

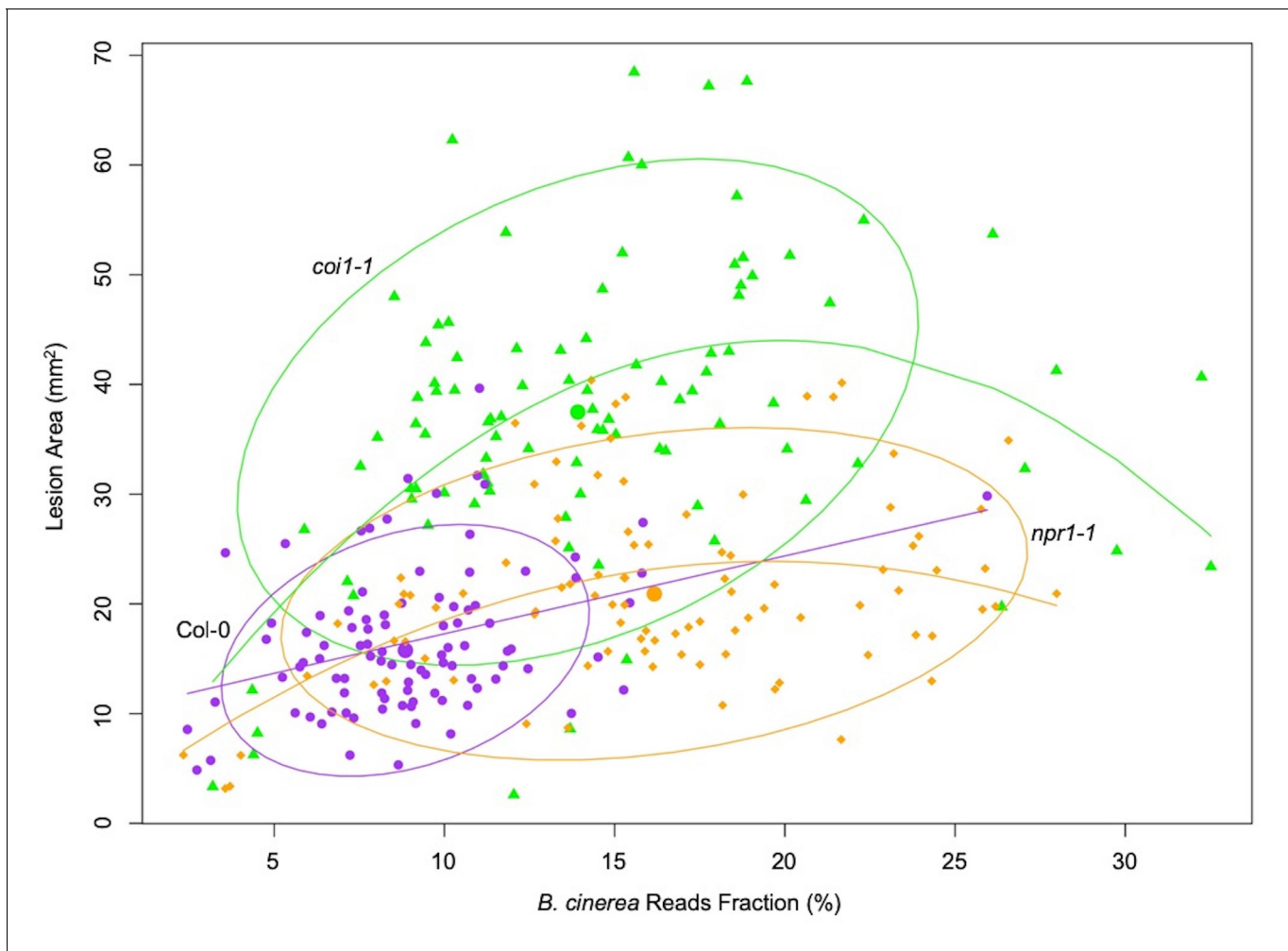


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## Figures and figure supplements

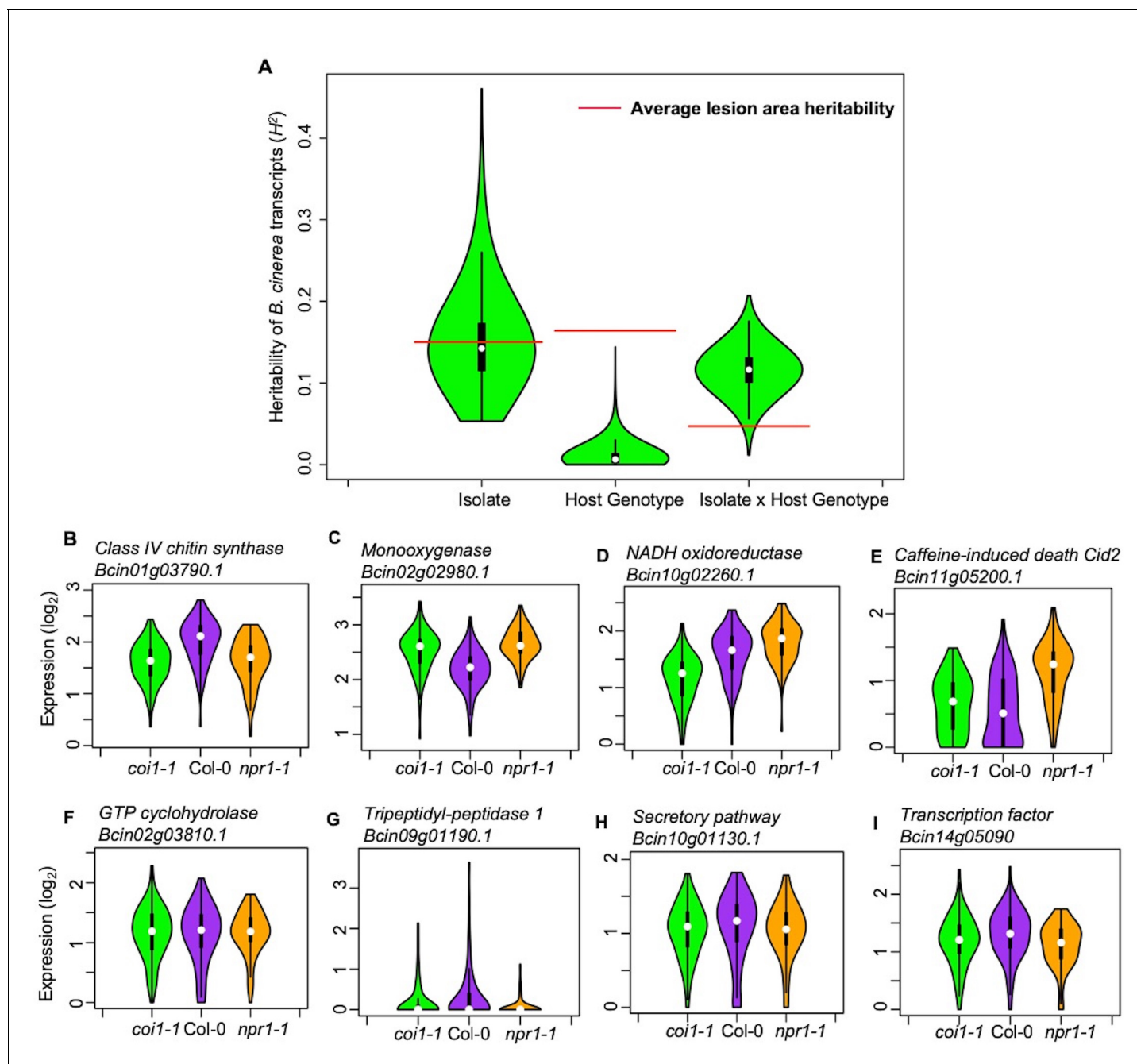
Plant–necrotroph co-transcriptome networks illuminate a metabolic battlefield

**Wei Zhang *et al***



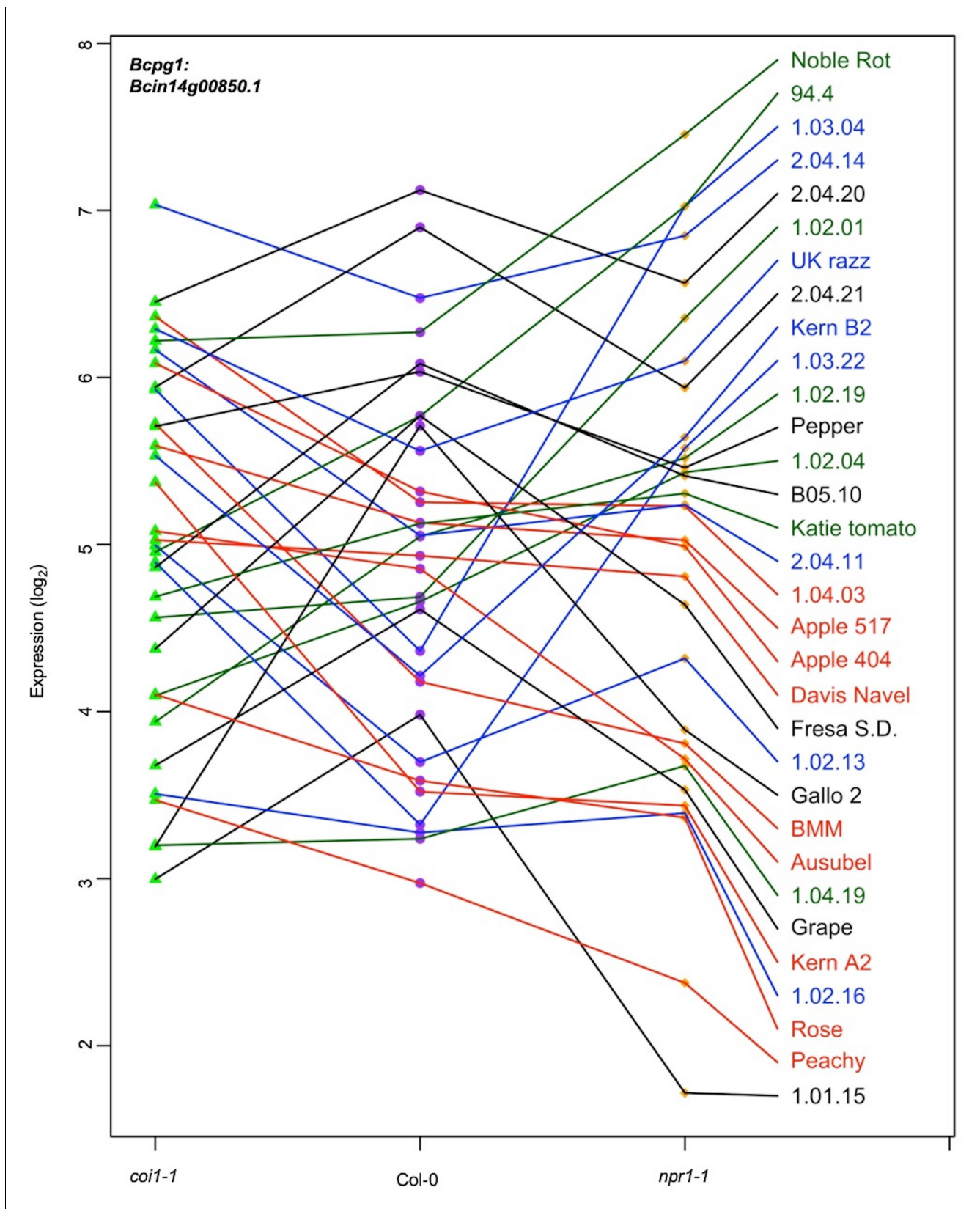
**Figure 1.** Correlation between earlier estimated *B. cinerea* biomass and later lesion area. Model-corrected lesion area means were estimated using the linear model on the six replicates data from three *Arabidopsis* genotypes at 72 hr post-infection with 96 *B. cinerea* isolates. Estimated biomass of *B. cinerea* was calculated using the linear model-corrected fraction of *B. cinerea* mapped reads against total mapped reads to *Arabidopsis* and *B. cinerea* reference genomes. RNA-Seq analysis was conducted at 16 hr post-infection for each pathosystem. Three *Arabidopsis* genotypes are wild-type Col-0 (purple dot), jasmonate insensitive mutant *coi1-1* (green triangle), and salicylic acid insensitive mutant *npr1-1* (orange diamond). The 90% confidence ellipse intervals are plotted for each *Arabidopsis* genotype for references. Quadratic regression lines are: Col-0:  $y = -0.00059x^2 + 0.729x + 10.037$ ,  $p = 0.0016$ , adjusted  $R^2 = 0.1101$ ; *coi1-1*:  $y = -0.117x^2 + 4.44x - 0.1585$ ,  $p = 3.914e-07$ , adjusted  $R^2 = 0.2562$ ; *npr1-1*:  $y = -0.0579x^2 + 2.26x + 1.673$ ,  $p = 0.0001$ , adjusted  $R^2 = 0.161$ .

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**Figure 2.** Transcriptomic responses of *B. cinerea* on Arabidopsis are controlled by genetic variation in pathogen population, host genotypes, and their interaction. (A) Distribution of broad-sense heritability ( $H^2$ ) of *B. cinerea* transcripts contributed by genetic variation in the *B. cinerea*, Arabidopsis genotypes, and the interaction between pathogen and host. Violin plots illustrating the distribution of  $H^2$  for transcripts from 96 *B. cinerea* isolates infecting on Arabidopsis genotypes. Heritability is partitioned across the different sources, 96 pathogen genotypes = 'Isolate', plant genotypes Col-0, *coi1-1* and *npr1-1* plant genotypes = 'Host', and the corresponding interaction. The transcriptomic analysis was conducted by sequencing mRNA extracted from *B. cinerea* infected Arabidopsis leaves at 16 hr post-infection. Red lines indicate the average broad-sense heritability values of lesion area caused by isolates, Arabidopsis genotypes, and their interaction. (B) to (E) Expression profiles of *B. cinerea* transcripts significantly influenced by host genotypes. The model-corrected means ( $\log_2$ ) for *B. cinerea* transcript were used for plotting. The Arabidopsis genotypes, wild-type Col-0 (purple), jasmonate insensitive mutant *coi1-1* (green), and salicylic acid mutant *npr1-1* (orange), are shown on the x axis. *B. cinerea* transcripts are: (B) *Bcin01g03790.1*, class IV chitin synthase; (C) *Bcin02g02980.1*, Monooxygenase; (D) *Bcin10g02260.1*, NADH oxidoreductase; (E) *Bcin11g05200.1*, caffeine-induced death *Cid2*; (F) to (I) Expression profiles of *B. cinerea* transcripts significantly influenced by the interaction between pathogen and host genotypes. (F) *Bcin02g03810.1*, GTP cyclohydrolase; (G) *Bcin09g01190.1*, Tripeptidyl-peptidase 1; (H) *Bcin10g01130.1*, in secretory pathway; (I) *Bcin14g05090.1*, a transcription factor.

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**Figure 3.** Expression profiles of an endopolygalacturonase gene *Bcp1* from diverse *B. cinerea* isolates across Arabidopsis genotypes. Rank plot shows the relationship of *Bcp1* expression from 32 diverse *B. cinerea* isolates (right) across three Arabidopsis genotypes (x axis). Three Arabidopsis

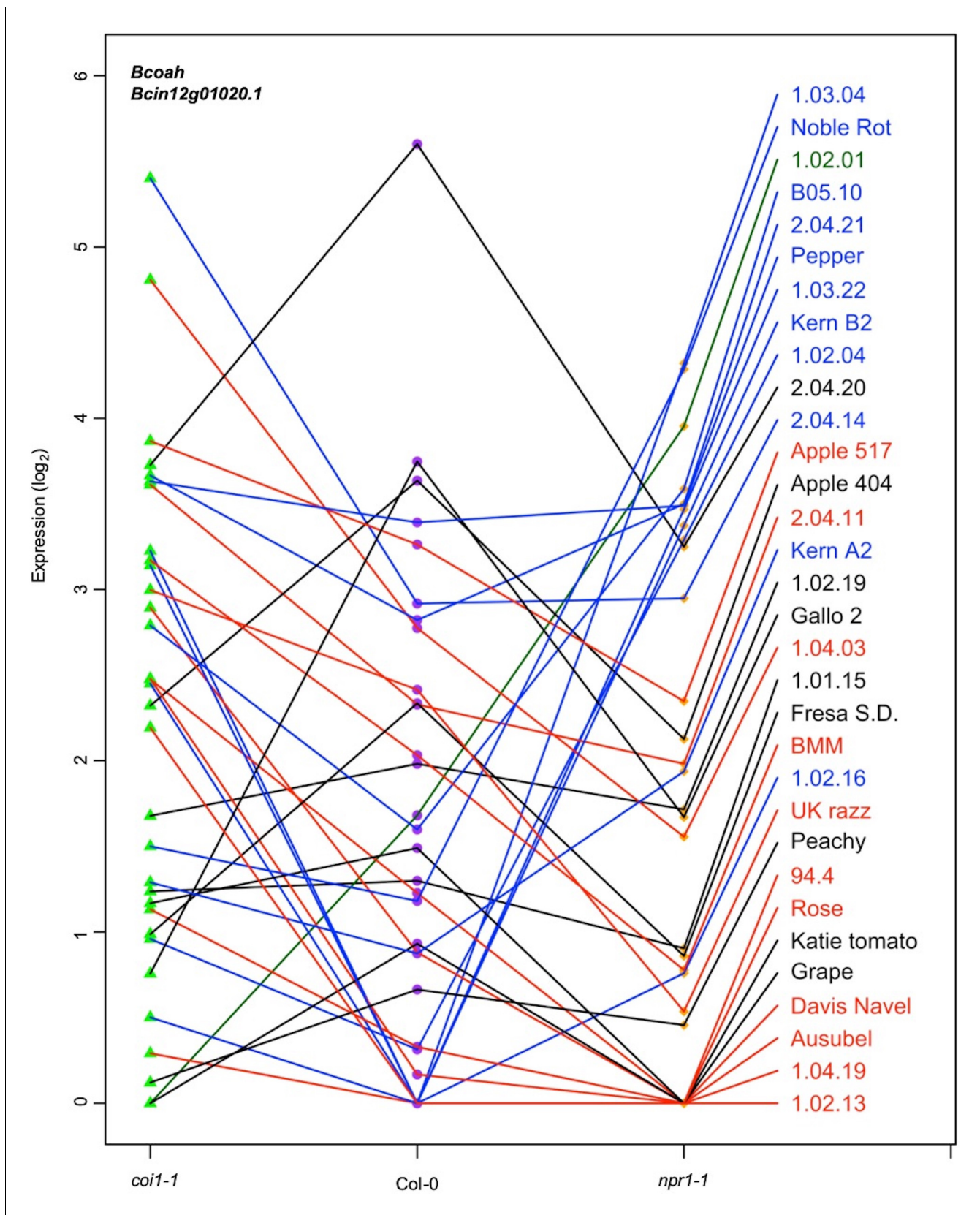
Figure 3 continued on next page



## Figure 3 continued

genotypes are wild-type Col-0 (purple dot), jasmonate insensitive mutant *coi1-1* (green triangle), and salicylic acid mutant *npr1-1* (orange diamond). The model-corrected means ( $\log_2$ ) for the transcript of *Bcpg1* (*Bcin14g00850.1*) encoding an endopolygalacturonase gene are utilized for plotting. The transcript expression levels from the same isolate across three *Arabidopsis* genotypes are connected with a colored line. The names of 32 isolates are represented with the same colored lines as induced *Bcpg1* expression levels. Black lines indicate the expression levels of *Bcpg1* are higher in *coi1-1* and *npr1-1* than in Col-0. Red lines indicate the higher expression levels of *Bcpg1* in *coi1-1* but lower in *npr1-1*. Blue lines indicate the highest expression levels of *Bcpg1* are in Col-0. Dark green lines indicate the higher expression levels of *Bcpg1* in *npr1-1* but lower in *coi1-1*.

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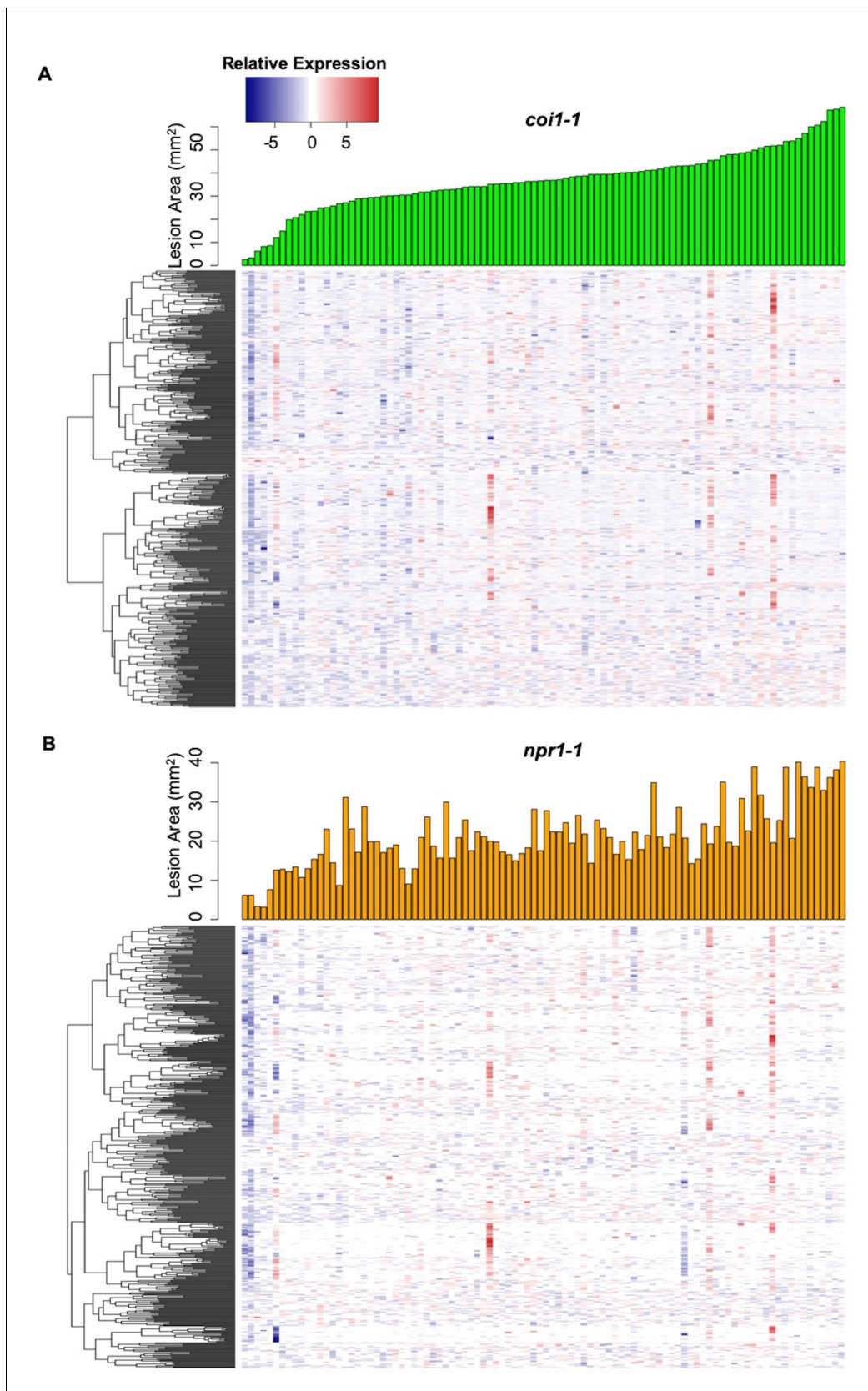


**Figure 3—figure supplement 1.** Expression profiles of an oxaloacetate hydrolase gene *Bcoah* from diverse *B. cinerea* isolates across Arabidopsis genotypes. Rank plot shows the relationship of *Bcoah* expression from 32 diverse *B. cinerea* isolates (right) across three Arabidopsis genotypes (x axis).  
Figure 3—figure supplement 1 continued on next page

## Figure 3—figure supplement 1 continued

Three Arabidopsis genotypes are wild-type Col-0 (purple dot), jasmonate insensitive mutant *coi1-1* (green triangle), and salicylic acid mutant *npr1-1* (orange diamond). The model-corrected means ( $\log_2$ ) for the transcript of *Bcoah* (*Bcin12g01020.1*) encoding an oxaloacetate hydrolase gene are utilized for plotting. The transcript expression levels from the same isolate across three Arabidopsis genotypes are connected with a colored line. The names of 32 isolates are represented with the same colored lines as induced *Bcoah* expression levels. Black lines indicate the expression levels of *Bcoah* are higher in *coi1-1* and *npr1-1* than in Col-0. Red lines indicate the higher expression levels of *Bcoah* in *coi1-1* but lower in *npr1-1*. Blue lines indicate the highest expression levels of *Bcoah* are in Col-0. Dark green lines indicate the higher expression levels of *Bcoah* in *npr1-1* but lower in *coi1-1*.

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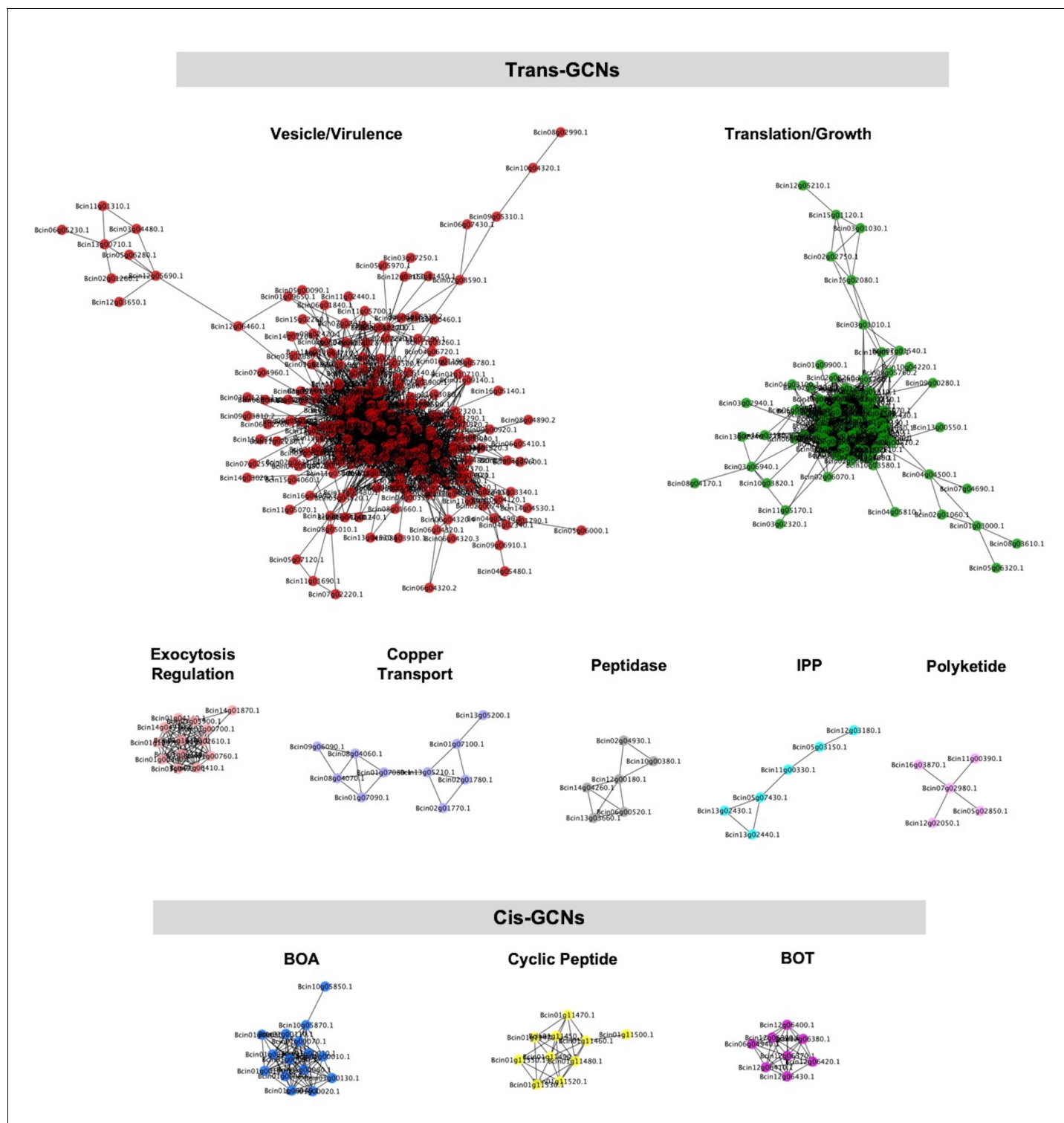
**Figure 4.** Interaction effects of host genotypes and pathogen isolates on *B. cinerea* transcriptome. Hierarchical clustering of relative expression of 500 genes from 96 *B. cinerea* isolates infection on *Arabidopsis* mutants *coi1-1* (A) or *npr1-1* (B) are plotted based on pairwise comparison of pathogen gene expression. Figure 4 continued on next page

*Figure 4 continued*

expression under Col-0. The 500 *B. cinerea* genes with highest broad-sense heritability ( $H^2$ ) of host X pathogen were used for analysis. Lesion area induced by 96 isolates are compared under *coi1-1* (green bar plot) and *npr1-1* (orange bar plot).

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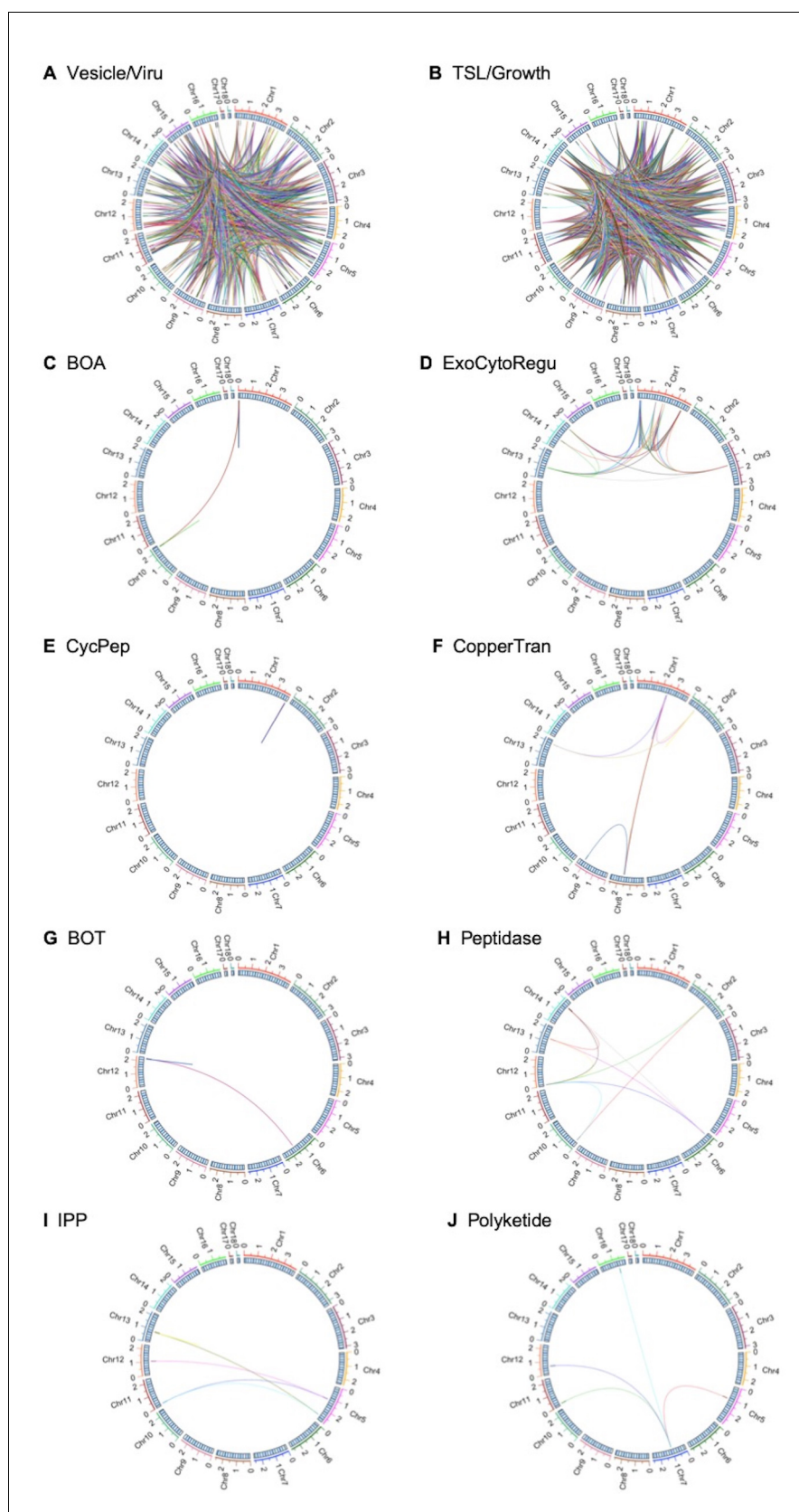


**Figure 5.** Gene co-expression networks identified from *B. cinerea* transcriptomic responses to Arabidopsis wild-type Col-0 immunity. Ten gene co-expression networks (GCNs) with more than five nodes were identified from 96 *B. cinerea* isolates infecting on Arabidopsis wild-type Col-0. The similarity matrix is computed using Spearman's rank correlation coefficient. Nodes with different colors represent *B. cinerea* genes condensed in GCNs with different biological functions. Edges represent the Spearman's rank correlation coefficients between gene pairs. Trans- and cis-GCNs means GCNs are regulated by trans- and cis-regulatory elements, respectively. GCNs were named after their biological functions, which were determined by hub and bottleneck genes within each network. GCNs are: vesicle/virulence (red), translation/growth (green), exocytosis regulation (pink), cyclic peptide (yellow), Figure 5 continued on next page

*Figure 5 continued*

peptidase (gray), isopentenyl pyrophosphate (IPP, turquoise), polyketide (violet), botcinic acid (BOA, blue), copper transport (slate blue), botrydial (BOT, purple).

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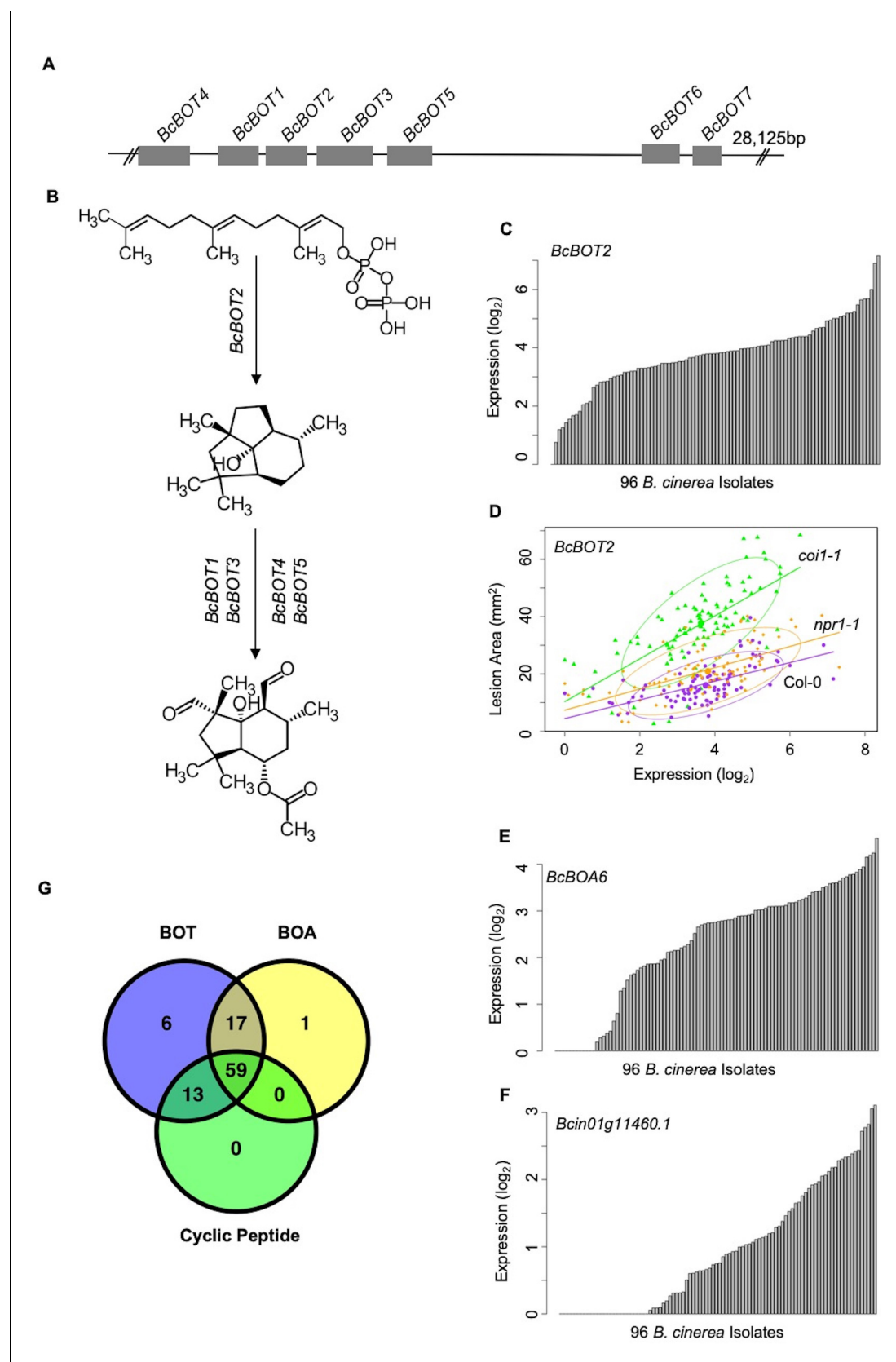
**Figure 5—figure supplement 1.** Genomic location of *B. cinerea* gene co-expression networks. The circle diagrams showed the genome-wide distribution of gene pairs identified by *B. cinerea* gene co-expression

Figure 5—figure supplement 1 continued on next page

*Figure 5—figure supplement 1 continued*

networks (GCNs) under Arabidopsis wild-type Col-0. (A) to (J) Genomic locations of gene pairs identified by GCNs: (A) Vesicle/virulence, (B) translation/growth, (C) botcinic acid (BOA), (D) exocytosis regulation, (E) cyclic peptide, (F) copper transport, (G) botrydial (BOT), (H) peptidase (gray), (I) isopentenyl pyrophosphate (IPP), (J) polyketide. The rings show 18 *B. cinerea* chromosomes on a Mb scale. Genomic locations of co-expressed gene pairs are connected by the colored lines.

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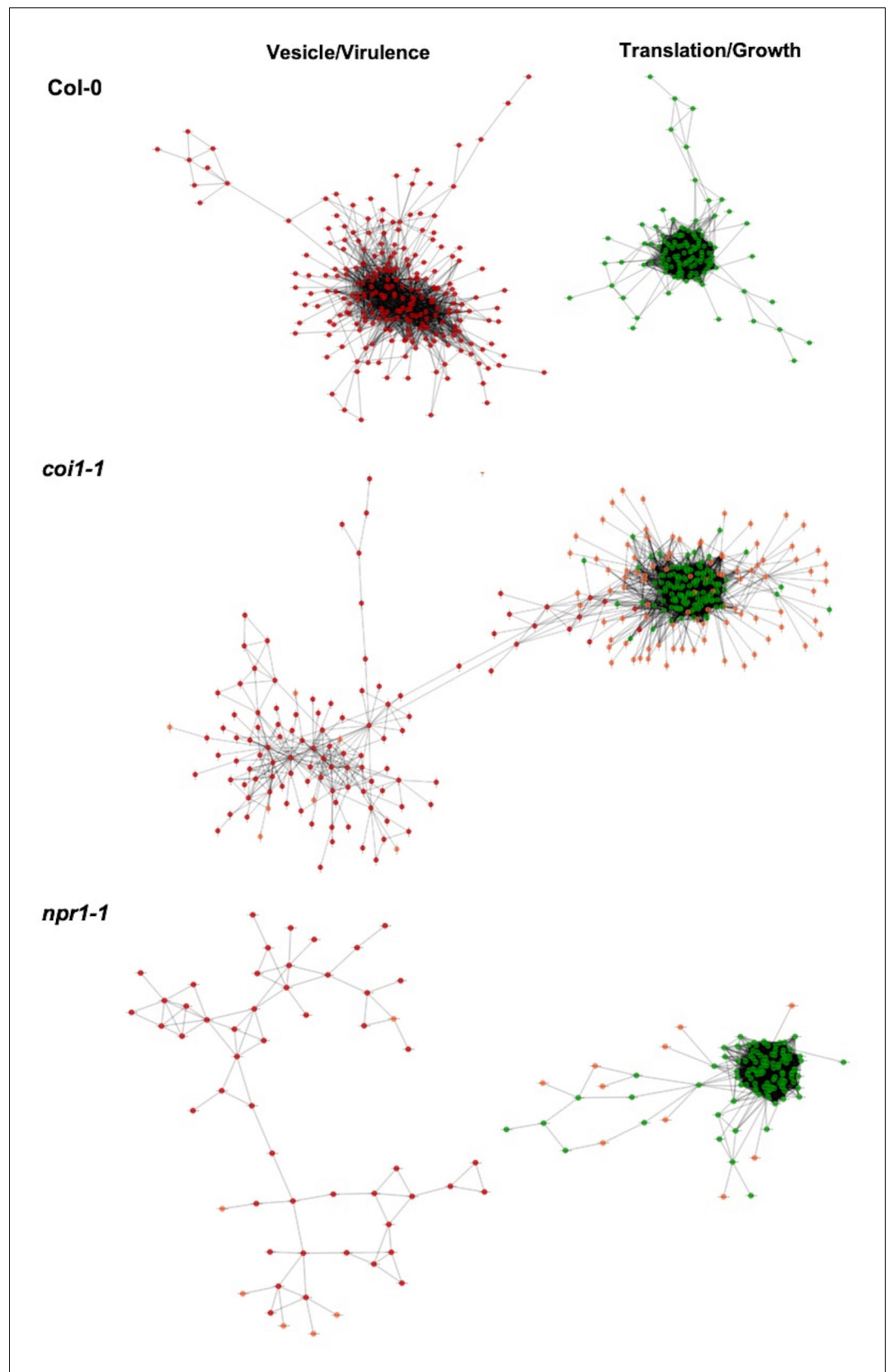
**Figure 6.** Variation of transcripts accumulation for secondary metabolites production across diverse *B. cinerea* isolates. Expression profiles of genes responsible for botrydial, botcinic acid, cyclic peptide production across 96 isolates under Arabidopsis wild-type Col-0 are shown. (A) Schematic shows Figure 6 continued on next page



## Figure 6 continued

the genomic locus of seven botrydial (BOT) biosynthesis genes clustered together. Exons are represented by gray boxes. Introns and intergenic regions are represented by the grey line. Seven BOT genes are: *BcBOT1*, *BcBOT3* and *BcBOT4*, encoding a cytochrome P450 monooxygenase, respectively; *BcBOT2* encoding a sesquiterpene cyclase; *BcBOT5* encoding an acetyl transferase; *BcBOT6* encoding a Zn(II)2Cys6 transcription factor, *BcBOT7* encoding a dehydrogenase reductase. (B) BOT biosynthesis pathway in *B. cinerea*. (C) Bar plots compare expression variation of *BcBOT2* across 96 *B. cinerea* isolates in responding to Arabidopsis wild-type Col-0 immunity. The model-corrected means ( $\log_2$ ) of transcripts were used for plotting. (D) Scatter plot illustrates the positive correlations between lesion area and accumulation of *BcBOT2* transcript across the 96 isolates in response to varied Arabidopsis immunities. Model-corrected lesion area means were estimated for three Arabidopsis genotypes at 72 hr post-infection with 96 *B. cinerea* isolates. The three Arabidopsis genotypes are labeled next to the confidence ellipse curves: wild-type Col-0 (purple dot), jasmonate insensitive mutant *coi1-1* (green triangle), and salicylic acid mutant *npr1-1* (orange diamond). The 90% confidence ellipse intervals are plotted for each Arabidopsis genotype for reference. Linear regression lines: Col-0:  $y = 3.2532x + 4.4323$ ,  $p = 1.008e-10$ , Adjusted  $R^2 = 3.3537$ ; *coi1-1*:  $y = 7.4802x + 10.3289$ ,  $p = 7.895e-15$ , adjusted  $R^2 = 0.4700$ ; *npr1-1*:  $y = 3.7086x + 7.3487$ ,  $p = 2.425e-11$ , adjusted  $R^2 = 0.3726$ . (E) and (F) Bar plots compare expression variation of *BcBOA6* in botcinic acid (BOA) pathway and *Bcin01g11460* in cyclic peptide pathway across 96 *B. cinerea* isolates in response to Arabidopsis wild-type Col-0 immunity. (G) Venn diagram illustrates the number of *B. cinerea* isolates with the ability to induce BOT, BOA, and cyclic peptide.

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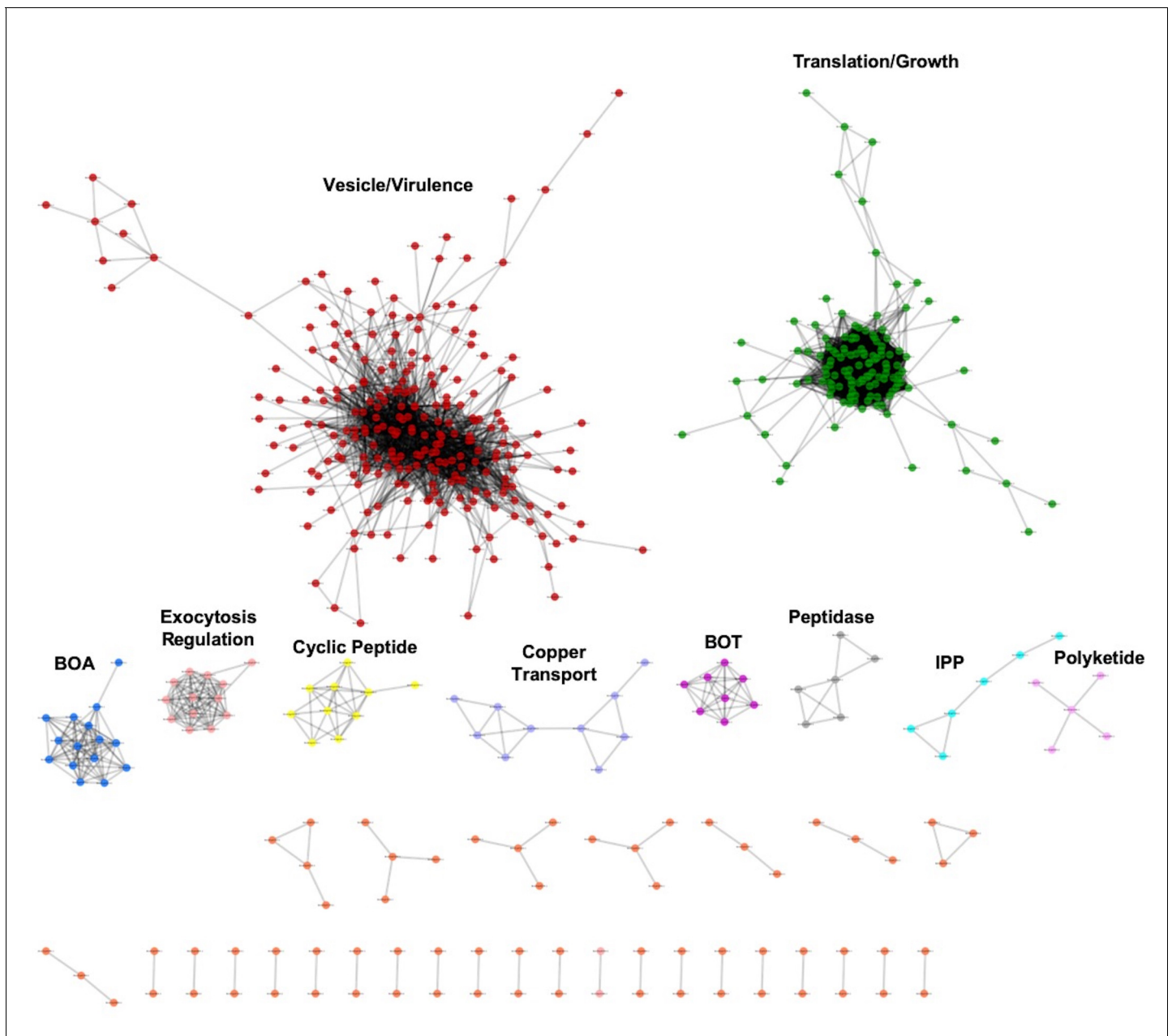


**Figure 7.** Comparison of plasticity of *B. cinerea* gene co-expression network under varied host immunity. *B. cinerea* gene co-expression networks (GCNs) of vesicle/virulence (red) and translation/growth (green) identified  
Figure 7 continued on next page

*Figure 7 continued*

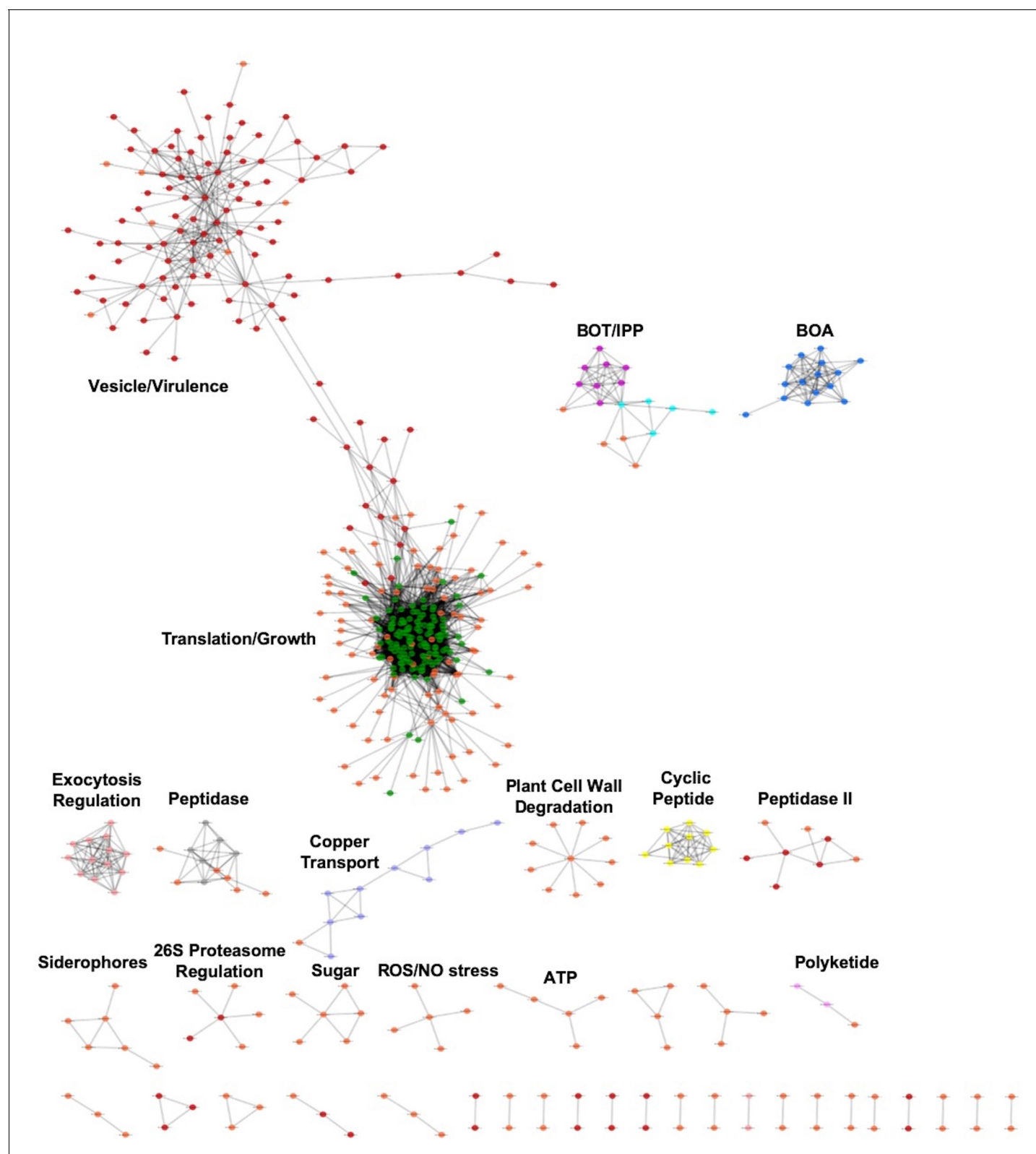
under three *Arabidopsis* genotypes are compared. Three *Arabidopsis* genotypes are wild-type Col-0, jasmonate insensitive mutant *coi1-1*, and salicylic acid mutant *npr1-1*. Nodes marked with red and green colors represent *B. cinerea* genes condensed in GCNs with different biological functions. The same node condensed in GCNs across three *Arabidopsis* genotypes was marked with same color. Nodes specifically condensed in GCNs under two mutants *coi1-1* and *npr1-1* background are marked with orange color. Edges represent the Spearman's rank correlation coefficients between gene pairs.

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**Figure 7—figure supplement 1.** Gene co-expression networks identified from *B. cinerea* transcriptomic responses to Arabidopsis wild-type Col-0 immunity. *B. cinerea* gene co-expression networks (GCNs) were identified from 96 *B. cinerea* isolates infecting on Arabidopsis wild-type Col-0. The similarity matrix is computed using Spearman's rank correlation coefficient. All co-expressed gene pairs with correlation greater than 0.85 were shown. Nodes with different colors represent *B. cinerea* genes condensed in GCNs with different biological functions. Edges represent the Spearman's rank correlation coefficients between gene pairs. GCNs were ordered as number of nodes within each network. GCNs were named after their biological functions, which were determined by hub and bottleneck genes within each network: vesicle/virulence (red), translation/growth (green), botcinic acid (BOA, blue), exocytosis regulation (pink), cyclic peptide (yellow), copper transport (slate blue), botrydial (BOT, purple), peptidase (gray), isopentenyl pyrophosphate (IPP, turquoise), polyketide (violet).

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**Figure 7—figure supplement 2.** Gene co-expression networks identified from *B. cinerea* transcriptomic responses to Arabidopsis jasmonate-compromised immunity. *B. cinerea* gene co-expression networks (GCNs) were identified from 96 *B. cinerea* isolates infecting on Arabidopsis jasmonate insensitive mutant *coi1-1*. The similarity matrix is computed using Spearman's rank correlation coefficient. All co-expressed gene pairs with correlation greater than 0.8 were shown. Nodes with different colors represent *B. cinerea* genes condensed in GCNs with different biological functions. Nodes

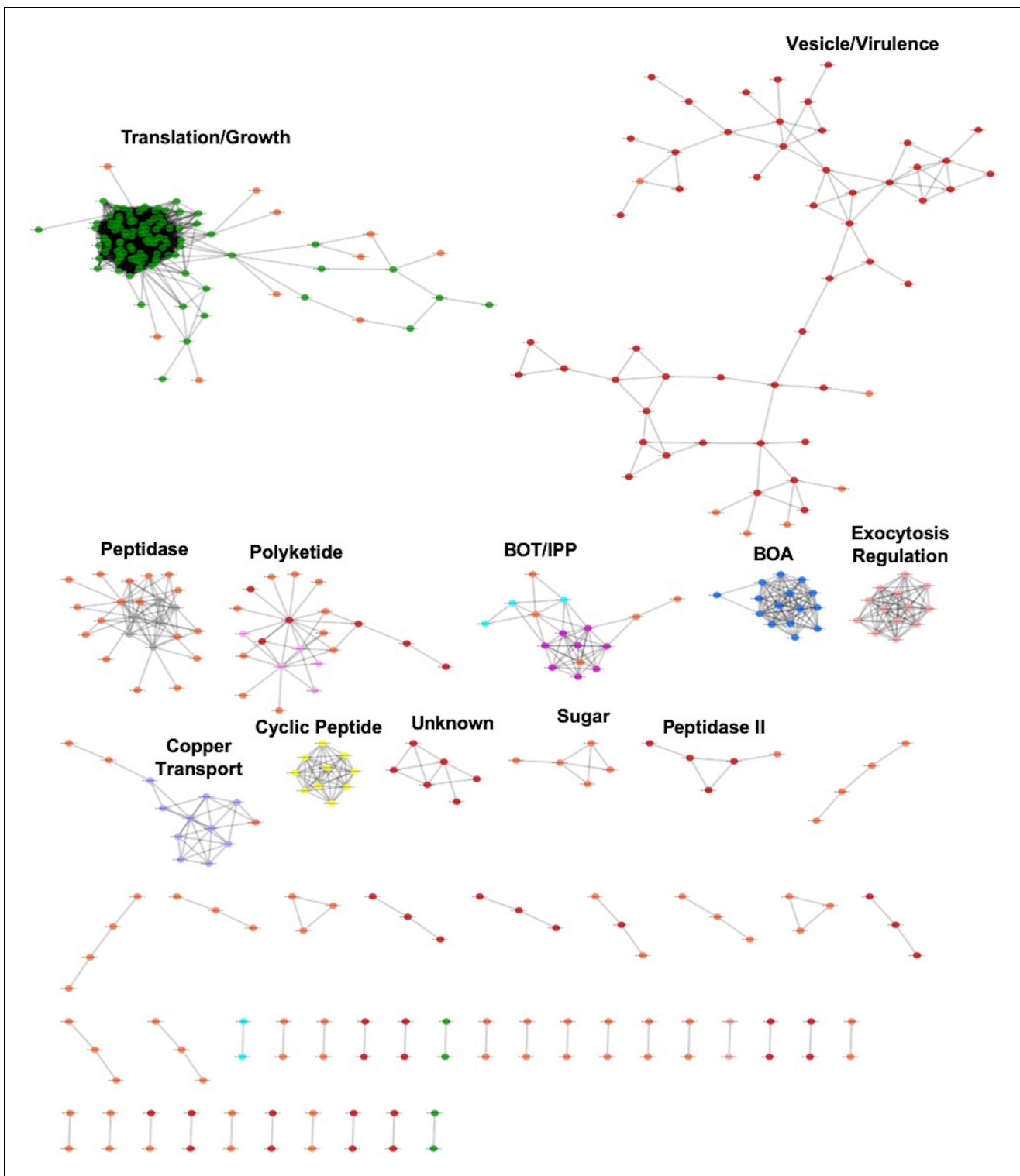
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*Figure 7—figure supplement 2 continued*

were marked with same color as under *Arabidopsis* wild-type Col-0 background. Nodes specifically condensed in GCNs under *Arabidopsis* mutant *coi1-1* background are marked with orange color. Edges represent the Spearman's rank correlation coefficients between gene pairs. GCNs were ordered as number of nodes within each network. GCNs were named after their biological functions, which were determined by hub and bottleneck genes within each network: vesicle/virulence (red/orange), translation/growth (green/orange), botrydial/isopentenyl pyrophosphate (BOT/IPP, blue/turquoise/orange), botcinic acid (BOA, blue), exocytosis regulation (pink), peptidase (gray/orange), copper transport (slate blue/orange), plant cell wall degradation (orange), cyclic peptide (yellow), peptidase II (red/orange), siderophores (orange), 26S proteasome regulated protein degradation (red/orange), sugar, ROS/NO stress (orange), ATP (orange), polyketide (violet/orange).

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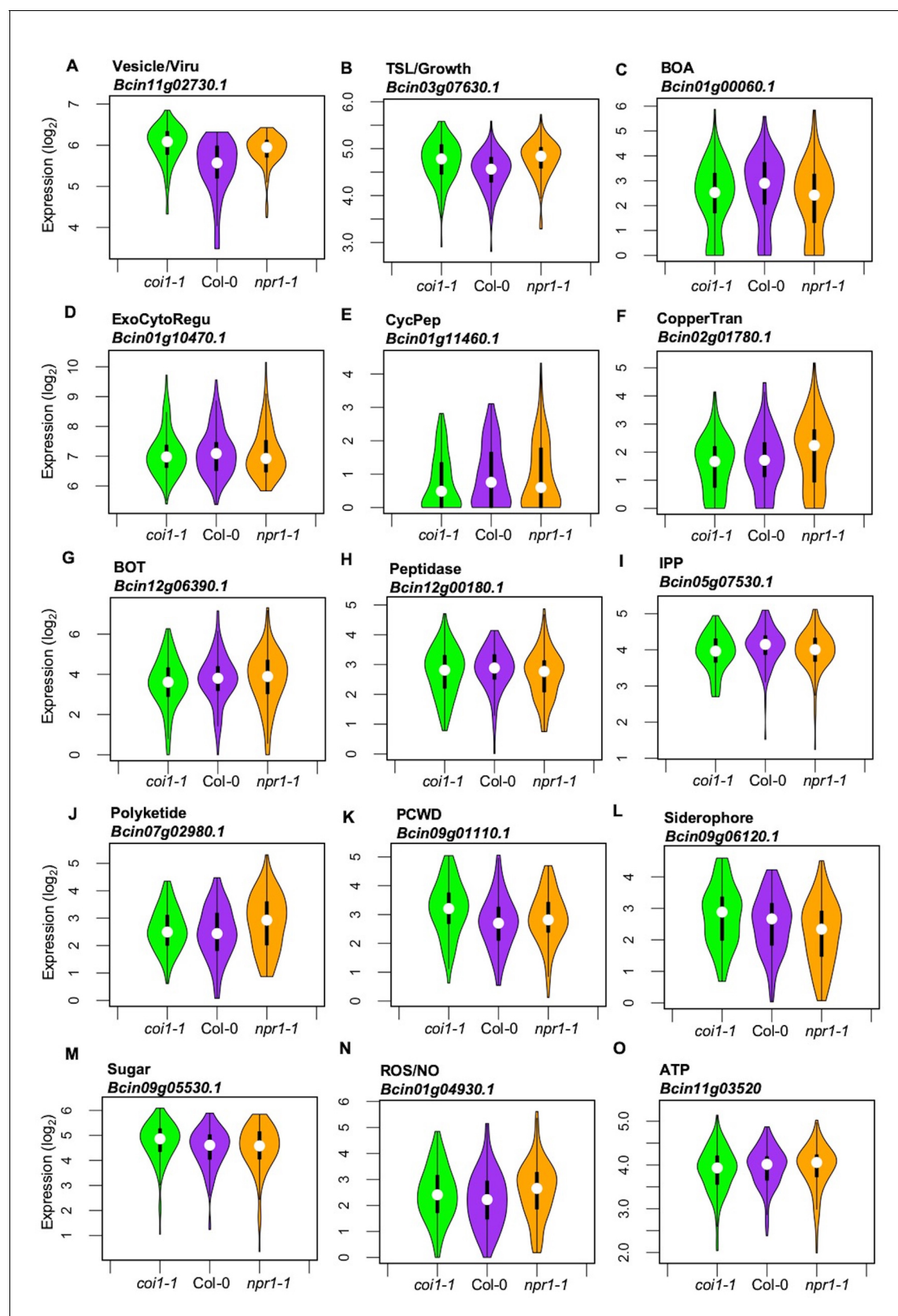


**Figure 7—figure supplement 3.** Gene co-expression networks identified from *B. cinerea* transcriptomic responses to Arabidopsis salicylic acid-compromised immunity. *B. cinerea* gene co-expression networks (GCNs) were identified from 96 *B. cinerea* isolates infecting on Arabidopsis salicylic acid insensitive mutant *npr1-1*. The similarity matrix is computed using Spearman's rank correlation coefficient. All co-expressed gene pairs with correlation greater than 0.8 were shown. Nodes with different colors represent *B. cinerea* genes condensed in GCNs with different biological functions. Nodes were marked with same color as under Arabidopsis wild-type Col-0 background. Nodes specifically condensed in GCNs under Arabidopsis mutant *npr1-1* background are marked with orange color. Edges represent the Spearman's rank correlation coefficients between gene pairs. GCNs were ordered as number of nodes within each network. GCNs were named after their biological functions, which were determined by hub and Figure 7—figure supplement 3 continued on next page

Figure 7—figure supplement 3 continued

bottleneck genes within each network: translation/growth (green/orange), vesicle/virulence (red/orange), peptidase (gray/orange), polyketide (violet/orange), botrydial/isopentenyl pyrophosphate (BOT/IPP, blue/turquoise/orange), botcinic acid (BOA, blue), exocytosis regulation (pink), copper transport (slate blue/orange), cyclic peptide (yellow), unknown (red), sugar (orange), peptidase II (red/orange).

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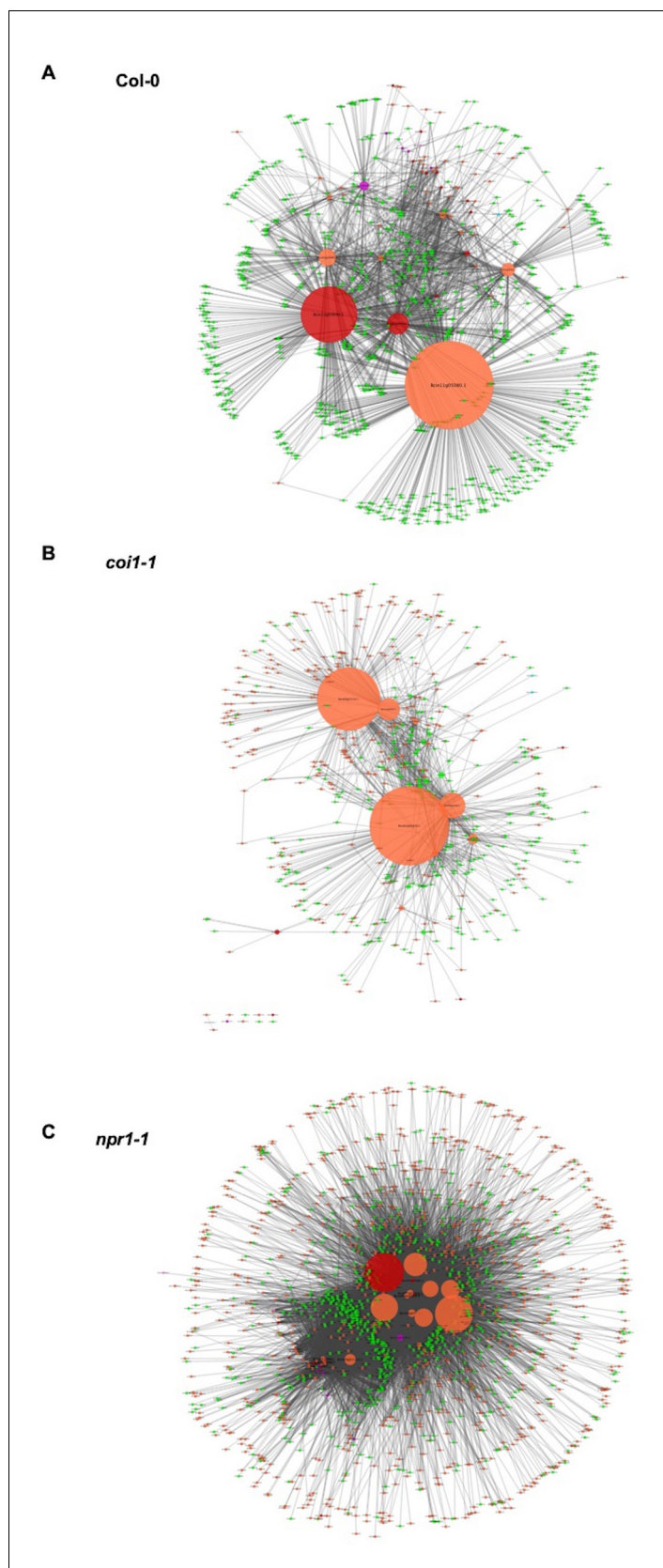
**Figure 7—figure supplement 4.** Plasticity in expression profiles of genes identified by *B. cinerea* gene co-expression networks under varied Arabidopsis immunities. Violin plots of (A) to (O) show the expression profiles of *B. cinerea* genes in response to variation of Arabidopsis immunity. Figure 7—figure supplement 4 continued on next page

*Figure 7—figure supplement 4 continued*

Genes shown are condensed in *B. cinerea* gene co-expression networks (GCNs). The model-corrected means ( $\log_2$ ) for *B. cinerea* transcript were used for plotting. The Arabidopsis genotypes, wild-type Col-0 (purple), jasmonate insensitive mutant *coi1-1* (green), and salicylic acid mutant *npr1-1* (orange), are shown on the x axis.

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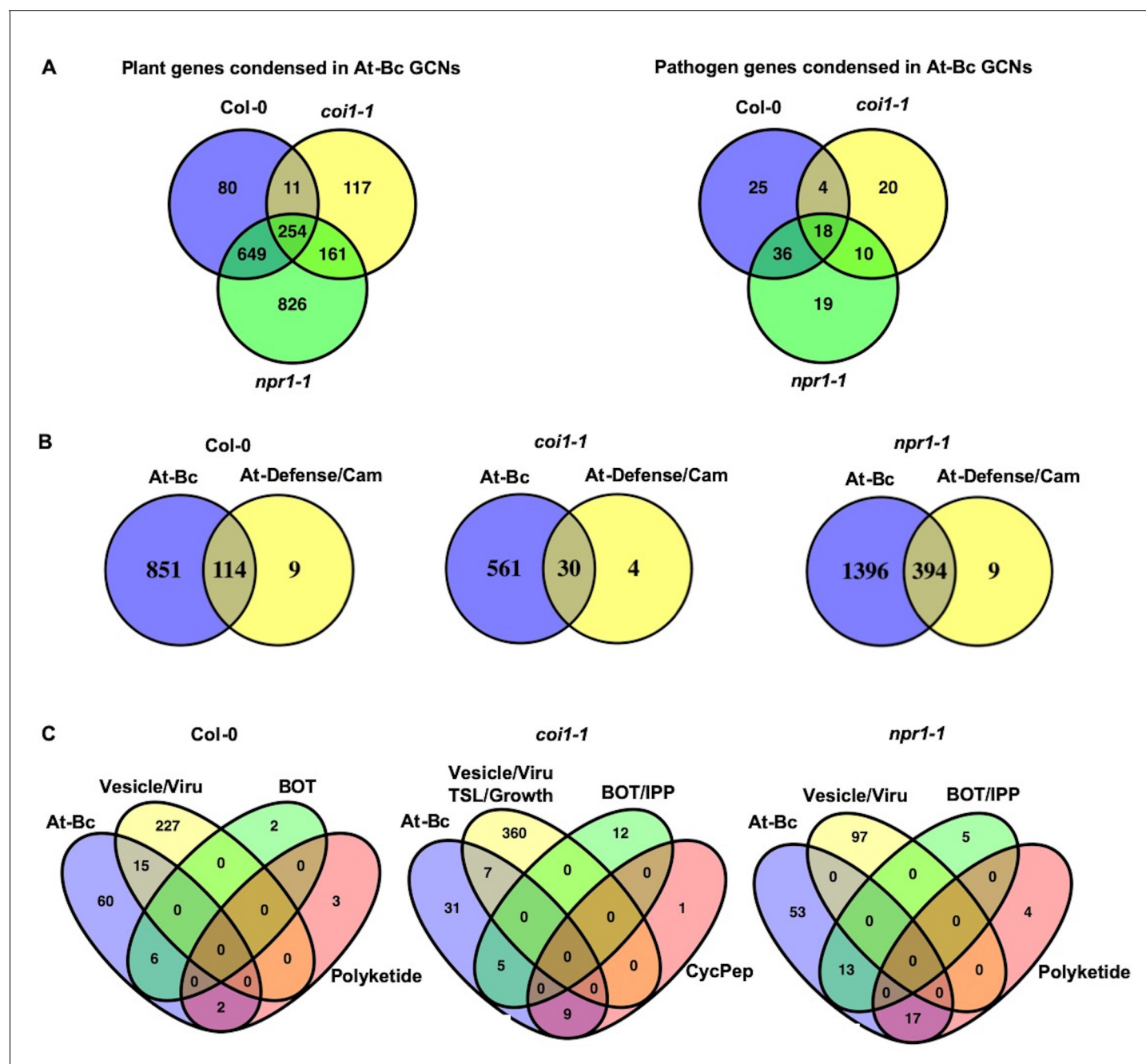


**Figure 8.** Cross-kingdom *Arabidopsis*-*B. cinerea* gene co-expression networks. Networks showing the co-expression connectivity between *Arabidopsis* and *B. cinerea* genes within three *Arabidopsis* genotypes are shown. *Figure 8 continued on next page*

*Figure 8 continued*

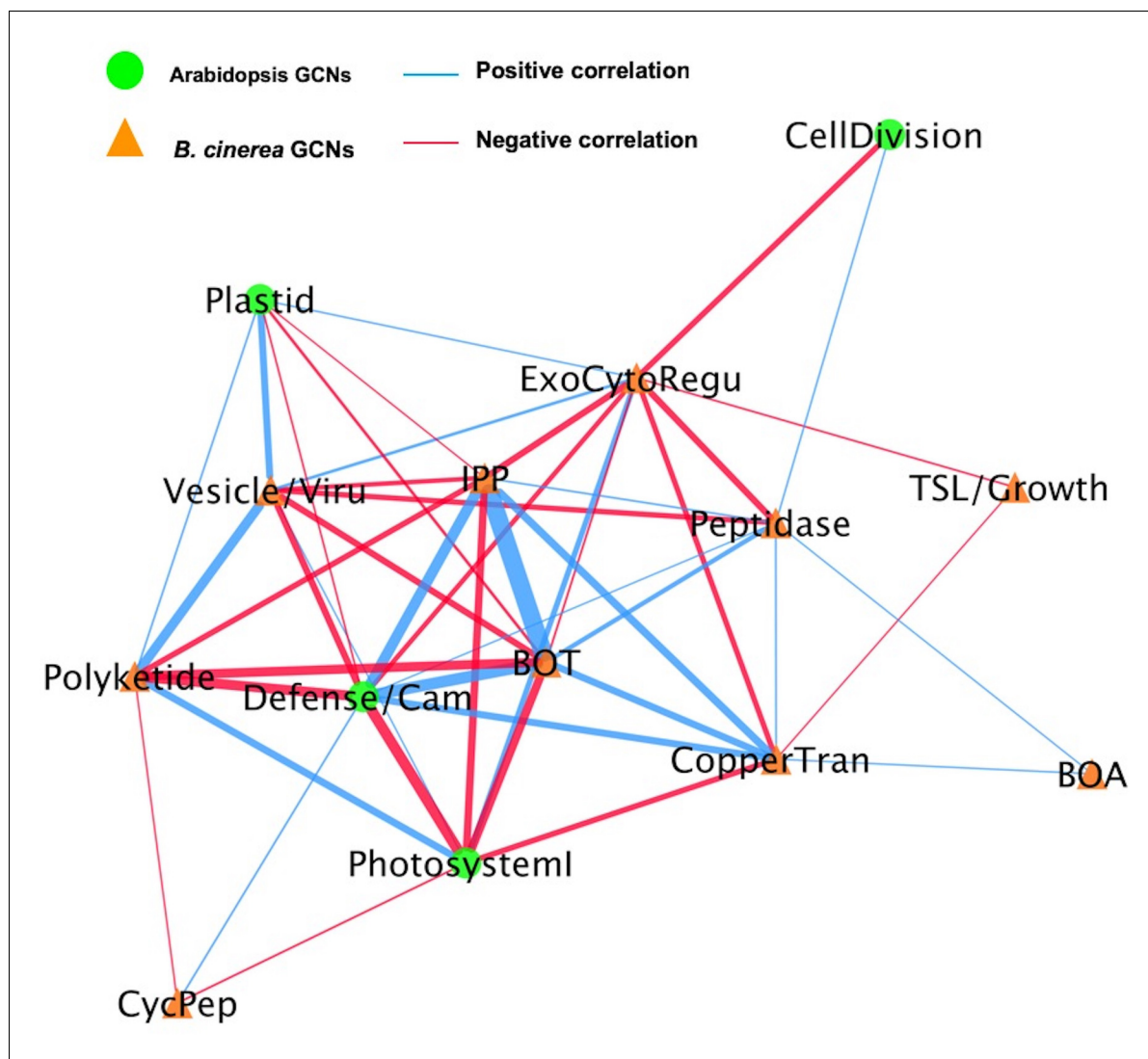
(A) shows connectivity within Arabidopsis wild-type Col-0, (B) shows connectivity within the Arabidopsis jasmonate insensitive mutant *coi1-1*, and (C) shows connectivity within the Arabidopsis salicylic acid insensitive mutant *npr1-1*. Within each connectivity plot, orange and green nodes show transcripts from *B. cinerea* and Arabidopsis, respectively. Nodes with red and violet colors represent the *B. cinerea* transcripts that were found to be members of the *B. cinerea* membrane/vesicle virulence network and BOT network, respectively. Node size shows the number of interactions with a specific gene. The connectivity between the nodes was derived using Spearman's rank correlation analysis.

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**Figure 8—figure supplement 1.** Associations between gene co-expression networks identified from co- and single-transcriptome. Venn diagram highlights: (A) the overlap of plant (left) and pathogen (right) genes condensed in *Arabidopsis-B. cinerea* gene co-expression networks (GCNs) across three *Arabidopsis* genotypes, (B) the overlap of plant genes in *Arabidopsis-B. cinerea* GCNs and *Arabidopsis* GCNs across three genotypes, (C) the overlap of pathogen genes in *Arabidopsis-B. cinerea* GCNs and *B. cinerea* GCNs across three genotypes.

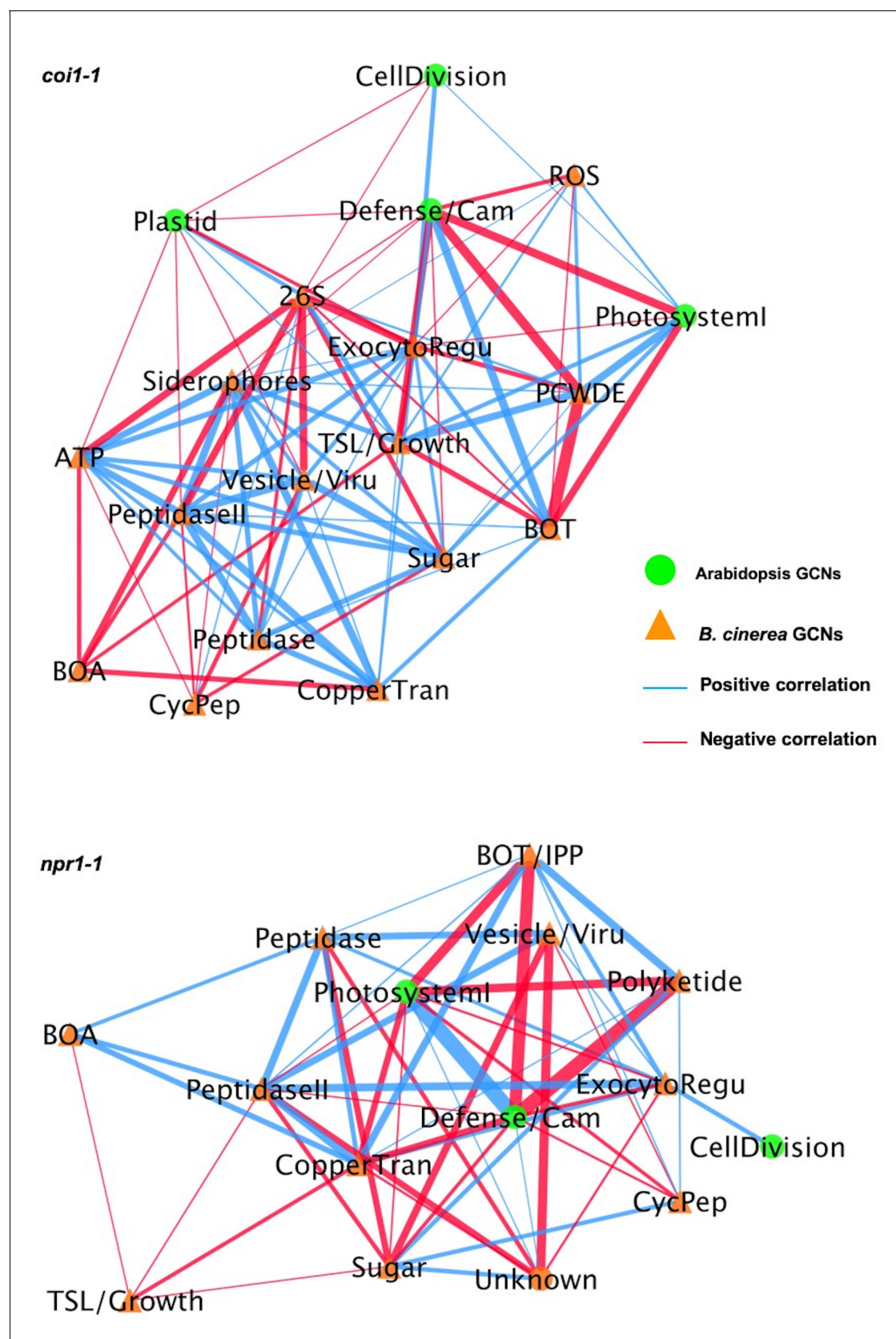
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**Figure 9.** A dual interaction network reveals links between Arabidopsis immunity and *B. cinerea* virulence. A dual interaction network was constructed using gene co-expression networks (GCNs) from Arabidopsis and *B. cinerea* co-transcriptome. The first eigenvectors were derived from individual GCNs and used as input to calculate Spearman's rank correlation coefficient between GCN pairs. Green dots and orange triangles represent Arabidopsis immune- and *B. cinerea* virulence-GCNs, respectively. Blue and red lines (edges) represent the positive and negative Spearman's rank correlation coefficients between GCN pairs, respectively. The thickness of line signifies the correlational strength.

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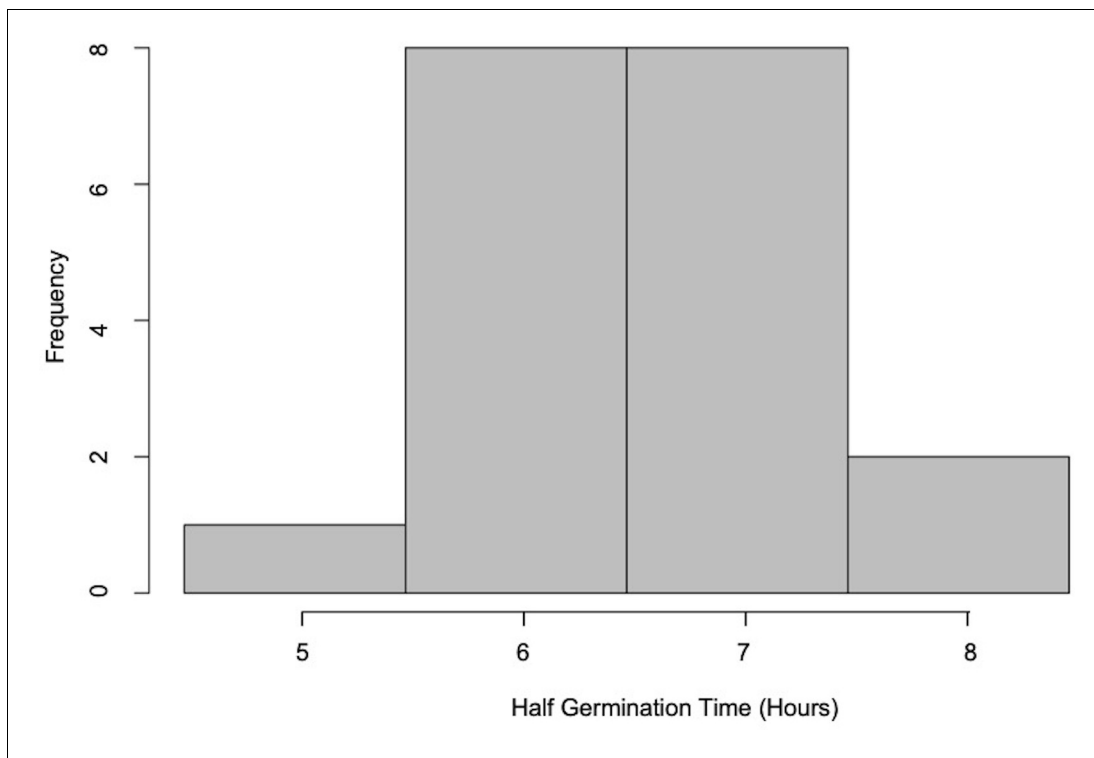
**Figure 9—figure supplement 1.** Dual networks reveal links between Arabidopsis immunity and *B. cinerea* virulence under *coi1-1* and *npr1-1*. Dual interaction networks were constructed using gene co-expression networks (GCNs) from Arabidopsis and *B. cinerea* co-transcriptome under JA-  
Figure 9—figure supplement 1 continued on next page

*Figure 9—figure supplement 1 continued*

insensitive mutant *coi1-1* and SA-insensitive mutant *npr1-1*, respectively. The first eigenvectors were derived from individual GCNs and used as input to calculate Spearman's rank correlation coefficient between GCN pairs. Green dots and orange triangles (nodes) represent *Arabidopsis* immune- and *B. cinerea* virulence-GCNs, respectively. Blue and red lines (edges) represent the positive and negative Spearman's rank correlation coefficients between GCN pairs, respectively. The thickness of line signifies the correlational strength.

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**Figure 9—figure supplement 2.** Variation of *B. cinerea* spore germination time under in vitro condition.  
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