Plant defense activation of two contrasting soybean genotypes in response to *Diaporthe caulivora*

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INTRODUCTION

Soybean (*Glycine max* (L.) is an important crop worldwide, whose production is limited by soybean stem canker (SSC) caused by the fungal pathogens *Diaporthe caulivora, D. aspalathi and D. longicolla.* Plants perceive pathogens and trigger cellular and molecular modifications associated with defense responses such as mitogen-activated protein kinases (MAPKs) cascades, calcium influx, reactive oxygen species accumulation, callose deposition, hormone synthesis and transcriptional reprograming. During soybean-*D. caulivora* interaction, plant cells activate the expression of genes encoding pathogenesis-related proteins (PR-1, PR-2, PR-3, PR-4, PR-10), and enzymes involved in phenylpropanoid and oxylipin synthesis (phenylalanine-ammonia lyase, chalcone synthase and lipoxygenases). However, limited information related to the molecular mechanisms underlying soybean resistance to *Diaporthe* species is available. In the present work, the defense responses to *D. caulivora* in two contrasting soybean genotypes were analyzed.

RESULTS

1. Disease symptoms after *D.caulivora* infection in Williams (susceptible) and Génesis 5601 (resistant) cultivars

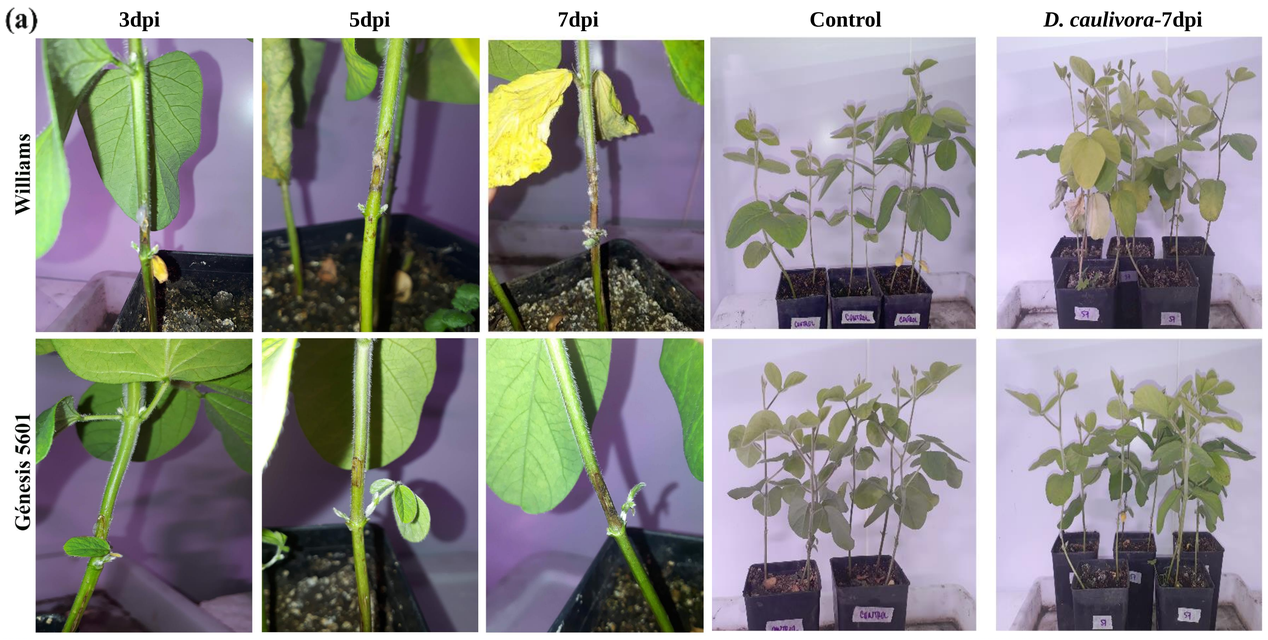
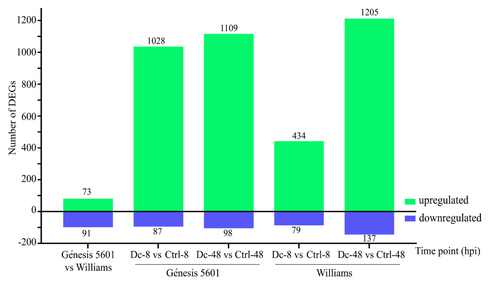


Figure 1. Soybean stem canker disease progress after *D. caulivora* inoculation. Asterisks in b-e indicate a statistically significant difference between soybeans cultivars, t de Student, p<0.01.

Disease symptoms appear on the stem of both cultivars as 1-2 mm spots that expand as elongated brown lesions, however greater lesion length and withered leaves were observed in Williams and disease progressed earlier in this susceptible cultivar.
Génesis 5601 was more resistant to fungal infection than Williams, evidenced by smaller lesion length, reduced disease severity index and pathogen biomass.

2. Transcriptomes of soybean plants inoculated with *D.caulivora*



In total, 2322 and 1855 DEGs were identified in Génesis 5601 and Williams, respectively.

At 8 hpi a more extensive and complete defense response was activated in Génesis 5601, demonstrated by upregulation of 1028 compared to 434 genes in Williams.

Figure 2. Differentially expressed genes (DEGs) identification in susceptible (Williams) and resistant (Génesis 5601) soybean plants without treatment and after *D. caulivora* inoculation.

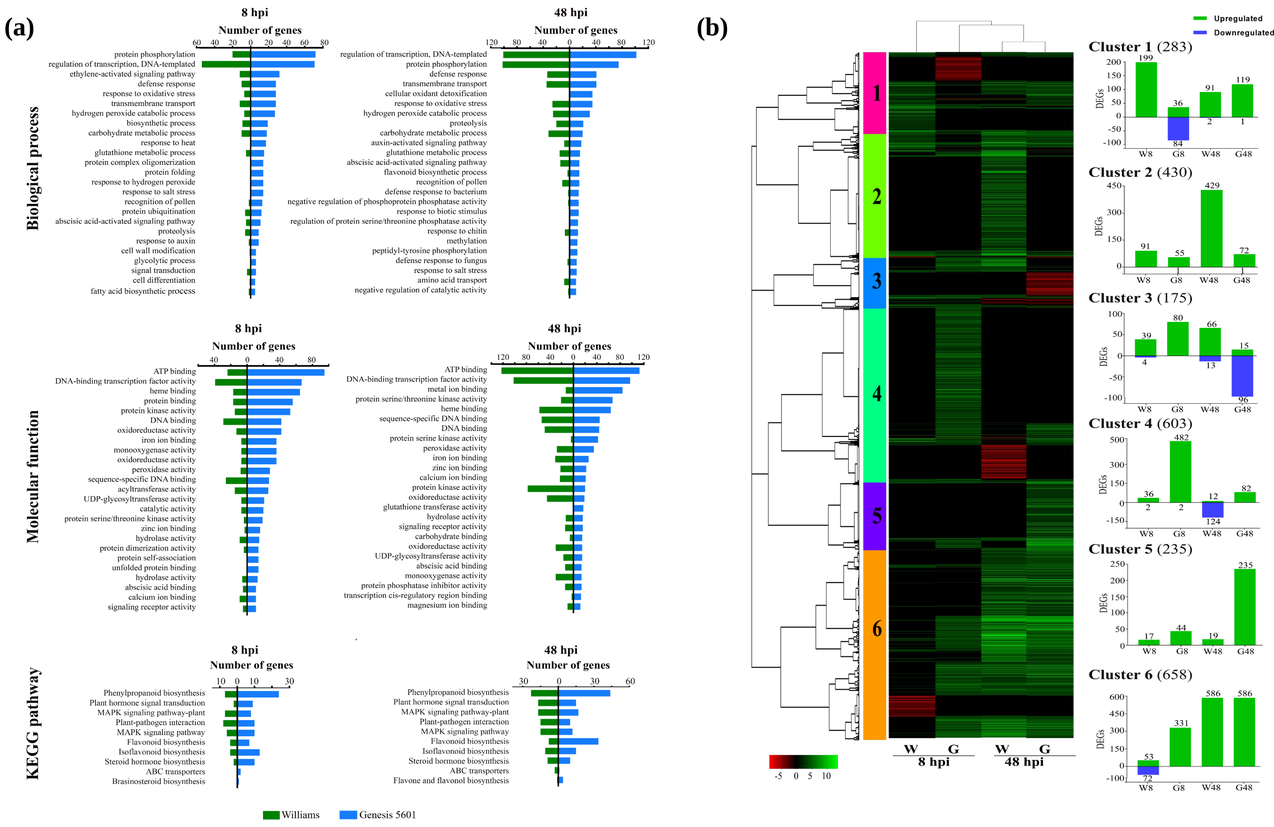


Figure 3. (a) Enriched GO and KEGG pathway of upregulated genes (b) Hierarchical clustering of all DEGs in soybean plants inoculated with *D. caulivora.* Green (upregulated) and Red (downregulated).

In total, 2215 sequences are identified with GO terms. The most represented Biological Process in both genotypes at 8 and 48 hpi were protein phosphorylation, regulation of transcription and defense. GO terms in Génesis 5601 at 8 hpi, included ethylene-activated signaling pathway, response to oxidative stress, response to heat, salt stress, auxins and cell wall modification. The majority of enriched KEGG pathways were phenylpropanoid, flavonoid, and isoflavonoid biosynthesis, MAPK signaling, plant hormone signal transduction and plant-pathogen interaction.

Clusters 4, 5, and 6 were related to defense activation in Génesis 5601 after *D. caulivora* inoculation. 400 DEGs were only upregulated in Génesis 5601 at 8 hpi , including genes involved in perception of the pathogen, hormonal signaling, transcription factors, phenylpropanoid biosynthesis, pathogenesis related proteins, transporters and oxidative stress detoxification.

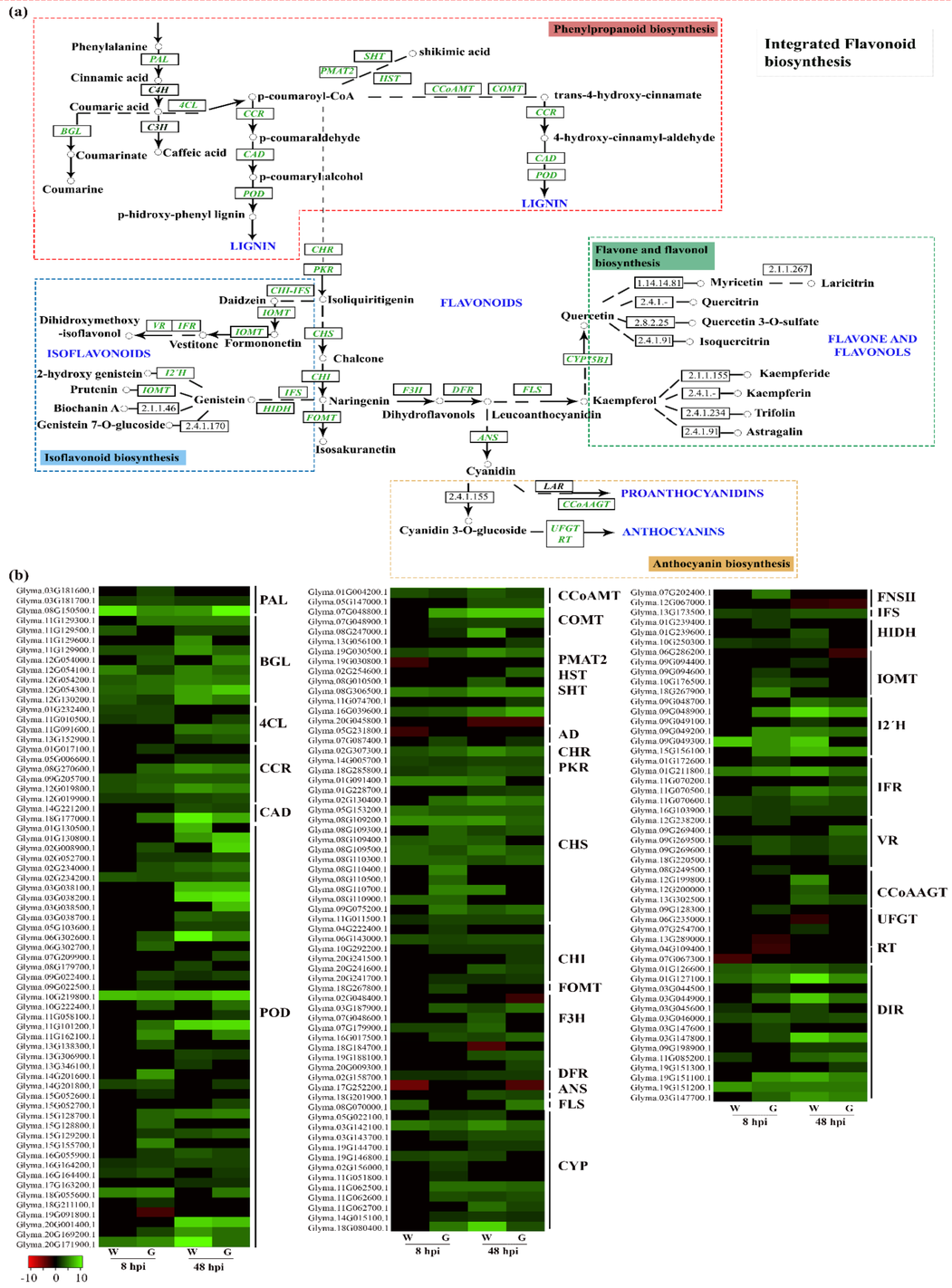


Figure 4. Activation of the phenylpropanoid and flavonoids pathways in response to *D. caulivora*. (a) Integrated and simplified scheme of biosynthesis KEGG pathway. (b) Heatmap of DEGs encoding genes of the phenylpropanoid and flavonoids biosynthetic pathway. Green (upregulated) and red (downregulated).

In total, 169 DEGs related to this pathway were identified during *D. caulivora* infection. At 8 hpi 117 genes were upregulated in Génesis 5601, while only 50 genes were induced in Williams.

3. Defense response activation in soybean plants inoculated with *D. caulivora*

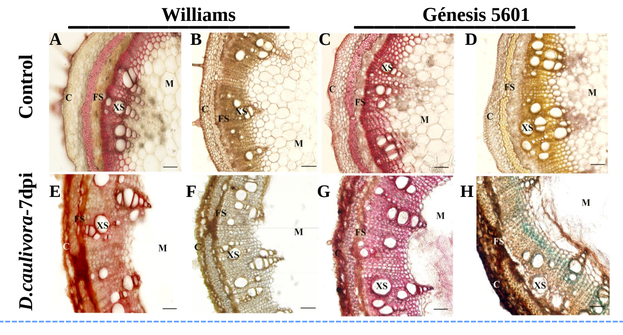


Figure 5. Cell wall associated defense responses in *D. caulivora* infected tissues at 7 dpi stained with safranin-O (A,C,E,G) and nitro blue tetrazolium (NBT) (B,D,F,H). Abbreviations: cortex (C), secondary phloem (FS), secondary xylem (XS), and pith (M).

In contrast to control tissues, cell wall modifications related to defense often include the incorporation of phenolic compounds into the cell walls and reactive oxygen species.
All infected tissues were stained with safranin-O, including the cortex and the secondary phloem, showing an intense red-brownish coloration.
Superoxide was detected in Génesis 5601 soybean plants inoculated with *D. caulivora* and stained with NBT at 7 dpi

CONCLUSIONS

This comparative study between contrasting soybean genotypes revealed that the observed resistance of Génesis 5601 to *D. caulivora* infection, is likely based on a rapid recognition of the pathogen and induction of genes related to plant immunity. Future studies involving functional characterization of soybean candidate genes and target genes of *D. caulivora* effectors will contribute to a valuable comprehension of soybean-*Diaporthe* interactions. These findings provide novel molecular insights into soybean molecular defense developed to control this pathogen, and a foundation for improving resistance in breeding programs.

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