Bulked segregant RNA-seq reveals key pathways associated with potato resistance to *Dickeya solani*

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Introduction

Potato resistance to pectinolytic bacteria, including *Dickeya solani*, is polygenic. We selected diploid progeny with contrasting phenotypes from a diploid mapping population obtained by crossing a highly resistant form of *D. solani* with a susceptible form. Pooled samples representing extreme phenotypes were collected 8 hours post-inoculation (hpi) and subjected to transcriptome analysis. Two strong, reproducible QTLs for resistance to *D. solani* were discovered on potato chromosomes IV and II. The aim of this study was to identify the key pathways responsible for the high resistance of potato tubers to *D. solani*. Bulked segregant RNA-seq was performed using tissue obtained from potatoes at an early stage of infection (8 hpi). This time point marks the transition from the early latent phase to the symptomatic phase of infection.

Materials

Ten 2*n* potato interspecific hybrids with contrasting levels of tuber resistance to infection with *D. solani* (IFB0099)..

Methods

Potato tubers wound-inoculated with bacteria, mock–inoculated and not wounded were sprayed with water and kept in closed boxes at a temperature 27℃. Tubers were cut after 8 h and tissue was cut out along the wound and immediately frozen in liquid nitrogen. RNA was isolated from three tubers per genotype, resulting in six bulked samples (Fig.1.), prepared in three replicates. The quality criteria for RNA samples: RNA yield: ≥ 2 µg, volume: ≥ 20 µl concentration: ≥ 40 ng/µl, purity: OD260/280 = 2.0–2.2, RNA integrity: 28S:18S ratio ≥ 1.0; RIN ≥ 7.0.

Sequencing was performed on Illumina NovaSeq6000 sequencing platform. Reads were mapped to the reference genome of *Solanum tuberosum* L. (NCBI accession: GCF_000226075.1_SolTub_3.0). Differential gene expression analysis was performed with DESeq2. Biological pathways were determined using the KEGG database with the clusterProfiler package for statistically significant genes ($p \leq 0.01$) and of log2 fold change ≥ 2, with assigned Entrez Ids.

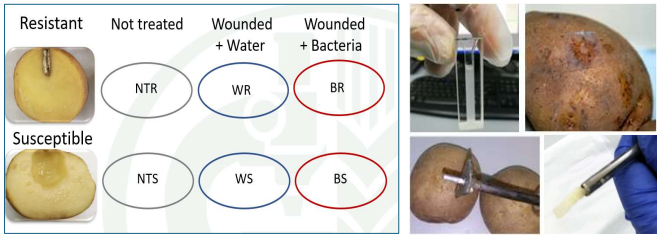

Fig. 1. The experimental design comprised three treatment groups with each group consisting of five potato genotypes that were either resistant or susceptible to *D. solani* infection. Each bulk consisted of five genotypes. Three tubers were sampled from each genotype, for each treatment. NT, non-treated; W, wounded tubers treated with sterile water; B, wounded tubers inoculated with *D. solani*.

Results

A principal component analysis revealed that the 8 hpi time point was the most discriminative for identifying defence-related gene expression, compared with the 24 and 48 hpi time points. Differences among treatments explained 77 % of the variance, while differences in resistance level explained 15%.

DEGs between resistant and susceptible samples, across the three treatments (B: red; W: blue; N: grey) at 8 hpi, selected based on the applied thresholds $p \leq 0.01$, log2 ≥ 2, are shown n Figure 2.

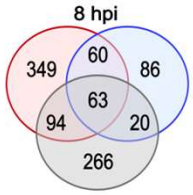

Fig. 2. Venn diagram showing the overlap of significantly upregulated DEGs at 8 hours post-inoculation (hpi) in BS vs. BR (red), WS vs. WR (blue), and NTS vs. NTR (grey) comparisons.

A total of 566 DEGs were found to be significantly up-regulated between the BR and BS bulks at 8 hpi. Figure 3 shows the KEGG pathways enriched with these DEGs.

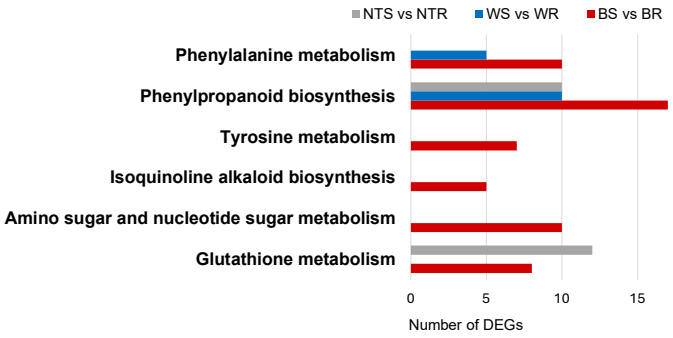

Fig. 3. Top six KEGG pathways enriched among differentially expressed genes (DEGs) at 8 hours post inoculation (hpi) in BS vs. BR (red), against WS vs. WR (blue), and NTS vs. NTR (grey) comparisons.

Conclusions

The present study demonstrates that in the 2*n* interspecific hybrids of *Solanum* spp., secondary metabolic pathways are associated with tuber resistance to *D. solani*. It is hypothesised, that the rapid activation of structural and immune-related pathways may contribute to the higher level of resistance observed in potato tubers. In the present study, a total of 17 differentially expressed genes (DEGs) were identified in the phenylpropanoid pathway. Of these 17 DEGs, five were found to be associated with key suberin biosynthetic pathways and assembly steps, including phenylalanine ammonia lyase-like, three 4-coumarate ligases and cinnamoyl-CoA reductase. In the BR vs. BS comparison at 8 hpi, 566 DEGs were identified. Of these, 18 were found to be located within QTL regions on chromosomes II and IV, as identified in previous studies (Lebecka et al. 2021, Plant Pathology 70: 1745–1756).

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