Differentially expressed genes in potato tubers inoculated with virulent and avirulent races of *Phytophthora infestans*.

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Introduction

Late blight caused by *Phytophthora infestans* is the most destructive potato disease worldwide and there is consequently strong market demand for resistant cultivars. Resistance breeding is based mainly on resistance genes (R-genes) from wild *Solanum* species. However, some R-genes protect the leaves and tubers of potatoes, while others protect the leaves but not the tubers. The most likely explanation of this phenomenon is different levels of R-genes expression, but more general differences in the defence response can also be expected. For our research we chose two R-genes: *Rpi-phu1*, which provides protection of both foliage and tubers, and *R2*, which fails to provide tuber resistance.

Materials & Methods

Plant material was a tetraploid potato clone DC 69 possessing both examined genes: *R2* and *Rpi-phu1*. Double round slices (~27 mm diameter and ~9 mm thick) were cut out from middle part of healthy tubers. The tuber tissue was inoculated by introducing of one droplet of *P. infestans* inoculum (50 sporangia/mm^3) between the slices. Two isolates were used for inoculation: MP 324x (virulent to *Rpi-phu1* and avirulent to *R2*) and 213/20 (virulent to *R2* and avirulent to *Rpi-phu1*). Inoculated slices were placed in glass covered plastic trays and stored for six days at 16 °C in dark.

Fig. 1. Tuber slices test and collection of tuber tissue samples.

Samples of tuber tissue for molecular analyses were collected at days 1, 2, 3, 4 and 5 post inoculation (dpi). The collected tissue was frozen in liquid nitrogen and stored in a deep-freezer until RNA extraction. The relative expression level of *R2* and *Rpi-phu1* was assessed based on the method described by Stefańczyk et al. (2017). The 18SrRNA was used as reference gene. The RNA-seq analyses were performed by external services on Illumina NovaSeq6000 sequencing platform. Differential gene expression analysis was performed with DESeq2 for two time points: 24 and 48 hours post inoculation (hpi).

Results

After six days of incubation tuber slices of DC 69 were assessed as highly resistant to both races of *P. infestans.* Regardless of which isolate was used for inoculation no significant symptoms of *P. infestans* infection were observed.

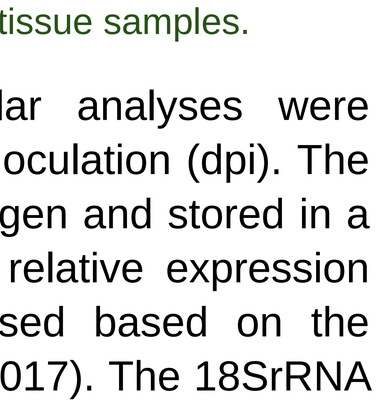

Fig. 2. Tuber slices of DC 69 inoculated with MP 324x (left) and 213/20 (right) after six days of incubation.

The *R2* and *Rpi-phu1* genes exhibited differential expression in tuber tissue. The relative expression level of both genes changed over course of the experiment, but the relative expression of *R2* was generally significantly lower than that of *Rpi-phu1*.

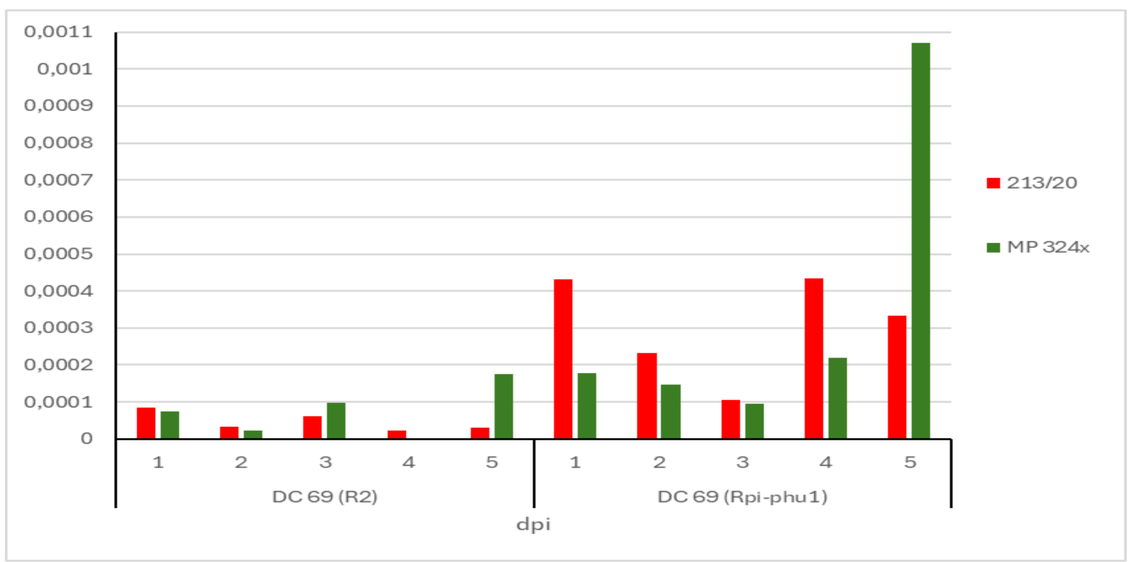

Fig. 3. Level of relative expression of *R2* (left side) and *Rpi-phu1* (right side) genes in tuber slices of DC 69 inoculated with MP 324x (virulent to *Rpi-phu1* and avirulent to *R2*) and 213/20 (virulent to *R2* and avirulent to *Rpi-phu1*) at 1, 2, 3, 4 and 5 dpi.

A comparison of the transcriptome profiles of DC 69 tuber tissue inoculated with two *P. infestans* isolates 24- and 48-hours post inoculation showed significant differences between these samples. At 24 hpi only 92 DEGs (differentially expressed genes) were identified, whereas at 48 hpi 1006 DEGs were identified.

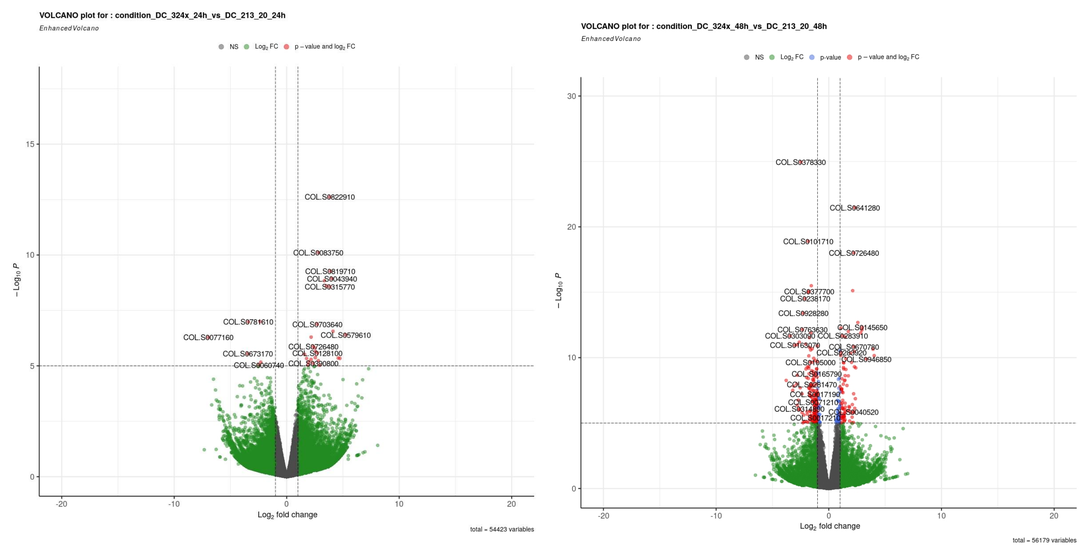

Fig. 4. ‚Volcano' plots illustrating DEGs - differentially expressed genes (both, up- and downregulated) in case of comparisons of samples of tuber tissue inoculated with 213/20 and MP 324x isolates at 24 and 48 hpi.

Conclusions

The tuber slices of potato clone DC 69 were highly resistant after inoculation with both examined races of *P. infestans.* Results of preliminary studies on the relative expression level of *R2* and *Rpi-phu1* genes indicate much lower expression level of *R2*. A comparison of the transcriptome profile in tuber tissue samples of potato clone DC 69 inoculated with MP 324x and 213/20 isolates showed slight differences at 24 hpi (92 DEGs) and more pronounced at 48 hpi (1006 DEGs).

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