region of South America, while the Mesoamerican beans were domesticated from wild populations in Mexico and the rest of Central America.

Using phenotypic (virulence diversity) and genotypic (Random Amplified Polymorphic DNA - RAPD markers) analyses of 90 *U. appendiculatus* isolates from thirteen Latin American countries, Araya et al. (2004) were able to divide them into two major groups, namely the Andean and the Mesoamerican, and one intermediate group. In general, Andean and Mesoamerican isolates showed virulence specificity to landraces belonging to their respective gene pools. However, the Andean isolates tended to display greater geographic specificity than the Mesoamerican isolates. This phenomenon, previously observed by Sandlin et al. (1999), suggests a parallel evolution in the bean rust pathosystem. The intermediate virulence group of *U. appendiculatus* races, observed by Braithwaite et al. (1994), Maclean et al. (1995), Sandlin et al. (1999), and Araya et al. (2004), provide evidence of a transition area between these two gene pools in both the common bean host and the rust pathogen. It is therefore possible that ongoing adaptation between pathogen and host will eventually include isolates from the intermediate group into the other two major groups (Araya et al., 2004).

The virulence pattern analysis of 41 *U. appendiculatus* isolates from Brazil demonstrated they were compatible with both Mesoamerican and Andean common bean cultivars (Souza et al., 2005a), suggesting that these isolates belong to the intermediate virulence group.

#### Genetics of the rust resistance in common bean

Resistance to bean rust is mainly controlled by major single dominant genes (Augustin et al., 1972; Ballantyne, 1978; Christ & Groth, 1982a; Sayler et al., 1995; Corrêa et al., 2000; Faleiro et al., 2000a, 2000b; Alzate-Marin et al., 2004; Souza et al., 2007b, 2007c). However, it can be also controlled by single recessive genes (Zaiter et al., 1989), two genes (Finke et al., 1986), two complementary dominant genes (Grafton et al., 1985), or by many genes with minor effect (Edington et al., 1994). The gene-to-gene relationship proposed by Flor (1971) has been shown to occur in the *P. vulgaris*-*U. appendiculatus* interaction (Christ & Groth, 1982a, 1982b). Resistance genes effective against multiple races of the pathogen can also be organized in clusters or complex loci (Stavely, 1984b; Stavely & Grafton, 1985).

To date at least 14 major dominant RR genes have been identified (genes *Ur-1* to *Ur-14*), which are described in Table 3. These genes are named according to a nomenclature proposed by Kelly et al. (1996). In addition to these 14 genes, other important unnamed RR genes have also been identified, e.g. those present in 'Montcalm' (McClellan & Myers, 1990), 'BAC6' (Jung et al., 1996), 'Dorado' (Miklas et al., 2000, 2002), 'CNC' (Rasmussen et al., 2002), and 'PI 260418' (Pastor-Corrales, 2005; Pastor-Corrales et al., 2008) (see Table 3).

The proper characterization of new RR genes from Mesoamerican and Andean gene pools is essential for the future of the common bean breeding aiming rust resistance. It will broaden the spectra of the RR genes presently used (Liebenberg et al., 2006; Pastor-Corrales et al., 2008).

### MAPPING OF RESISTANCE GENES

Several RAPD markers associated with genes conferring resistance to rust in common bean have been identified, as described in Table 3. Many Sequence Characterized Amplified Region - SCAR markers have been developed to increase the reproducibility of the RAPD markers (Table 3). These molecular markers have been used for mapping *Ur* genes in the integrated common bean map (Freyre et al., 1998; Miklas et al., 2002; Kelly et al., 2003; Miklas et al., 2006).

The groups of Mesoamerican genes *Ur-5/Ur-Dorado53/Ur-14* and *Ur-3/Ur-7/Ur-11/Ur-Dorado108/Ur-BAC6* have been mapped in linkage groups (LG) B4 and B11, respectively. *Ur-3* and *Ur-11*, and also *Ur-Dorado108* map to the end of LG B11, next to the *Co-2* locus, which is related to resistance to anthracnose. *Ur-BAC6* is located near to the *Ur-7* locus, and they do not appear to be close to *Ur-Dorado108*, *Ur-3*, and *Ur-11*. The Andean genes *Ur-4*, *Ur-6*, *Ur-9*, *Ur-12*, and *Ur-13* were mapped to LG B6, B11, B1, B7, and B8, respectively (Miklas et al., 2002; Kelly et al., 2003; Miklas et al., 2006; Wright et al., 2008). Park et al. (2008) observed a possible allelic relationship between *Ur-7* present in Mesoamerican cultivar 'Great Northern 1140' and *Ur-6* present in Andean cultivar 'Olathe', based on the fact that the band generated by SCAR AD12 linked to *Ur-7* was also present in cultivar 'Olathe'.

Clustering is also observed between RR genes and those conferring resistance to anthracnose (*Co*) and Bean Common Mosaic Virus - BCMV (Miklas et al., 2006). For instance, the Andean RR gene *Ur-9* and the anthracnose resistance gene *Co-1* co-localize on LG B1 (Kelly & Vallejo, 2004; Miklas et al., 2006). The Mesoamerican genes *Ur-5* and *Co-3/Co-9*, and genes *Ur-14* and *Co-10* both from cultivar 'Ouro Negro' co-localize on LG B4 (Faleiro et al., 2000b; Alzate-Marin et al., 2003), and *Ur-3* co-localize with *Co-2* on LG B11, suggesting that these genes derived from common ancestral gene sequences

(Geffroy et al., 1999; Faleiro et al., 2000b, 2003; Miklas et al., 2006). Awale et al. (2008) show that SCAR SQ4 linked to the *Co-2* anthracnose resistance gene is closely linked to *Ur-11*.

The linkage groups designated as B1-to-B11 in the BJ common bean core map correspond to the chromosomes 1-to-11, respectively (Pedrosa et al., 2003, 2006, 2008).

## DEVELOPMENT OF COMMON BEAN CULTIVARS RESISTANT TO RUST

### Breeding philosophy and strategy

The use of resistant cultivars is certainly the main component of the integrated bean rust management. Pyramiding of resistance genes from both Andean and Mesoamerican gene pools is an important strategy for developing complementary and durable resistance to a large number of *U. appendiculatus* races (Stavelly & Pastor-Corrales, 1989; Pastor-Corrales & Stavelly, 2002; Araya et al., 2004). The large number of virulence patterns of *U. appendiculatus*, some of which are unique to certain countries, requires the use of specific resistance genes in different regions (Ballantyne, 1978; Araya et al., 2004; Souza et al., 2005a; Liebenberg et al., 2006; Acevedo et al., 2008; Alleyne et al., 2008).

Gene pyramiding using only conventional breeding methods has not been effective mainly due to the difficulties in selecting genotypes harboring different resistance genes which demand multiple or serial inoculations of the same plant or population (Michelmore, 1995). This limitation affects the breeding process as a whole and also decreases the accuracy and efficiency of the selection process (Bigirimana & Höfte, 2001; Souza et al., 2005c). Epistatic interactions between different resistance genes can also affect the selection process (Singh et al., 2001).

These limitations can be overcome by the use of molecular markers linked to the resistance genes. With the use of molecular markers not only the multiple and sequential inoculations can be avoided but also the confounding effect of potential epistatic interactions among the different resistance genes present in the same genetic background (Michelmore, 1995; Bigirimana & Höfte, 2001; Singh et al., 2001; Toenniessen et al., 2003). However, for each resistance allele a specific marker or markers need to be identified. The use of flanking markers tightly linked to the locus of interest makes selection even more robust (Faleiro et al., 2003).

Molecular markers can also be used to accelerate the recovery of the recurrent parent's genome in backcross breeding programs. Simulation studies and real data indicate that only three or four backcrosses are necessary to recover the recurrent parent's genome when molecular markers are used (Openshaw et al., 1994; Faleiro et al., 2004; Ragagnin et al., 2009; Souza et al., 2009b). Separate backcross programs assisted by molecular marker fingerprinting can

**TABLE 3** - Major common bean rust resistance genes and linked molecular markers

Resistance Gene	Gene Pool <sup>b</sup>	Cultivar Source	LG <sup>d</sup>	Molecular Marker	Note
<i>Ur-1</i>	MA	B 1627 (Gallaroy Genotype I)	?	-	Identified by Ballantyne (1978). ‘Gallaroy’ derives from ‘643’ x ‘Sanilac’. <i>Ur-1=Ur-A</i> .
<i>Ur-2</i>	MA	B2090 (Gallaroy Genotype II)	?	-	Identified by Ballantyne (1978). ‘Gallaroy’ derives from ‘643’ x ‘Sanilac’. <i>Ur-2=Ur-B</i> .
<i>Ur-2</i> <sup>2</sup>	MA	B2055 and AxS 37	?	-	‘B2055’ possesses only the gene <i>Ur-E</i> derived from ‘AxS 37’ (‘Actopan’ x ‘Sanilac Selection 37’, with genes <i>Ur-E</i> and <i>Ur-F</i> ). <i>Ur-B</i> and <i>Ur-E</i> are allelic (Ballantyne, 1978).
<i>Ur-3</i>	MA	Aurora <sup>c</sup>	B11	-	Identified by Ballantyne (1978). ‘Aurora’ possesses two linked genes ( <i>Ur-M</i> and <i>Ur-N</i> ); <i>Ur-M=Ur-3</i> .
<i>Ur-3</i>	MA	NEP 2	B11	RAPD-K14 <sub>620</sub> (Haley et al., 1994) and SCAR-K14 <sub>620</sub> (Nemchinova and Stavely, 1998) – 2.2 cM/Coupling	‘NEP 2’ possesses the genes <i>Ur-F</i> , <i>Ur-I</i> , <i>Ur-J</i> and <i>Ur-K</i> . <i>Ur-J</i> is allelic or closely linked in repulsion phase to gene <i>Ur-H</i> of ‘Cornell 49242’. Gene <i>Ur-I</i> is allelic to <i>Ur-3</i> (Ballantyne, 1978).
<i>Ur-3</i> <sup>+</sup>	MA	Mexico 235 <sup>c</sup>	B11	-	It is distinct from the other <i>Ur-3</i> sources (‘Aurora’, ‘Nep-2’, and ‘51051’) because it presents slightly different reaction profile across a differential set of <i>U. appendiculatus</i> races (Miklas et al., 2002).
<i>Ur-4</i>	A	Early Gallatin <sup>c</sup>	B6	RAPD-A14 <sub>1,100</sub> – 0.0 cM/Coupling (Miklas et al., 1993), and SCAR-A14 <sub>1079/800</sub> – 0.0 cM/Codominant (Mienie et al., 2004)	Identified by Ballantyne (1978). <i>Ur-4=Ur-C</i> .
<i>Ur-5</i>	MA	Mexico 309 <sup>c</sup> and B-190	B4	RAPD-F10 <sub>970</sub> – 2.1 cM/Coupling (Haley et al., 1993); RAPD-I19 <sub>460</sub> (Haley et al., 1993) and SCAR-I19 <sub>460</sub> (Melloto and Kelly, 1998) – 0.0 cM/Coupling; SCAR-I19 <sub>460</sub> – 3.3 cM/Coupling (Souza et al., 2007b)	Block of eight tightly linked rust resistance genes identified by Stavely (1984a).
<i>Ur-6</i>	A	Golden Gate Wax <sup>c</sup> and Olathe	B11	RAPD-BC06 <sub>308</sub> and SCAR-BC06 <sub>308</sub> – 1.3 cM/Coupling (Park et al., 2003a, 2004); RAPD-AG15 <sub>300</sub> – 2.0 cM/ Coupling, and RAPD-AY15 <sub>200</sub> – 7.7 cM/ Repulsion (Park et al., 2003a, 2004)	Identified by Ballantyne (1978) and Grafton et al. (1985). <i>Ur-6=Ur-G</i> .
<i>Ur-7</i>	MA	Great Northern 1140 <sup>c</sup>	B11	RAPD-AD12 <sub>537</sub> and SCAR-AD12 <sub>537</sub> – 0.0 cM/Coupling; RAPD-AA11 <sub>500</sub> – 0.0 cM/Coupling (Park et al., 1999a, 2003b); RAPD-AF17 <sub>900</sub> – 0.0 cM/Coupling, RAPDAB16 <sub>850</sub> – 2.2 cM/Coupling, RAPD-AD9 <sub>550</sub> – 2.2 cM/Coupling, and RAPD-AB18 <sub>650</sub> – 2.4 cM/Repulsion (Park et al., 1999a)	Identified by Augustin et al. (1972). Also found in cultivar ‘Pinto US-5’.
<i>Ur-8</i>	A	U.S. 3	?	-	Identified by Christ and Groth (1982a, b).
<i>Ur-9</i>	A	PC-50 <sup>c</sup>	B1	RAPD-J13 <sub>1,100</sub> – 5.0 cM/Coupling (Jung et al., 1998); RAPD-A04 <sub>1,050</sub> – 8.6 cM/Coupling (Park et al., 1999b)	Identified by Finke et al. (1986).
<i>Ur-10</i>	A/MA	Cape and Resisto	?	-	Identified by Webster and Ainsworth (1988).

...cont.

Resistance Gene	Gene Pool <sup>b</sup>	Cultivar Source	LG <sup>d</sup>	Molecular Marker	Note
<i>Ur-11</i>	MA	PI 181996 <sup>c</sup>	B11	RAPD-AC20 <sub>490</sub> – 0.0 cM/Coupling, and RAPD-AE19 <sub>890</sub> – 6.2 cM/Repulsion (Johnson et al., 1995); RAPD-AE19 <sub>890</sub> (Souza et al., 2002) and SCAR-AE19 <sub>890</sub> (Queiroz et al., 2004; Liebenberg et al., 2008) – 1.0 cM/Repulsion; RAPD-GT02 <sub>450</sub> and SCAR-GT02 <sub>450</sub> – 0.0 and 5.4 cM/Coupling (Boone et al., 1999)	Identified by Stavely (1998) as <i>Ur-3</i> <sup>2</sup> . Tightly linked to <i>Ur-3</i> .
<i>Ur-12</i>	A	PC-50 <sup>c</sup>	B7	RAPD-O13 <sub>1350</sub> – 34.6 cM/Coupling (Jung et al., 1998)	Identified by Jung et al. (1998). Conditions adult plant resistance (APR).
<i>Ur-13</i>	A	Kranskop	B8	SCAR-KB126 <sub>430/405</sub> – 1.6 cM/Codominant, SCAR-KB85 <sub>310/288</sub> – 9.2 cM/Codominant, and SCAR-KB4 <sub>250/186</sub> – 13.8 cM/Codominant (Mienie et al., 2005)	Identified by Liebenberg and Pretorius (2004). ‘Kranskop’ shares an ancestor with ‘Redlands Pioneer’ (Liebenberg et al., 2006).
<i>Ur-13</i>	A/MA(?)	Redlands Pioneer <sup>c</sup>	B8	-	Described by Liebenberg and Pretorius (2004). Although ‘Redlands Pioneer’ has been considered as an Andean cultivar (Steadman et al., 2002), <i>Ur-13</i> appears to be of Mesoamerican origin (Liebenberg et al., 2006).
<i>Ur-14</i>	MA	Ouro Negro	B4	RAPD-BA08 <sub>530</sub> and SCAR-BA08 <sub>530</sub> – 6.0 cM/Coupling, RAPD-F10 <sub>1,072</sub> and SCAR-F10 <sub>1,072</sub> – 7.0 cM/Coupling (Corrêa et al., 2000; Faleiro et al., 2000a); RAPD-X11 <sub>550</sub> – 5.8 cM/Coupling (Faleiro et al., 2000a)	Identified by Faleiro et al. (2000a, b) and fully characterized by Souza et al. (2011). It has shown a wide resistance spectrum in Brazil (Faleiro et al., 1999a) and USA (Alzate-Marin et al., 2004). It was temporarily named as <i>Ur-OuroNegro</i> or <i>Ur-ON</i> . It is the main rust resistance source used in Brazil.
<i>Ur-?</i> <sup>a</sup>	A	PI 260418 <sup>c</sup>	?	Two flanking Simple Sequence Repeat - SSR markers - 20.0 cM of genetic interval/ /Coupling (Pastor-Corrales et al., 2008)	Important Andean source from Bolivia (Pastor-Corrales, 2005).
<i>Ur-?</i>	A	Montcalm <sup>c</sup>	?	-	Pedigree: ‘Great Northern #1’ × ‘Dark Bed Kidney’ (McClellan and Myers, 1990).
<i>Ur-?</i>	MA	CNC <sup>c</sup>	?	-	Composite of Guatemalan black beans (McClellan and Myers, 1990). One single gene conferring resistance to race 49 was detected by Rasmussen et al. (2002). Confers resistance to Andean <i>U. appendiculatus</i> races (Sandlin et al., 1999).
<i>Ur-?</i>	MA	Dorado (DOR 346)	B4	-	Reported by Miklas et al. (2000). Temporarily named as <i>Ur-Dorado108</i> (Miklas et al., 2002).
<i>Ur-?</i>	MA	Dorado (DOR 346)	B11	-	Reported by Miklas et al. (2000). Temporarily named as <i>Ur-Dorado53</i> (Miklas et al., 2002).
<i>Ur-?</i>	MA	BAC6	B11	RAPD-AJ16 <sub>250</sub> – 12.5 cM/Coupling (Jung et al., 1996)	Described by Jung et al. (1996). Temporarily named as <i>Ur-BAC6</i> (Miklas et al., 2002).

<sup>a</sup>*Ur-?* = unnamed gene.

<sup>b</sup>Andean (A) and Mesoamerican (MA) *P. vulgaris* gene pools.

<sup>c</sup>Differential cultivar for *U. appendiculatus* (Steadman et al., 2002).

<sup>d</sup>The linkage groups (LG) designated as B1-to-B11 in the BJ (‘BAT 93’ × ‘Jalo EEP 558’) common bean core map (Freyre et al., 1998; Miklas et al., 2002; Kelly et al., 2003; Miklas et al., 2006) correspond to the *P. vulgaris* chromosomes 1-to-11, respectively (Pedrosa et al., 2003, 2006, 2008). ? = information not available.

be used for the individual introgression of resistance genes in commercial lines. This strategy can be the initial step for pyramiding of RR alleles (Ragagnin et al., 2009; Souza et al., 2009b).

Experimental evidence demonstrates that gene pyramiding confers more effective resistance to the host plant than that conferred by the sum of the resistance present in the progenitor plants (Yoshimura et al., 1995; Huang et al., 1997; Singh et al., 2001). According to Schafer & Roelfs (1985), the probability that a pathogen will overcome a gene pyramid of four to six genes is extremely low. In order for this to happen, independent mutations in the pathogen genome must occur and they should be combined in the same genetic background, or they could occur simultaneously or sequentially in the genome of a specific pathogen isolate. Nelson (1979) argues that resistance resulting from the partial action of several resistance genes exerts a low selection pressure on the pathogen and for this reason it tends to last for a long period of time. Although this concept is not fully accepted, there are experimental data supporting the existence of partial effects of different resistance genes in some pathosystems (Brondy et al., 1986; Pedersen & Leath, 1988). According to the theory presented the duration of resistance will depend on the number of genes to be overcome by the pathogen.

Epidemiology data also support the use of gene pyramiding as an effective strategy for disease control. According to Thrall & Burdon (2003), there is an inverse correlation between pathogen fitness, as measured by the number of spores produced, and the number of avirulence genes present in its genome. The authors observed that the pathogen populations which were able to infect a greater number of host populations were less aggressive than pathogen populations which were able to infect a lower number of host populations. This indicates that the inactivation of several avirulence genes in the pathogen compromises its adaptability. This is a positive aspect from the epidemiological perspective because it indicates that gene pyramiding can potentially keep the disease below an economical damage level and also prevent its fast dissemination.

The main steps of a Marker-Assisted Selection - MAS gene pyramiding breeding program aiming at disease resistance are: (i) identification of the most prevalent and virulent races of the pathogen in the region of interest and characterization of the most promising resistance sources for that region; (ii) determination of the disease resistance inheritance mode by crossing the resistance sources and the susceptible cultivar; (iii) identification of molecular markers tightly linked to the various disease resistance alleles; (iv) development of lines harboring the R genes and the molecular markers of interest; this process is often done by backcrossing; (v) identification of markers that can specifically identify the resistance alleles to avoid false positives; and (vi) pyramiding of resistance alleles by intercrossing the lines obtained. During this process, the

following activities must also be considered: (i) continuous characterization of the variability of the pathogen and the host; (ii) characterization and introduction of new resistance sources in the breeding program; and (iii) identification of molecular markers linked to the resistance genes present in the new resistance sources (Alzate-Marin et al., 2005; Souza et al., 2008).

A possible limitation of the presented breeding strategy which is based on backcrosses to develop lines harboring single R genes followed by intercrosses between these lines aiming to pyramid different R alleles in a same genetic background is the time spent, even using MAS to accelerate this process. The developed advanced lines should present a wide and durable resistance spectrum to the target disease but probably with some yield penalty in relation to modern cultivars.

In Brazil, the Bioagro/UFV Common Bean Breeding Program has used a molecular fingerprinting approach based on the RAPD-PCR technique to accelerate the development of common bean lines resistant to rust, anthracnose and angular leaf spot. Molecular marker-assisted selection has been also used to combine the different resistance genes in commercial genetic backgrounds (Faleiro et al., 2004; Ragagnin et al., 2009; Souza et al., 2009b).

In the specific case of rust, the breeding program conducted at Bioagro/UFV is also using the MAS for developing lines with specific RR genes (*Ur-5*, *Ur-11*, and *Ur-14*) aiming at their introgression and pyramiding in Brazilian modern cultivars (Alzate-Marin et al., 2004; Faleiro et al., 2004; Souza et al., 2005c, 2007b, 2009b; Ragagnin et al., 2009). Initially, the gene *Ur-14* has been used as the only source for resistance to *U. appendiculatus* in that breeding program. The RAPD marker X11 (Faleiro et al., 2000a) and the SCAR markers F10 and BA08 have been used for the indirect selection of *Ur-ON* and its introgression in the genetic background 'Rudá' (Corrêa et al., 2000). Later on, another RR gene was characterized, the gene *Ur-11*, which was then also introgressed into 'Rudá' (Souza et al., 2002). To assist the selection of *Ur-11*, the RAPD marker AE19 was validated in a F<sub>2</sub> population derived from the cross 'Rudá' × 'Belmidak RR-3' (Alzate-Marin et al., 2004). Then, this marker was converted into a SCAR marker (SCAR AE19) by Queiroz et al., (2004). In the study of Souza et al., (2007b) the SCAR marker SI19 was validated as linked to gene *Ur-5* from cultivar 'Mexico 309'. It was also verified that this marker can be used for the indirect selection of gene *Ur-5* in the presence of genes *Ur-11* and *Ur-14*.

Using separate backcross programs and gene pyramiding approaches assisted by molecular markers, advanced "carioca-type" common bean lines with wide and potentially durable resistance to *U. appendiculatus* were developed. These lines show the same resistance spectra present in the donor parents and no yield penalty in relation to the commercial cultivars used as recurrent parents (Souza et al., 2005b, 2009b).



### Evaluation of agronomical performance

The yield components of the common bean resistant lines regularly developed in Brazil are initially evaluated under field conditions by their respective owner breeding programs, in at least two distinct growing seasons. The evaluation assays include resistant control lines and high yielding reference cultivars widely planted across the country. Randomized complete block or lattice designs with three repetitions (plots) are commonly used. Each plot consists of two-four rows each 2.0-4.0 m long, spaced by 0.5 m, with about 12-15 seeds per meter. Fertilizer application is done according to the recommendations for dry bean crop in Brazil. Harvest of the whole plot is regularly done manually. The yield components usually evaluated are: grain yield, determined for each plot and expressed as kg/ha; plant height, the mean height of the plants at stage R8 in each plot; mean number of seeds per pod in each plot; mean number of pods per plant in each plot.

The superior lines selected based on molecular markers and yield components are also tested against the pathogen under greenhouse (specific races) and field (natural infection) conditions. The advanced lines with resistance spectra similar to those of the donor parents and as productive as the recurrent parents and control cultivars are evaluated in different regions by a Brazilian bean assay network coordinated by Embrapa Arroz e Feijão, during at least two years and three growing seasons. If the superior agronomic performance of these lines is confirmed they can be recommended as new cultivars. At the moment, the advanced “carioca-type” common bean lines harboring three different rust resistance genes developed by Bioagro/UFV are being already used as genitors in crossing blocks of the breeding program conducted by Embrapa Arroz e Feijão and research partners. The main objective is to add this resistance gene pyramid to “carioca” type high yielding and modern lines and also transfer it to other bean market classes.

### PERSPECTIVES

Yield and disease resistance are the main foci of common bean breeders throughout the world. The large number of pathogens affecting this crop is one of the main causes of yield losses observed in many bean growing regions.

The concept of gene pyramiding assisted by molecular markers has been successfully used in Brazil to create common bean advanced lines with wide and durable resistance to pathogens, including resistance to rust. Separate backcross programs assisted by molecular markers have allowed the individual introgression of resistance genes in recurrent cultivars after only three or four backcross cycles. The advanced lines obtained have shown the same resistance spectra present in the donor parents. Evaluation of yield components of the advanced lines has demonstrated that no yield penalty in relation to recurrent cultivars was

observed after the pyramiding process (Faleiro et al., 2004; Ragagnin et al., 2009; Souza et al., 2005b, 2009b).

To be effective, the gene pyramiding strategy must be a continuous effort. Permanent monitoring for the presence of new virulent races in the field and search for new resistance sources are inherent steps in this breeding strategy.

The use of recurrent selection assisted by molecular markers as a breeding strategy to combine different target alleles into a same progeny also presenting other desirable agronomic traits and still use this and other superior progenies to form a promissory new base population has already been adopted in common bean (Souza et al., 2012). This strategy could be very useful to develop high agronomic performing and modern common bean lines harboring multiple R genes.

A significant variability of *U. appendiculatus* isolates has been observed in Brazil (Faleiro et al., 1998, 1999a; Souza et al., 2005a, 2007a). Isolates collected by these cited authors have been used to support bean breeding programs of different research institutions across the country. As a result of this work, it was possible to select resistant cultivars, like ‘Ouro Negro’ (Faleiro et al., 2000b; Vieira et al., 2005), and to develop other cultivars and advanced lines harboring rust resistance genes (Faleiro et al., 2004; Ragagnin et al., 2009; Souza et al., 2009b). Periodical collection, maintenance and classification of *U. appendiculatus* isolates are essential steps of the bean breeding program as these steps inform about the variability of the pathogen in a growing area over time. Thus new sources of resistance can be identified and validated with adequate monitoring and identification of the most frequent races of the pathogen (Souza et al., 2007a).

Frequent monitoring of prevalent races of pathogens presenting high variability is very helpful in studies involving genetic dynamics, as the geographical origin of the isolates may provide indirect evidences of their diversity, because of their adaptation to different environment conditions.

In this way, a consistent effort of monitoring and classification of *U. appendiculatus* isolates has been established in Minas Gerais, Brazil, to attend the breeding programs of the “Minas Gerais Common Bean Covenant”. This agreement includes four institutions: UFV, Universidade Federal de Lavras - UFLA, Empresa de Pesquisa Agropecuária de Minas Gerais - Epamig, and Embrapa Arroz e Feijão. This effort also includes analyses of the geographical variability of *U. appendiculatus* in association with molecular markers.

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