RUST RESISTANCE GENE PRESENT IN COMMON BEAN CULTIVAR OURO NEGRO (UR-ON) DOES NOT CORRESPOND TO UR-3+

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Rust, incited by the fungus *Uromyces appendiculatus*, can cause serious damage to common bean (*Phaseolus vulgaris* L.) crops in humid tropical and subtropical areas of the world. Resistance gene pyramiding has been used as a strategy to overcome this problem. Identification of different rust resistance genes with wide resistance spectra and determination of the allelic relationships among them are basic steps for works aiming at developing new bean commercial cultivars with durable resistance.

Cultivar Ouro Negro is the main rust resistance source used in Brazil (Faleiro *et al.*, 2004). It is resistant to several pathotypes of *U. appendiculatus* collected in central, northern and southern Brazil (Rios *et al.*, 2001; Souza *et al.*, 2005). The temporary symbol *Ur-ON* was assigned to the rust resistance gene present in 'Ouro Negro' because it has not been fully characterized. Allelism studies reported by Alzate-Marin *et al.* (2004) showed that *Ur-ON* does not correspond to genes *Ur-5* (cv. Mexico 309) or *Ur-11* (line Belmidak RR-3). To aid the characterization of the rust resistance locus present in 'Ouro Negro', in this work we determined the allelic relationships between *Ur-ON* and the gene $Ur\text{-}3^+$ from cultivar Mexico 235. Initially, the reactions of 'Ouro Negro' and 'Mexico 235' to eight selected races of *U. appendiculatus* from the state of Minas Gerais (central Brazil) were determined. In addition, the presence/absence of SCAR and RAPD markers reported as linked in coupling phase to the gene *Ur-ON* was tested in 'Mexico 235'. Finally, we analyzed the inheritance of rust resistance in an F_2 population derived from crosses between 'Mexico 235' and 'Ouro Negro'.

Ten days after sowing the primary leaves of ten plants each of cultivars Ouro Negro, Mexico 235 and U.S. Pinto 111 (susceptible control) were inoculated with spore suspensions (2.0×10^4 spores/ml) of eight selected races of *U. appendiculatus* (Table 1). The plants were then incubated for two days in a mist chamber kept at 20-22°C and 100% relative humidity (Souza *et al.*, 2005). Fifteen days after inoculation the plants were scored visually for the disease symptoms using a 1-to-6 scale (Stavely *et al.*, 1983). Resistance reaction was assigned to plants with no or limited symptoms (grades 1 to 3), whereas plants graded 4 or greater were considered to be susceptible. Leaf DNA from plants of these three cultivars was extracted by a procedure based on Doyle & Doyle (1990). Amplification reactions were according to Faleiro *et al.* (2000) for the RAPD marker $OPX11_{550}$ and according to Côrrea *et al.* (2000) for SCAR markers $SF10_{1050}$ and $SBA08_{560}$.

Eighty-one F_2 seeds (Mexico 235 x Ouro Negro) were obtained and sowed in the greenhouse. Using the same inoculation and disease symptom evaluation procedures described the F_2 plants and ten plants each of cultivars Ouro Negro, Mexico 235 and U.S. Pinto 111 were inoculated with *U. appendiculatus* race 63-3. The phenotypic frequencies observed in the F_2 population were tested for goodness-of-fit to theoretical ratios with chi-square test.

Cultivars Ouro Negro and Mexico 235 presented a similar resistance spectra. 'Ouro Negro' was resistant to all eight races and 'Mexico 235' was susceptible only to race 29-15 (Table 1). These results did not allow an accurate distinction between the resistance loci present in these two cultivars, but they show that both are good resistance sources to races from the state of Minas Gerais. Two of the three tested molecular markers were polymorphic between 'Ouro Negro' and 'Mexico 235' (Table 1). This indicates that the resistance allele of the locus *Ur-ON* is not present in cultivar Mexico 235. It also shows that the two polymorphic markers ($OPX11_{550}$ and $SF10_{1050}$) can be useful for monitoring the pyramiding of *Ur-ON* and *Ur-3*$^{+}$ in the same genetic background.

The results of the allelism test are shown in Table 2. The segregation ratio was of 15 resistant to 1 susceptible plant in the F_2 population (Mexico 235 x Ouro Negro) indicating that two independent genes govern resistance in this population. These results confirm that the gene (or complex gene locus) present in 'Ouro Negro' does not correspond to gene *Ur-3*$^{+}$. Thus, cultivars Ouro Negro and Mexico 235 can be used simultaneously as rust resistance sources in common bean breeding programs in Brazil.

Table 1. Phenotypic and molecular characterization of cultivars Ouro Negro (*Ur-ON*), Mexico 235 (*Ur-3*$^{+}$) and U.S. Pinto 111 (susceptible control) regarding the resistance to selected races of *U. appendiculatus* and the presence/absence of RAPD (OP) and SCAR (S) markers linked in coupling phase to the rust resistance gene *Ur-ON*

Cultivar	Gene	Reaction to races of *U. appendiculatus*[a]								Markers (*Ur-ON*)[b]		
		21-3	29-3	29-15	53-3	53-19	61-3	63-3	63-19	OPX11	SF10	SBA08
Ouro Negro	*Ur-ON*	R	R	R	R	R	R	R	R	1	1	1
Mexico 235	*Ur-3*$^{+}$	R	R	S	R	R	R	R	R	0	0	1
U.S. Pinto 111	-	S	S	S	S	S	S	S	S	0	0	0

[a] Resistance (R) and susceptibility (S). [b] Presence (1) and absence (0) of molecular markers.

Table 2. Evaluation of resistance to race 63-3 of *U. appendiculatus* in the F_2 population derived from crosses between cultivars Mexico 235 (*Ur-3*$^{+}$) and Ouro Negro (*Ur-ON*)

Genetic material	Locus tested	N° of plants	Expected ratio (R:S)[a]	Observed ratio (R:S)[a]	χ^2	P(%)[b]
Ouro Negro	*Ur-ON*	10	1:0	10:0	-	-
Mexico 235	*Ur-3*$^{+}$	10	1:0	10:0	-	-
F_2 population	*Ur-ON* and *Ur-3*$^{+}$	81	15:1	75:6	0.185	66.7

[a] Resistant (R) and susceptible (S) plants. [b] Probability in percentage.

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References

Alzate-Marin *et al.* (2004) Allelism tests between the rust resistance gene present in common bean cultivar Ouro Negro and genes *Ur-5* and *Ur-11*. Journal of Phytopatology, 152:60-64.

Corrêa *et al.* (2000) Sequence characterized amplified regions linked to rust resistance genes in the common bean. Crop Science, 40:804-807.

Doyle & Doyle (1990) Isolation of plant DNA from fresh tissue. Focus, 12:13-15.

Faleiro *et al.* (2000) RAPD markers linked to a block of genes conferring rust resistance to the common bean. Genetics and Molecular Biology, 23:399-402.

Faleiro *et al.* (2004) Use of molecular markers to accelerate the breeding of common bean lines resistant to rust and anthracnose. Euphytica, 138:213-218.

Rios *et al.* (2001) Avaliação da resistência de cultivares e linhagens de feijoeiro comum a diferentes populações de *Uromyces appendiculatus*. Fitopatologia Brasileira, 26:128-133.

Souza *et al.* (2005) Análise da variabilidade patogênica de *Uromyces appendiculatus* em algumas regiões brasileiras. Fitopatologia Brasileira, 30:143-149.

Stavely *et al.* (1983) The 1983 bean rust workshop. Annual Report of the Bean Improvement Cooperative, 26:iv-vi.