



Fig. S4 Optimization of autoactivation inhibition conditions prior to verifying protein-protein interactions between GhMYB3_A08, GhMYB5_A11, GhMYB308_A01 and GhbHLH130D, GhTT2-3A, GhMYB113. Optimization of 3-aminotriazole (3-AT) inhibition concentration for GhMYB3_A08, GhMYB5_A11, and GhMYB308_A01. In the yeast two-hybrid system, 3-AT can inhibit histidine synthesis and is used to eliminate false positives caused by autoactivation of transcription factors. The results showed that GhMYB308_A01 had no autoactivation activity, and no yeast growth was observed on the SD/-Trp-His-Ade screening medium even when 0 mM 3-AT was added. In contrast, GhMYB3_A08 and GhMYB5_A11 exhibited autoactivation, and 25 mM 3-AT was required to completely inhibit their autoactivation activity (to ensure the specificity of subsequent interaction detection). AD and BD indicate pGADT7 and pGBKT7 vectors respectively. pGBKT7 was used as negative control, pGBKT7-GhFSN1 was used as positive control.