Molecular and metabolomic analysis of resistant potato varieties as a way forward to generate resistance to Ralstonia solanacearum

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INTRODUCTION

Ralstonia solanacearum (*Rs*), an A2 quarantine pathogen, is known to cause Bacterial wilt in potato and some 200 other plant species. The disease has reportedly resulted in severe economic loss in potato, tobacco and tomato leading to complete crop failures. Several strategies, including the molecular analysis of naturally resistant/tolerant plant varieties can be followed in order to understand their resistance mechanism and confer resistance against this devastating pathogen.

Here, we compared two known *Rs*-resistant potato varieties with the susceptible commercial cultivar 'Désirée' by first confirming their resistance/susceptibility, followed by transcriptome sequencing, metabolomic analysis and qPCRs validate gene expression patterns. Our understanding of the resistance pathways particularly in 'Calalo Gaspar' (CG) and 'Cruza' (CR) has ignited potential ideas for generating a gene-edited resistant potato.

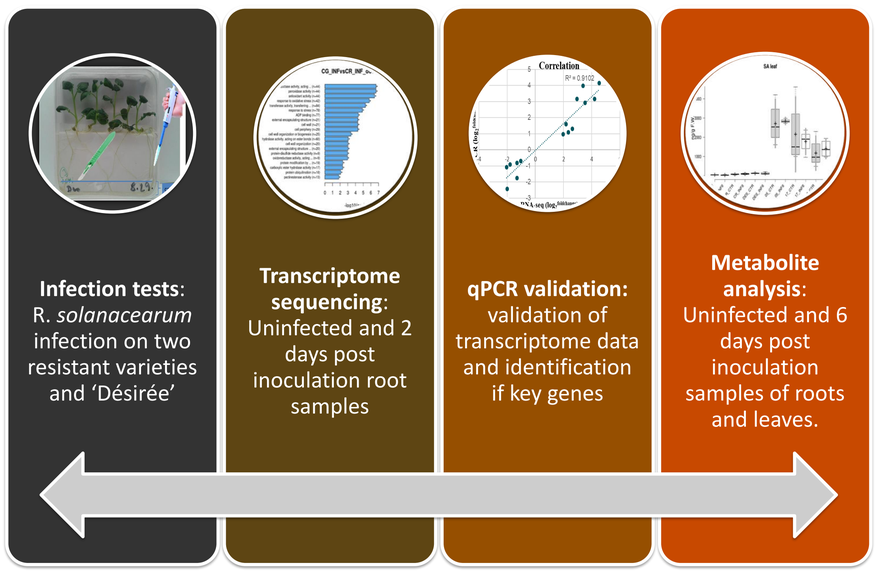
AIMS & OBJECTIVES

Compare the resistant lines with susceptible cultivar 'Désirée'

Analyze differentially expressed genes and find correlation to resistance

Use the knowledge of the mode of resistance to identifiy targets for gene editing

METHODOLOGY



CONCLUSIONS

RESULTS

Inoculation tests

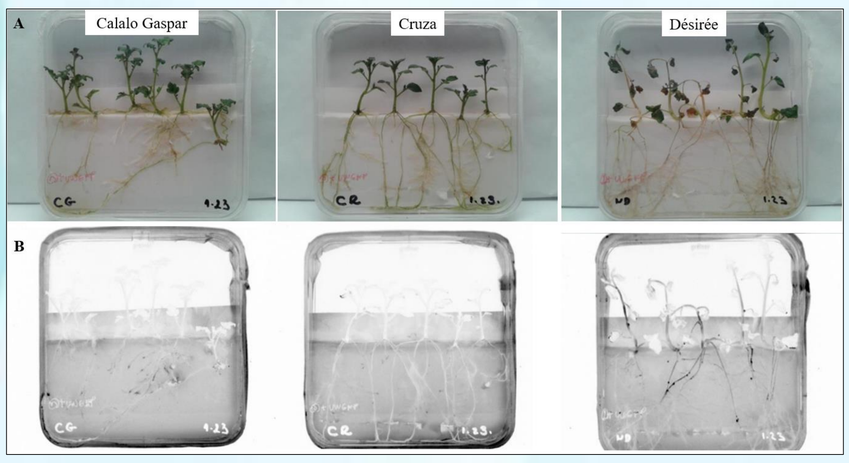


Figure 1. Symptoms of the engineered *Ralstonia solanacearum* strain UW551 expressing GFP at 19 dpi on 'Calalo Gaspar' (left), 'Cruza' (middle) and 'Désirée' (right) under **A)** normal light and **B)** UV light.

Transcriptome analysis

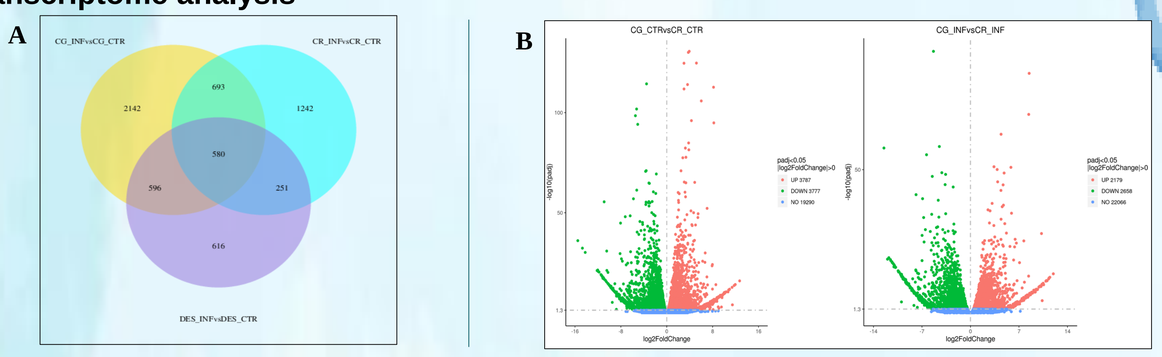


Figure 2. A) Venn diagram of the differentially regulated genes between control (CTR) and infected (INF) root samples of 'Calalo Gaspar' (CG), 'Cruza' (CR) and 'Désirée' (DES) with padj<0.05. **B)** Volcano plot of the upregulated and downregulated genes between control samples of 'Calalo Gaspar' and 'Cruza' (CG_CTRvsCR_CTR), and their infected samples (CG_INFvsCR_INF).

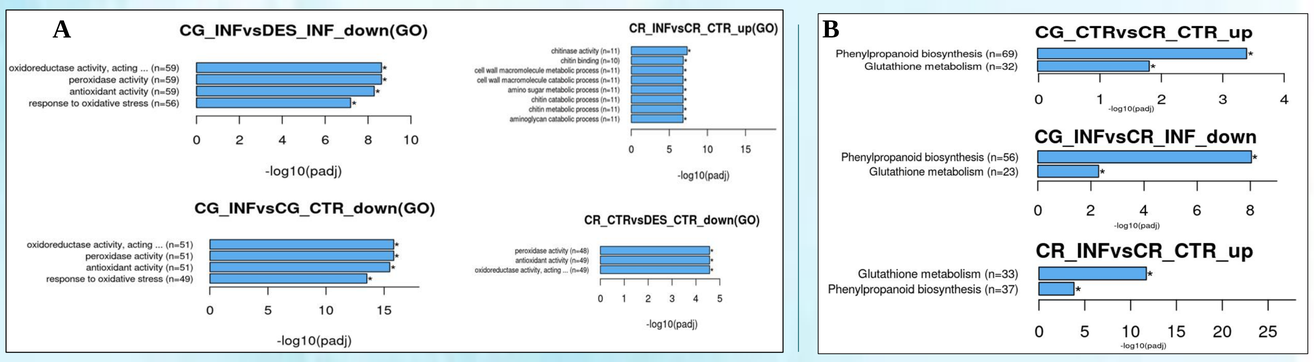


Figure 3. A) Gene ontology analysis of the differentially (up or down)regulated genes between control (CTR) and infected (INF) root samples of 'Calalo Gaspar' (CG), 'Cruza' (CR) and 'Désirée' (DES) with padj<0.05. **B)** KEGG analysis of the differentially regulated pathways between control root samples of 'Calalo Gaspar' and 'Cruza' (CG_CTRvsCR_CTR), their infected samples (CG_INFvsCR_INF) and 'Cruza' infected and control samples (CR_INFvsCR_CTR).

qPCR validation

A

Gene name
Hydroxycinnamoyl transferase
Pathogenesis-related protein STH-2
Wound-induced protein
ABA-inducible protein TAS14
Ascorbate peroxidase

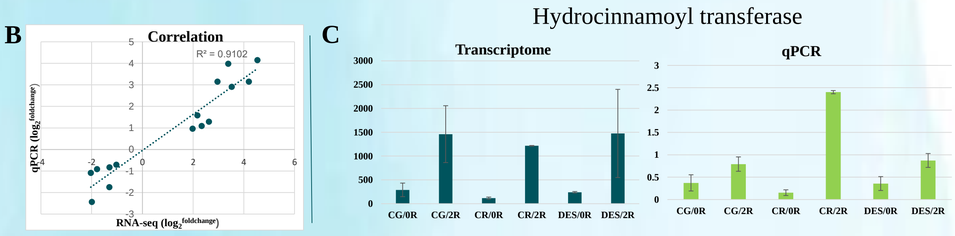


Figure 4. A) List of selected genes that were used to validate transcriptome data. **B)** Correlation of the qPCR (y-axis) and the transcriptome data (x-axis) for 5 selected genes of each variety tested **C)** Hydrocinnamoyl transferase gene expression at 0 and 2 dpi in the three varieties, 'Calalo Gaspar' (CG), 'Cruza'(CR) and 'Désirée' (DES).

Metabolite analysis

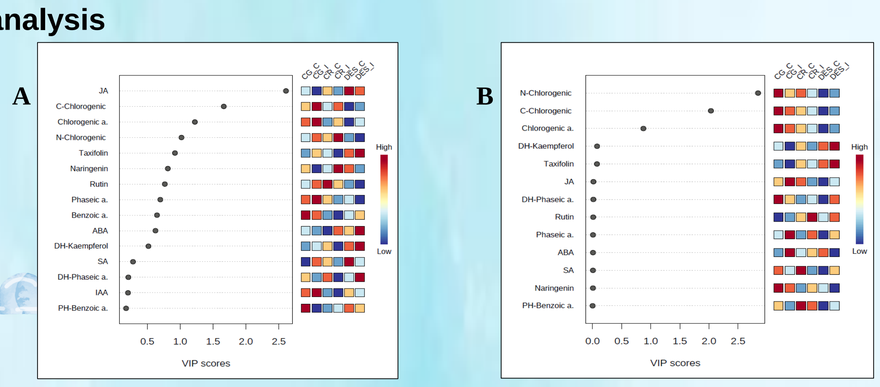


Figure 5. Variable importance projection (VIP) plots and heat maps showing the phytochemical differences in **A)** roots and **B)** leaves of infected (I) and control (C) samples of 'Calalo Gaspar' (CG), 'Cruza' (CR) and 'Désirée' (DES) with significance levels on the x-axis. VIP scores of >1.0 indicate the compounds (y-axis) with major quantitative differences between the tested varieties.

- The two test varieties ('Calalo Gaspar' and 'Cruza'; CG and CR) were verified to be tolerant against *Ralstonia* in comparison to susceptible 'Désirée' control.
- GO analysis showed a strong decrease of oxidoreductase, peroxidase and antioxidant activity in CG, whereas cell wall reinforcement seemed to be upregulated in CR upon *Rs* infection.
- KEGG analysis indicated the involvement of the phenylpropanoid and glutathione metabolic pathways: both types were downregulated in CG upon infection, but upregulated in the CR samples.
- The transcriptome data were validated by qPCRs on five selected genes.
- Targeted metabolite analysis revealed significantly higher quantities of chlorogenic acid, neochlorogenic acid and cryptochlorogenic acid in the resistant varieties than the control.
- The research will enable us in identifying potential targets to generate edited lines resistant to *Ralstonia*.

National Research, Development and Innovation Office

Support by grant No. 132829, K-132967 and ATK-SZIE no. 0205K0036P acknowledged