



**Evolutionary, transcriptomic and functional analyses revealed a GST cluster played an important role in Verticillium wilt resistance in *Gossypium hirsutum***

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# Homologous identification and evolution analysis of GST genes

- A total of 123 putative GST genes were identified in *G. hirsutum*
- The colinearity analysis showed that whole-genome duplication event and tandem duplication have more contribution to tau class genes expansion than other eight class genes
- PMAL analysis showed that the selective pressure was different among branches in each class GST. Especially, group 2 undergone the positive selection acting relative to the integral tau class genes
- Circle mapping showed that the higher selection pressure for the cluster (*Gh\_A09G1508*, *Gh\_A09G1509* and *Gh\_A09G1510*) was exerted on chromosome 09 in A -subgenome than in D -subgenome

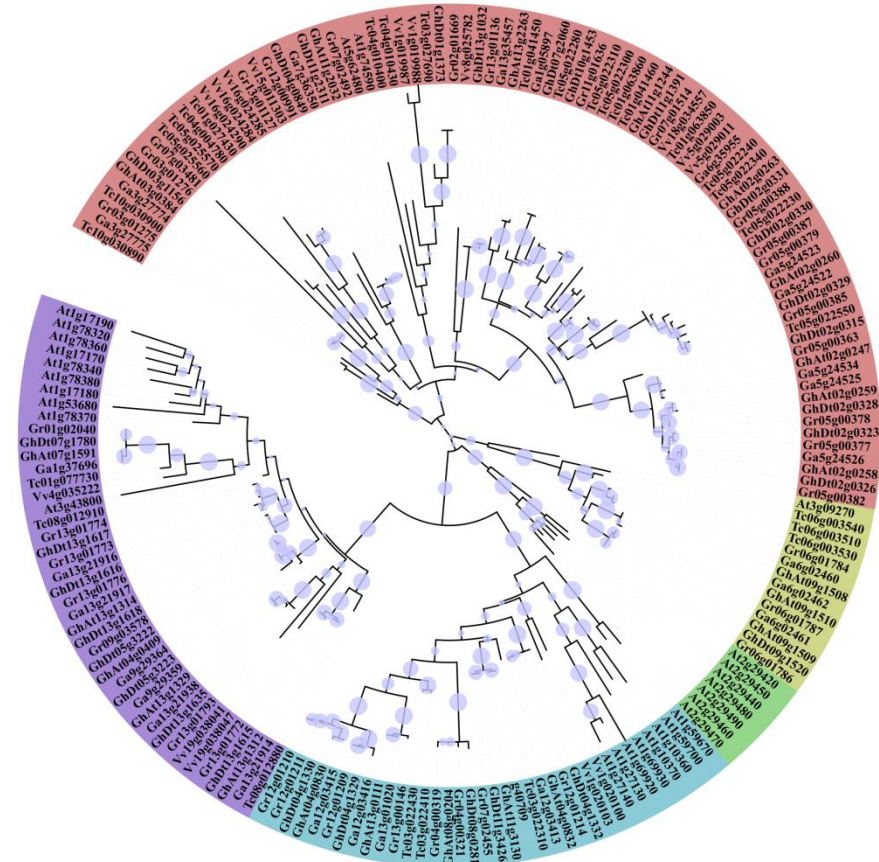


Figure 1 The phylogenetic tree of tau class genes in six species

(Unpublished data)



# Transcriptome analysis of GST genes under *V. dahliae* stress

- 17 GST genes were significantly changed more than 2.0 fold under *V. dahliae* stress in *G. hirsutum* cv. ND601 and CCRI8 and *G. barbadense* cv. Pima90-53
- The tau cluster (including *Gh\_A09G1508*, *Gh\_A09G1509* and *Gh\_A09G1510*) was up-regulated expression under *V. dahliae* stress
- The gene *Gh\_A09G1509* was notably differentially expressed in resistant variety ND601
- So, we chosen the *Gh\_A09G1509* gene as representative of the tau cluster, and operated the following assays

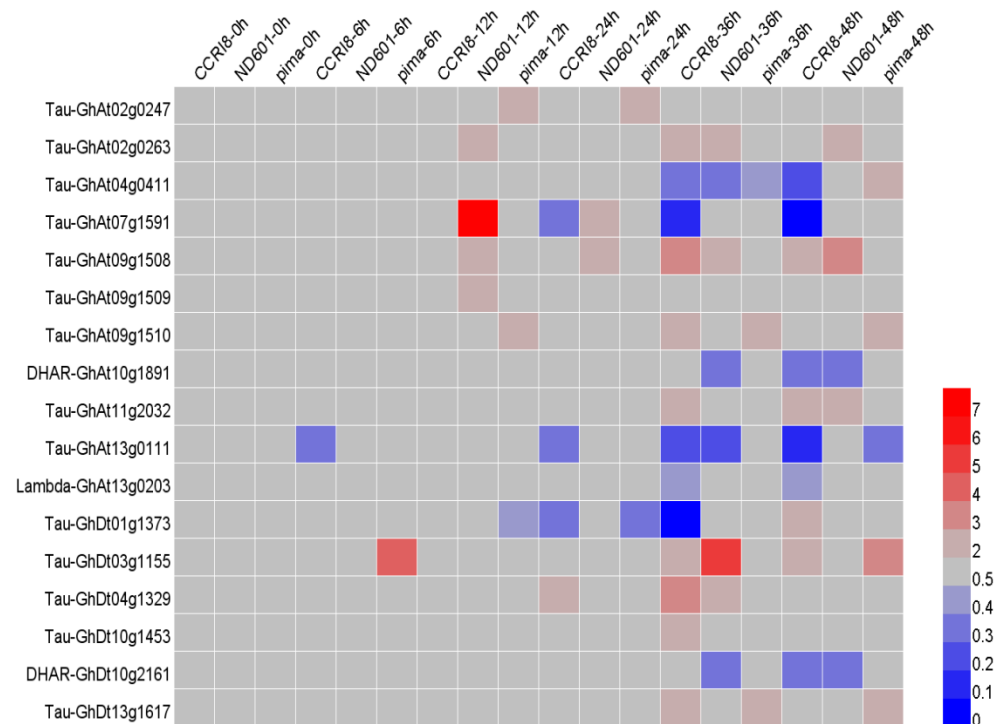


Figure 2 The heatmap of differential expression of GST genes in cotton under *V. dahliae* stress

(Unpublished data)



# Function analysis of the cluster

- The observation of the phenotype of *35S:GhGST* showed that resistance to *V. dahliae* of the transgenic tobacco was greatly enhanced (Figure 3 A and 3 C)
- Recovery assay showed that the *35S:GhGST* contained fewer *V. dahliae* (Figure 3 B)
- The real-time PCR result showed that the silence efficiencies of *GhGST*, *Gh\_A09G1508* and *Gh\_A09G1510* were 97%, 86% and 60% in VIGS seedlings
- disease grade analysis showed that the WT seedlings exhibited Verticillium wilt resistant phenotype (Figure 4 A and 4 B)
- More colonies in PDA agar medium containing the stem sections from silencing plants than that of WT plants with *V. dahliae* treatment (Figure 4D and 4E)
- Taken together, the tau GST cluster played a positive role in cotton to Verticillium wilt resistance

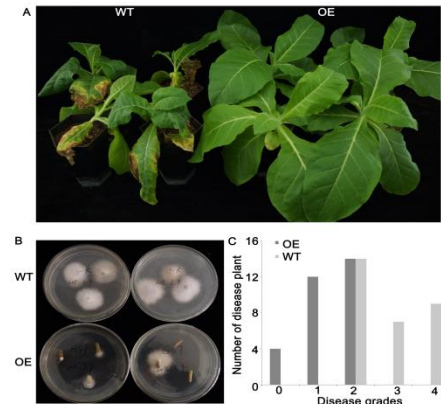


Figure 3 Identification of Verticillium wilt resistance of *35S:GhGST* transformants under *V. dahliae* stress

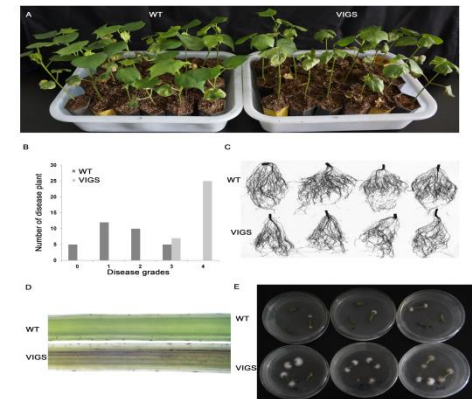


Figure 4 Identification of Verticillium wilt resistance of resistant cultivar plants under *V. dahliae* stress (Unpublished data)