Transcriptomic analysis of two tomato isolines in response to the fungus *Stemphylium lycopersici*: resistance mechanisms mediated by the Sm locus

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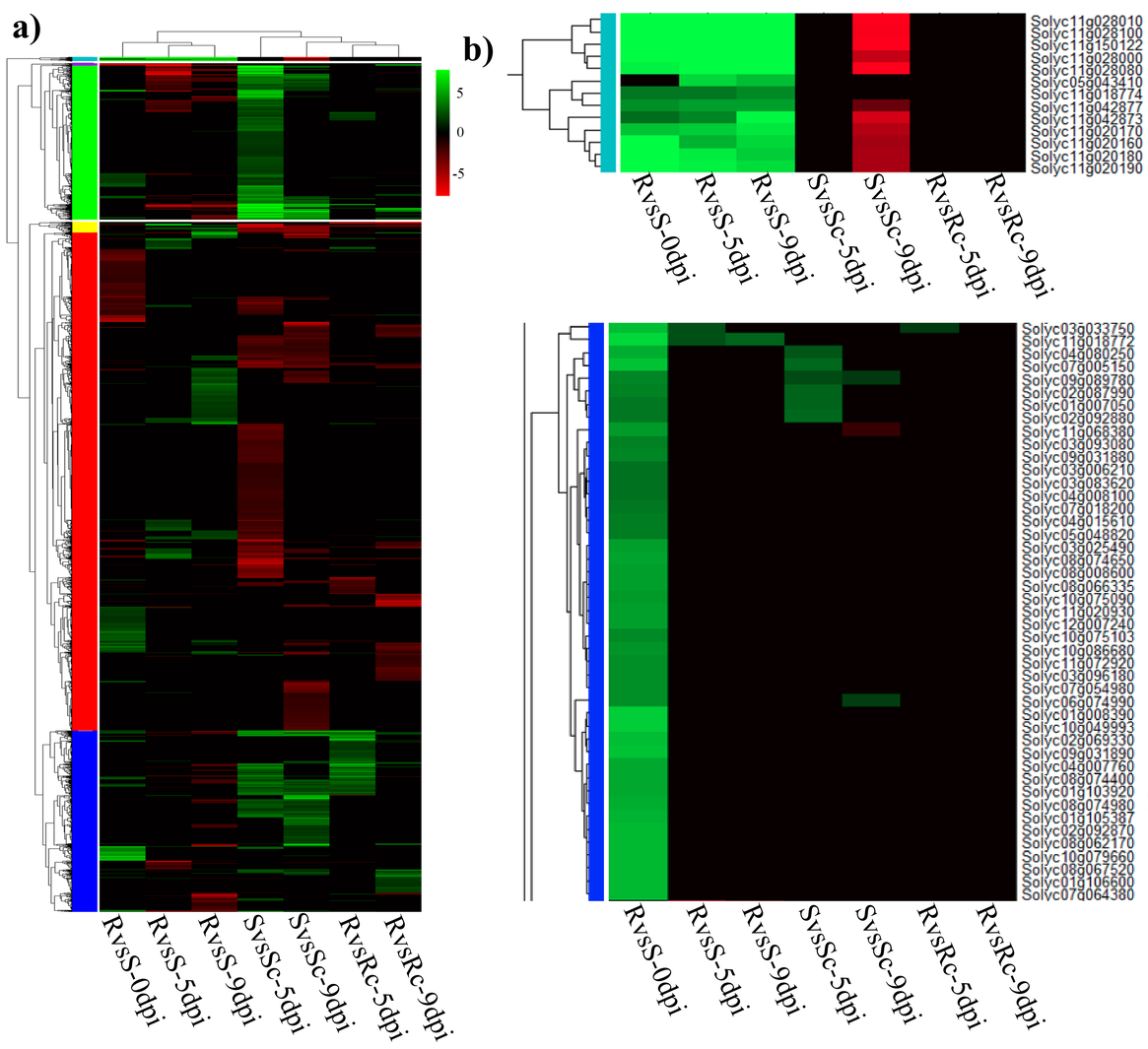
INTRODUCTION

Tomato (*Solanum lycopersicum L.*) is a crop of great importance worldwide and it is the second most important horticultural crop in Uruguay. Fungi of the genus *Stemphylium* cause tomato gray leaf spot, a destructive disease responsible for large production losses in susceptible tomato varieties. Through the INIA breeding program, tomato isolines with different levels of resistance to Stemphylium were generated by incorporating the Sm resistance locus. To identify the mechanisms associated with this resistance, the isolines were inoculated with a virulent isolate of *S. lycopersici* (UYSLS32), which produced clear symptoms in the susceptible isoline by 5 days post inoculation (dpi), whereas the resistant one showed only small spots by 9 dpi.

RESULTS

Hierarchical clustering of DEGs reveals distinct transcriptional profiles between resistant and susceptible isolines



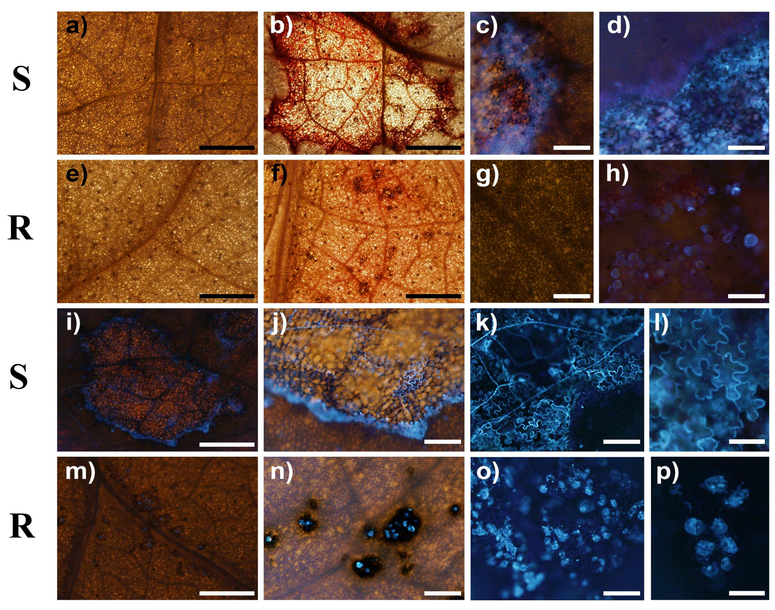
IAN9 (immune signaling), **lectin/glucanase** (pathogen recognition and wall degradation), **PERX** (lignification/ROS) and **NUP88** (nuclear transport of defense regulators).

Several genes located across different loci showed defense-related functions, including receptors (**RLP6**, **Cf-9 homolog**), kinases and E3 ligases (**PBL15**, **PUB21/24**), transcription factors (**MYC4**, **MYB4**, **ORG2**, **SPATULA-like**), and cell wall–modifying enzymes (**XTH23**, **PMEI**, **PERX**, **PPOF**, **NLTPs**).

Genes overexpressed in the resistant line include multiple transcription factors (**bHLH**, **MYB**, **ERF**, **NAC**), hormone-responsive genes (**ABA**, **ethylene**, **auxin**), and enzymes involved in pathogen degradation (**chitinases, β-1,3-glucanases**, **peroxidases**, **subtilisins**). Additional components related to detoxification (**GSTs**, **DTX14**), redox control (**ALD1**, **PERs**), and structural reinforcement (**LTPs**, **expansins**, **KCS11**) indicate a coordinated activation of signaling, metabolic, and structural defense layers.

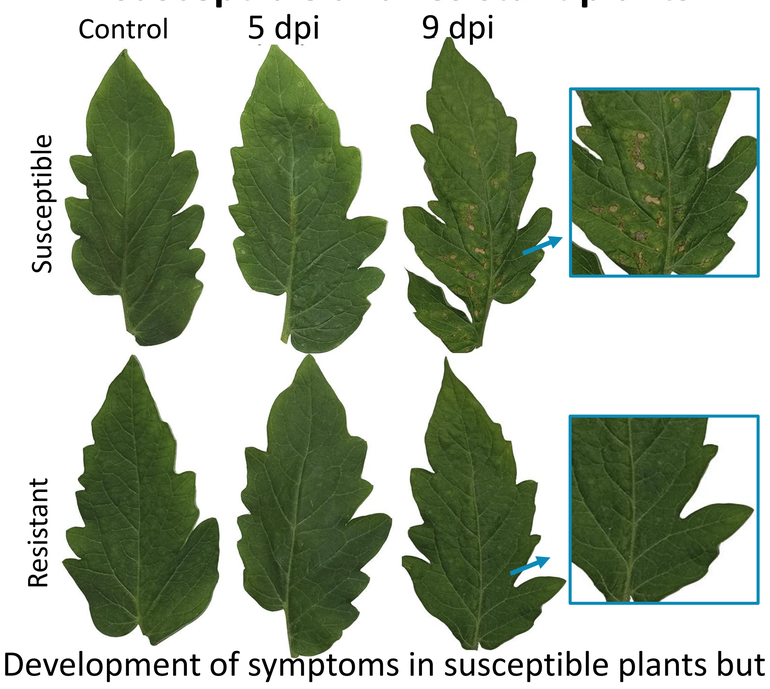
a) Heatmap showing hierarchical clustering of DEGs. **b)** Groups of genes upregulated in the resistant isoline at 0 dpi. **c)** Groups of genes upregulated in the resistant isoline at 5 dpi.

Presence of callose in mesophyll cells in resistant plants



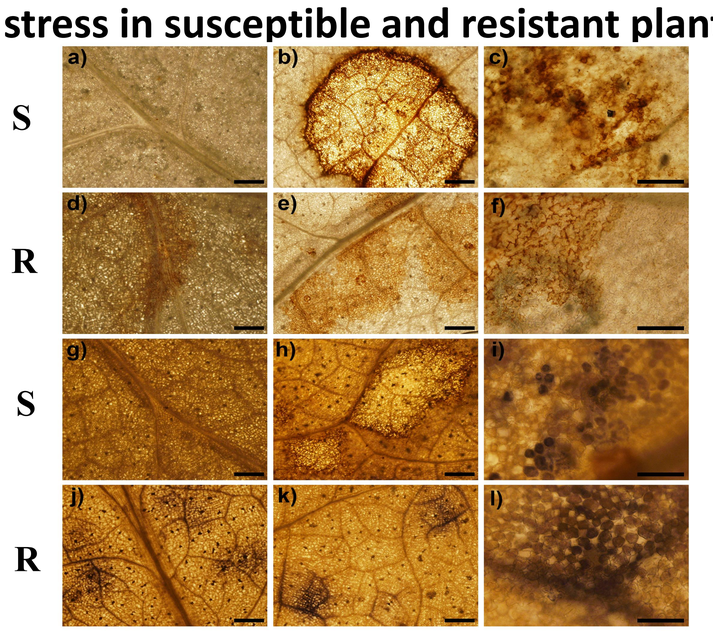
a-h) Safranin and solophenyl staining.
i-p) Callose staining. Panels **a, e)** controls

Symptom differences between susceptible and resistant plants



Development of symptoms in susceptible plants but are absent in resistant plants following infection.

DAB and NBT staining to assess oxidative stress in susceptible and resistant plants



a-f) DAB (3, 3'-diaminobenzidine) staining;
g-l) NBT (Nitro blue tetrazolium) staining.
Panels **a, d, g, j)** Controls

CONCLUSIONS

- The resistant isoline showed, by epifluorescence microscopy, accumulation of callose in mesophyll cells after infection and basal deposits of reactive oxygen species (O_2^- and H_2O_2).
- We saw an increase in gene expression at 0 dpi in the resistant which probably come from *Solanum pimpinellifolium*, which was used for crossing and obtaining the isolines. The genes located near the **Sm** locus include components potentially involved in defense signaling and cell wall reinforcement.
- At 5 dpi in the resistant isoline we see an increase in genes of transcription factors, hormone-response, enzymes involved in pathogen degradation, detoxification, redox control and structural reinforcement.

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