TRANSCRIPTOME ANALYSIS UNCOVERS THE GENES REGULATING THE APPLE ROOTSTOCK RESPONSE TO *PHYTOPHTHORA CACTORUM*

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1. Objectives

Considering the plant molecular mechanism of apple rootstock response to *P. cactorum* is not clear, the main aim of the study was to identify specific genes involved in plant pathogen resistance regulation. In this research we have used the Next Generation Sequencing technique to uncover new genes, potentially involved in the mechanism of rootstock response to apple collar and root rot disease.

Phytophthora cactorum is a polyphagous oomycetal pathogen infecting many host plant species. It is the principal agent of root rot in the strawberry and collar rot in the apple trunk, causing inhibition of plant growth and root system development. The motile zoospores are spread through water in soil between the root system of plants, but also through splashing rainfall water on the above ground parts of plants.

❑ **INOCULUM PREPARATION AND PLANT INFECTION**

- *P. cactorum* - fresh PDA medium - 25° C / 14 days,
- medium with oat flakes (5g of oat flakes / 15 ml of water) - 14 days of incubation,
- mixed with a soil – incubation 10 days / 23-25° C,
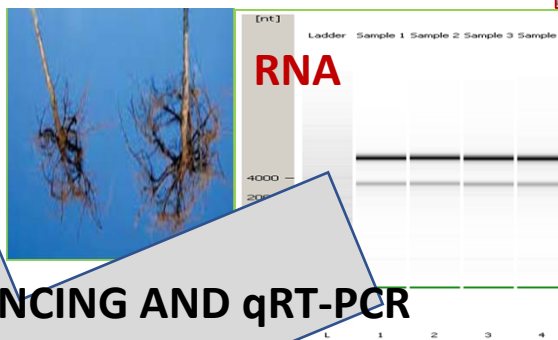- InHort selections PJ-173/2012, PJ-191/2016, M.9, P 59, P 60 (differ in plant response to *P. cactorum*).

❑ **RNA ISOLATION AND REVERSE TRANSCRIPTION**

BAIT TEST – plant selection P173/2012(2022,2023)
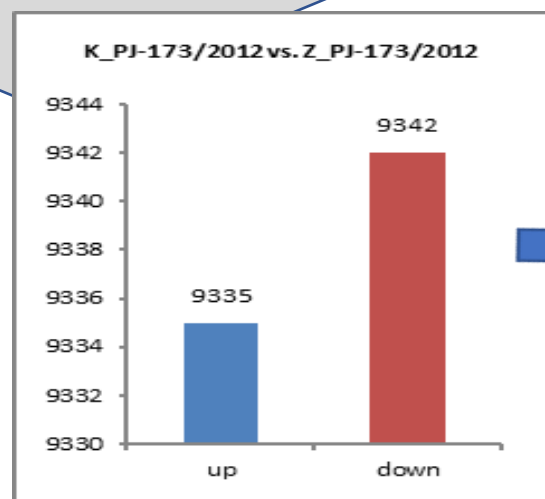Roots - infected and control plants

RNA

❑ **TRANSCRIPTOME SEQUENCING AND qRT-PCR**

NGS

Sequence raw reads / ref. genome
https://www.ncbi.nlm.nih.gov/data-hub/genome/GCF_002114115 .1/

K_PJ-173/2012 vs. Z_PJ-173/2012

up 9335
down 9342

❑ **qRT-PCR DATA ANALYSIS AND STATISTICS**

- GOI relative expression / reference *Md18sRNA* gene (RotorGene 6000 Series Software 1.7)
- The relative change - $2^{-\Delta\Delta Ct}$ method.
- The average relative gene expression, compared to the controls, normalized Md18sRNA (± SEM, GraphPad PRISM 8.1)

Plant potting and growing

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3. RESULTS AND DISCUSSIONS

✓ cDNA libraries - control: K_PJ173/2012 and infected: Z_PJ173/2012, over **34G** clean data

✓ KEGG enrichment analysis, functional groups assignment:

▪ biological process, BP (7 681),
▪ cellular components, CC (2 724)
▪ molecular factors, MF (4 938).

BP group, 323 mapped in **protein processing of endoplasmic reticulum pathway (KEGG - mdm4141).**
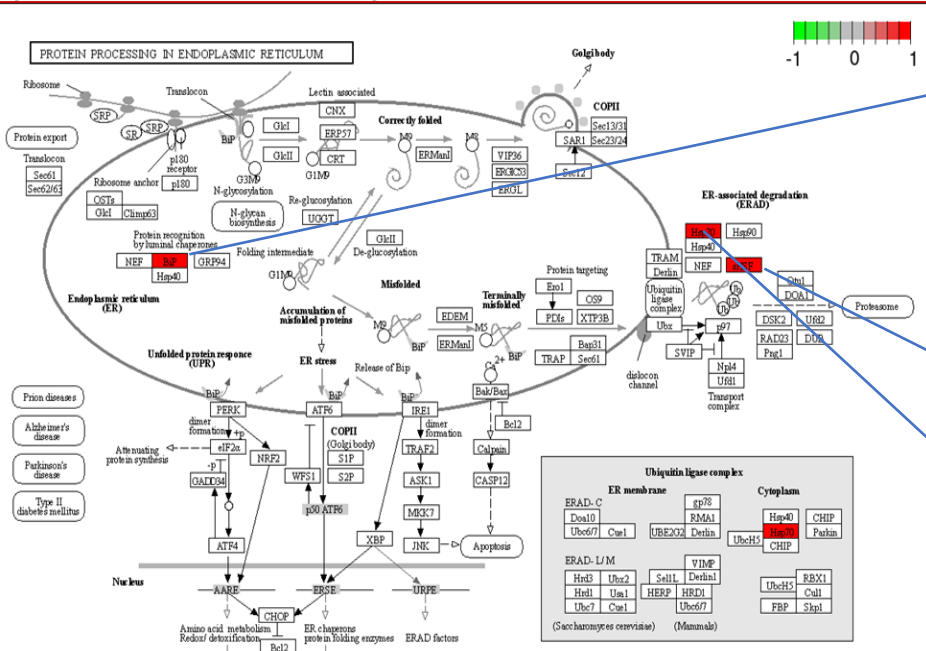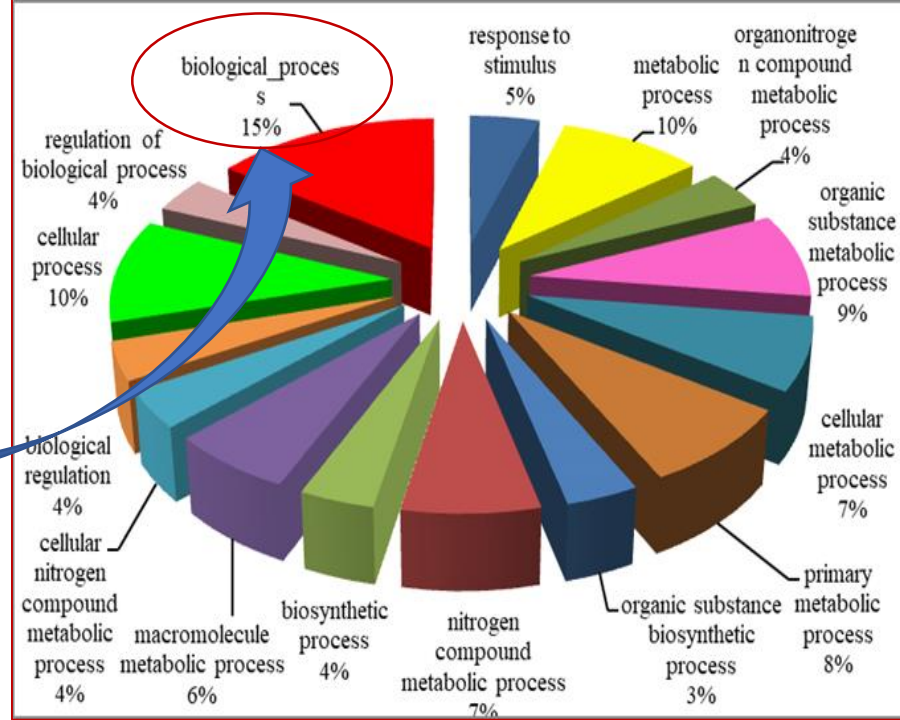
GOI	Gene function	Localization
BiP	Binding protein 5-like endoplasmic reticulum chaperone BiP, Genetic Information Processing.	LOC103412527 (chr 2)
sHSF	class II heat shock protein-like. Genetic Information Processing	LOC103411069 (chr 8)
Hsp70	class IV heat shock protein-like, mediator of RNA polymerase II transcription subunit. Genetic Information Processing.	LOC103435785 (chr 5)

Expression profiling of selected GOI

LOC103411069 (*sHSF*)

LOC10345785 (*Hsp70*)

LOC103412527 (*BiP*)
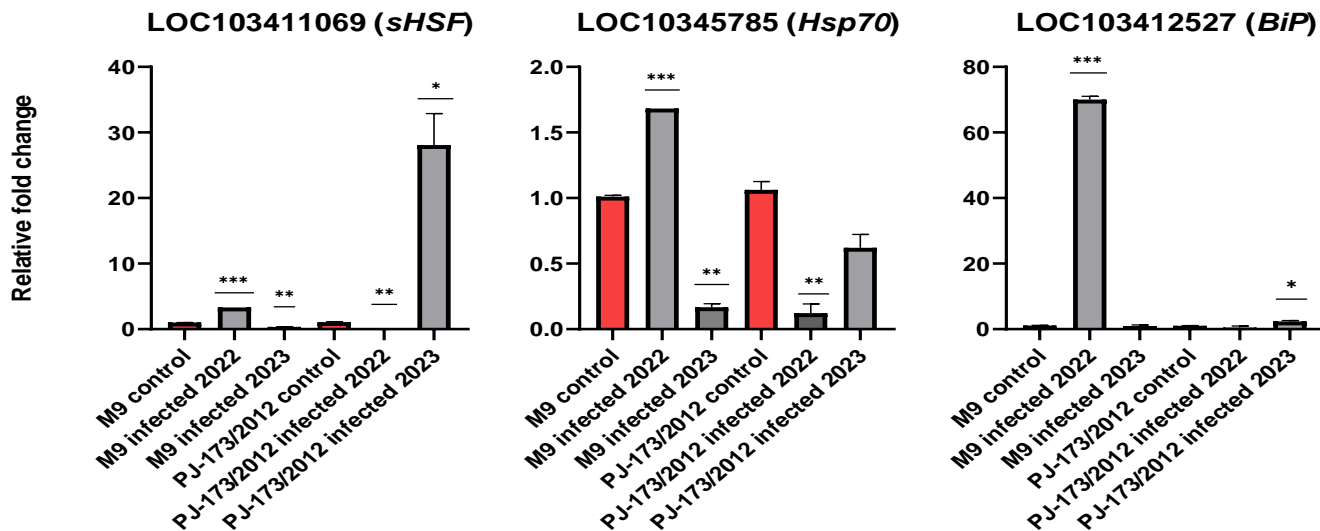
- **sHSF , BiP** - high expression in inoculated PJ-173/2012 in season 2023.
- **All genes** - up regulation in M.9 in the season of 2022.
- **Hsp70** - down regulation in infected PJ-173/2012 - season of 2022.

4. CONCLUSIONS AND PERSPECTIVES

❑ Genes, could be considered as being the first activated in the plant response to the infestation by *P. cactorum*, might be applied as potential functional molecular markers for the selection of newly developed apple rootstocks in breeding programs.

❑ **Perspectives - verification**
Possibility of marker application for the early selection process of rootstocks for apple trees resistant to root rot disease (MAS, Marker Assisted Selection).

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