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SCRENNING OF ISSR PRIMERS TO STUDY THE POPULATION GENETIC STRUCTURE OF *Botrytis cinerea* FROM *Eucalyptus* IN BRAZIL/ Seleção de *primers ISSR* para estudo da estrutura genética de populações de *Botrytis cinerea* de *Eucalyptus* no Brasil. D.M.Q. AZEVEDO[1,2]; S.D.S. MARTINS[1,3]; M.D. MARTINS[1,3]; L.M.S. GUIMARÃES[1]; L.S. BORGES[1,2]; R. BELISÁRIO.[1,3]; A.C. ALFENAS[1]; G.Q. FURTADO[1] [1]Departamento de Fitopatologia da Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, Viçosa, MG, Brasil 36570-900 / [2]Bolsista CAPES/ [3]Bolsista CNPq. E-mail: daiana.azevedo@ufv.br

Botrytis cinerea Pers. is a necrotrophic and polyphagous pathogen responsible for considerable losses in many important agricultural and forest crops in Brazil. In *Eucalyptus,* the gray mold disease caused by *B. cinerea* lead to seedling and cutting death resulting in losses in commercial forest nurseries. Despite its importance, little is known about the genetic structure of the fungus from eucalypt. Thus, in this study, we selected Inter-Simple Sequence Repeats (ISSR) primers and evaluate their potentials as molecular markers to analyse the genetic structure of *B. cinerea* populations from *Eucalyptus* in Brazil. Five *B. cinerea* isolates were used for primers screening and optimization of the amplification protocol. The DNA extraction was performed with the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. Thirty-two primers were tested to amplify the DNA ISSR regions by PCR. Each ISSR reaction was carried out in a total volume of 20 µL, containing 2 µL of DNA (15 ng), 2 µL of primer (10 nmol/mL), 10 µL de GoTaq® (Promega) and 6 µL of ultra-pure water. PCR conditions were one cycle of 3 min at 94 °C; 40 cycles of 1 min at 92 °C, 1min at annealing temperature of primer and 2 min at 72 °C; followed by one cycle of 7 min at 72 °C and a 4 °C soak. The annealing temperatures of primers ranged from 33.7° to 50°C. PCR products were separated by electrophoresis in 1.5 % agarose gels in TAE 1X buffer, at 80V for 120 min, stained with ethidium bromide and visualized by UV fluorescence. Seventeen primers (UBC 807, UBC 808, UBC 835, UBC 841, P1, P7, P8, P9, P10, P12, P14, P15, P20, P120, Jonh, Omar e SSR7) amplified ISSR fragments in all isolates, generating clear and polymorphic bands. After the selection, the amplifications were repeated to confirm the robustness and the repeatability of the *loci* and markers. The selected primers will be useful in future studies on the genetic diversity of *B. cinerea*.

Key words: Grey mold; Populations biology; Forest diseases; Genetic diversity.

Apoio: CAPES; FAPEMIG.