

Comparative transcriptomics reveals accession and time-point-specific gene expression patterns in grass pea (*Lathyrus sativus*) upon *Erysiphe pisi* infection

Rita Marques¹, Pedro Barros¹, Susana T. Leitão¹, Diego Rubiales², M Carlota Vaz Patto¹ and Carmen Santos^{1*}

¹ Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal; ² Institute for Sustainable Agriculture, CSIC, Córdoba, E-14004, Spain. *e-mail: css@itqb.unl.pt

INTRODUCTION

Grass pea (*Lathyrus sativus*) is a robust and promising crop for a more sustainable agriculture, combining dietary advantages with exceptional agronomic properties, such as drought tolerance¹. However, its yield stability has been constrained by foliar diseases, such as **powdery mildew** caused by *Erysiphe pisi*².

Aiming to advance our knowledge on the grass pea defence molecular mechanisms against *E. pisi*, a comparative transcriptomics approach was performed using four grass pea accessions showing contrasting responses to *E. pisi* infection.



Figure 1. *Lathyrus sativus* flowers (on the left), seeds (on the center) and a *L. sativus* plant infected by *Erysiphe pisi* (on the right).

MATERIALS AND METHODS

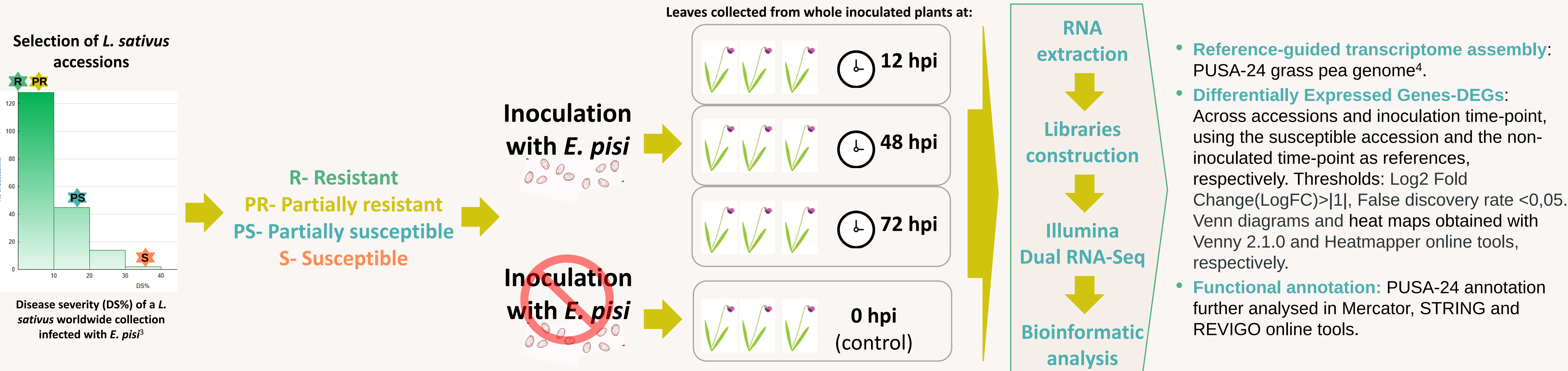


Figure 2. **Workflow of RNA-seq experiment.** An Illumina RNA-seq study was performed for 48 cDNA libraries: 4 accessions (resistant, partially resistant, partially susceptible, and susceptible) in non-inoculated conditions (0h) and 12, 48 and 72 hours post inoculation (hpi) with *E. pisi* (Ep-CO-01) using a settling tower.

RESULTS

RNA-seq output

Table 1. Read counts and alignment rate (PUSA-24 genome*) obtained for each library of Illumina RNA-seq.

Sample	Nr reads	Alignment rate
S_72h	9869267	89.1
S_48h	10026809	87.7
S_12h	10900261	87.6
S_0h	10032103	87.4
PS_72h	10670927	89.6
PS_48h	10701233	87.0
PS_12h	10379206	88.9
PS_0h	10167911	87.8
PR_72h	10091867	85.5
PR_48h	10327110	86.3
PR_12h	10604027	86.5
PR_0h	10531146	87.5
R_72h	10254383	84.9
R_48h	10200620	87.9
R_12h	10654057	87.7
R_0h	10080851	86.0

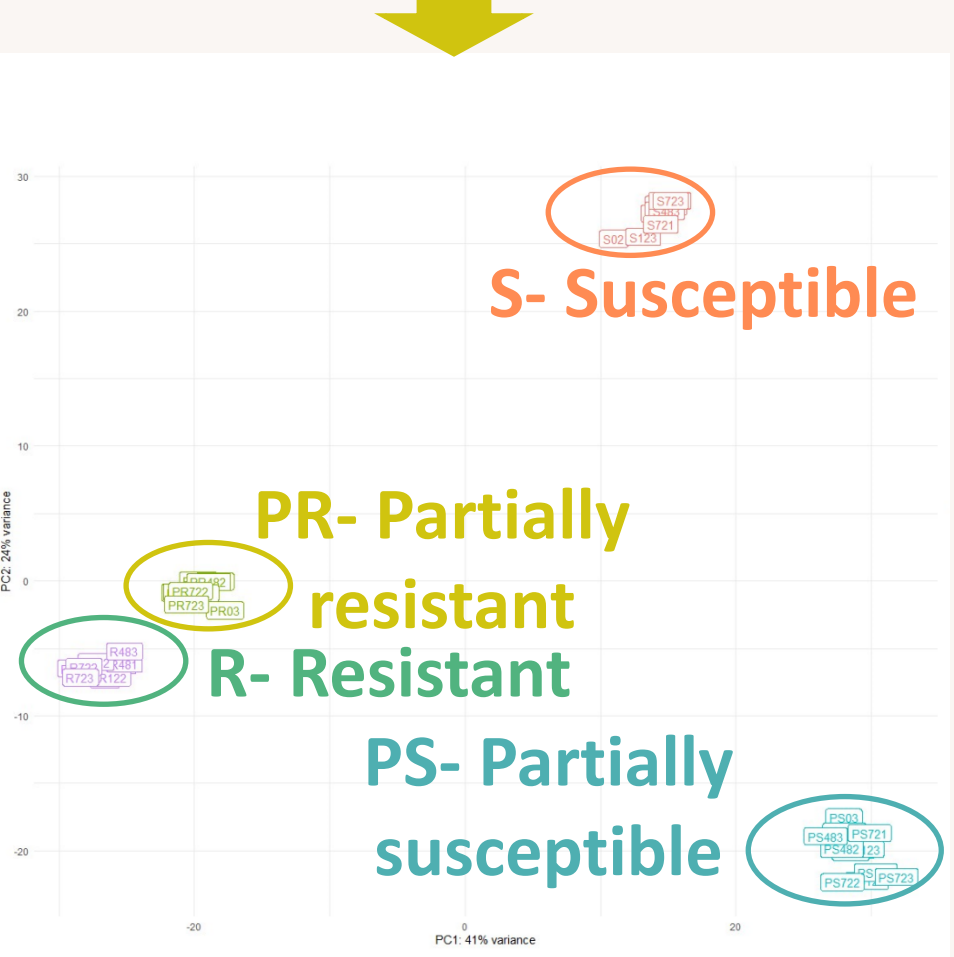


Figure 3. **Principal component analysis** based on gene counts average values for each accession and time point.

Differentially Expressed Genes

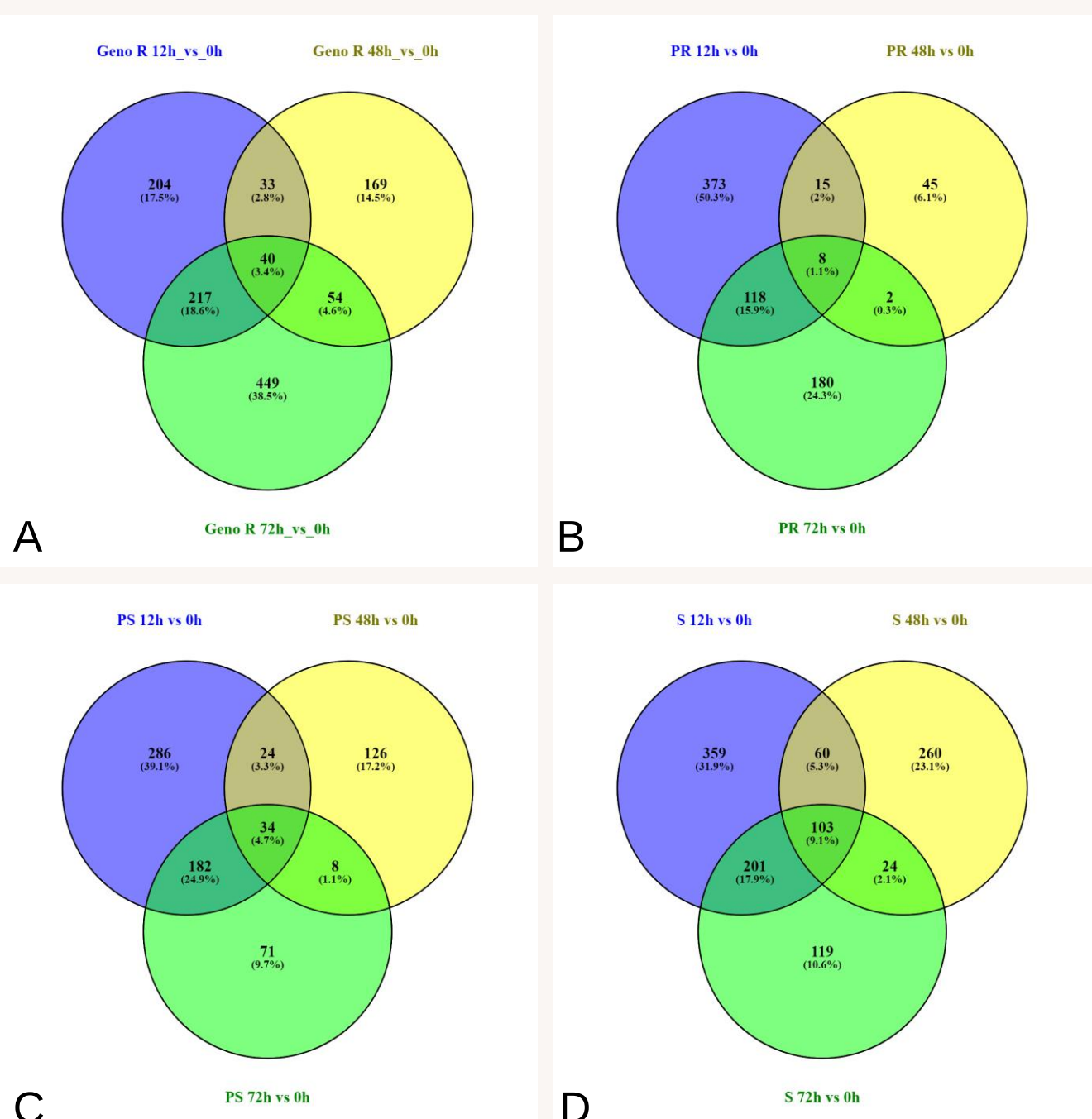


Figure 4. **Venn diagrams** with unique and shared DEGs for all comparisons (0hvs12h, 0hvs24h and 0hvs72h) in each accession.

- Early stages of infection (12hpi):** more DEGs in **PR**, **PS** and **S** accessions.
- Less DEGs identified** at 48hpi for all accessions, and a **downregulation trend** comparing to 12hpi and 72hpi observed.
- Later stages of infection (72hpi):** more DEGs in **R** accession (71% upregulated).

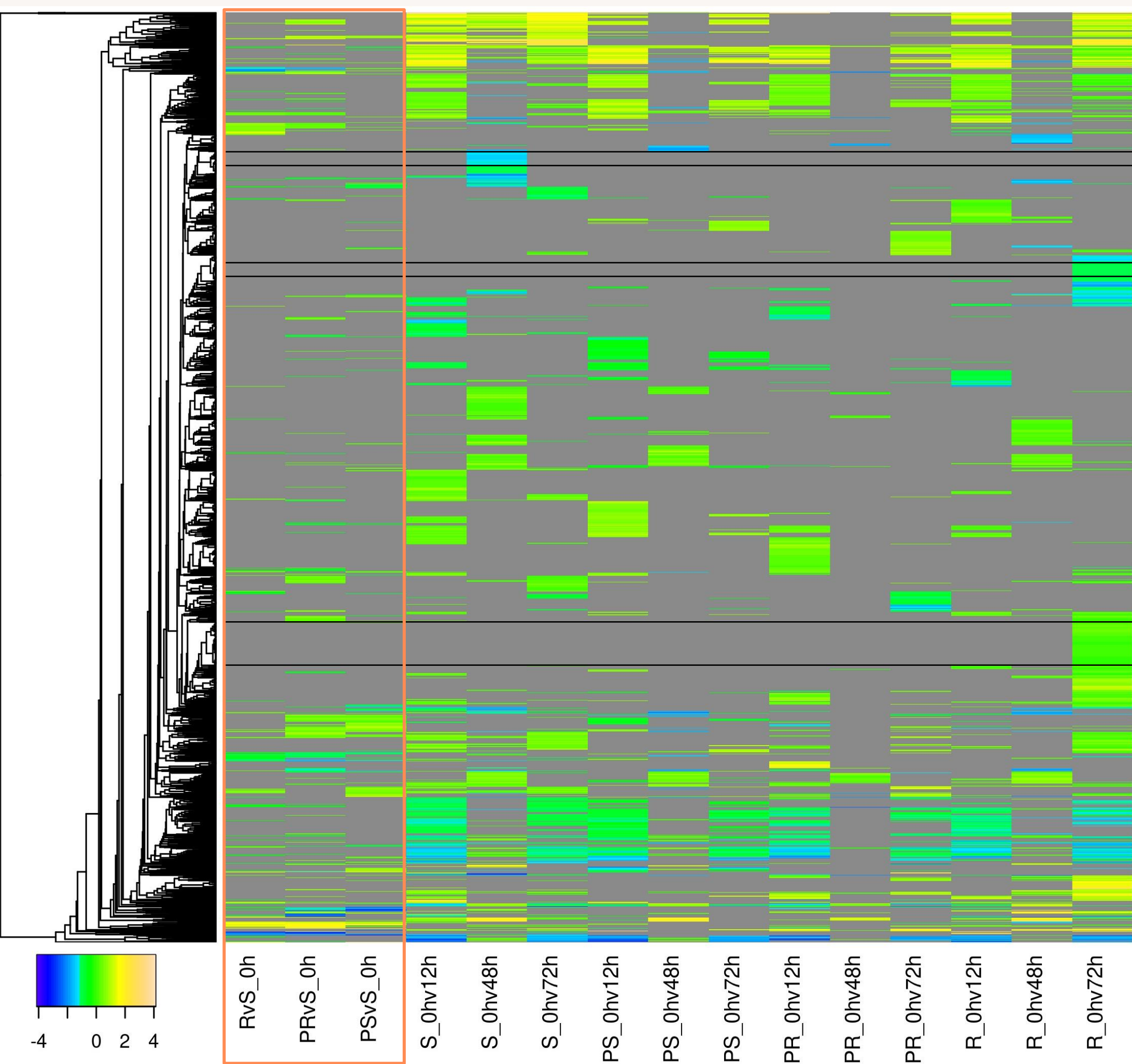


Figure 5. **Heat map** of annotated⁴ DEGs identified at least in one inoculated comparison (n= 1573). Grey lines indicate no DEG.

- At **non-inoculated conditions (0h)**, genes related to **defence response**, were more abundant in **R** and **PR** accessions.
- Example of **Resistance genes** identified in **R_0hvs72h**:
 - Disease resistance response protein 206** (Ls_pschr7.g69780, LogFC=5.67)
 - Disease resistance response protein Pi49** (Ls_pschr4.g32378, LogFC=4.79)

Functional annotation

Exclusive DEGs downregulated in S_0hvs48h involved in: Cell wall biogenesis/degradation, such as pEARL1-like (Ls_pschr6.g53229, LogFC=-4.10), a lipid transfer protein (LTP). LTPs play a crucial role in synthesizing lipid barrier polymers and are involved in **defense signaling during pathogen attacks**⁵.

Exclusive DEGs upregulated in R_0hvs72h involved in:

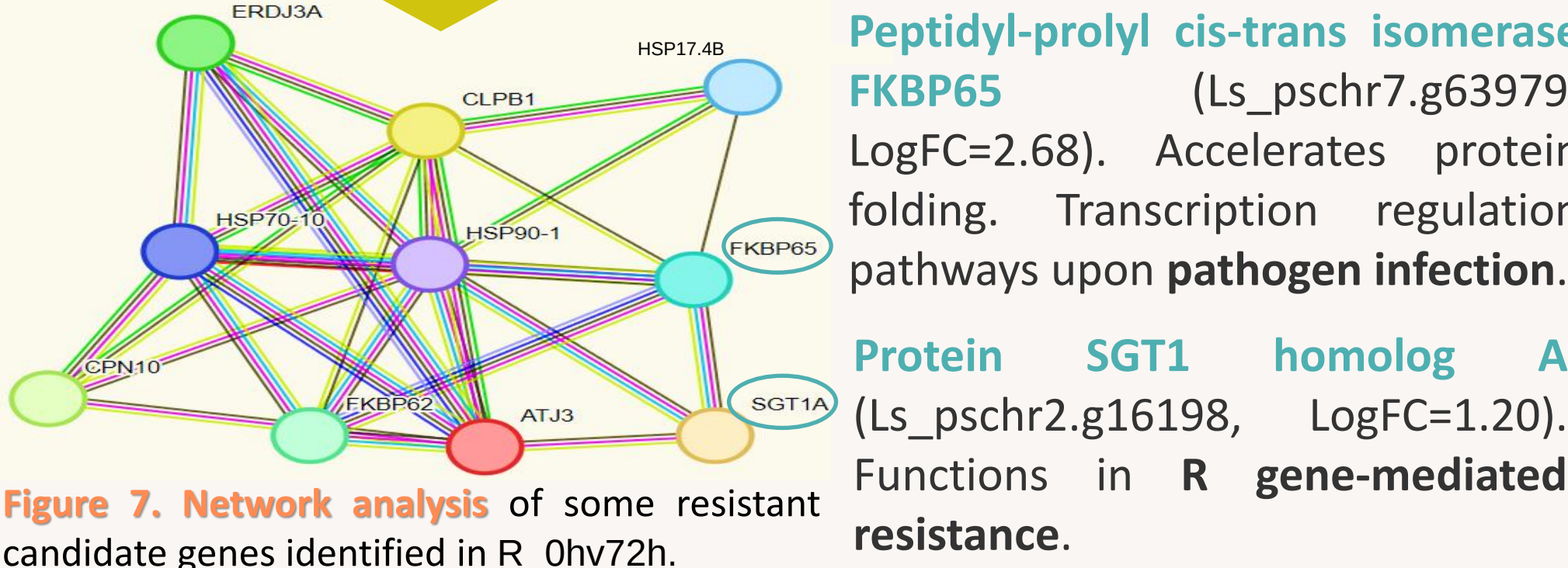
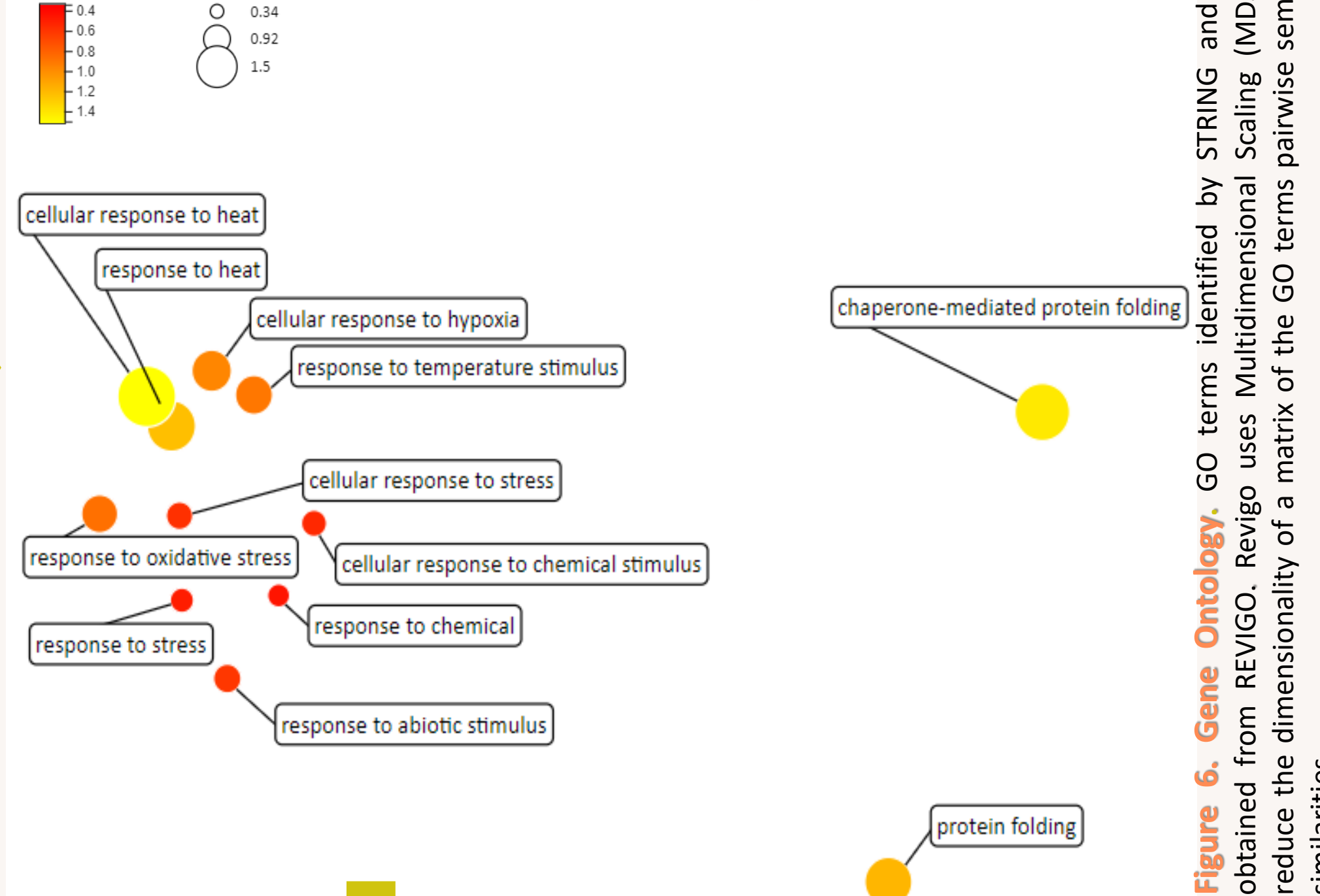


Figure 7. **Network analysis** of some resistant candidate genes identified in R_0hvs72h.

CONCLUSIONS AND FUTURE PERSPECTIVES

- At gene count level, bigger differences identified among grass pea accessions than among infection time points;
- Genes related to **defence response** more abundant at **72hpi** in **R** and **PR** grass pea accessions;
- Susceptibility to *E. pisi*** infection may be related with downregulation of genes involved in the **integrity of host cell wall** (physical barrier to pathogen entrance);
- At 48hpi, DEGs identified were generally downregulated comparing to 12hpi and 72hpi, suggesting a possible counter-attack of *E. pisi*. Pathogen effectors will be predicted for each time point to validate this hypothesis;
- Candidate-resistance genes against powdery mildew will be selected from the transcriptomic data, to be functionally validated and to develop new precision screening tools for breeding in grass pea and related species.

REFERENCES

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