

Identification of Races of Selected Isolates of *Uromyces appendiculatus* from Minas Gerais (Brazil) Based on the New International Classification System

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ABSTRACT

Twelve single-pustule isolates of *Uromyces appendiculatus*, the etiological agent of common bean rust, were collected in the state of Minas Gerais, Brazil, and classified according to the new international differential series and the binary nomenclature system proposed during the 3rd Bean Rust Workshop. These isolates have been used to select rust-resistant genotypes in a bean breeding program conducted by our group. The twelve isolates were classified into seven different physiological races: 21-3, 29-3, 53-3, 53-19, 61-3, 63-3 and 63-19. Races 61-3 and 63-3 were the most frequent in the area. They were represented by five and two isolates, respectively. The other races were represented by just one isolate. This is the first time the new international classification procedure has been used for *U. appendiculatus* physiological races in Brazil. The general adoption of this system will facilitate information exchange, allowing the cooperative use of the results obtained by different research groups throughout the world. The differential cultivars Mexico 309, Mexico 235 and PI 181996 showed resistance to all of the isolates that were characterized. It is suggested that these cultivars should be preferentially used as sources for resistance to rust in breeding programs targeting development lines adapted to the state of Minas Gerais.

Additional keywords: common bean rust, *Phaseolus vulgaris* L., source of resistance.

RESUMO

Identificação de raças de isolados selecionados de *Uromyces appendiculatus* oriundos de Minas Gerais (Brasil) com base no novo sistema internacional de classificação

Doze isolados monopustulares de *Uromyces appendiculatus*, agente causal da ferrugem do feijoeiro comum, coletados no estado de Minas Gerais, Brasil, foram classificados com base na nova série de cultivares diferenciadoras e no sistema binário de nomenclatura propostos no “3rd Bean Rust Workshop”. Tais isolados têm sido utilizados para selecionar os genótipos resistentes à ferrugem no âmbito do programa de melhoramento do feijoeiro conduzido pela nossa equipe. Os doze isolados foram classificados em sete raças fisiológicas distintas: 21-3, 29-3, 53-3, 53-19, 61-3, 63-3 e 63-19. As raças 63-19 e 63-3 foram as mais frequentes, sendo representadas por cinco e dois isolados, respectivamente. As outras raças foram representadas por apenas um isolado. Esta é a primeira vez que se utiliza o novo sistema internacional de classificação de raças fisiológicas de *U. appendiculatus* no Brasil. A ampla adoção desse sistema poderá facilitar o intercâmbio de informações e o uso cooperativo dos resultados obtidos pelos diferentes grupos de pesquisa em todo o mundo. As cultivares diferenciadoras Mexico 309, Mexico 235 e PI 181996 foram resistentes a todos os isolados caracterizados, indicando que estas cultivares devem ser preferencialmente utilizadas como fontes de resistência à ferrugem pelos programas de melhoramento do feijoeiro em Minas Gerais.

Palavras-chave adicionais: ferrugem do feijoeiro, *Phaseolus vulgaris* L., fontes de resistência.

INTRODUCTION

Uromyces appendiculatus F. Strauss (sin. *U. phaseoli* G. Winter), the etiological agent of the common bean (*Phaseolus vulgaris* L.) rust, is distributed throughout the world. However, its infection is favored by temperatures between 17-27 °C and relative humidity around 95% during 10 to 18 hours/day (Augustin *et al.*, 1972). This is the reason

why the highest losses resulting from this rust occur in the humid tropical and subtropical areas (Staveland *et al.*, 1989). This is the case of Brazil, where this disease is among the most damaging for this crop (Jesus Junior *et al.*, 2001).

In addition to being harmless to the environment, the use of resistant cultivars is an economically advantageous strategy when compared to chemical control. However, the wide variability of *U. appendiculatus* represents an

obstacle to breeding programs aimed at resistance to rust. The simultaneous introgression (pyramiding) of different resistance genes (R) in the same genetic background has been proposed in order to obtain bean cultivars with durable resistance and wide resistance spectrum (Coyne & Schuster, 1975; Miklas *et al.*, 1993).

The classification of *U. appendiculatus* physiological races and the consequent knowledge of its local pathogenic variability is a basic stage in breeding programs. In addition, it is at this stage that the pathotypes used to monitor the pyramiding process are identified.

One of the main difficulties hampering the advances in rust resistance breeding programs was the inadequate definition of the differential cultivars used in the classification of the physiological races of *U. appendiculatus*. Between 1941 and 1983, classification was performed on the basis of the differential series proposed by Harter & Zaumeyer (1941). However, this series was later modified in order to facilitate the discrimination of certain isolates (Fisher, 1952; Dias Filha & Costa, 1968; Augustin & Costa, 1971; Pereira & Chaves, 1977; Ballantyne, 1978).

During the "Bean Rust Workshop" (BRW), held in Puerto Rico 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). In 1984, cv. Mountainer White Half Runner was eliminated from this series because it was very similar to the Kentucky Wonder 780 (Stavely, 1984). 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.* (2005). 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, Pinto 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 state of Minas Gerais.

Another aspect hindering the study of common bean rust was the use of different scales for evaluating the symptoms incited by the pathogen. Several authors proposed different evaluation scales (Harter & Zaumeyer, 1941; Crispín & Dongo, 1962; Davison & Vaughan, 1963; Stavely *et al.*, 1983; Faleiro *et al.*, 1999b). The scale proposed by Davison & Vaughan (1963) was the most widely used throughout the world. In Brazil, modifications in this scale were proposed (Junqueira Netto *et al.*, 1969; Pereira & Chaves, 1977; Carrijo *et al.*, 1980). A scale containing 37 reaction grades was also proposed by Stavely *et al.* (1983).

Besides the distinct differential series and evaluation scales, another factor hindering the classification of the physiological races of the fungus was the nomenclature attributed to them. The terminology used for this purpose was not uniform. Most authors arbitrarily designated the races by successive numbers (Harter & Zaumeyer, 1941;

Fisher, 1952; Zúñiga & Victoria, 1975; Stavely, 1984). In Brazil, the nomenclature was usually given by a number preceded by a capital letter that represented the geographical area where the races were identified (Dias Filha & Costa, 1968; Junqueira Netto *et al.*, 1969; Augustin & Costa, 1971; Coelho & Chaves, 1975; Carrijo *et al.*, 1980). In Australia, Ballantyne (1978) attributed a lower case letter to each differential cultivar, whereas the designation was given by the letters corresponding to the differential cultivars to which the races were compatible.

In an attempt to facilitate the classification of *U. appendiculatus* races, Faleiro *et al.* (1999b) developed a simplified procedure that considered only the eight cultivars proposed by Mora-Nuñez *et al.* (1992). In addition, the authors proposed the use of an evaluation scale with three reaction degrees and a numerical system for the nomenclature of the races. By using this procedure, the authors grouped the 86 races that had been previously identified by Stavely (1984), Mora-Nuñez *et al.* (1992) and Faleiro *et al.* (1999a) into 66 races.

During the 3rd BRW held in South Africa in 2002, a new differential series was proposed for *U. appendiculatus*. This series contained six Andean and six Mesoamerican bean cultivars. In addition, a new binary nomenclature system was proposed for designation of the races, in which the evaluation scale of the disease was codified in only two reaction degrees: resistant and susceptible (Steadman *et al.*, 2002). In the new differential series, the cultivars Early Gallatin, Redlands Pioneer, Golden Gate Wax (GG Wax), Aurora, Mexico 309, Mexico 235 and Compuesto Negro Chimaltenango (CNC), pertaining to the series proposed in 1983 BRW, were maintained. The cultivars Montcalm, PC-50, PI 260418, Great Northern 1140 (GN 1140) and PI 181996 were added to the new series.

The main goal of the present work was to apply the new international classification procedure (Steadman *et al.*, 2002) to selected isolates of *U. appendiculatus* collected in different bean-producing areas in the state of Minas Gerais. These isolates have been used by our group in a program aimed at selecting rust-resistant genotypes.

MATERIAL AND METHODS

Differential cultivars

The seeds of the differential cultivars Early Gallatin, Redlands Pioneer, GG Wax, Aurora, Mexico 309, Mexico 235 and CNC, as well as of cv. Ouro Negro (resistant control) and cv. US Pinto 111 (susceptible control) (Faleiro *et al.*, 1999a; Souza *et al.*, 2005), were obtained from the germplasm bank of BIOAGRO/UFV bean breeding program. The other five cultivars (Montcalm, PC-50, PI 260418, GN 1140 and PI 181996) were supplied by the Agricultural Research Service, United States Department of Agriculture (USDA), Beltsville, Maryland, USA. The seeds were multiplied under greenhouse conditions before their use in the assays for rust race classification.

Single-pustule isolates

Uromyces appendiculatus single-pustule isolates were obtained from 12 isolates maintained in the fungal collection of BIOAGRO/UFV. Eleven of these isolates belong to the group of thirteen pathotypes previously identified by Faleiro *et al.* (1999a). They were collected in four bean producing municipalities (Coimbra, Lavras, Lambari and Patos de Minas) in MG. The 12th pathotype, identified as 'C', was recently collected in the UFV experimental station located in the municipality of Coimbra.

Cultivar US Pinto 111 was used for obtaining single-pustule isolates based on the method described by Carrijo *et al.* (1980). In our procedure, a lower than usual inoculum concentration (1.0×10^4 uredospores/mL) was used to increase the chance of obtaining separate pustules. The single-pustule isolates were multiplied in US Pinto 111 for three consecutive cycles, using the standard concentration of the inoculum (2.0×10^4 uredospores/mL). After this procedure, the uredospores were collected and stored in glass ampoules covered with aluminum foil, under controlled conditions (5 ± 1 °C and relative humidity < 50 %), according to Zambolim & Chaves (1974).

Isolate inoculations

Individual assays were conducted in order to classify each one of the 12 single-pustule isolates. In each assay, ten seeds from each differential cultivar and from the controls were sown in plastic trays (60 x 40 x 10 cm) containing a 4:1 mixture of soil and tanned manure. The mixture was fertilized with 5 kg per m³ of 4-14-8. This experiment was carried out during the winter and repeated during the summer.

Inoculation was performed according to Carrijo *et al.* (1980). When the primary leaves reached approximately 2/3 of their full development, about 10 days after sowing, they were inoculated with 2.0×10^4 uredospores/mL. The spores were suspended in a Tween-20 (0.05 %, v:v) solution. The inoculum suspension was sprayed on both leaf surfaces with a manual atomizer (De Vilbiss n° 15) adapted to an electric compressor. After inoculation, the plants were transferred to a mist chamber (20 ± 1 °C and relative humidity > 95 %) where they were kept for 48 h under a 12-hour light regime. In order to avoid contamination, plants inoculated with each isolate were kept in separate compartments of the mist chamber. After 48 h, the plants were transferred to a greenhouse (20 ± 5 °C), where they were kept until symptom evaluation.

Evaluation of disease symptoms

In all assays, the reaction degrees to the disease were determined on the basis of the scale adopted for the new procedure of the international classification (Steadman *et al.*, 2002). This scale considers 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.

The infection degrees were determined approximately 14 days after inoculation when up to 50 % of the pustules were sporulating. The lesions in both surfaces of the primary leaves were determined with the aid of a graphic representation diagram (Castaño, 1985). The cultivars that predominantly presented degrees 3 or lower were classified as resistant, whereas those with predominant degrees 4 or higher were considered to be susceptible.

Physiological races

The physiological race of each isolate was determined based on the binary nomenclature system proposed by Steadman *et al.* (2002). 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 set. The second number is obtained by the sum of the binary values of the susceptible Mesoamerican cultivars.

RESULTS AND DISCUSSION

The infection degrees presented by differential and control cultivars inoculated with the single-pustule isolates of *U. appendiculatus* classified in this study are shown in Table 1. The application of the new international differential series and the binary nomenclature system to the group of 12 selected isolates resulted in their classification into seven different physiological races (Table 2). The following races were represented by only one isolate in the group: 21-3, 29-3, 53-3, 53-19 and 63-19. Five isolates belonged to race 61-3 and two to 63-3.

Besides the resistant control 'Ouro Negro', the differential cultivars Mexico 309, Mexico 235 and PI 181996 of Mesoamerican origin were resistant to all the isolates included in the test. Cultivar CNC was resistant to ten out of the 12 isolates, being susceptible to isolates 2 and 7 (races 63-19 and 53-19, respectively). The other two Mesoamerican differential cultivars, GN 1140 and Aurora, were susceptible to all the isolates tested. The Andean differential cultivars, 'Early Gallatin', 'Montcalm' and 'GG Wax' were susceptible to all isolates included in the test. 'Redlands Pioneer' was susceptible only to isolates 2 (races 63-19), 9 and 13 (race 63-3); 'PC-50' was susceptible to all isolates, except for C, 7 and 8 (races 21-3, 53-19, and 53-3, respectively). PI 260418 was susceptible to all isolates, except for C and 10 (races 21-3 and 29-3, respectively).

The differential cultivars Redlands Pioneer, PC-50, PI 260418 and CNC were the most important ones for the classification of the isolates evaluated in the present study. The reactions of the other differential cultivars did not contribute to distinction among the isolates (Table 2).

The wide adoption of the differential series and the binary nomenclature system for the *U. appendiculatus*

TABLE 1 - Infection degrees of common bean cultivars belonging to the international rust differential series (Steadman *et al.*, 2002), and of control cultivars inoculated with *U. appendiculatus* isolates collected in the state of Minas Gerais, Brazil

Gene pool	Differential cultivars ^a	<i>U. appendiculatus</i> isolates ^b											
		C	1	2	4	6	7	8	9	10	11	12	13
Andean	Early Gallatin	4, 3 ^c	6, 2	6, 3	5, 6, 4	6, 5	4, 6	6, 3	6, 5	6	6, 5, 4	6, 5	4, 6
	Redlands Pioneer	3, 5	1, 2, 3	4, 3, 2	3, 4	3, 4	1, 4	1, 2	6, 4, 2	1, 4, 2	2, 4	2, 4, 5	4, 2
	Montcalm	5	6, 4, 5	6, 5	6	6	6, 2	6	6, 4, 5	6	6, 5	6, 5	6
	PC-50	3	4, 3	6, 5, 3	4, 5	6, 4, 5	3, 1	2, 3	5, 3	5	5, 3	6, 2	4, 5
	GG Wax	6	6, 5	6, 5	6	6	5, 6	6	6	6	6	6	6
	PI 260418	3	6, 2	5, 3	5, 3, 4	4, 6	4, 2	5, 3	6, 4	3, 4, 2	5, 3	5, 3, 4	6, 4, 3
Mesoamerican	GN 1140	4	6, 5	5, 4, 1	6	6, 5	6, 4	6, 4, 5	6, 5, 3	6	6, 2	6	6, 3, 4
	Aurora	6	6, 5	6	6	6	6, 4	6, 5	6, 5	6	6	6	4, 6
	Mexico 309	1	1	1	1, 2	1	1	1	1	1	1, 2	1	1
	Mexico 235	1, 2	1, 2	1	1, 2	1, 2	1	1	1, 3	1	1	1	1
	CNC	2	1, 4, 3	5, 2	2, 1, 4	2, 4	4, 2	1, 4	2, 1, 4	1, 3, 2	1, 4	2, 4	1, 4
	PI 181996	1	1, 2	1	1	1	1, 2	1	1	1	1	1	1, 2
	Ouro Negro (R)	3, 4	1, 2	2, 3	2, 3	1, 2, 3	1	1	1	1, 3	1, 3	1, 3	1, 3
	US Pinto 111 (S)	6	6, 5	6	6	6	6	6	6	6, 5	6	6	6

^aCultivars considered resistant: predominance of grade 3 or lower. Cultivars considered susceptible: predominance of grade 4 or higher. Resistant (R) and susceptible (S) controls.

^bThe isolates 1, 2, 4, 6, 7, 8, 9, 10, 11, 12 and 13 were previously collected by Faleiro *et al.* (1999a) and kept in the Rust Fungal Collection of BIOAGRO/UFV. The C (Coimbra) isolate has been recently collected in the municipality of Coimbra, Minas Gerais, Brazil.

^cWhen several infection grades are present, they are recorded according to their predominance, the most prevalent being listed first.

physiological races proposed by Steadman *et al.* (2002) can contribute to the elaboration of an internationally standardized classification methodology. This procedure would facilitate race identification and the designation process, as well as the exchange of information and the cooperative use of the results obtained by different research groups throughout the world. Re-characterization of *U. appendiculatus* isolates collected in the USA, South Africa, Honduras, Argentina and Mozambique has already been accomplished with this system (Steadman *et al.*, 2002; Acevedo *et al.*, 2004; Jochua *et al.*, 2004).

Faleiro *et al.* (1999a) classified 11 of the isolates analyzed in this study into 11 different races. However, according to our results, these same isolates were classified into six races. Faleiro *et al.* (1999a) used a simplified differential series composed of eight cultivars based on Mora-Nuñez *et al.* (1992). According to these authors this modified series would be adequate for discriminating among isolates collected in Brazil. Although the modified series contained some of the differential cultivars of the present series, the two series differ considerably, and this might explain the differences between our results and those of Faleiro *et al.* (1999a).

Another possible cause for this incongruence is the subjectivity of the symptom scale used by these authors (Stavely *et al.*, 1983). The present study adopted a scale in which degrees 1 to 3 denote resistance and degrees 4 to 6, susceptibility (Steadman *et al.*, 2002). Although this scale

is less subjective than those previously proposed, it still presents some subjectivity for the classification of plants with degrees 3 and 4.

Our results clearly show that the *U. appendiculatus* isolates maintained in the fungal collection used by the Bean Breeding Program of BIOAGRO/UFV do not represent the variability present in the state of Minas Gerais. New collecting expeditions are being programmed to obtain more isolates from a large number of municipalities of the state. In addition, isolates maintained in other fungal collections in the state have been requested.

An important contribution of this study was the identification of races with potential use during the resistance gene pyramiding process. Races were identified that are able to discriminate specific R genes. For instance, considering the pyramiding of the rust resistance genes found in the differentials Redlands Pioneer (gene *Ur-13*) and CNC (gene *Ur-?* – not officially classified), which provide a wide rust resistance spectrum in Brazil (Souza *et al.*, 2005), races 53-19 and 63-3 could be respectively used for the identification of genotypes that contain simultaneously these two genes (Table 2).

In the present study, the reactions of the new differentials 'Montcalm', 'PC-50', 'PI 260418', 'GN 1140', and 'PI 181996' to Brazilian *U. appendiculatus* isolates were demonstrated for the first time (Table 2). These data will be useful in studies concerning the geographical distribution of the pathogen (Linde *et al.*, 1990). Some promising sources

TABLE 2 - Designation of Minas Gerais State *U. appendiculatus* physiological races using the international differential series and the binary system of nomenclature reported by Steadman et al. (2002)

Gene pool	Binary value	Differential cultivar (Resistance gene)	Reaction to the <i>U. appendiculatus</i> isolates ^a											
			C	1	2	4	6	7	8	9	10	11	12	13
Andean	1	Early Gallatin (<i>Ur-4</i>)	+	+	+	+	+	+	+	+	+	+	+	+
	2	R. Pioneer (<i>Ur-13</i>)	-	-	+	-	-	-	-	+	-	-	-	+
	4	Montcalm (<i>Ur-?</i>)	+	+	+	+	+	+	+	+	+	+	+	+
	8	PC-50 (<i>Ur-9, Ur-12</i>)	-	+	+	+	+	-	-	+	+	+	+	+
	16	GG Wax (<i>Ur-6</i>)	+	+	+	+	+	+	+	+	+	+	+	+
	32	PI 260418 (<i>Ur-?</i>)	-	+	+	+	+	+	+	+	-	+	+	+
Mesoamerican	1	GN 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> [†])	-	-	-	-	-	-	-	-	-	-	-	-
	16	CNC (<i>Ur-?</i>)	-	-	+	-	-	+	-	-	-	-	-	-
	32	PI 181996 (<i>Ur-11</i>)	-	-	-	-	-	-	-	-	-	-	-	-
Nomenclature of the races			21-3	61-3	63-19	61-3	61-3	53-19	53-3	63-3	29-3	61-3	61-3	63-3

^aResistant (-) and susceptible (+).

were also identified for use in the breeding programs aiming to develop cultivars that are resistant to rust races present in MG. Besides cv. Ouro Negro (Table 1), the most used rust resistance source in Brazil, cultivars Mexico 309, Mexico 235, and PI 181996 were also resistant to all isolates included in the test (Table 2). 'CNC' also showed a wide resistance spectrum, being susceptible to only two of the isolates (Table 2). Therefore it is suggested that these sources should be preferentially used as donors of R genes while breeding for rust resistance in this region.

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