Analysing the role of pathogen effector targets in the plant immune response

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Introduction

- It is well accepted that agricultural production must be increased considerably in the foreseeable future to meet the food and feed demands of a rising human population and increasing livestock production. Crop protection plays a key role in safeguarding crop productivity against competition from weeds, animal pests, pathogens and viruses [2]. For this purpose, disentangling the complex mechanisms and signaling networks in plant immunity are of major importance.
- How do plants defend? [1]

Pathogen stimuli		Immunity mode
Pathogen-associated molecular patterns (PAMPs)	⟹	Pattern-trigger immunity (PTI)
Effectors	⟹	Effector-trigger immunity (ETI)

Pathogen type		Hormone response		Effect	Relationship
Biotrophs	⟹	Salicilic acid (SA) dependent	⟹	Long term immunity + Hypersensitive Response (HR)	Antagonistic
Necrotrophs	⟹	Jasmonic acid (JA) dependent	⟹	Long-term systemic defense response + metabolites	Cross-talk

Figure 1:Schematic representation of systemically induced immune responses [4]

- Under HR, some members of the hypersensitive induced reaction (HIR) gene family are induced. The HIR family genes encode proteins that contain the SPFH domain. Proteins with this domain are usually involved in the formation of lipid rafts [5]. Immune receptors are localized in lipid rafts and HIR2 has been found physically associated with the resistance protein RPS2 [6].
- TCPs are a transcription factor family with roles in both development and hormonal regulation. Several TCPs have been implicated as a target for pathogen effectors, suggesting a function in immune response.
- Our target: Assessing the role of two proteins with postulated roles in immunity, TCP14 and HIR2, as an attempt to characterize a portion of the complex mechanism of the immune response which have been previously studied using microarray.

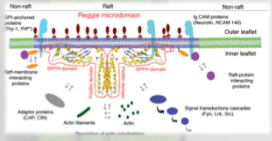
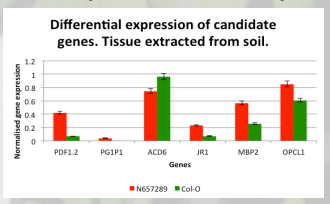


Figure 2: Model of a lipid raft [5]

Results

1. sqRT-PCR: Microarray validation



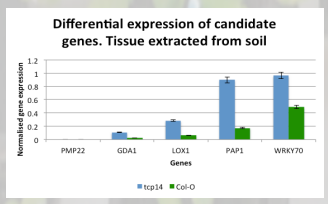
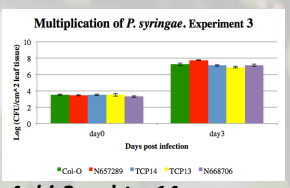


Figure 3:Gene expression levels of *tcp14* and *hir2*

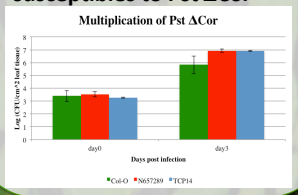
Initial expectations

- JA markers are up-regulated in the mutants
- Knowing the antagonistic relationship between SA and JA it was expected
  - Enhanced susceptibility to biotrophos (*H. arabidopsidis* and *P. syringae*)
  - Opposite effect when exposition to necrotroph (*B. cinerea*)

2. *hir2*, more susceptible to Pst DC3000.



4. *hir2* and *tcp14* more susceptibles to Pst *ΔCor*



3. *hir2*, more susceptible to *Hyaloperonospora arabidopsidis.*

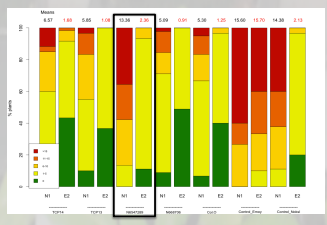


Figure 4: Varying susceptibility of KO lines to *Hyaloperonospora arabidopsidis*. Showing high susceptibility in red and low in green. Susceptibility based on number of sporangiophores per plant after spraying them with one incompatible and one compatible strains. The mutant *hir2* presents and enhanced susceptibility.

Conclusions and future work

TCP14

- In normal circumstances TCP14 interacts with WRKY70 that modulates cross-talking. It also interacts with GRX480-TGA which is involved in suppression of some JA-responsive genes [7]. Working with *tcp14*, JA-responsive genes are not suppressed and also the master regulator of cross talk WRK70 is upregulated provoking a disequilibrium in the immune responses.
- Future work: elucidating whether markers suppressed by WRK70 are down regulated in *tcp14* mutant.

HIR2

- HIR2 is not only important in immunity as its loss causes an increase in the susceptibility of all kind of pathogens, but also it may be essential for the formation of lipid rafts where the receptors required for pathogen detection are congregated. The absence of HIR2 in conjunction with its suggested scaffold function may cause increased susceptibility to many pathogens due to interference with initial signal transductions
- Future work: further characterization of HIR2 and investigation into its role in the plant membrane and specifically in lipid rafts.

5. *hir2* and *tcp14*, more susceptible to *Botrytis cinerea*

Figure 5: Lession of *B. cinerea*. Order of lines: Col-O, *tcp13, tcp14, hir2-1, hir2-2.*

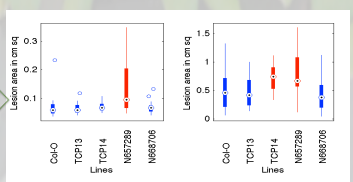


Figure 6: Box plots graph showing the variation of the lesion of *B. cinerea*

References

1. Katagiri, F., and Tsuda, K. (2010). Understanding the Plant Immune System.
2. Oerke, E. C., and Dehne, H. W. (2004). Safeguarding production—losses in major crops and the role of crop protection. Crop Protection *23*, 275–285.
3. Smith, J. L., De Moraes, C. M., and Mescher, M. C. (2009). Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. Pest. Manag. Sci. *65*, 497–503.
4. Pieterse, C. M. J., Leon-Reyes, A., Van der Ent, S., and Van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. Nat Chem Biol *5*, 308– 316.
5. Rivera-Milla, E., Stuermer, C., and Málaga-Trillo, E. (2006). Ancient origin of reggie (flotillin), reggie-like, and other lipid-raft proteins: convergent evolution of the SPFH domain. Cell. Mol. Life Sci. *63*, 343–357.
6. QI, Y., TSUDA, K., GLAZEBROOK, J., and KATAGIRI, F. (2011). Physical association of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) immune receptors in Arabidopsis - QI - 2011 - Molecular Plant Pathology - Wiley Online Library. Molecular Plant Pathology *12*, 702–708
7. Ndamukong, I., Abdallat, A. A., Thurow, C., Fode, B., Zander, M., Weigel, R., and Gatz, C. (2007). SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription. The Plant Journal *50*, 128–139.

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