SIMULTANEOUS TRANSFER OF RESISTANCE GENES FOR RUST, ANTHRACNOSE AND ANGULAR LEAF SPOT TO CULTIVAR PEROLA ASSISTED BY MOLECULAR MARKERS

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The common bean (*Phaseolus vulgaris*) is susceptible to several diseases under field conditions. The best strategy to overcome this situation is the use of resistant cultivars. The development of cultivars which are simultaneously resistant to several types of pathogens depends on a multidisciplinary effort. One of the strategies to reach this goal is the pyramiding of resistance genes. Usually in this process resistance genes are transferred through backcrosses from cultivars or lines not adapted for commercial use to elite cultivars. Molecular markers can be a useful tool in this process to facilitate selection of specific alleles and to accelerate the recovery of the recurrent progenitor's genome. In this sense, there is a great expectation on the success of the use of molecular markers in combination with conventional breeding methods to transfer resistance genes to commercial bean varieties. This work aimed at selecting bean progenies containing resistance genes to rust (caused by *Uromyces appendiculatus*), anthracnose (caused by *Colletotrichum lindemuthianum*) and angular leaf spot (caused by *Phaeoisariopsis griseola*), with the aid of molecular markers.

Materials and Methods

Cultivar Pérola ("carioca type") was first crossed to an advanced line ("carioca type") from the Common Bean Breeding Program from BIOAGRO/UFV containing genes for resistance to rust (from cv. Ouro Negro), anthracnose (genes *Co-6*, *Co-4* and *Co-10*) and angular leaf spot (gene *Phg-1*). The F_1 plants were then selfed until F_5. The resistance genes were selected in generations F_1 to F_4 based on molecular markers and inoculations with the pathogens causing the three diseases of interest. The inoculations were made sequentially, that is, after inoculation and evaluation of the symptoms of one disease, the other pathogen was inoculated, and so on. Evaluation of the disease symptoms were according to Stavely et al. (1983) for rust; Pastor-Corrales (1992) for anthracnose; and Pastor-Corrales and Jara (1995) for angular leaf spot.

The markers previously identified in our laboratory, were tested to determine if they were polymorphic between the resistant donor parent and cultivar Pérola. DNA was extracted from leaves according to Doyle and Doyle (1990). RAPD amplification reactions were according to Williams et al. (1990); microsatellite analysis was according to Routman and Cheverud (1994) and the SCAR protocol was described by Corrêa et al. (2000).

Results and Discussion

DNA amplification revealed that most resistance loci analyzed were polymorphic between the recurrent parent (Pérola) and the donor parents except for marker SSR Satt174 (Figure 1). Therefore, it was not possible to use this marker in the selection process.

In the beginning of the pyramidation process, we had nine F_1 plants resistant to the three pathogens and harboring the corresponding molecular markers. Generations F_2 to F_4 were conducted by the genealogical method, with inoculation of the pathogens for the three diseases and verification of the presence of the markers associated with the resistance genes. At the moment, we have approximately 60 resistant F_5 families and in a next step they will undergo field tests for productivity.

The selection of these families with "carioca-type" grains, resistant to rust, anthracnose and angular leaf spot demonstrates the viability of the use of molecular markers to select resistance genes in a pyramiding

program. The larger the number of genes involved the more useful the molecular markers become. However, inoculation is still important along the pyramiding process to confirm the data obtained through molecular markers.

Table 1. Molecular markers linked to resistance genes of the common bean to rust, anthracnose and angular leaf spot identified at BIOAGRO/UFV

Markers	Distance	Phase	Gene of Resistance	Disease	Cultivar
$OPB03_{1800r}$	3.7 cM	repulsion	*Co-4*	Anthracnose	TO
$OPY20_{830a}$	0.0 cM	coupling	*Co-4*	Anthracnose	TO
$OPAZ20_{940a}$	7.1 cM	coupling	*Co-6*	Anthracnose	AB 136
$OPH13_{490a}$	5.5 cM	coupling	*Phg-1*	Angular leaf spot	AND 277
$OPBA8_{560a}$	6.0 cM	coupling	*Ur-Ouro Negro*	Rust	Ouro Negro
$OPF10_{1050a}$	6.9 cM	coupling	*Ur-Ouro Negro, Co-10*	Rust and Anthracnose	Ouro Negro
$OPX11_{550a}$	5.8 cM	coupling	*Ur-Ouro Negro*	Rust	Ouro Negro
$Satt174_{300}$	4.1 cM	coupling	*Ur-Ouro Negro*	Rust	Ouro Negro
$SCARF10_{1050a}$	6.9 cM	coupling	*Ur-Ouro Negro, Co-10*	Rust and Anthracnose	Ouro Negro

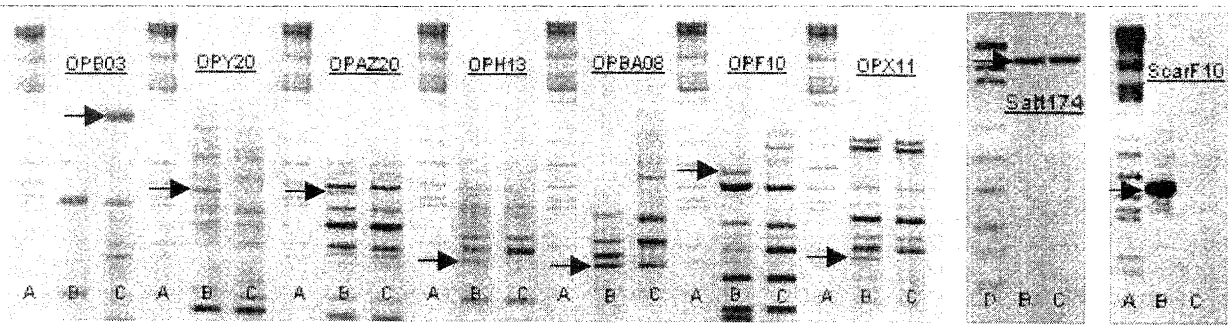

Figure 1. Molecular markers for different common bean resistance genes amplified in the resistant line and in cultivar Pérola. (**A**) - Size markers (lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII); (**B**) - resistant Line; (**C**) - cultivar Pérola; (**D**) - size markers (pUC18 digested with *Msp*I). The arrows indicate the marker bands for the resistance genes listed in Table 1.

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