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Phakopsora pachyrhizi secretome expressed during soybean interaction and characterization of effector candidate families

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Asian soybean rust (ASR), caused by the obligate biotrophic fungus *Phakopsora pachyrhizi*, can cause losses greater than 80%. Despite its economic importance, there is no soybean cultivar with durable ASR resistance. In addition, the *P*.

pachyrhizi genome is not yet available. However, the availability of other rust genomes, as well as the development of sample enrichment strategies and bioinformatics tools, has improved our knowledge of the ASR secretome and its potential effectors. In this context, we used a combination of laser capture microdissection (LCM), RNAseg and a bioinformatics pipeline to identify a total of 36,350 P. pachyrhizi contigs expressed in planta and a predicted secretome of 851 proteins. Some of the predicted secreted proteins had characteristics of candidate effectors: small size, cysteine rich, do not contain PFAM domains (except those associated with pathogenicity) and strongly expressed in planta. A comparative analysis of the predicted secreted proteins present in Pucciniales species identified new members of soybean rust and new Pucciniales or *P. pachyrhizi*-specific families (tribes). Members of some families were strongly upregulated during early infection, starting with initial infection through haustorium formation. Effector candidates selected from two of these families were able to suppress immunity in transient assays, and were localized in the plant cytoplasm and nuclei. Host induced gene silencing has been used to transiently silence effectors candidates demonstrating its involvement in pathogenicity.