

Genome wide analysis of quantitative disease resistance against *Verticillium* wilt in the model legume *Medicago truncatula*

M. Mazurier¹, M. Toueni¹, A. Sbeiti¹, M.C. Tardin², M.C. Gras², M. Rickauer¹, L. Gentzbitte¹, C. Ben^{1*}

¹ *EcoLab, Université de Toulouse, CNRS, Toulouse INP, UPS, Toulouse, France*

² *R2n – Centre de Recherche Le Bourg, Druelle, France*

* e-mail: ben@ensat.fr

Key words: GWAS, quantitative trait loci (QTL), candidate gene, root disease, biodiversity, functional validation, biotechnology

Motivation and Aim: Legumes are one of the most important crop families with high nutritional value thanks to their nitrogen-fixing symbiosis with rhizobia. However, they are prone to many diseases which reduce yields. The interaction between the model legume *Medicago truncatula* and the root pathogen *Verticillium alfalfae* is studied to investigate the genetic mechanisms involved in quantitative disease resistance against *Verticillium* wilt in Legumes.

Methods and Algorithms: Genome-wide association studies (GWAS) were conducted on disease parameters assessing disease development and plant colonization in a collection of 242 *M. truncatula* accessions. The disease parameters were obtained after fitting non-linear mixed models to the time-course disease symptoms. The analyses on adjusted means from an augmented-block experimental design were performed with the TASSEL v5 software by implementing a linear mixed model including kinship as the variance-covariance matrix of the random genetic effects, and population structure as fixed effects (EMMAX algorithm). A set of 5M SNPs was used.

Results: Symptom scoring and fungus reisolation in 242 *M. truncatula* ecotypes highlighted a large biodiversity of the response to *V. alfalfae* (*Va* V31-2 strain) with a continuous range from fully resistant to susceptible lines. The genome wide association study (GWAS) on various modeled disease parameters pinpointed quantitative trait loci (QTL) on chromosome 1, 7 and 8. Both phenotypic and genetic analyses thus suggest the occurrence of different resistance mechanisms in *M. truncatula* populations towards *V. alfalfae*. Among five candidate genes localized under a previously described major QTL for *V. alfalfae* resistance on chromosome 7 [1], only two genes, encoding for proteins involved in ubiquitination and lipid metabolism, were found to be expressed in response to *V. alfalfae*. The latter one was validated as a disease susceptibility gene towards *Va* V31.2 in *M. truncatula*. Gene silencing using artificial microRNA in A17 (resistant) and F83005.5 (susceptible) lines decreases *Va* V31-2 colonization rate on transgenic roots whereas overexpression of the gene increases the colonization rate in A17.

Conclusion: Understanding *Verticillium* resistance and its genetic control in the model legume *M. truncatula* will help to develop breeding strategies for legume plants which are important components of sustainable agriculture and ecosystems.

Acknowledgements: M. Mazurier was supported by a PhD scholarship from the French “Ministère de la Recherche et de l’Enseignement Supérieur”, M. Toueni by a doctoral grant from Mayotte department, and A. Sbeiti by a PhD grant from “Association des Jeunes de KFARSIR”, managed by Toufik Sbeiti et Mariam Malek, Lebanon. We thank J.M. Prospero for providing seeds and maintaining a large part of the *Medicago truncatula* collections. We thank the *Medicago* Hapmap international consortium, mainly funded by the Plant Genome Program of the National Science Foundation (USA), for providing *M. truncatula* genome resequencing data.

References

1. Ben C. et al. (2013) Natural diversity in the model legume *Medicago truncatula* allows identifying distinct genetic mechanisms conferring partial resistance to *Verticillium* wilt. *J Exp Bot.* 64(1):317-332.