Genetic Variability in *Puccinia psidii* as Revealed in Commercial Plantations of *Eucalyptus* Species

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Abstract—The climates of central and southern regions of São Paulo state (Brazil) are suitable for pathogens such as *Puccinia psidii*, which causes a common and serious disease in eucalyptus plantations less than 2 years old. We studied the genetic variability of *P. psidii* in commercial plantations of *Eucalyptus* species by random amplified polymorphic DNA molecular markers. All 56 isolates of *P. psidii* analyzed showed high polymorphism and there was no evidence of preferential infection in different plant tissues. In the analysis of pooled pustules according to the clone of origin, we observed the formation of two groups, one with isolates from susceptible clones and the second group only with isolates from resistant clones, indicating that isolates infecting resistant clones are more genetically similar than isolates infecting susceptible clones.

INTRODUCTION

Puccinia psidii was originally described from common guava (*Psidium guajava*) in eastern Brazil in 1884. It became notorious for its host jumps to nonnative Eucalyptus, first observed in Brazil in the 1940s. It caused substantial economic damage to large Brazilian plantations in the 1970s (Coutinho et al. 1998). The wide host range of the rust fungus and its potential threats to many native or introduced species in Myrtaceae has become a major concern to many countries, including New Zealand, Australia, South Africa, and Brazil, where Myrtaceae species are the major components of the forests and ecosystem (Coutinho et al. 1998; Glen et al. 2007; Tommerup et al. 2003).

The host jump in Brazil is currently cited as a prime example in the literature illustrating the potential danger for host jumps following anthropocentric movement of potential hosts (Slippers et al. 2005; Wingfield et al. 2001). The probability of a new race is particularly high when a particular resistance gene is used extensively in breeding (Keiper et al. 2003).

Breeding programs are underway to improve resistance of eucalypts to *P. psidii*. In *E. grandis*, resistance is controlled by a locus of main effect, which makes this species useful in breeding programs (Junghans et al. 2003). However, based on the pattern of inheritance, slight changes in the pathogen's genetic structure can overcome this host resistance gene and render it ineffective (Graça et al. 2011).

The lack of information about the origin and genetic variation of the pathogen population has hindered choosing the best form of management aimed at reducing disease.

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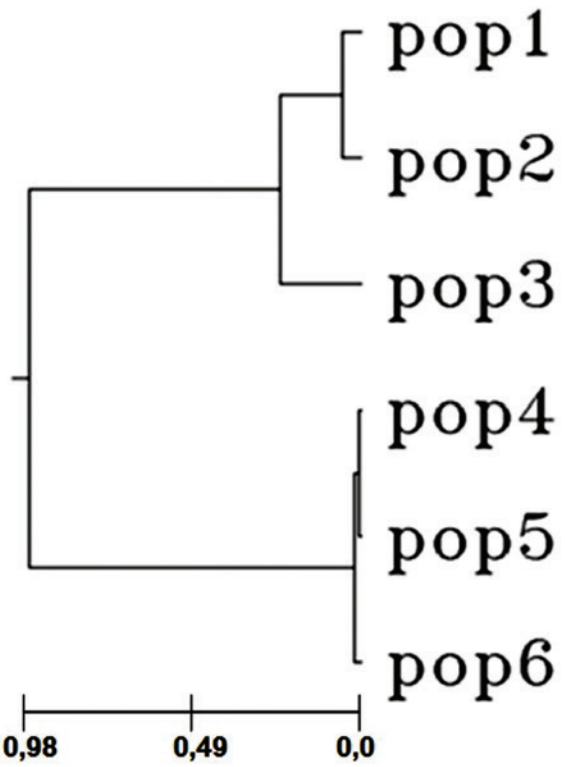

Figure 1—Cluster diagram based on genetic distance calculated from the presence/ absence of polymorphic markers across the pustules grouped by clone. Pop 1, 2, 3, 4, 5 and 6: name of each group of pustules in the clones.

Our aim is to determine the genetic relationships among *P. psidii* isolates from *Eucalyptus* species in Brazil with random amplified polymorphic DNA (RAPD) markers, allowing us to investigate whether the pathogen has undergone a genetic change that could enable it to break the barrier of plant resistance. Results of this research are expected to assist in developing effective control measures.

METHODS

Fungal Isolates

Single uredinial isolates of *P. psidii* were collected from six different Eucalyptus clones, totaling 56 pustules (table 1). These clones are located in the city of Jacarei, São Paulo (Brazil) and were provided by the Fibria SA breeding program.

DNA Extraction

Genomic DNA was extracted by using Chelex® (Sigma-Aldrich Co., St. Louis, Missouri, USA) resin. The protocol is to add to each sample 100 µl of 5 percent Chelex, then to soak the spores until the entire contents are homogenized, and to add 80 mg of proteinase K. Samples were placed in a water bath overnight at 65 °C. The next day, the samples were exposed to a temperature of 95 °C water bath for 15 minutes, removed, and centrifuged for 1 minute at 14,000 rpm. The supernatant was transferred to new tubes.

RAPD Genotyping

We genotyped 56 isolates with the primers OPAD-01, OPM-16, OPM-10, OPX-06 by Operon Technologies (Olive Branch, Mississippi, USA). Each 13-µL polymerase chain reaction (PCR) contained 10 ng total genomic DNA, 0.8 mM of dNTP, 1923 mM of MgCl2, 0.36 percent of bovine serum albumin (BSA), 1 unit of Taq DNA polymerase (Invitrogen®; Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.3 mM of primer, and autoclaved deionized water. The PCR reaction was performed in a PT 100™ thermocycler (MJ Research, Waltham, Massachusetts) with one cycle at 95 °C for 3 minutes, followed by 40 cycles at 92 °C for 1 minute, 35 °C for 1 minute, and 72 °C for 2 minutes; a final elongation step of 5 minutes at 72 °C; and finally a holding step at 10 °C. The PCR products were separated by using a 2.5 percent agarose gel.

Data Analysis

A binary matrix recording specific RAPD fragments as present (1) or absent (0) was generated for each genotype, and the data obtained plotted on a Microsoft® Excel® spreadsheet.

Analysis of diversity and genetic distance was performed with the software PopGene 1.32. A dendrogram was generated based on UPGMA analysis of array-based genetic distance of Nei (1978).

Table 1—Plant location of *Puccinia psidii* pustules collected from six *Eucalyptus* sp. clones obtained from crosses between susceptible and resistant trees of *E. grandis* located in Jacarei-SP, Brazil, from Fibria SA breeding program.

Sample Number	Description
1	Clone A, Plant 1, Leaf 1, Tree top
2	Clone A, Plant 1 ,Leaf 2 , Pock 1 ,Tree top
3	Clone A, Plant 1, Leaf 2 ,Pock 2 ,Tree top
4	Clone A ,Plant 1, Leaf 3 ,Tree bottom
5	Clone A, Plant 1, Leaf 4 , Tree bottom
6	Clone A, Plant 2, Leaf 1, Tree top
7	Clone A, Plant 2, Leaf 2, Tree bottom
8	Clone A, Plant 2, Leaf 3, Pock 1, Tree bottom
9	Clone A, Plant 2, Leaf 3, Pock 2, Tree bottom
10	Clone A, Plant 2, Leaf 4, Tree top
11	Clone B, Plant 1, Leaf 1, Pock 1, Tree top
12	Clone B, Plant 1, Leaf 1, Pock 2, Tree top
13	Clone B, Plant 1, Leaf 1, Pock 3, Tree top
14	Clone B, Plant 1, Leaf 1, Pock 4, Tree top
15	Clone B, Plant 1, Leaf 2, Pock 1, Tree top
16	Clone B, Plant 1, Leaf 2, Pock 2, Tree top
17	Clone B, Plant 1, Leaf 2, Pock 3, Tree top
18	Clone B, Plant 1, Leaf 2, Pock 4, Tree top
19	Clone B, Plant 1, Leaf 2, Pock 5, Tree top
20	Clone B, Plant 1, Leaf 3, Pock in the stem, Tree top
21	Clone B, Plant 2 ,Leaf 1, Pock in the petiole, Tree top
22	Clone B, Plant 2, Pock in the stem, Tree top
23	Clone B, Plant 3, Leaf 1, Pock 1, Tree top
24	Clone B, Plant 3, Leaf 1, Pock 2, Tree top
25	Clone C, Plant 1, Leaf 1, Tree top
26	Clone C, Plant 1, Leaf 2, Tree top
27	Clone C, Plant 1, Leaf 3, Pock 1, Tree top
28	Clone C, Plant 1, Leaf 3, Pock 2, Tree top
29	Clone C, Plant 1, Leaf 3, Pock 3, Tree top
30	Clone C, Plant 2, Leaf 1, Tree top
31	Clone C, Plant 2, Leaf 2, Pock in veins, Tree top
32	Clone C, Plant 4, Leaf 1, Pock in veins, Tree top
33	Clone D, Plant 1, Leaf 1, Pock 1, Tree top
34	Clone D, Plant 1, Leaf 1, Pock 2, Tree top
35	Clone D, Plant 1, Leaf 2, Pock 1, Tree top
36	Clone D, Plant 1, Leaf 2, Pock 2, Tree top
37	Clone D, Plant 2, Leaf 1, Pock in the petiole, Tree top
38	Clone D, Plant 2, Leaf 2, Tree top
39	Clone D, Plant 2, Leaf 3, Pock in the petiole, Tree top
40	Clone D, Plant 2, Leaf 4, Pock in the petiole, Tree top
41	Clone D, Plant 3, Leaf 1, Tree top
42	Clone D, Plant 3, Leaf 2, Tree top
43	Clone E, Plant 1, Leaf 1, Tree top
44	Clone E, Plant 1, Leaf 2, Tree top
45	Clone E, Plant 1, Leaf 3, Pock 1, Tree top
46	Clone E, Plant 1, Leaf 3, Pock 2, Tree top
47	Clone E, Plant 1, Leaf 3, Pock 3, Tree top
48	Clone E, Plant 2, Leaf 1, Tree top
49	Clone E, Plant 2, Leaf 3, Pock 1, Tree top
50	Clone E, Plant 2, Leaf 3, Pock 2, Tree top
51	Clone E, Plant 2, Leaf 4, Tree top
52	Clone F, Plant 1, Leaf 1, Tree top
53	Clone F, Plant 1, Leaf 2, Tree top
54	Clone F, Plant 1, Leaf 3, Tree top
55	Clone F, Plant 1, Leaf 4, Pock 1, Tree top
56	Clone F, Plant 1, Leaf 4, Pock 2, Tree top

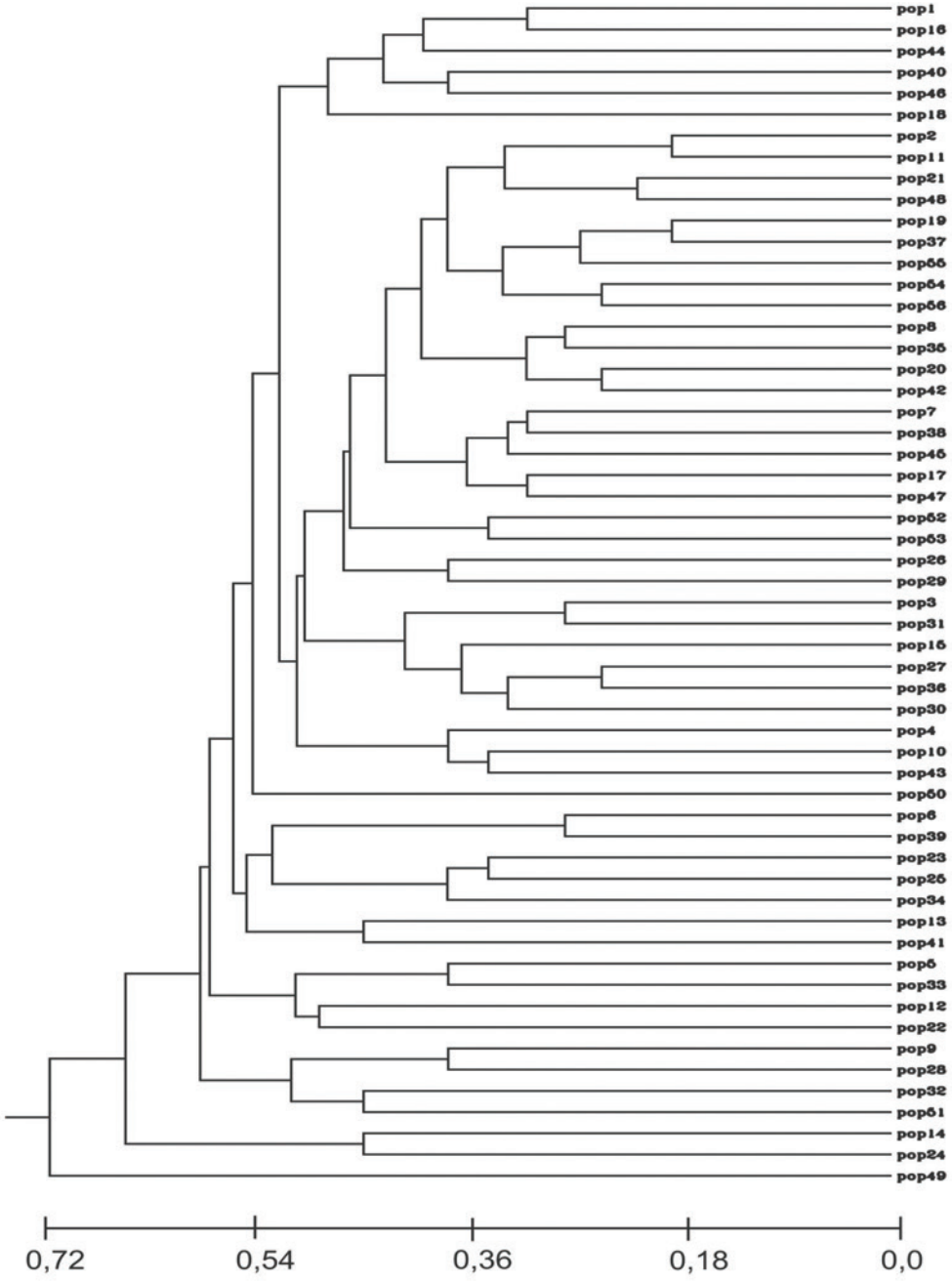

Figure 2—Cluster diagram based on genetic distance calculated from the presence or absence of polymorphic markers across the pustules not grouped by clone. Pop 1 until 56: name of each pustule.

RESULTS AND DISCUSSION

Two dendrograms were built, one with the pustules grouped by clone and another with the pustules separated without regard to the origin clone. In the dendrogram with pustules grouped by clone (fig. 1), we observed two main groups. Group I with isolates from pop1, pop2, and pop3 was subdivided into a cluster formed by pop1 and pop2 with a similarity coefficient of 0.1. Group II is an isolate of pop3. The two groups were correlated, with a similarity coefficient of 0.2. In group II, formed by pop4, pop5, and pop6, two clusters were observed with a few genetic polymorphisms (fig. 1). Note that the first group contains only

susceptible clones and the second group, only resistant clones, which means that *Puccinia* isolates from resistant clones are genetically more similar than isolates from susceptible clones.

The analysis of pustules separately (unrelated to the clone of origin) showed high genetic diversity. We observed the formation of many groups with no apparent relationship between the pustules taken from the same clone forest (fig. 2). There was no relationship between the pustule positions along the tree, showing no fungus preference to infect a specific tree tissue. This high diversity was expected because the fungus is native to Brazil.

These results open a favorable perspective to the development of specific fungus markers for each *Eucalyptus* clone. These and similar results can provide a basis for choosing new strategies for improving plant resistance.

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Competing Interests: The study presented in the manuscript is a collaboration between the University of São Paulo State and Fibria Pulp and Paper. The collaboration did not involve any financial agreement between the two parties. Juliana Érika de Carvalho Teixeira Yassitepe was employed by Fibria at the time of the study, but is no longer with the company. This does not alter the policies on sharing data and materials.

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