

Genetic Diversity of *Phakopsora pachyrhizi*

Stuerm C; Bernasconi A; Haas HU; Sierotzki H; Torriani SFF

Syngenta Crop Protection Münchwilen AG, Schaffhauserstrasse 215, 4332 Stein, Switzerland

Email: carolina.stuerm@syngenta.com

INTRODUCTION

The sensitivity of *Phakopsora pachyrhizi* Syd. and *P. Syd.* to Qo inhibitors (QoIs) has rapidly shifted since 2013 in Brazil. In order to check whether the quick resistance to QoIs (conferred by the F129L mutation in *cytb* gene) is associated with other genetic changes in the *P. pachyrhizi* populations in Brazil, sequences from the nuclear ribosomal internal transcribed spacer (ITS) regions and 5 intergenic regions of the mitochondrial genome from different regions and different years (collected before and after the QoI resistance in Brazil) have been analysed.

MATERIALS AND METHODS

The protocol developed by Freire et al. 2008 was used to amplify DNA from the ITS 1 and ITS 2 regions. A total of 186 sequences from mostly Brazilian samples collected during the 2014-2015 season were analysed together with 642 additional sequences from previous studies (Freire et al. 2012; Zhang et al. 2012; Jorge et al. 2015). Overall 828 global ITS sequences spanning four decades of *P. pachyrhizi* populations were analysed.

RESULTS AND DISCUSSION

The analysis inferred from five intergenic mitochondrial regions and the *cytb* for a total of 6'624 bp (about the 20% of the total mtDNA) showed that the mitochondrial genome of *P. pachyrhizi* is highly conserved. The only polymorphisms detected, were a microsatellite and the mutation F129L in the *cytb* gene. The population study based on the temporal network analysis inferred from ITS sequences identified 28 ribotypes (Fig.1). Four ribotypes (H1, H12, H21, and H27) were considered as most frequent and were shared between populations from different continents and temporal origins. The same population structure was monitored before and after the emergence of QoI resistance in Brazil (2013). All together these results indicate that the Brazilian population structure of *P. pachyrhizi* did not change substantially after the rapid evolution of QoI resistance. The quick spreading of the mutation F129L in multiple ITS ribotypes of the Brazilian *P. pachyrhizi* populations and the most probable absence of sexual recombination in nature (Bromfield 1984) might indicate that this mutation have emerged independently in different genotypic backgrounds. Further studies in this direction are needed to clarify the previous assumption. The four most frequent ribotypes globally shared could be explained by strong wind-dispersal of spores and/or transport of contaminated plant material.

Geographically unique and rare haplotypes were found worldwide. Further studies are necessary to define if these rare haplotypes reflect locally adapted genotypes.

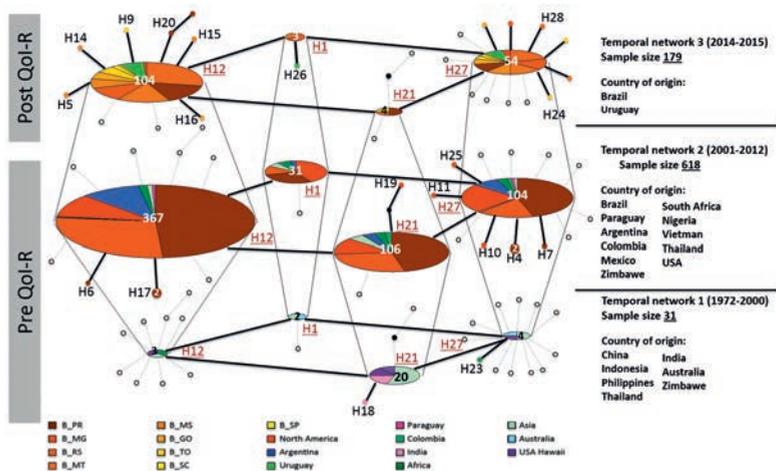


Figure 1 Temporal ribotype network of *P. pachyrhizi* inferred with 828 ITS sequences excluding insertions and deletions (indels). The ITS sequences were collapsed into 3 temporal networks. The 28 ribotypes (H1- H28) explaining the global genetic diversity are presented as circles. The four most frequent ribotypes (H1, H12, H21, and H27) are underlined. The size of the circle within each temporal network is proportional to the sequences number collapsing into the ribotypes. The number of sequences per ribotype is written if this is bigger than 1. Empty circles are missing ribotypes within the temporal networks. For each ribotype is indicated in colour the different geographic origin. B_ = Brazil, PR=Paraná, MG=Minas Gerais, MT=Mato Grosso, MS = Mato Grosso do Sul, RS=Rio Grande do Sul; GO= Goiás, SC= Santa Catarina, TO= Tocantins, SP= São Paulo. Lines connecting each ribotype refer to a single mutation. Vertical lines connecting the 3 temporal networks highlight shared ribotypes.

REFERENCE

Bromfield KR (1984). *Soybean rust*. St.Paul, MN: American Phytopathological Society.

Freire MCM; Oliveira LO; Almeida AMR; Schuster I; Moreira MA; Liebenberg MM; Mienie CMS (2008). Evolutionary history of *Phakopsora pachyrhizi* (the Asian soybean rust) in Brazil based on nucleotide sequences of the internal transcribed spacer region of the nuclear ribosomal DNA. *Genetics and Molecular Biology* 31, 920-931.

Freire MCM; Silva MR; Zhang X; Almeida AMR; Stacey G; Oliveira LO (2012). Nucleotide polymorphism in the 5.8s nrDNA gene and internal transcribed spacers in *Phakopsora pachyrhizi* viewed from structural models. *Fungal Genetics and Biology* 49, 95-100.

Jorge VR; Silva MR; Guillin EA; Freire MCM; Schuster I; Almeida AMR; Oliveira LO (2015). The origin and genetic diversity of the causal agent of Asian soybean rust, *Phakopsora pachyrhizi*, in South America. *Plant Pathology* 64, 729-737.

Zhang XC; Freire MCM; Le MH; de Oliveira LO; Pitkin JW; Segers G; Concibido VC; Baley GJ; Hartman GL; Upchurch G; Pedley KF; Stacey G (2012). Genetic diversity and origins of *Phakopsora pachyrhizi* isolates in the United States. *Asian Journal of Plant Pathology* 6, 52-65.