

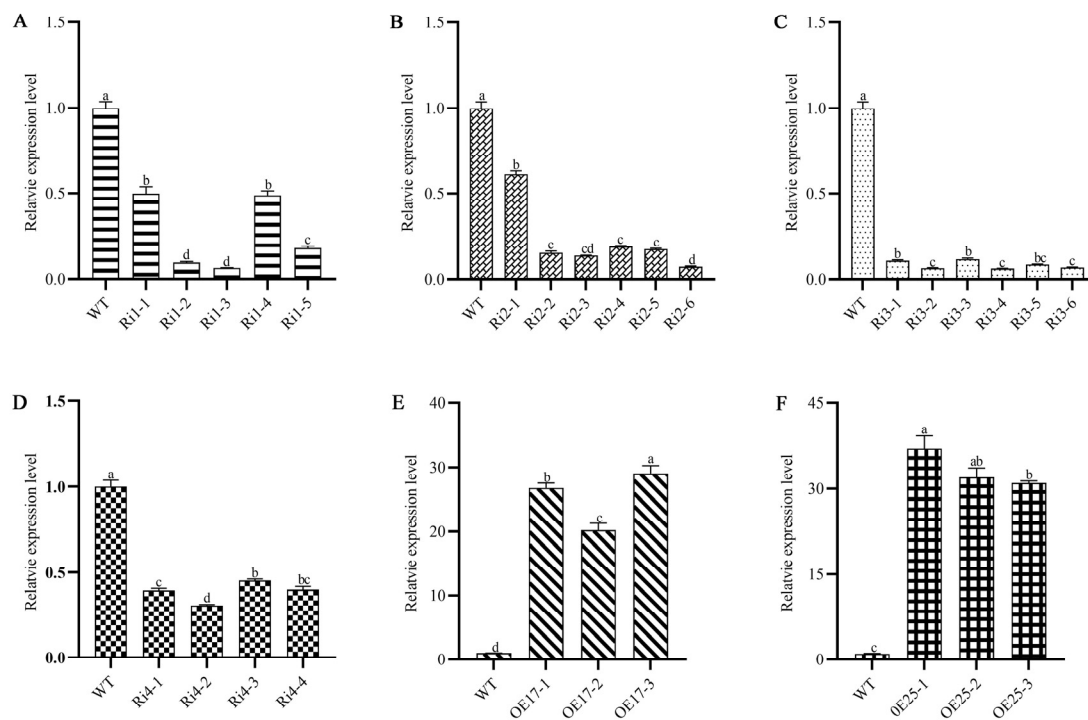
# Downregulation of the *GhROD1* gene Improves Cotton Fiber Fineness by Decreasing Acyl Pool Saturation, Stimulating Small Heat Shock Proteins (sHSPs), and Reducing H<sub>2</sub>O<sub>2</sub> Production

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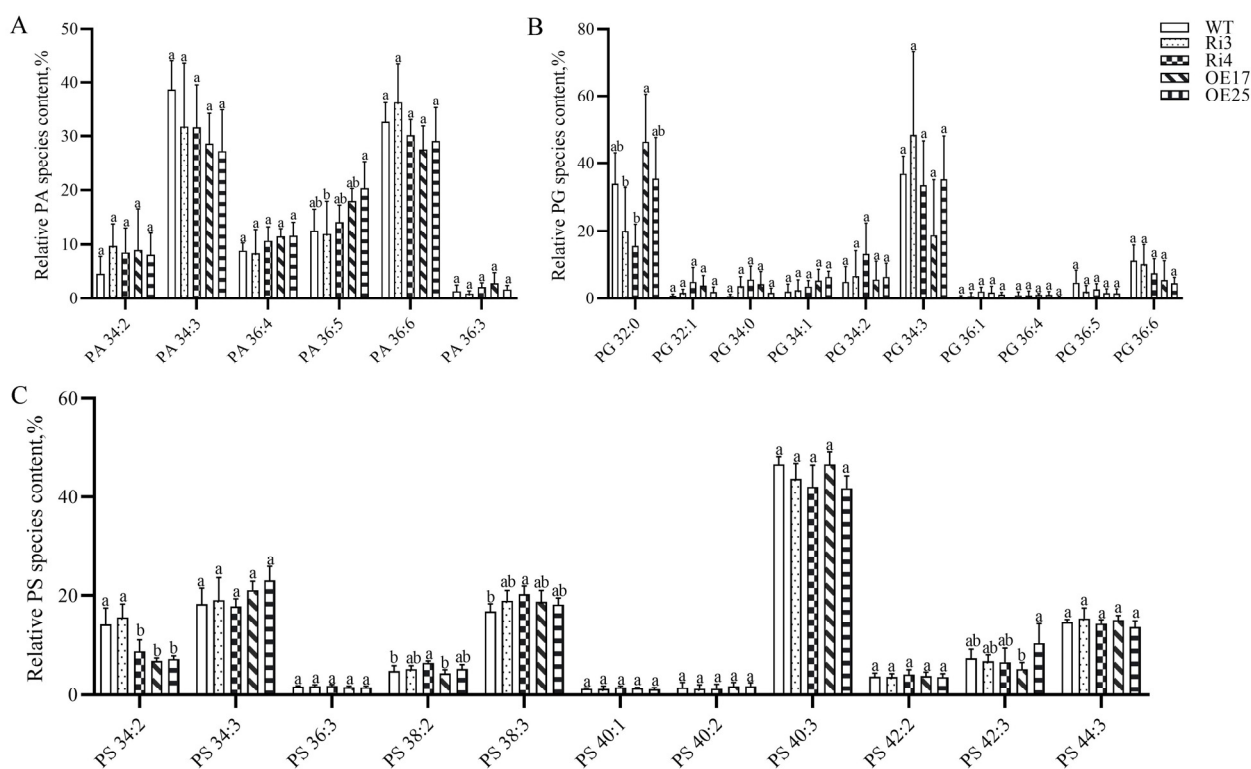
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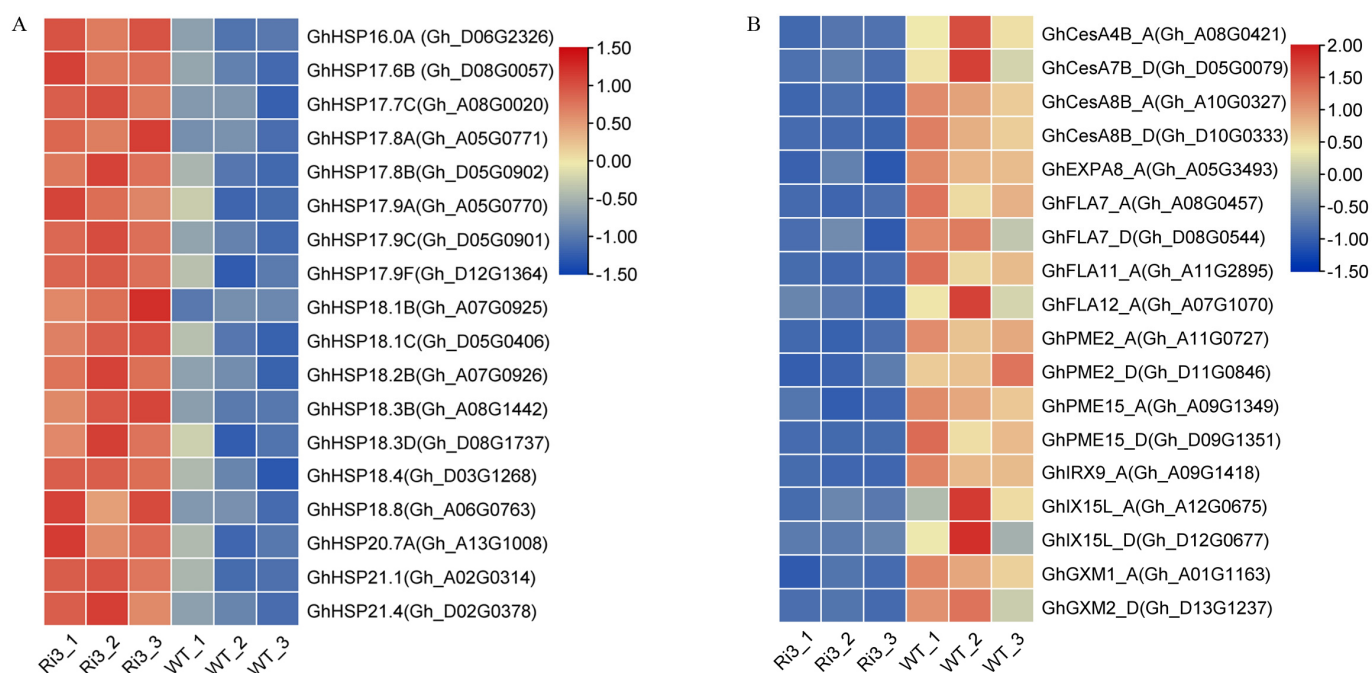
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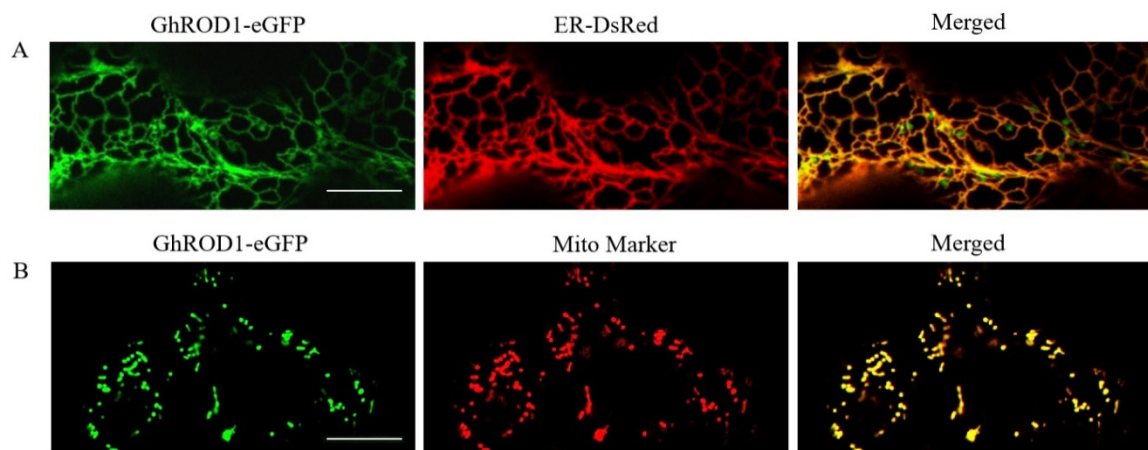
**Figure S1:** Screening of *GhROD1* transgenic plants. (A-D) Relative expression level of *GhROD1* in transgenic plants. (A) Ri1, (B) Ri2, (C) Ri3, and (D) Ri4. (E and F) Relative expression level of *GhROD1* in transgenic plants. (E) OE17 and (F) OE25. *GhHis3* served as the internal reference gene. Data are presented as mean  $\pm$  SD (n = 3). Differences between transgenic lines and wild-type were analyzed by one-way ANOVA followed by Tukey's test. Different lowercase letters indicate significant differences among groups.



**Figure S2:** Individual molecular species profiles quantified by ESI-MS/MS for PA, PG, and PS in extracts from transition stage (20 DPA) fibers. (A), (B), (C) PA, PG, PS molecular species profile. WT: wild-type (*Gossypium hirsutum* L. cv. Jimian14). Ri3 and Ri4: *GhROD1*-downregulated lines. OE17 and OE25: *GhROD1*-upregulated lines. PA: phosphatidic acid. PG: phosphatidylglycerol. PS: phosphatidylserine. Numerical designations represent the total number of acyl carbons: total number of double bonds. Data for each molecular species represent the mean and SD of five independent extractions and are presented as mol% of total glycerophospholipids. Differences between transgenic lines and wild-type were analyzed by one-way ANOVA followed by Tukey's test. Different lowercase letters indicate significant differences among groups. Components with lipid molecular species below 1% are not shown in the figure.



**Figure S3:** Heatmaps of *sHSPs* and cell wall synthesis-related genes in 16-DPA fibers of Ri3 and wild-type. (A) Heatmap of *sHSPs* expression in Ri3 and wild-type 16-DPA fibers. (B) Heatmap of cell wall synthesis-related genes expression in Ri3 and wild-type 16-DPA fibers. Note: All FPKM values are log2 transformed. Blue indicates relatively lower expression levels, while red indicates relatively higher expression levels.



**Figure S4:** Subcellular localization of GhROD1. (A) GhROD1-eGFP colocalizes with the ER marker protein. The *proGhROD1::GhROD1-eGFP* and *ER-DsRed* constructs were co-expressed in leaves of *Nicotiana benthamiana*. (B) GhROD1-eGFP colocalizes with the mitochondrial marker protein. The *proGhROD1::GhROD1-eGFP* and mitochondrial marker constructs were co-expressed in leaves of *Nicotiana benthamiana*. Scale bar = 10  $\mu$ m.