External application of dsRNA targeting a *Phakopsora pachyrhizi* effector candidate attenuated fungal pathogenicity

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Introduction

Asian soybean rust (ASR) is currently the most severe soybean disease and it is caused by the obligate biotrophic fungus *Phakopsora pachyrhizi*. Only in Brazil, the second main producer, costs in dealing with ASR were estimated in US$2.2 billion in 2013/2014 (Consórcio Antiferrugem, 2015) and, in the absence of control measures, yield losses can reach 90% (Hartman et al. 2015).

The pathogen secrets a set of effector proteins during infection to interfere with plant defense and successfully colonize the host. *P. pachyrhizi* families of effector candidates has been predicted and its ability in suppress plant immunity was previously demonstrated (Link et al. 2014; De Carvalho et al. 2016).

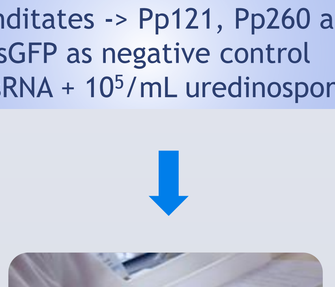
A potential strategy for controlling ASR is by using RNAi to silence *P. pachyrhizi* genes. Silencing constructs of pathogen genes, such as dsRNA targeting of effectors candidates, would be expected to be processed by the plant RNAi machinery to produce siRNA molecules that are taken up by the pathogen and interact with the RNA-induced silencing complex to target the pathogen mRNA for degradation or inhibition of the translation (Hannon, 2002). It is also possible that the RNAi machinery of the fungus is being activated (Hu et al. 2013). The down regulation of critical virulence and/or essencial genes can lead to compromised pathogenicity and infection, and, consequently, contribute to plant resistance (Godoy et al. 2016). Besides, external application of dsRNA has proven effective for the control of nematode and insect (Fire et al. 1998; Hunter et al. 2012).

Objective

Considering the ability of recently described of another biothrophic fungi uptake dsRNA from the environment, as well as the effectiveness of externally application of these molecules in inhibiting pathogen virulence, the aim of this study was to applied a similar approach to check the potential role of three *P. pachyrhizi* effector candidates in the pathogenicity.

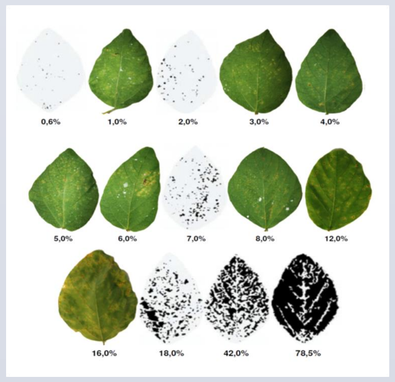
Methods

3 effector canditates -> Pp121, Pp260 and Pp5370
dsGFP as negative control
300 ng uL-1 dsRNA + 10^5/mL uredinospores solution



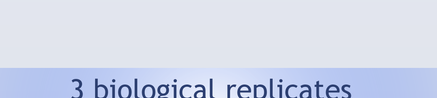
3 biological replicates
10 detached leaves
Evaluation 14 days after infection

Disease severity



Adapted (Godoy et al 2005)

Sporulation level
Nº of uredinia per lesion
Nº of open uredinia per lesion



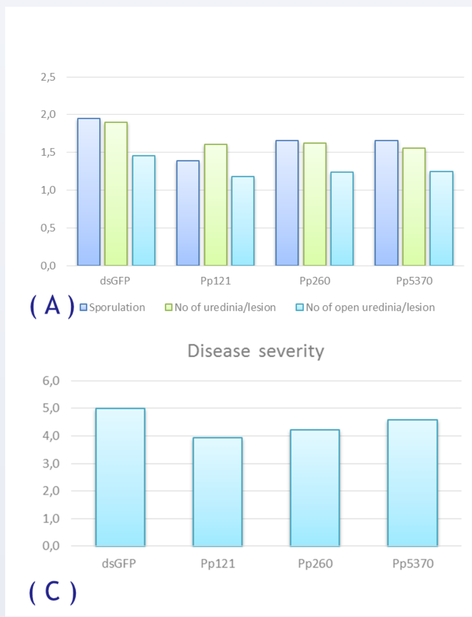
(Yamanaka et al 2015)

Infection frequency

✓ Classification criteria of rust reaction (Akamatsu et al 2013)

✓Statistical level of significance at 5% Dunnet test

Results



Treatment	Sporulation level	Average	N° uredinia	Average	Reaction type
GFP	1,34		1,08		
	1,77	1,99	1,04	1,49	Susceptible
	2,87		2,35		
121	0,63		0,48		
	1,69	1,44	1,40	1,23	Resistant
	2,00		1,82		
260	0,97		0,79		
	1,45	1,73	1,08	1,29	Intermediate
	2,76		1,99		
5370	0,91		0,73		
	1,59	1,74	1,31	1,30	Intermediate
	2,71		1,87		

(D)

Figure 1: Phenotypic parameters evaluation 14 days after inoculation: Sporulation level, number of uredinia per lesion, number of open uredinia per lesion (A); Infection frequency (B); Disease severity (C) and classification criteria of rust reaction (D)

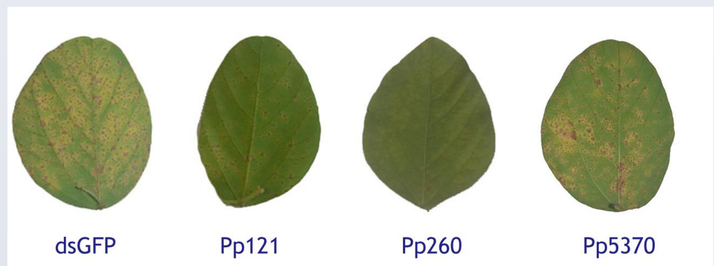


Figure 2: Phenotypic differences among the three effector candidates using dsRNA sprayed with spores solution 14 days after inoculation.

A statistical level of significance at 5% in a Dunnet test was applied and compared with a control, dsRNA targeting GFP, demonstrating attenuation on fungal pathogenicity when Pp121 and Pp260 were silenced (Figure 2). The effector candidate Pp5370 did note show any differences when compared with dsGFP control.

Conclusion

The identification and characterization of *P. pachyrhizi* effectors is expected to provide insight into the mechanisms by which this fungus manipulates soybean to promote infection and elicit Rpp-mediated defense. This study showed that Pp121 and Pp260 can play essencial role in the disease infection by attenuating it. Also, the results obtained here suggest the presence of a silencing machinery in the *P. pachyrhizi* fungus, which was observed in Basidiomycota by Hu et al (2013).

Moreover, there is no report on the application of HIGS in soybean for fighting ASR. Host induced gene silencing experiments mediated by virus silencing system in soybean is being conducted and might confirm the results and give additional evidence of an active machinery of gene silencing in *P. pachyrhizi*. It could also support the understanding of molecular role of fungal effectors.

References

Consórcio Antiferrugem. Ferrugem da soja. Disponível em: <ww.consorcioantiferrugem.net>

De Carvalho, M. C. C. G. et al. Prediction of the in planta *Phakopsora pachyrhizi* secretome and potential effector families. Molecular Plant Pathology, 2016.

Fire, A. et al. Potent and specific genetic interference by dsRNA in *Caenorhabditis elegans*. Nature, 1998.

Godoy, C. V. et al. Asian soybean rust in Brazil: past, presente and future. Pesq. agropec. bras., Brasília, 2016.

Hannon, G.J. RNA interference. Nature, 2002.

Hartman, G.L. et al. Compendium of soybean diseases and pests. 5th ed. St. Paul: American Phytopathological Society, 2015.

Hu, Y. et al. Evolution of RNA interference proteins dicer and argonaute in Basidiomycota, 2013.

Hunter, W. B. et al. Advances in RNA interference: dsRNa treatment in trees and grapevines for insect pest supression. Southwest Entomol., 2012.

Link, T. et al. The haustorial transcriptomes of *Uromyces appendiculatus* and *Phakopsora pachyrhizi* and their candidate effector families. Molecular Plant Pathology, 2014.