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Exploiting Wild Genetic Resources: Characterization of PR genes from *Sinapis alba* for Resistance to Alternaria Blight

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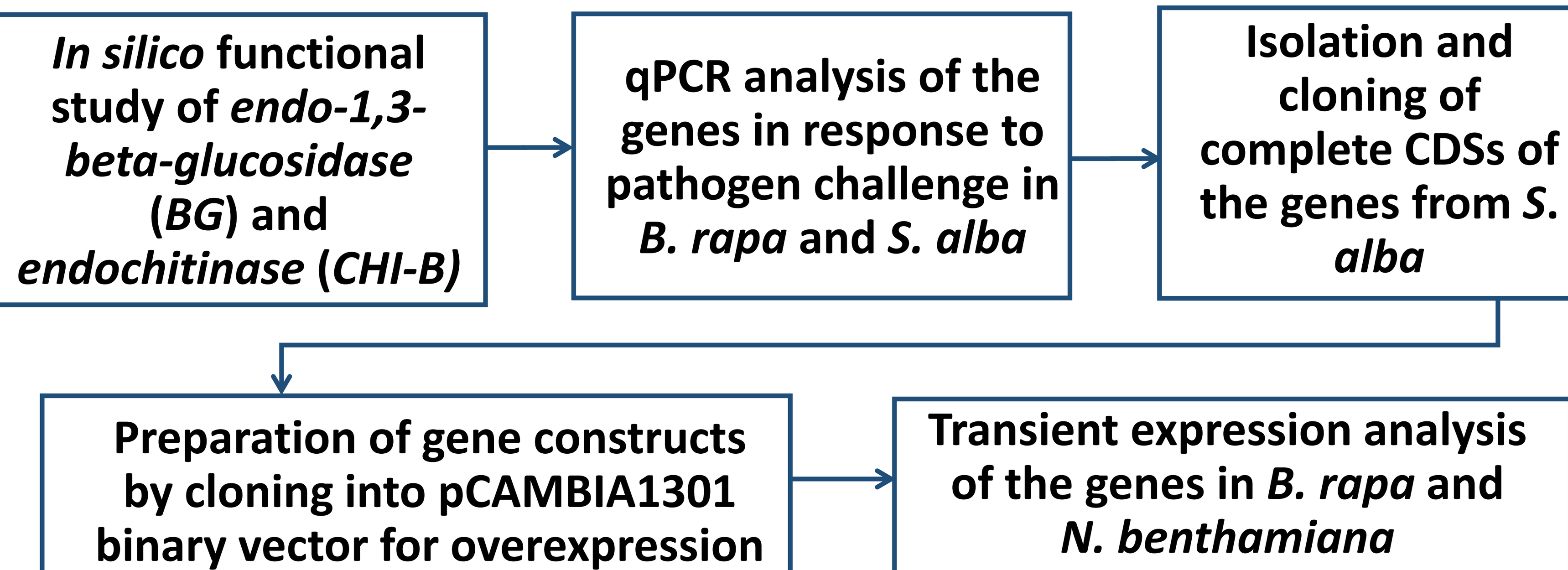
Background

Alternaria blight, primarily caused by the necrotrophic fungi *Alternaria brassicae* and *A. brassicicola,* is one of the most destructive biotic stressors of *Brassica* spp. Conventional breeding efforts to develop resistant cultivars have been unsuccessful due to the lack of suitable resistance sources among cultivated species. *Sinapis alba*, a crop wild relative (CWR) of *Brassica* spp., has been reported to exhibit considerable resistance to the disease [1]. However, strong cross-incompatibility, dominant gene interactions, polygenic background of resistance, and variation in ploidy among *Brassica* spp. pose challenges in transferring resistance trait to cultivated varieties from wild species. The present investigation aimed to clone, and characterize two pathogenesis-related (PR) genes, *endo-1,3-beta-glucosidase* and *endochitinase,* associated with defense against Alternaria blight in *S. alba*, and to develop constructs for functional validation through overexpression in the susceptible species *Brassica rapa*. The genes were selected from a transcriptomic dataset of differentially expressed genes, generated in a previous study [2], based on their expression patterns in *S. alba* and *B. rapa* following inoculation with *A. brassicicola*. The differential expression patterns of the two PR genes were validated through qPCR. Furthermore, gene ontology analysis and in silico protein–protein co-expression network studies provided insights into the functional roles of these genes in defense against the pathogen. The binary vector constructs prepared for the overexpression of *endo-1,3-beta-glucosidase* and *endochitinase genes* will further be used for stable transformation in *B. rapa* for functional validation through bioassays.

Methodology



Results

Fig. 1: Gene Ontology (GO) study

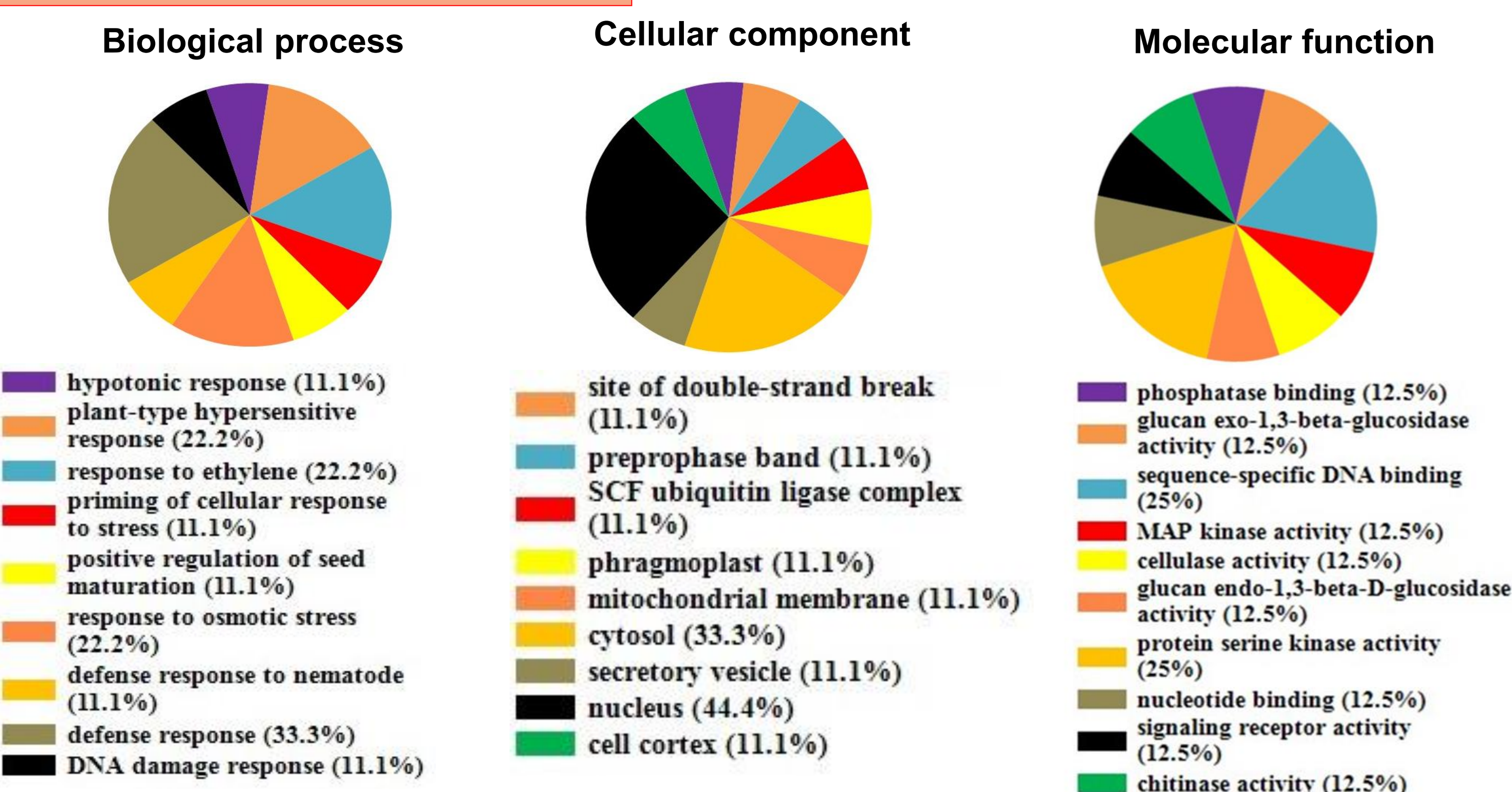


Fig. 2: Coexpression study

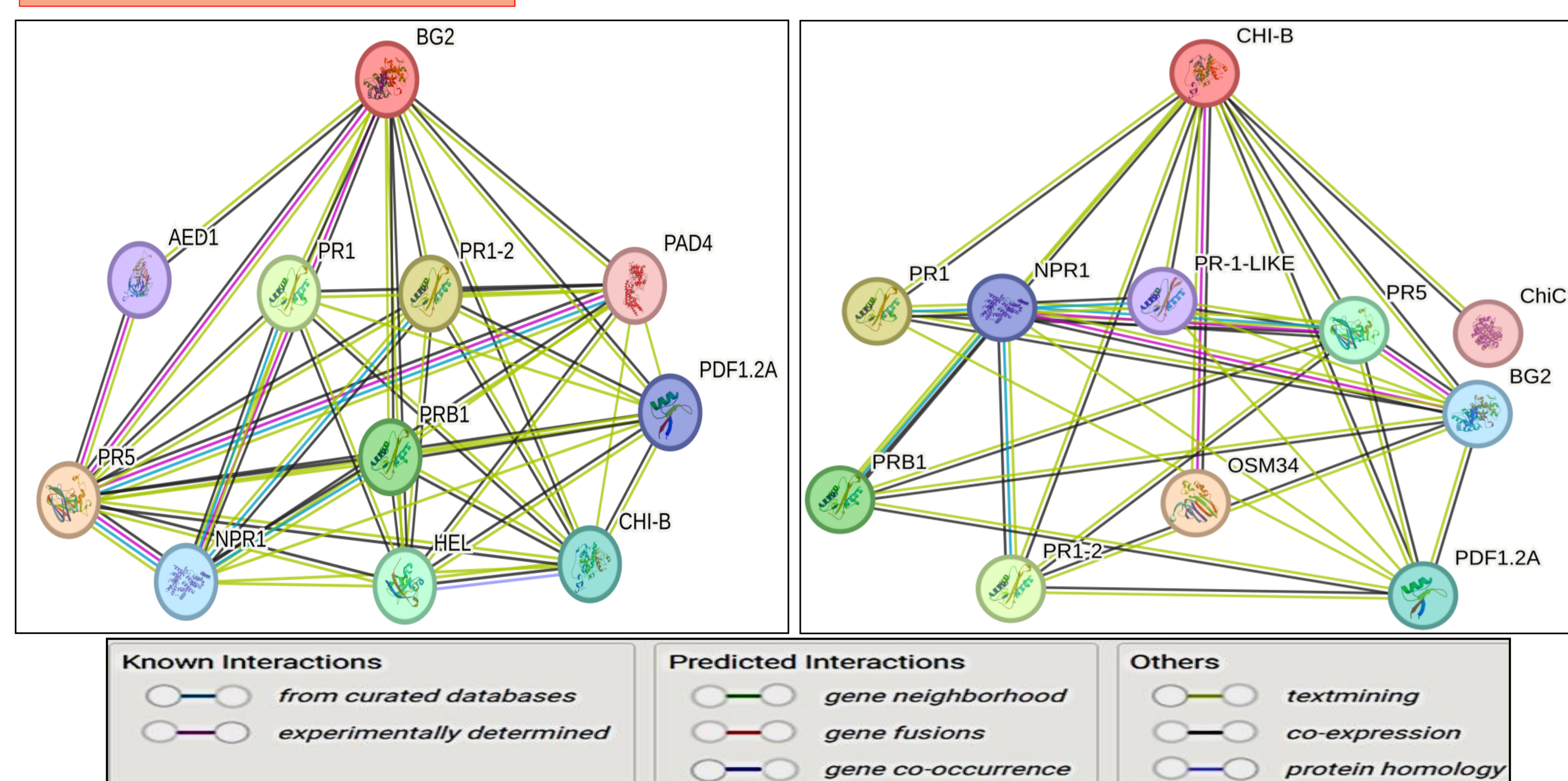
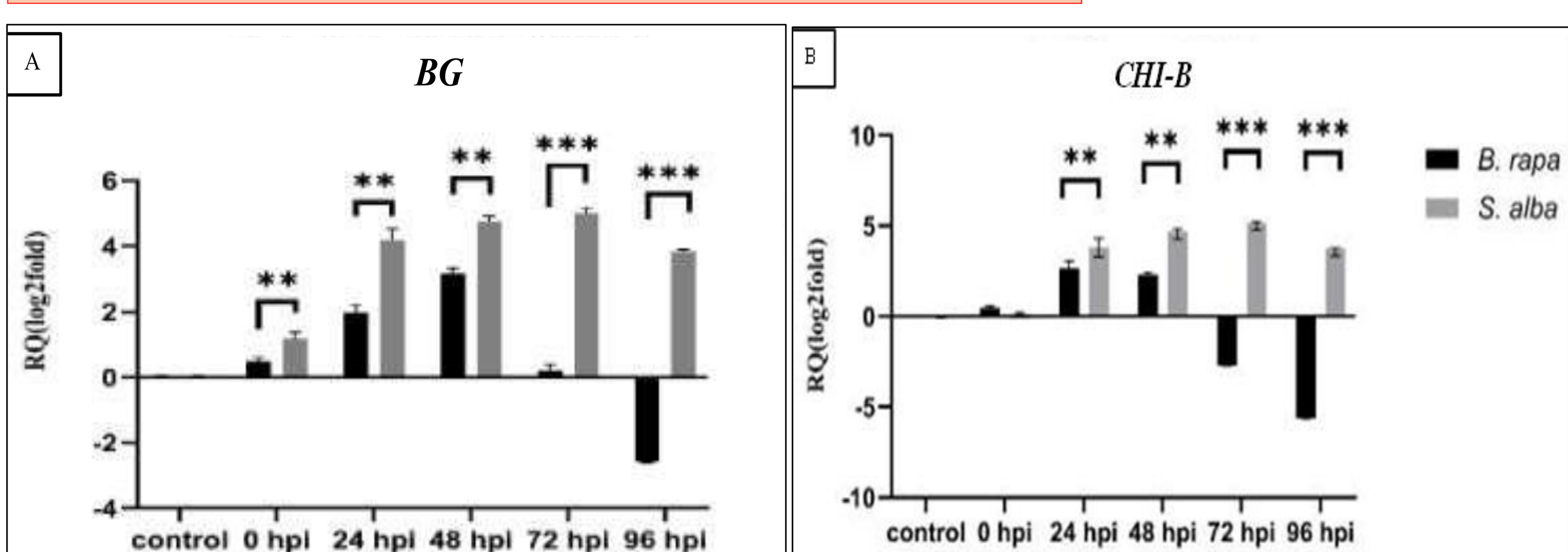


Fig. 3: qPCR based expression analysis of BG and CHI genes



Full length CDS isolation and cloning into pGEM®- T Easy cloning vector

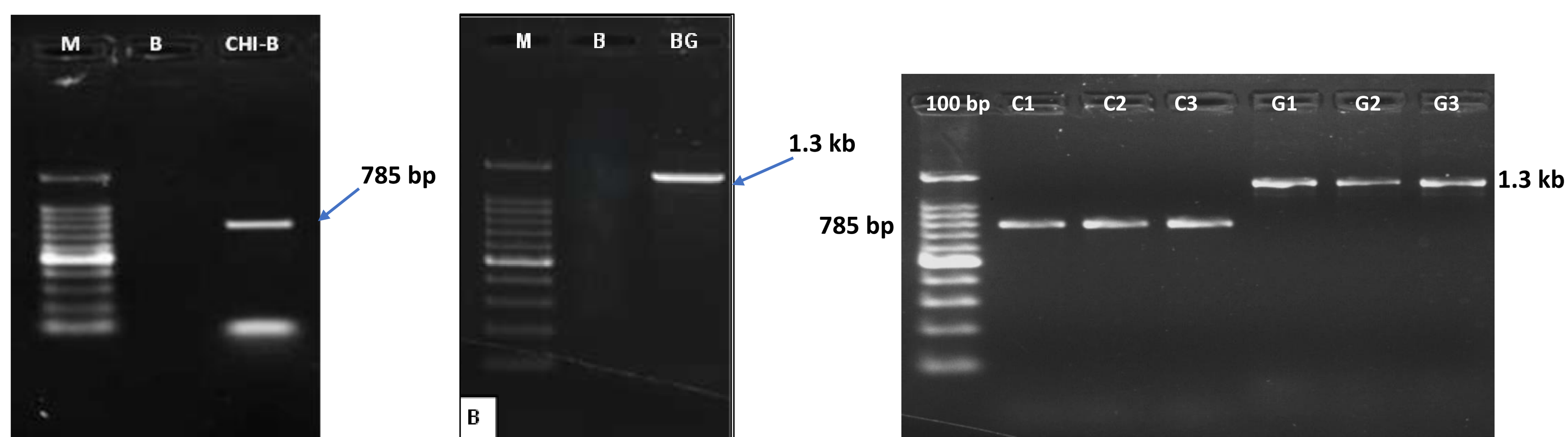


Fig. 4: PCR amplification of *CHI-B* and *BG* genes from *S. alba* infected with *A. brassicicola* (M- DNA marker; B- Blank)

Fig. 5: Colony PCR amplification of *endochitinase* and *endo-1,3-beta-glucosidase* cloned in pGEM®-T for confirmation

Cloning of *endo-1,3-beta-glucosidase* and *endochitinase* genes to pCAMBIA1301

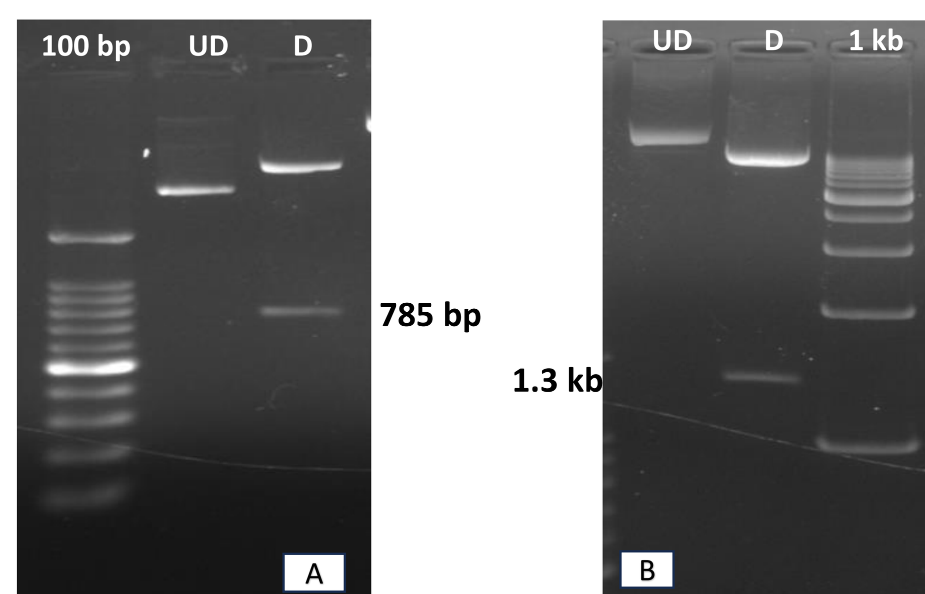


Fig. 6: Restriction analysis of recombinant pCAMBIA1301 binary vector carrying *endochitinase* (A) and *endo beta 1,3-beta-glucosidase* (B) gene constructs, using *NcoI* and *BstEII* restriction enzymes for confirmation (UD: Undigested vector; D: Digested vector)

Transient assay of gene constructs through Agroinfiltration

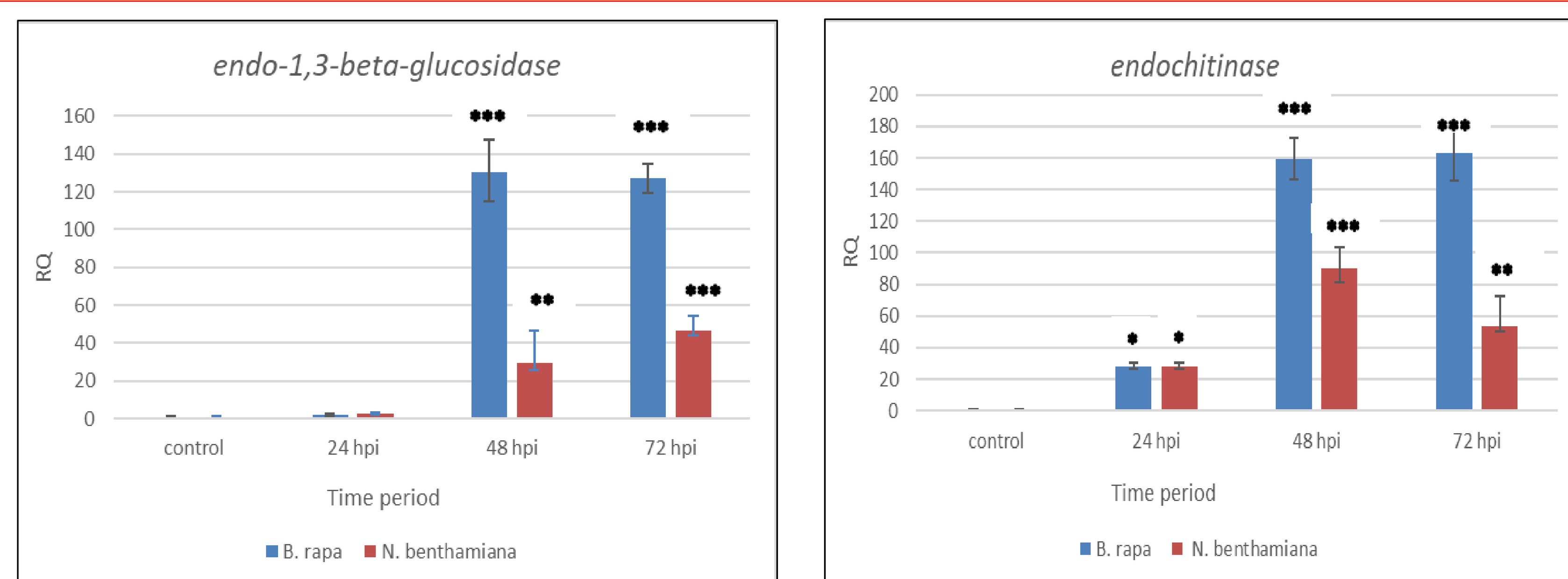


Fig. 7: qPCR analysis showing relative expression of *endo-1,3-β-glucosidase* and *endochitinase* genes, across different time points, in *B. rapa* and *N. benthamiana* after agroinfiltration with the gene constructs

Conclusions

- β-1,3-glucosidase and endochitinase genes are involved in key defense-related processes such as hypersensitive response, ethylene signaling, and overall pathogen defense.
- The genes show strong interaction with other PR proteins, suggesting synergistic defense roles.
- Transient expression assays in *B. rapa* and *N. benthamiana* confirmed successful overexpression of the genes, with peak expression at 48 and 72 hpi.
- These key defense genes isolated from *S. alba* represent promising candidates for Alternaria blight resistance in susceptible Brassica cultivars.

References

[1] Ahmed R, Saikia P, Aryasree R & Bhorali P (2024). Isolation and characterization of Alternaria brassicicola from Assam (India) and screening for resistance in Sinapis alba. Tropical Plant Pathology, 49(6):850-863.

[2] Ahmed R, Dey KK, Senthil-Kumar M, Modi MK, Sarmah BK & Bhorali P (2024). Comparative transcriptome profiling reveals differential defense responses among Alternaria brassicicola resistant Sinapis alba and susceptible Brassica rapa. Frontiers in Plant Science, 14:1251349.

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