DEVELOPMENT OF COMMON BEAN LINES RESISTANT TO RUST AND ANTHRACNOSE BY MOLECULAR MARKER-ASSISTED BACKCROSSING

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One of the main reasons for yield losses in the common bean (*Phaseolus vulgaris* L.) in Brazil and in other parts of the world is the great number of diseases affecting this crop. Rust and anthracnose, caused the fungi *Uromyces appendiculatus* and *Colletotrichum lindemuthianum*, respectively, are considered among the most important ones. They may cause losses of up to 100% depending on the environment and the use of susceptible cultivars. The use of resistant cultivars has been considered as an efficient, safe, and inexpensive alternative method for control of these pathogens. The backcrossing breeding method is largely used to transfer traits with high heritability, such as disease resistance, to elite genotypes. Based on the concept of graphic genotypes (Young and Tanksley, 1989), the use of molecular markers can speed up the recovery of the recurrent parent's genome. Molecular fingerprints can be used to aid the selection of individuals that bear the gene of interest and in addition have a high proportion of the recurrent parent's genome (Openshaw et al., 1994). The main goal of this work was to introduce resistance genes for rust and anthracnose in an adapted common bean cultivar through marker-assisted backcrossing. DNA fingerprint was used to select plants genetically closer to the recurrent parent which were also resistant to rust and to race 89 of *C. lindemuthianum*.

Cultivar Ouro Negro (derived from Honduras-35), which was used as donor parent, is a black seeded mesoamerican bean resistant to several races of *U. appendiculatus* and *C. lindemuthianum*. Cultivar Rudá, which was used as recurrent parent, is a "carioca-type" mesoamerican bean, highly recommended for several Brazilian regions, but susceptible to rust and to anthracnose. All crosses were performed in the greenhouse. Thirty-four BC_1, 44 BC_2, and 32 BC_3 seeds were obtained. The seeds from each backcross were sown in 2.5 L pots. Approximately ten days after sowing the BC seeds, the primary leaves were inoculed with a mixture of uredioniospores collected in the state of Minas Gerais, Brazil. Only plants with no pustules or very small pustules (<0.3 mm in diameter) were considered resistant. After evaluation for rust symptoms, the same plants were inoculated with *C. lindemuthianum* (pathotype 89) in the first trifoliate leaf. Only plants with no symptoms were considered resistant. Resistant BC_3 plants were used to generate BC_3F_2 individuals which were inoculated with the same two pathogens and analyzed for disease symptoms. These plants were submitted to progeny tests to identify those lines that did not segregate for resistance/susceptibility to the two diseases. Leaf DNA from the progenitors, BC_1 and BC_2 plants resistant to rust and anthracnose, and non-segregating BC_2F_3 resistant lines was extracted and amplified by RAPD-PCR. The polymorphic bands were used to build a binary matrix. The relative genetic distances were calculated by the euclydian method and used to cluster by the nearest neighbour method.

In the first backcross, 11 out of 34 BC_1 plants were resistant to *U. appendiculatus* and to *C. lindemuthianum* pathotype 89. Amplification of their DNA with nine primers generated 22 polymorphic bands. The relative genetic distances between these plants and the recurrent parent Rudá varied between 9 and 59% (Figure 1A). Plants number 6, 24, and 25, which were genetically closest to the recurrent parent were used in the second cycle of backcrosses. In the second backcross, 19 out of 44 BC_2 plants were resistant to *U. appendiculatus* and to *C. lindemuthianum* pathotype 89. Amplification of their DNA with fourteen primers generated 27 polymorphic bands. The relative genetic distances between these plants and the recurrent parent Rudá varied between 7 and 33% (Figure 1B). Plants number 3 and 18, which were genetically closer to the recurrent parent were used in the third cycle of backcrosses. In the third backcross, 15 out of 32 BC_3 plants were resistant to *U. appendiculatus* and to *C. lindemuthianum* pathotype 89. These 15 plants generated 50 BC_3F_2 plants, 35 of them being resistant to rust and anthracnose. Progeny tests with the resistant individuals identified 13 non-segregating lines. DNA

amplification of each one of these lines with 53 primers generated 43 polymorphic bands. The relative genetic distances between these lines and the recurrent progenitor varied between 0 and 7% (Figure 1C). Lines number 6, 25, 43, 45, and 48, which were genetically closer to the recurrent parent were selected. These five resistant lines are being presently tested for field performance. In all backcrosses, cluster analyses defined two distint groups: one containing the resistant progenitor and the other containing the resistant lines and the recurrent progenitor (Figure 2). It can be clearly noticed that the lines BC_3 are closer to the recurrent parent than plants BC_1 and BC_2. The breeding strategy we used considerably decreased the time normally required to recover the genome of the recurrent progenitor allowing the fast pyramidation of genes for resistance to rust and anthracnose in a commercial common bean variety.

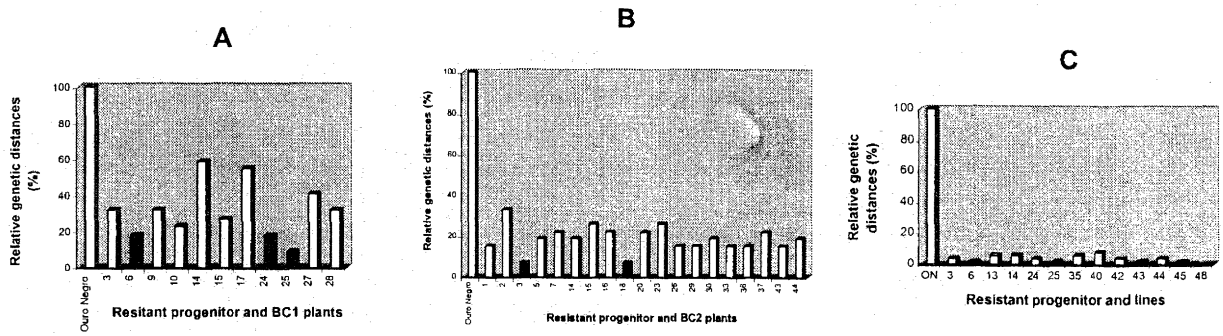

Figure 1. Relative genetic distances (%) between BC_1 plants (A), BC_2 plants (B), non-segregant lines (C) and the resistant progenitor (Ouro Negro), and the recurrent progenitor (Rudá). The black columns represent plants that were closer to the recurrent progenitor.

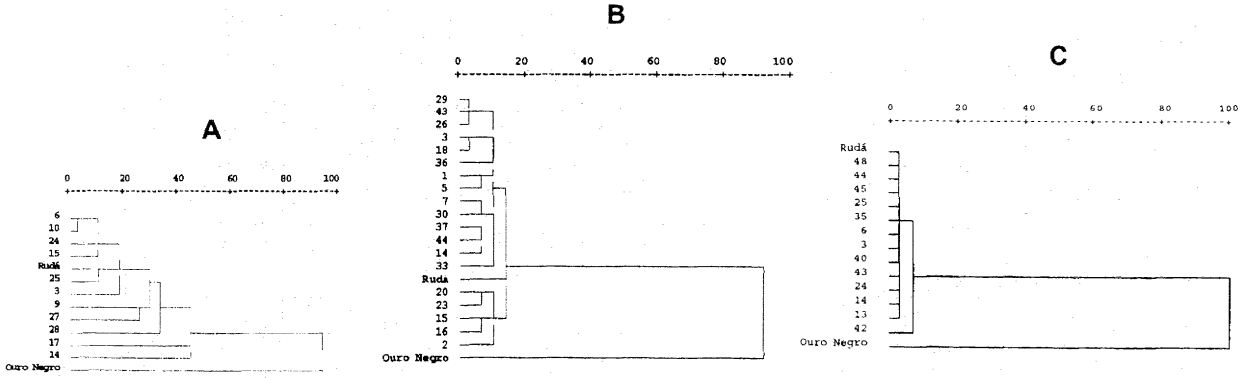

Figure 2. Cluster analysis (dendrogram) of BC_1 plants (A), BC_2 plants (B), non-segregant lines (C) and their progenitors, based on the relative genetic distances among the individuals.

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