From Wei Zhang 2018: **Genetic Variation in *Botrytis* Controls the Transcriptional Plasticity in *Planta***

**Genetic Variation in Pathogen, Host Showed Differing Impacts on Gene Expression of BOT and BOA Pathways**

The above observations show that at the transcriptomic level, genetic variation in pathogen and host have differing influences on *B. cinerea* virulence-related individual gene. To further study the role of genetic variation in *B. cinerea* virulence mechanism during plant infection, we initially focused on the transcriptional response of well-known toxin biosynthesis pathways responsible for two non-host specific phytotoxin sesquiterpene BOT and polyketide BOA (Siewers et al., 2005; Pinedo et al., 2008; Dalmais et al., 2011; Porquier et al., 2016). Previous studies on the BOT production in *B. cinerea* infection demonstrated that a biosynthetic pathway consisting of seven genes clustered within a region of 28,125 base pair in chromosome 12 (Figure 5A and Supplemental Data Set 7). One gene encodes a sequiterpene cyclase (*BcBOT2*) responsible for converting farnesyl diphosphate to the parent sesquiterpene in the earlier biosynthetic step (Pinedo et al., 2016). A group of cytochrome P450 monooxygenases encoded by three genes, *BcBOT1*, *BcBOT3*, *BcBOT4*, and an acertyl transferase encoded by *BcBOT5* responsible for downstream chemical modifications and transformation (Figure 5A, 5B, and Supplemental Data Set 7) (Siewers et al., 2005; Urlacher and Girhard, 2012; Moraga et al., 2016). The analysis of the expression profiles revealed all clustered genes were induced with varied expression levels during infection on Arabidopsis WT Col-0 (Figure 5C to 5I and Supplemental Data Set 2). To better visualize the expression pattern of the seven genes involved in BOT biosynthesis, we calculated the correlations between transcripts’ abundance using a Spearman’s rank method. The heat map based on the Spearman’s rank correlations showed expression of the BOT genes were highly correlated with r > 0.85. Furthermore, the cluster analysis demonstrated the seven genes were condensed into two expression patterns that *BcBOT6* showed a distinguished expression pattern from other six genes (Figure 5J). This finding is consistent with the role of *BcBOT6* as a pathway-specific Zn(II)2Cys6 transcription factor that positively regulates the BOT biosynthetic gene cluster (Porquier et al., 2016). Since *BcBOT2* and *BcBOT6* transcript accumulations represented the two expression patterns of all BOT genes, we conducted scatter plots between lesion area and transcript abundances of *BcBOT2*/*BcBOT6* in 96 isolates infection on three Arabidopsis genotypes (Figure 5K and 5L). The scatter plots further supported our previous analysis that not only the individual gene involved in BOT biosynthesis but also the BOT production were highly correlated with lesion expansion during plant infection. We further investigated the broad-sense heritability of the whole BOT pathway by calculation the average of the heritability of each transcript in the gene cluster. Our analysis revealed that induction of the BOT biosynthesis is more dependent on the genetic variation in the pathogen (H2 isolate = 0.243 ± 0.016) and less by plant host (H2 host = 0.007 ± 0.001) or their interaction (H2 isolate x host = 0.075 ± 0.045) (average ± SD) (Supplemental Data Set 3).

Previous studies on the production of BOA and its derivates in *B. cinerea* demonstrated that 17 putative botcinic acid biosynthetic genes (*BcBOA1* to *BcBOA17*) are separated into two clusters in the wild-type (WT) strain B05. 10, including two polyketide synthases *BcPKS6* and *BcPKS9* (Dalmais et al., 2011). The GO analysis in our study identified 13 genes clustered within the *B. cinerea* chromosome 1, including seven genes with oxidoreductase activity, two genes with transferase, one gene encoding a transcription factor, one gene with hydrolase activity (Supplemental Data Set 7). Spearman’ rank correlation analysis on transcript accumulation on Col-0 showed the 13 genes condensed into three clusters, which is in line with their biological functions (Figure 6A). We further investigated the expression profiles of three genes, *BcBOA6*, *BcBOA10*, and *BcBOA13*, that represented the three clusters across 96 isolates infection on Arabidopsis wild-type Col-0. All the three genes were only induced in some of the 96 isolates with varied accumulation levels (Figure 6B to 6 D and Supplemental Data Set 2). This result is further support the previous researches that the BOA production is isolate-specific (Dalmais et al., 2011).

Using analysis of variance (ANOVA), expression variation of six BOT genes significantly altered the lesion development across three Arabidopsis genotypes induced by 96 isolates, except a gene (*BcBOT5*) encoding a transferase (Supplemental Data Set 6 and 8). This finding further supported our previous analysis that BOT production in *B. cinerea* facilities the fungal infection on plants by enhancing the lesion expansion. Additionally, four BOA genes were identified significantly altered the lesion development, including three genes encoding cytochrome P450 monooxygenase and one gene encoding trichothecence 3-O-acetyltransferase (Supplemental Data Set 6 and 8) **(Malmierca et al., 2016b; Malmierca et al., 2016a).** This result indicates BOA production is also play an important role during *B. cinerea* infection but not a necessary for all isolates’ virulence. In conclusion, both BOT and BOA productions are isolate-specific and contribute *B. cinerea* virulence during plant infection.