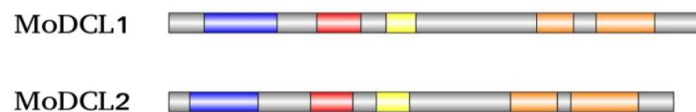
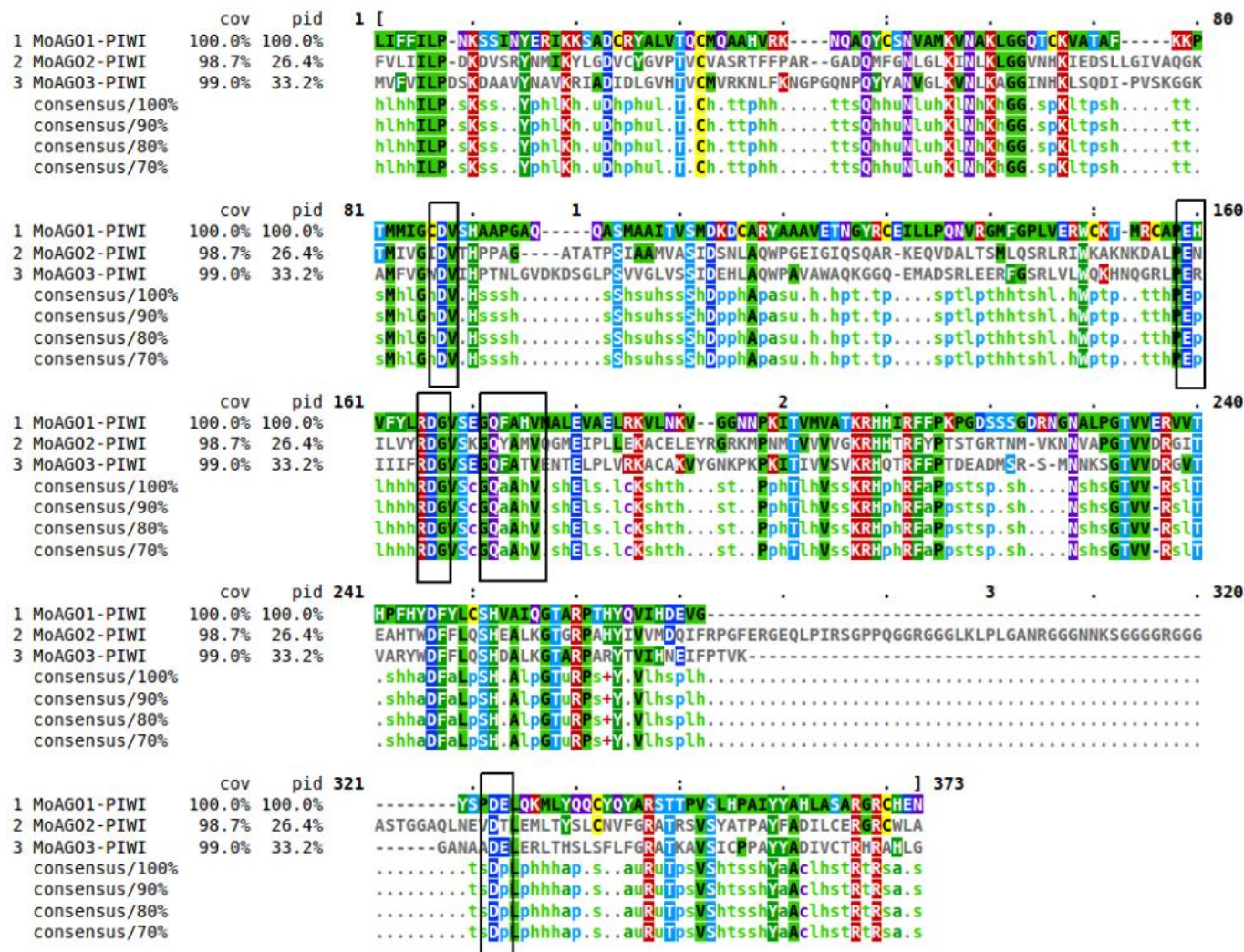


■ N domain  
 ■ DUF1785  
 ■ PAZ  
 ■ L2  
 ■ Piwi

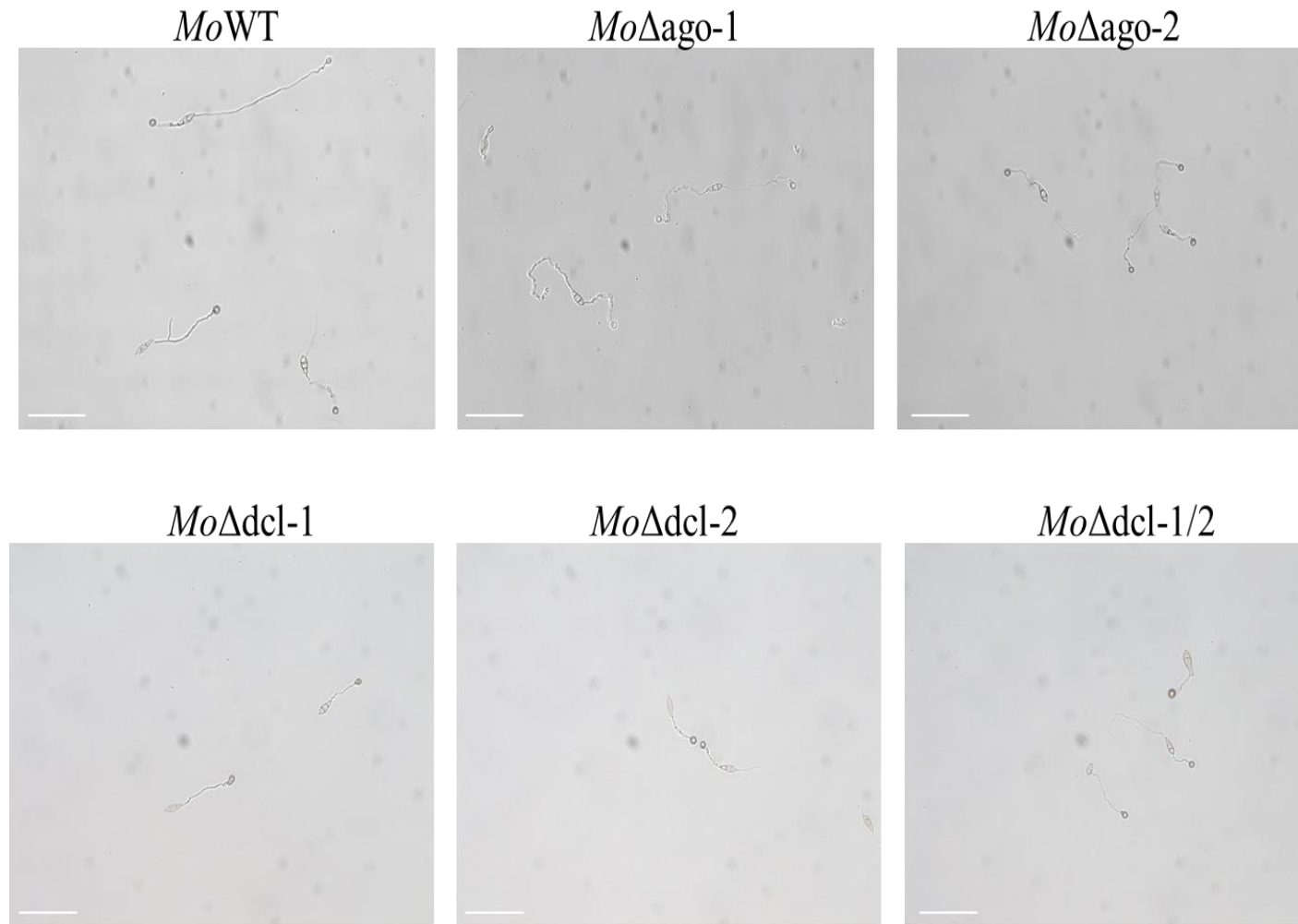


■ DEXDc  
 ■ HELICc  
 ■ dicer\_dimer  
 ■ RIBOc

**Figure S1. Analysis of MoAGO and MoDCL protein sequences.** Phylogram of (A) MoDCL and (B) MoAGO protein sequences. Branch support values are displayed, the scale bar defines the branch length. Ss= *Sclerotinia sclerotiorum*, Bc= *Botrytis cinerea*, Cp= *Cryphonectria parasitica*, Sp= *Schizosaccharomyces pombe*, Nc= *Neurospora crassa*, Ggt= *Gaeumannomyces graminis* var. *tritici*, Mc= *Mucor circinelloides*, Sn= *Parastagonospora nodorum*, Cg= *Chaetomium globosum*. Visual representation of domain structure of (C) MoAGO and (D) MoDCL proteins. Domains were identified by SMART and PFAM search and represented with IBS illustrator. Displayed domains of AGOs: N-domain, DUF1785, PAZ, L2, PIWI. Sequence with no domain predicted is colored in grey. Displayed domains of DCLs: DEXDc, HELICc, dicer\_dimer and RIBOc.



**Figure S2. Multiple sequence alignment of the PIWI domain of MoAGO proteins.** Sequences were selected based on SMART domain identification, aligned with Clustal Omega and visualized with Mview. The catalytic tetrad DEDD and the QF-V motifs are boxed.



**Figure S3. Development of appressoria from conidia of *Mo* WT and RNAi mutants.** Conidial suspensions at  $2 \times 10^3 \text{ ml}^{-1}$  of Mo70-15 and mutant strains in distilled water were inoculated onto poly-L-lysine-coated glass coverslips in a damp chamber and examined for appressorium formation after 24 HPI. The experiment was conducted 3 times with similar results. White scale bar = 80μm.

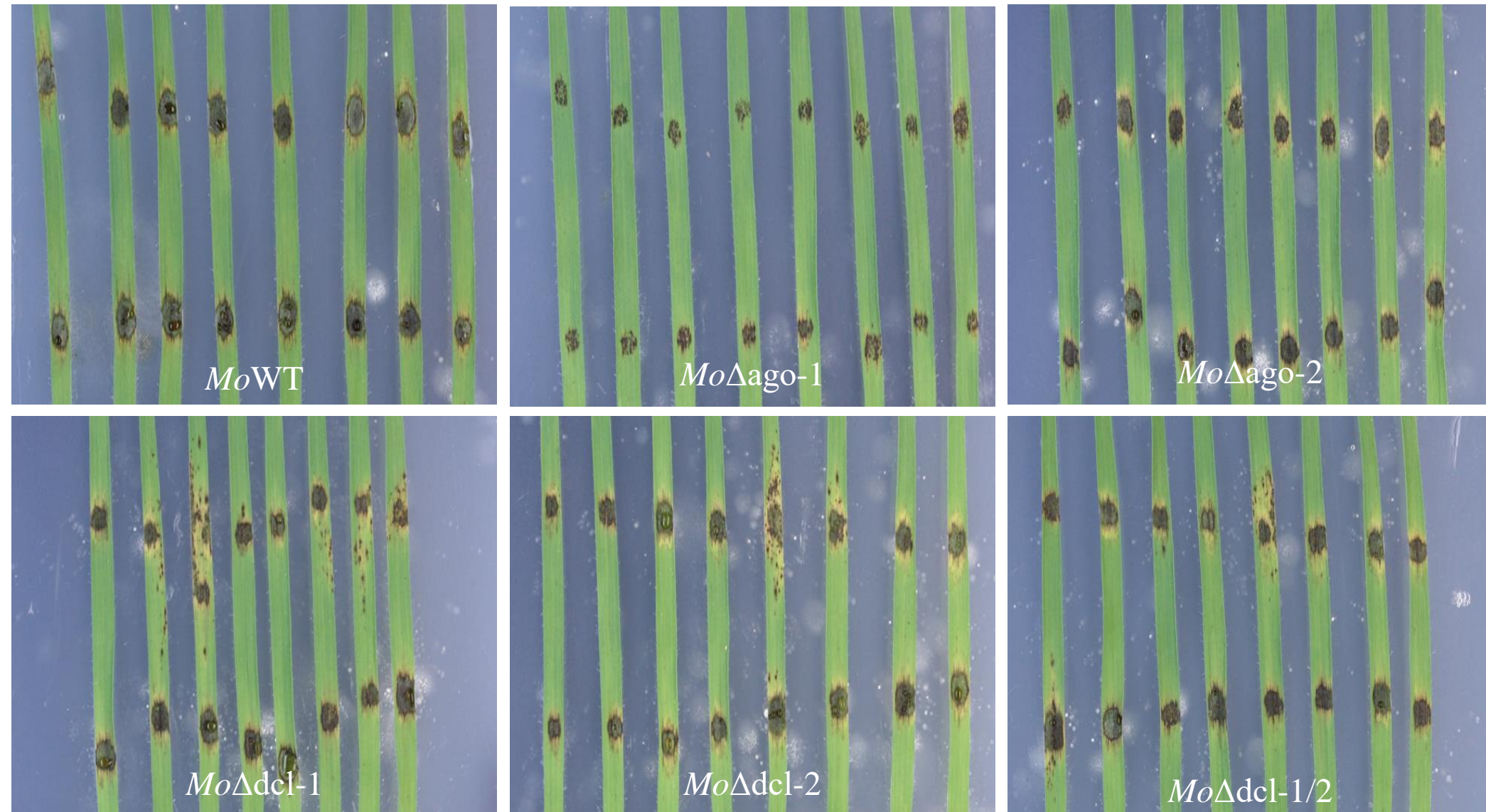


## *Whole seedling spray*



**Figure S4. Phenotypic analysis of Mo WT and RNA interference mutants.**

*Drop inoculation assay*



**Figure S4 continuation**



*Detached leaves spray*



*MoWT*

A photograph of a detached leaf from a wild-type (WT) plant. The leaf is green and shows numerous small, dark brown spots (lesions) distributed along its length, indicating a high level of pathogen infection after a spray treatment.



*MoΔago-1*

A photograph of a detached leaf from a plant with a mutation in the *ago-1* gene. The leaf shows a moderate number of dark brown lesions, suggesting a reduced level of infection compared to the wild type.



*MoΔago-2*

A photograph of a detached leaf from a plant with a mutation in the *ago-2* gene. The leaf shows a moderate number of dark brown lesions, similar to the *MoΔago-1* mutant.



*MoΔdcl-1*

A photograph of a detached leaf from a plant with a mutation in the *dcl-1* gene. The leaf shows a moderate number of dark brown lesions, similar to the *MoΔago* mutants.



*MoΔdcl-2*

A photograph of a detached leaf from a plant with a mutation in the *dcl-2* gene. The leaf shows a moderate number of dark brown lesions, similar to the other mutants.

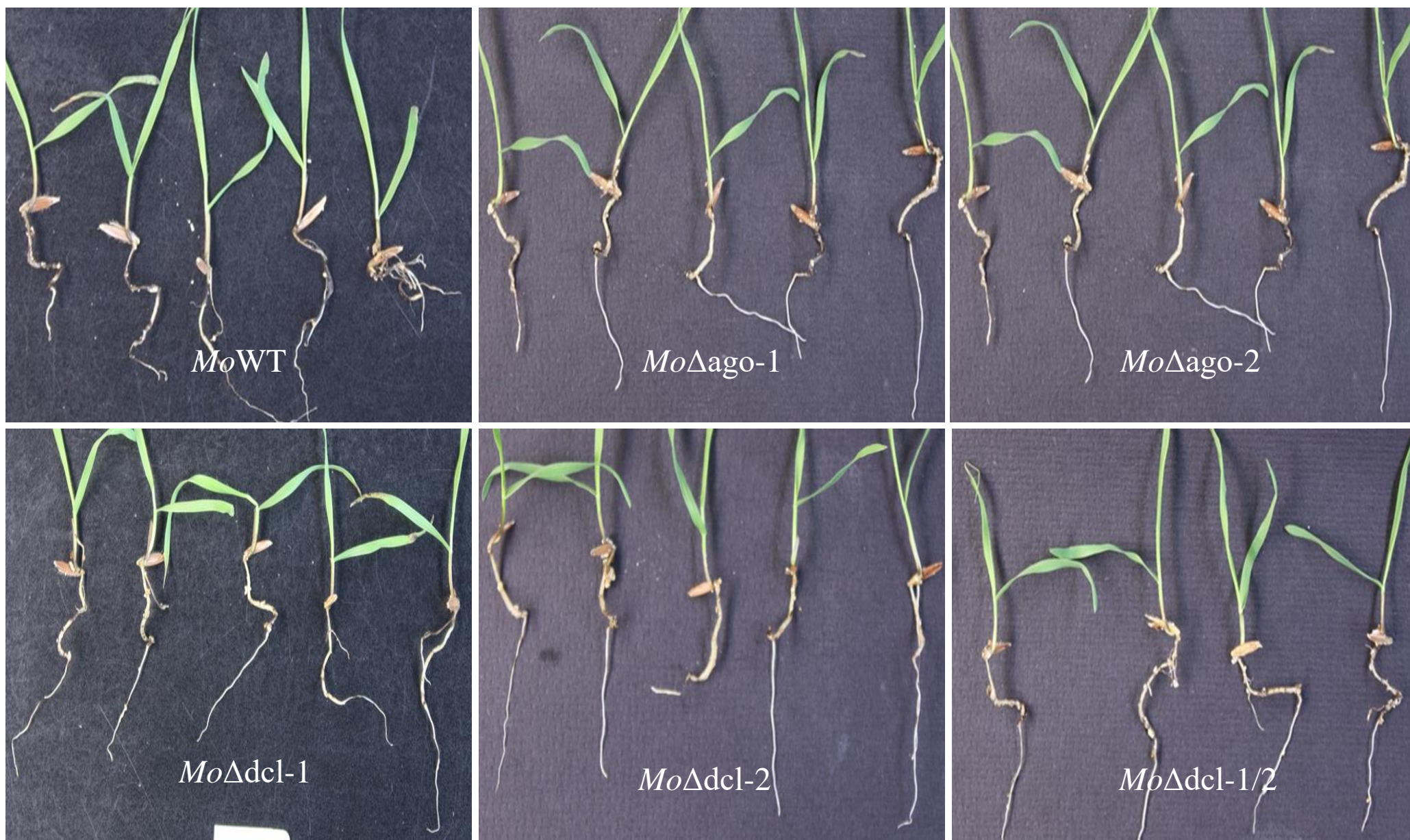


*MoΔdcl-1/2*

A photograph of a detached leaf from a plant with a mutation in the *dcl-1/2* gene. The leaf shows a moderate number of dark brown lesions, similar to the other mutants.

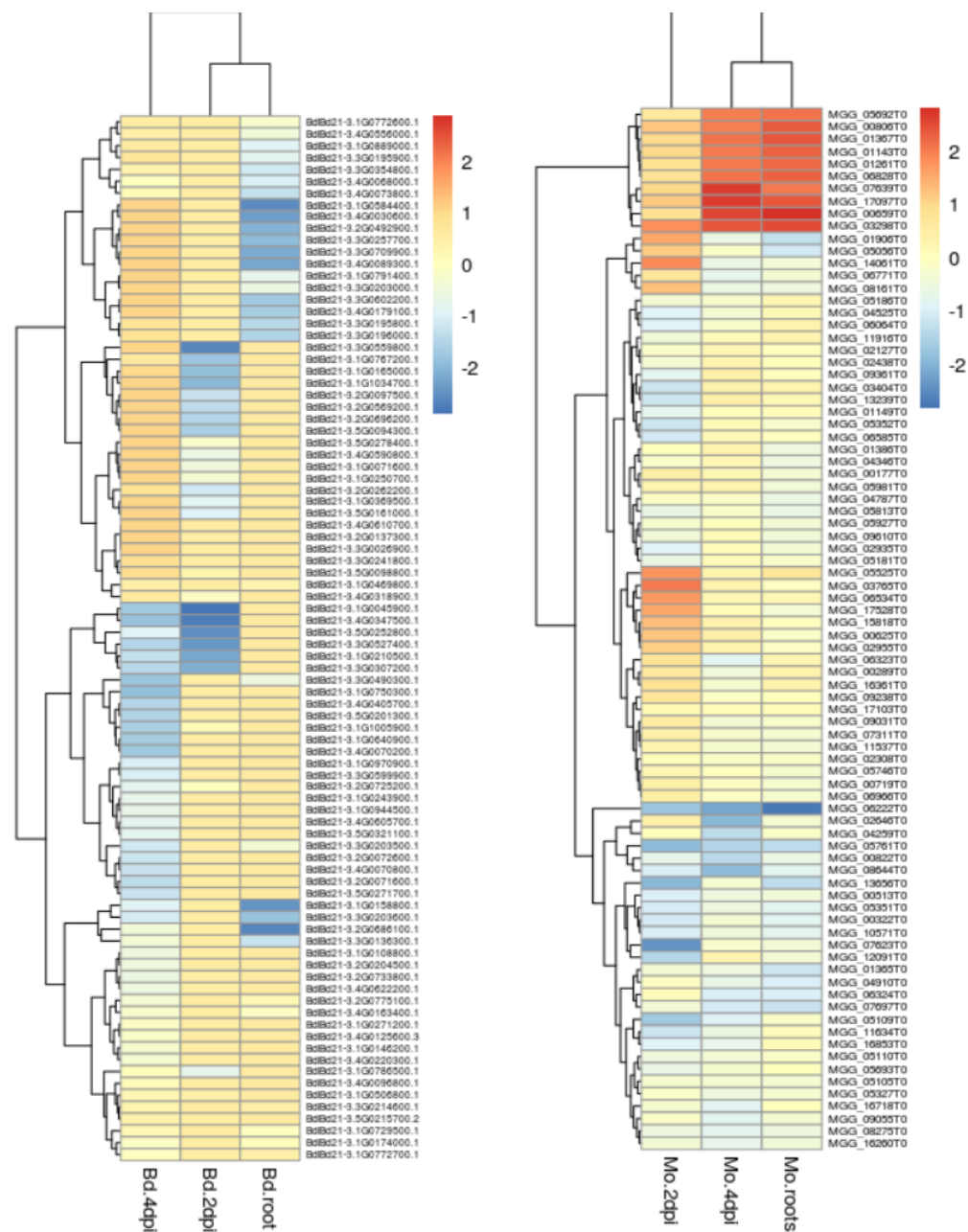
**Figure S4 continuation**

### *Root infection assay*



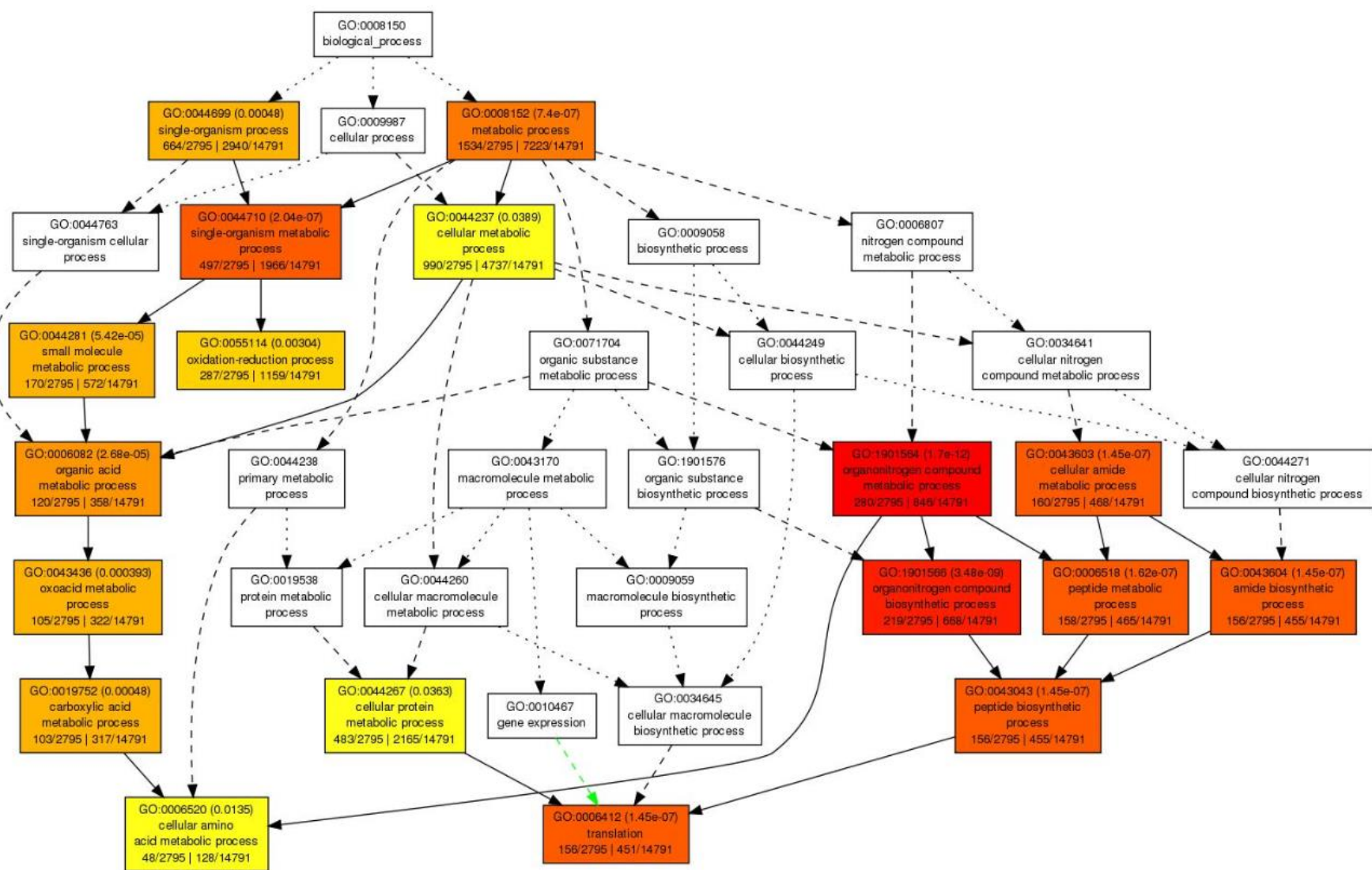
**Figure S4 continuation**



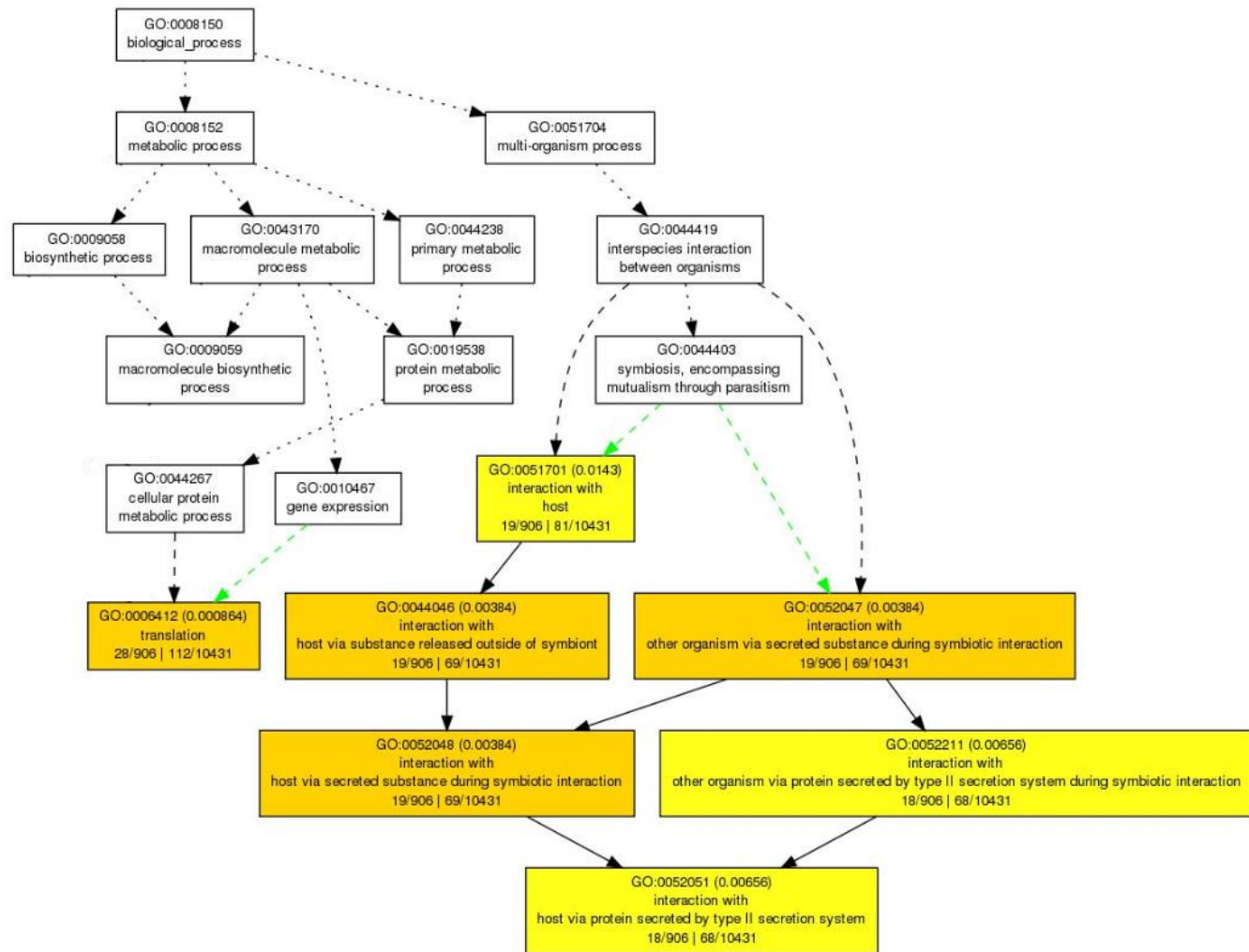


**Figure S5. Heatmap for Mo and Bd DEG calling with DESeq2.** Heatmap of expression levels (logFC) of the top Bd (A) and Mo (B) mRNAs in all 3 setups (leaf 2 DPI, leaf 4 DPI and root). Color gradient from red to blue indicative of log2FC of corresponding transcript as shown in the legend.

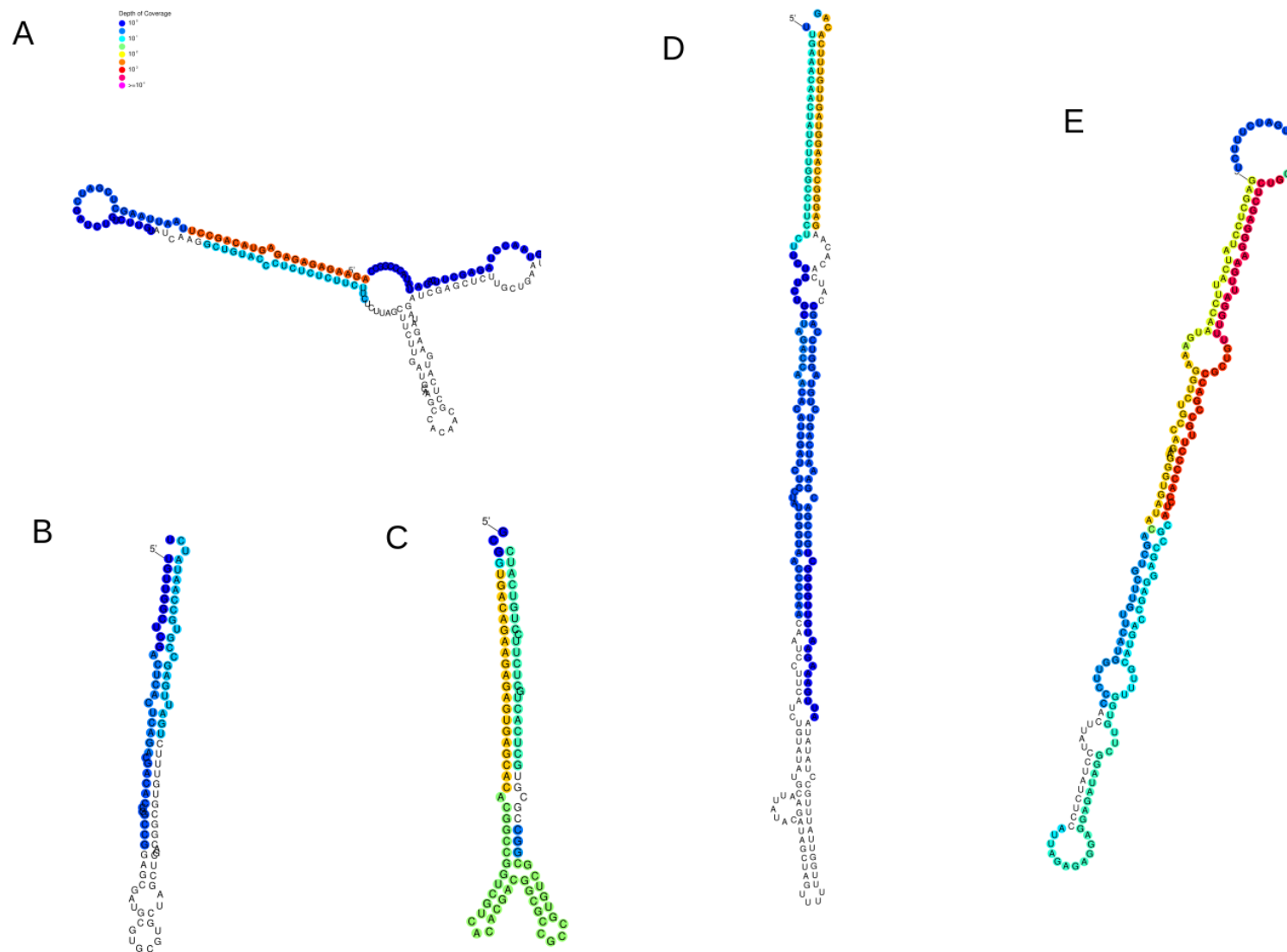




**Figure S6. Results of gene ontology enrichment (GOE) analysis for significantly DE Bd genes in the 4 DPI leaf setup.** GOE analysis done with AgriGO v2.



**Figure S7. Results of gene ontology enrichment (GOE) analysis for significantly DE Mo genes in the root setup.** GOE analysis done with AgriGO v2.



**Figure S8. Visual representation of the identified upregulated *Bd* clusters (miRNA precursors) structures:** (A) cluster\_7470, (B) cluster\_3162, (C) cluster\_7744, (D) cluster\_2384, (E) cluster\_3312.