

Exploring natural variation in tobacco nonhost immune responses to *Erwinia amylovora*

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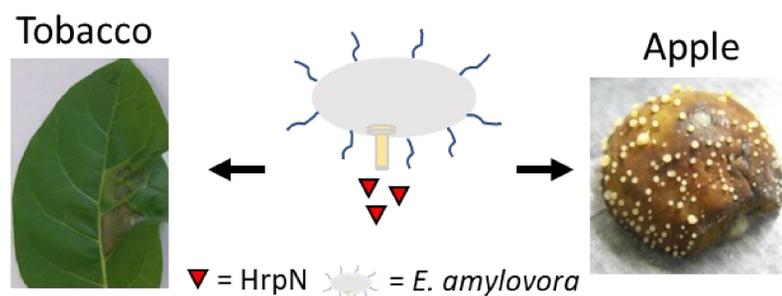
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Background

- The fire blight bacterium *Erwinia amylovora* secretes the protein HrpN ('harpin'), which triggers a strong hypersensitive cell death response (HR) in nonhost tobacco (*Nicotiana tabacum*). In apple, HrpN is a crucial virulence factor which facilitates disease development.



- The genetics underlying HR induction by HrpN in tobacco have remained unexplored.
- Understanding the genetics of this phenotype in tobacco can potentially aid in the development of fire blight resistant apple and pear varieties.

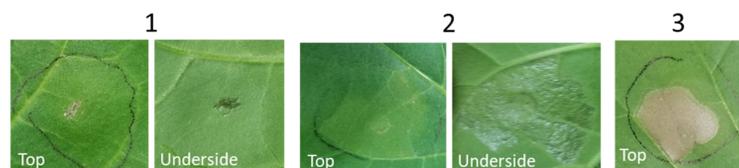
Methods

- An HR-inducing epitope of HrpN, representing amino acids 142-188 (Hrp47), was synthesized and syringe-infiltrated into the leaves of 127 tobacco accessions to assess phenotypic variation in the HrpN-induced HR.



Location of Hrp47 within the full-length 403 aa HrpN protein

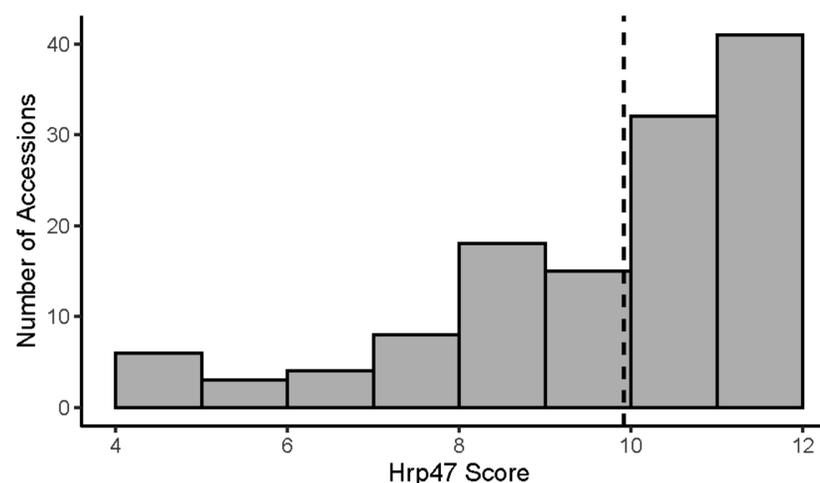
- HRs were scored 2 days after infiltration using a qualitative rating system.



- Quantitative phenotyping of select accessions was performed with electrolyte leakage.

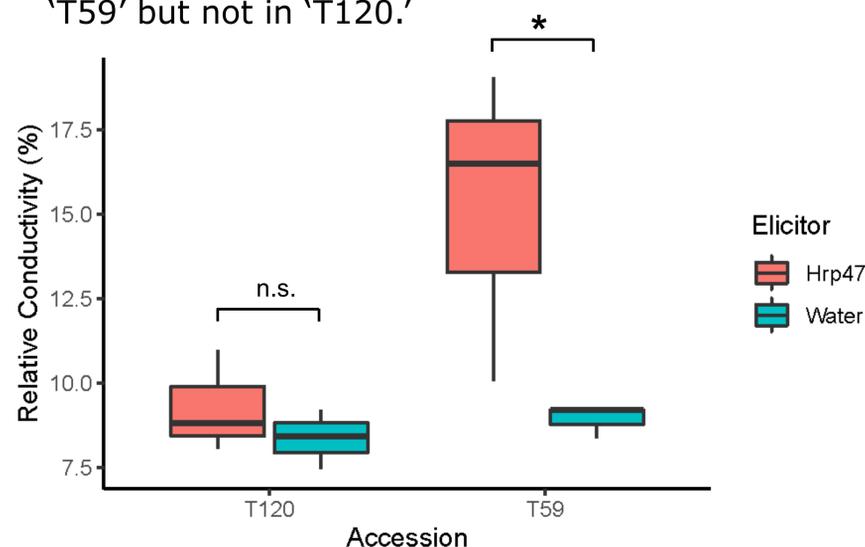
Results

- 65% of tobacco accessions were strongly responsive to Hrp47 (Hrp47 score ≥ 10), and 7% were unresponsive to Hrp47 (Hrp47 score < 6).



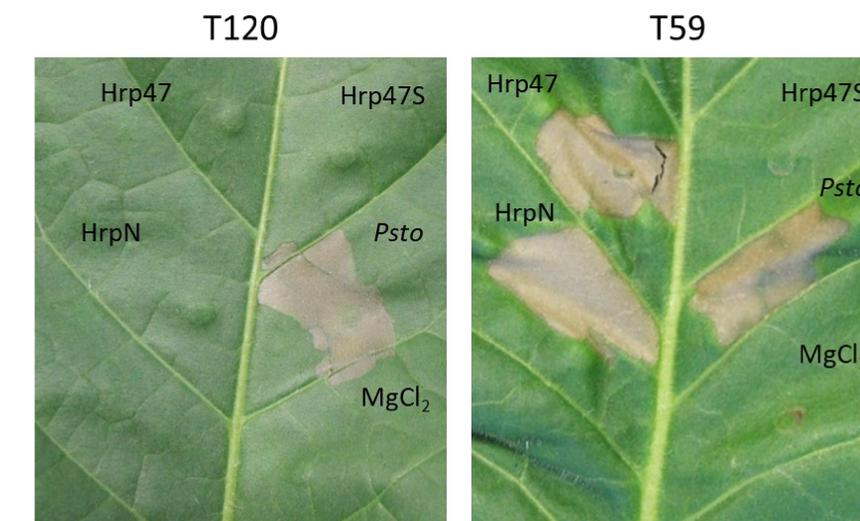
Hrp47 score distribution of 127 tobacco accessions. Three plants of each accession were each infiltrated in four leaves with 100 μ M Hrp47 peptide. Each leaf was scored 2 days later using the rating system described in 'Methods.' Leaf scores were summed for each plant to generate a Hrp47 score and averaged across the three plants for each accession. The dotted line is the experiment mean.

- Quantitative electrolyte leakage was performed on the Hrp47-responsive accession 'T59' and the unresponsive accession 'T120.' Relative Hrp47 leakage was significantly greater than water in 'T59' but not in 'T120.'



Electrolyte leakage in leaves of tobacco accessions infiltrated with water or 100 μ M Hrp47. 24 hours after infiltration, a leaf disc was harvested and placed in distilled water to record the initial conductivity. The discs were then autoclaved and a second reading was taken. The first value was divided by the second value to calculate relative conductivity. N=3 per accession. Brackets indicate comparisons for t-test. * $p < 0.1$; n.s., not significant

- The HR deficiency of T120 is specific to Hrp47 and full-length HrpN.



Representative leaves of tobacco accessions infiltrated with the indicated elicitors. Hrp47S is a peptide with the 47 amino acids of Hrp47 in a scrambled order. *Psto*, *Pseudomonas syringae* pv. *tomato*, OD₆₀₀=0.1. Peptides infiltrated at 100 μ M; HrpN at 25 μ M.

- To investigate the genetics of the Hrp47 HR, an F₂ mapping population of T59 σ x T120 ρ was generated. Segregation of Hrp47-responsive to Hrp47-unresponsive individuals was consistent with a 3:1 ratio.

Generation	Observed Individuals		Expected R:U	Chi-square	P-value
	Hrp47-R ^a	Hrp47-U ^b			
F ₁	20	0	NA	NA	NA
F ₂	174	46	3:1	1.96	0.16

R, responsive; U, unresponsive; NA, not applicable

^a Individuals had at least one '3' rating to Hrp47

^b Individuals had no '3' ratings

Conclusions

- Most tobacco accessions displayed a strong HR to *Erwinia amylovora* HrpN, but a small subset exhibited an HrpN-specific HR deficiency.
- This HR deficiency phenotype segregated as a single recessive allele in a mapping population.

Acknowledgements

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