Deficiencies in the formation and regulation of anther cuticle and tryphine contribute to male sterility in cotton PGMS line

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BMC Genomics BMC Series

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DOI:
10.21203/rs.3.rs-18152/v1

SUBJECT AREAS
Epigenetics & Genomics

KEYWORDS
cotton, photosensitive genetic male sterile (PGMS), exine, tryphine, anther cuticle, MYB TFs, ABA

Abstract

Background: Male sterility is a simple and efficient pollination control system that is widely exploited in hybrid breeding. In upland cotton, CCRI9106, a photosensitive genetic male sterile (PGMS) mutant isolated from CCRI040029, was reported of great advantages to cotton heterosis. However, the underlying molecular mechanism of CCRI9106 remains unclear.

Results: In this study, light and electron microscopy revealed that the male sterility phenotype of MT was mainly attributed to irregularly exine, lacking tryphine and immature anther cuticle. Based on the cytological characteristics of MT anthers, 18 RNA libraries were constructed from the anthers of MT and WT at tetrad (TTP), late uninucleate (LUNP) and binucleate (BNP) stages of anther development for transcriptomic analysis, therefore revealing a total of 870,4 differentially expressed genes (DEGs). By performing gene expression pattern analysis and protein-protein interaction (PPI) networks construction, we found down-regulation of DEGs in cluster 2, which enriched by the lipid biosynthetic process and the synthesis pathways of several types of secondary metabolites such as terpenoids, flavonoids and steroids, may crucial to the male sterility phenotype of MT, and resulting in the defects of anther cuticle and tryphine, even the irregularly exine. Furthermore, several lipid-related genes together with ABA-related genes and MYB transcription factors were identified as hub genes via weighted gene co-expression network analysis (WGCNA), such as NPC2, LTPG, LTP1, MAKR6, Ghir_D11G032630, Ghir_A01G008890, Ghir_D01G009320, MYB3, MYB7, MYB16 and MYB48. Additionally, the ABA content of MT anthers was reduced across all stage when compared with WT anthers.

Conclusions: In summary, we propose that the down-regulation of genes related to the assembly of anther cuticle and tryphine may lead to the male sterile phenotype of MT, and MYB transcription factors together with ABA play key regulatory role in these processes. These findings provide valuable information on the transcriptional level to anther and pollen development, and contribute to elucidate the mechanism of male sterility in upland cotton.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Figures

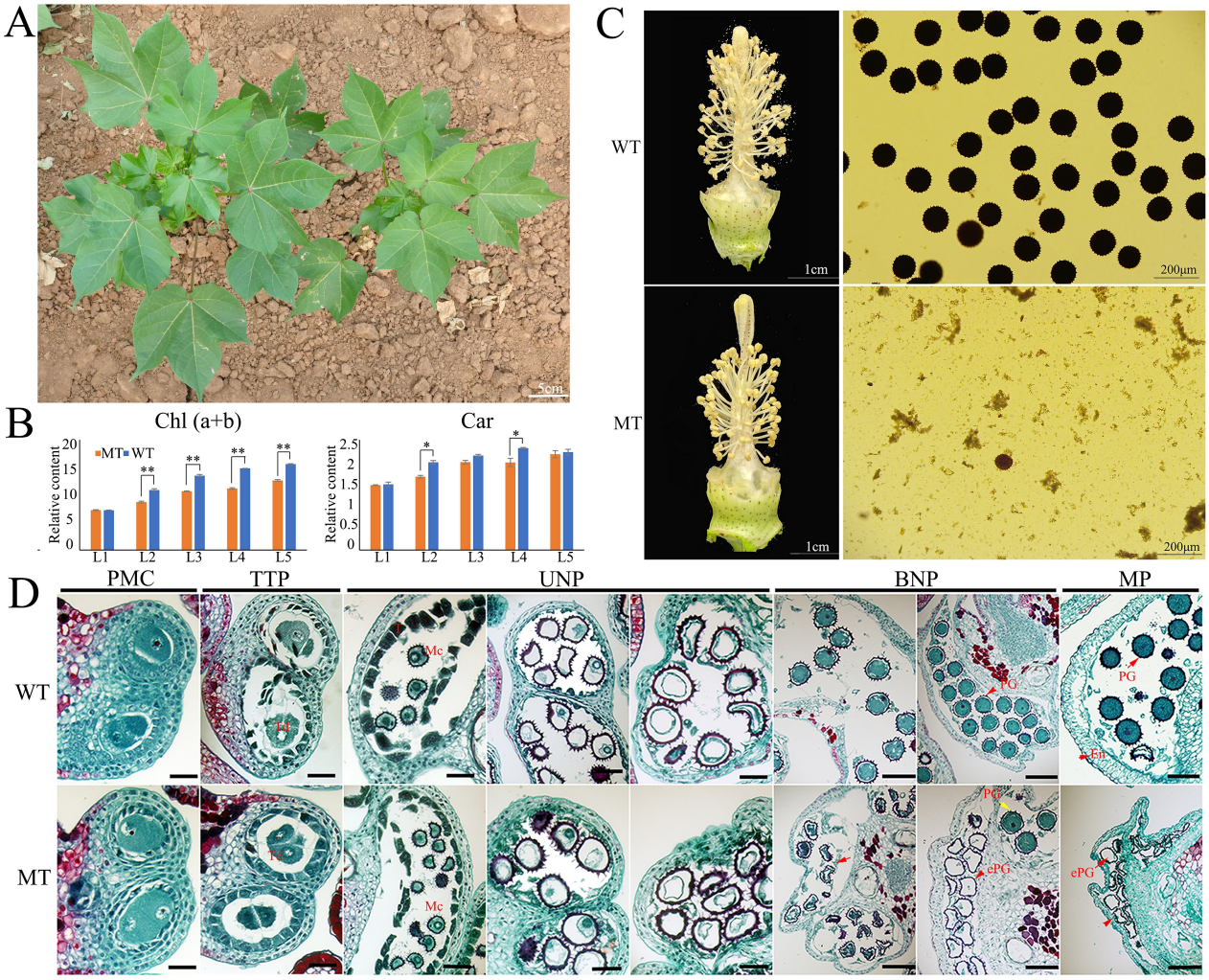

Figure 1

Morphological characteristics of the PGMS MT line CCRI9106 and its WT line CCRI040029. (A) Intact plants of MT (right) and WT (left). (B) Chl (a+b) and Car content of leaves in MT and WT (Student's t-test, *P<0.05, **P < 0.01). L1-L5, the first to the fifth leaf. (C) Flower and pollen phenotype of WT and MT. (D) Paraffin sections in virous development stages of WT and MT anthers. En, endothecium; ePG, empty pollen grain; Mc, mother cells; PG, pollen grain; Td, tetrad; T, tapetum. The red arrows point out the major differences of pollen grains between WT and MT. Bar=100 μm.

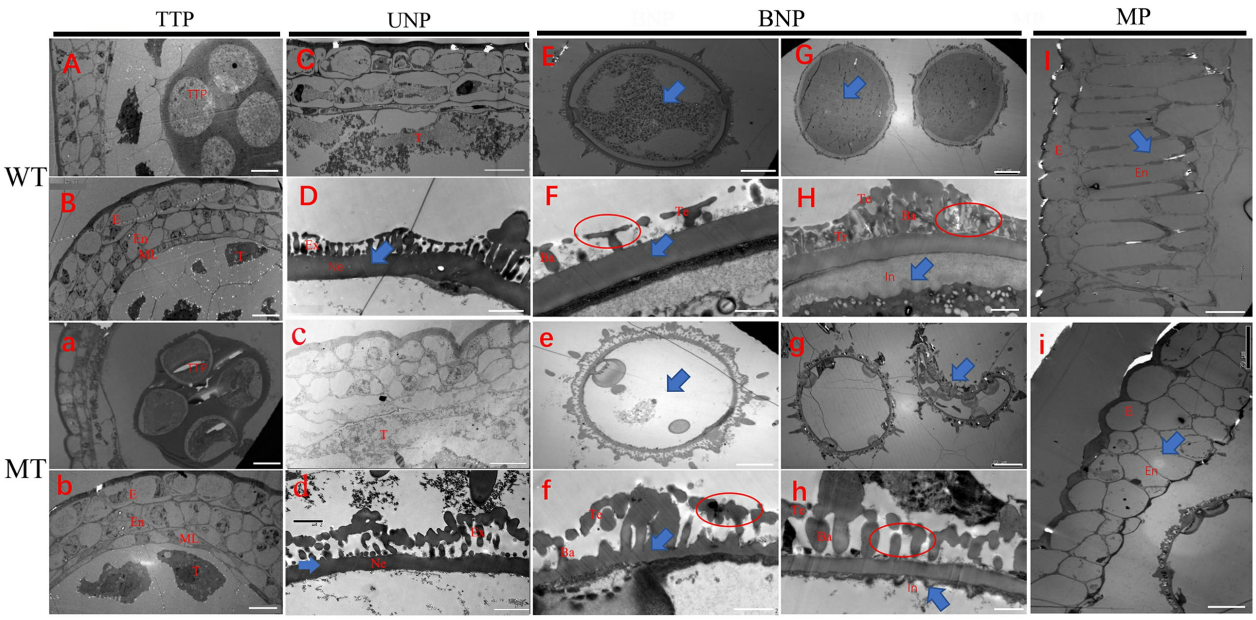

Figure 2

TEM analysis of anther development in WT (A-I) and MT (a-i) plants. Cross-sections of WT and MT anthers at the tetrad stage (A, B and a, b), uninucleate pollen stage (C, D and c, d), binucleate pollen stage (E-H and e-h) and mature pollen stage (I and i) are shown. Anther wall is showed in B, C, I (WT) and b, c, i (MT). Pollen is showed in E, G (WT) and e, g (MT). Pollen wall is showed in D, F, H (WT) and d, f, h (MT). Ba, bacula; E, epidermis; En, endothecium; Ex, exine; In, intine; ML, middle layer; Ne, nexine; T, tapetum; Te, tectum; Tr, tryphine. Bar=10μm in (A-C, and a-c), 20μm in (E, G, I, and e, g, i), 2μm in (D, F, H, and d, f, h). The ellipses and arrows point out the major differences between WT and MT in the same development stages.

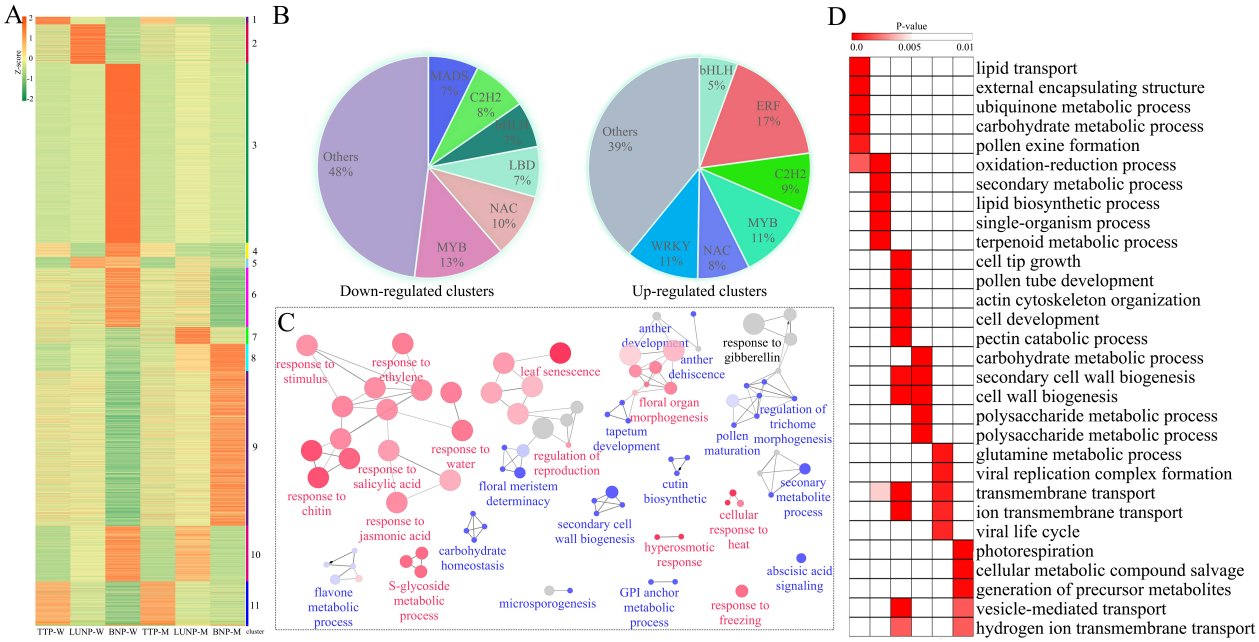

Figure 3

K-means clustering and functional analysis of clusters. (A) Eleven clusters representing 8,704 genes are shown with distinct expression patterns in different stage. Z-score of all DEGs were used to generate the heat map. TTP-W, LUNP-W, BNP-W together with TTP-M, LUNP-M, BNP-M represent different stages of WT and MT, respectively. (B) The number of genes from different TF families of down- or up-regulated clusters. (C) Significantly over-represented gene ontology (GO) terms of down- or up-regulated TFs. The size of the circles shows the significance of that node. Nodes that were specifically represented of down-regulated TFs are indicated as blue circles and as red circles for up-regulated TFs. Grey nodes were over-represented non-specifically. (D) GO functional categories enriched in cluster 1-6. Only top five terms are displayed.

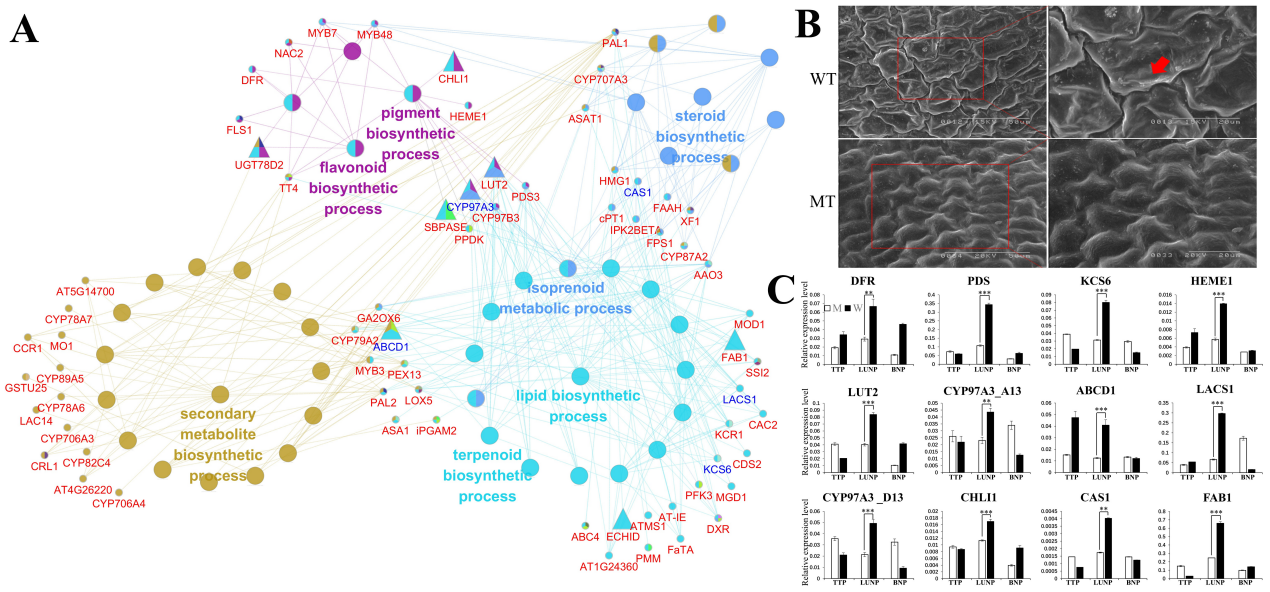

Figure 4

PPI network of cluster 2 DEGs. (A) GO modules enriched of PPI DEGs in cluster 2 visualized by the ClueGO plug-in in Cytoscape. Genes with blue font are MS genes reported in Arabidopsis. Trigonal node represents key genes in the network. (B) Appearance of the anther surface in WT and MT under scanning electron microscopy. The red arrow points out the major differences between WT and MT anther. (C) qRT-PCR expression analysis of DEGs in the network. TTSET is performed only in LUNP stage. **P<0.01, ***P<0.001.

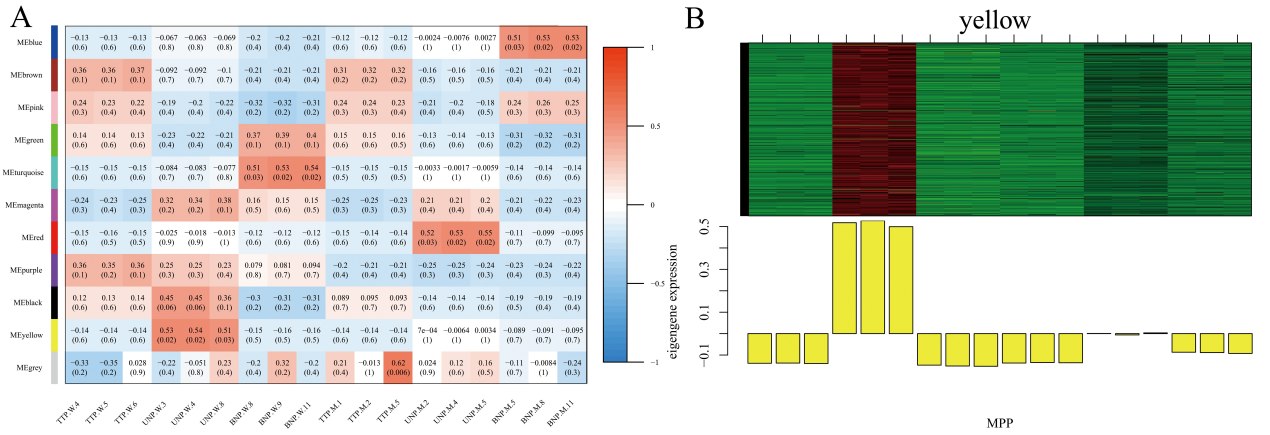

Figure 5

The co-expression modules of the valid DEGs analyzed via WGCNA. (A) The correlation coefficients between modules and samples. 10 module eigengenes are generated and named as the left lane presented. (B) The eigengene expression levels in the yellow module.

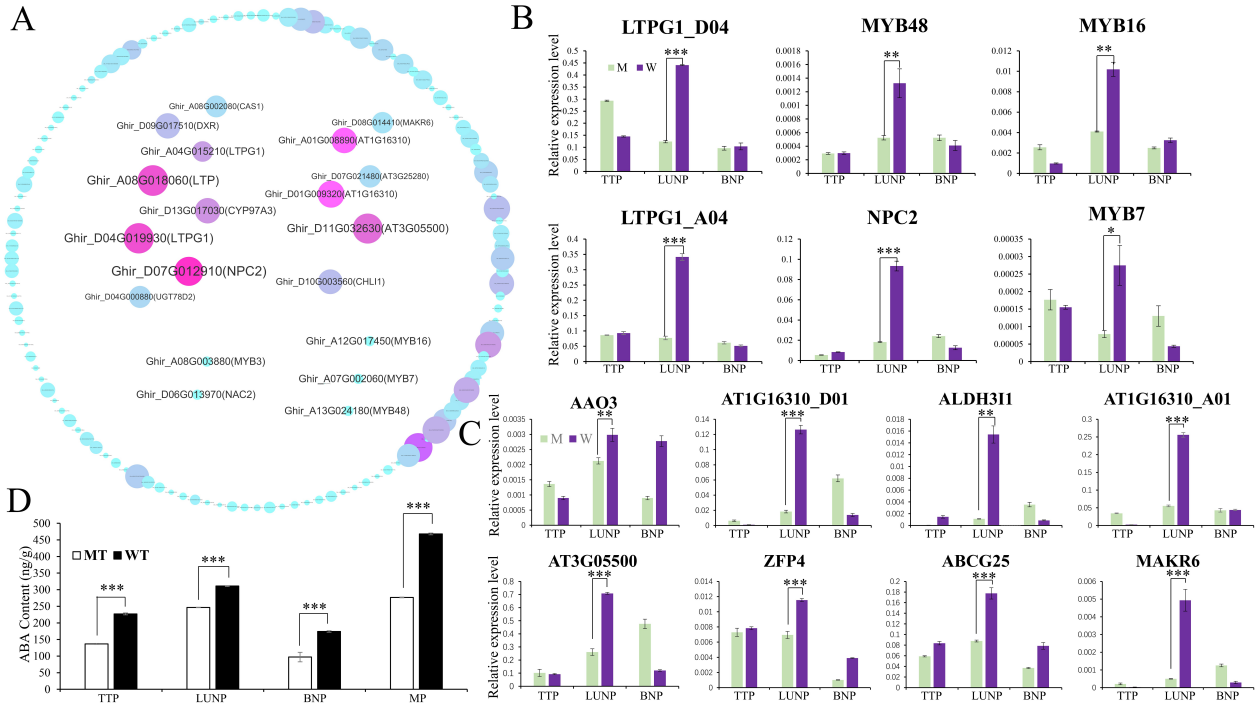

Figure 6

Information of yellow module network and DEGs expression characteristics. (A) The co-expression network of the DEGs in yellow module. Central genes are referred to as hub genes. The color gradation from turquoise to red and the increase of node size represents betweenness and degree from low to high, respectively. (B) and (C) qRT-PCR expression analysis of several hub genes or ABA-related genes in this network. TTSET is performed only in LUNP stage. *P<0.05, **P<0.01, ***P<0.001. (D) ABA content of anthers in virous development stages of MT and WT. ***P<0.001.

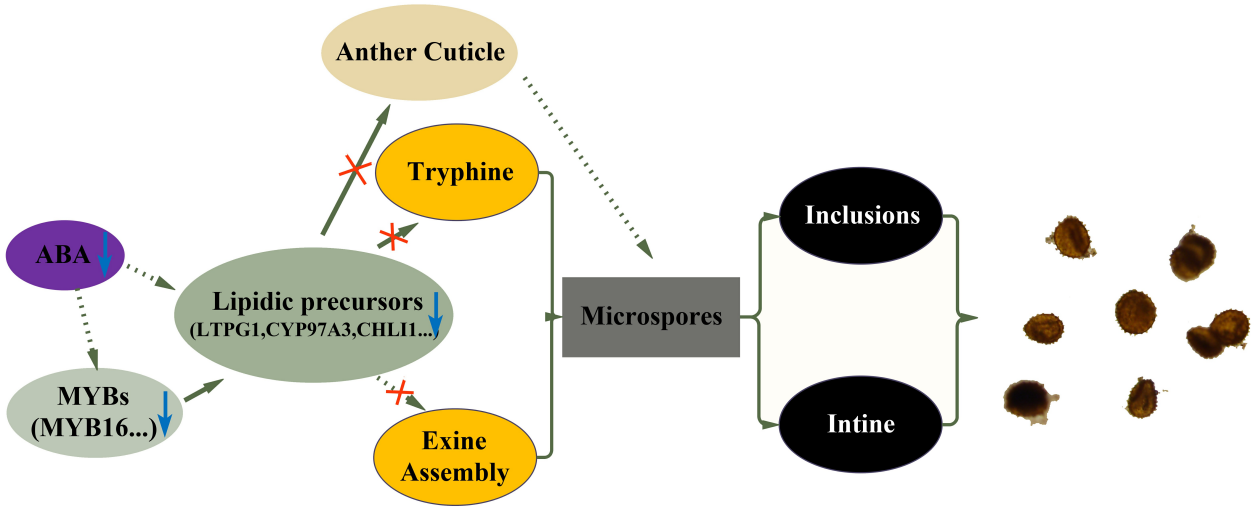

Figure 7

A possible gene regulation network in the male sterile line CCRI9106. Abnormal expression of lipidic genes disrupts the microsporogenesis and causes pollen abortion.

Supplementary Files

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