

Positive selection evidences on *Moniliophthora* PR-1 genes suggest evolution towards pathogenicity role

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Abstract

Moniliophthora perniciosa is a basidiomycete fungus with three known biotypes, being the C-biotype the one that infects *Theobroma cacao*, causing witches broom disease (WBD). The arrival of WBD in cocoa plantations in Bahia led to enormous economic and social damage. In order to aid the development of forms of pathogen control, the understanding of the plant defense mechanisms and virulence factors of *M. perniciosa* is necessary. Pathogenesis-related 1 (PR-1) proteins, which belong to the SCP/TAPS or CAP superfamily, are widespread markers of the induced defense response in plants against pathogens. Interestingly, *M. perniciosa*'s genome contains 11 PR-1-like genes (named MpPR-1a to k), many of them being highly up-regulated genes during WBD. In this study, we carried out the evolutionary analysis of *M. perniciosa*'s PR-1 genes, also searching for evidence of positive selection shaping those proteins. We used putative PR-1 gene families identified across the genomes of 22 *Moniliophthora* isolates (18 *M. perniciosa* and 4 *M. roreri*) for inference of the gene phylogenetic history within *Moniliophthora* and also for phylogenetic inference among orthologous PR-1 identified from other 17 species from Agaricales order. These analysis revealed that PR-1c, a highly expressed gene during WBD, is possibly exclusive to C-biotype isolates and is a recent paralog of PR-1j. Besides, the five most recent PR-1 genes in the gene phylogenetic tree are the ones expressed during infection (f, g, i, k, h). The phylogenetic inference with Agaricales PR-1 genes revealed that PR-1a, b, d, and j, were the gene families with most orthologous from the various species, while PR-1i, g and k are only represented in *Moniliophthora* isolates. PR-1a, b and d are ubiquitously expressed during *M. perniciosa* mycelium stages and PR-1j is expressed in basidiomata, suggesting that these proteins might have a role for fungi basal metabolism. Evolutionary models of positive selection were tested in all *Moniliophthora* PR-1 gene families using the dN/dS ratio with codeml package of PAML4. While branch-sites model using C-biotype branches as foreground did not detect evidence of positive selection, testing for sites model detected signals of sites under positive selection in four PR-1 families (PR-1f, g, h, i), which are strongly expressed during WBD. These proteins might have evolved from non-pathogenic PR-1 proteins and became advantageous for the pathogen's success in host infection during *Moniliophthora* recent evolution, revealing important proteins and codon-targets for the pathogenicity of *M. perniciosa* in cocoa.

Funding: Supported by FAPESP (2017/13015-7)