Copper tolerance of wheat plants mediated by TaGST1 gene and it upstream regulatory factor WRKY74

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Abstract

Copper (Cu) is an important plant micronutrient; however, excessive Cu can disturb the protein structure, affect plant growth and development, and pose as a potential human health risk. Glutathione S-transferase (GST) is the key enzyme in glutathione (GSH) synthesis; thus, it plays crucial role in Cu detoxification. Nonetheless, its regulatory mechanisms remain largely unclear. In this study, we identified a Cu-induced glutathione S-transferase 1 (TaGST1) gene in wheat. Yeast one-hybrid (Y1H) between TaGST1 promoter and Cu-stressed wheat leaf cDNA library was performed and screened out TaWRKY74 transcription factor. Their bind were further verified by using another Y1H and luciferase (LUC) assays Functions of TaWRKY74 were further tested by using transiently silence and overexpression methods. Under Cu stress, TaWRKY74 and TaGST1 expression, GST activity, and GSH content were significantly inhibited in transiently TaWRKY74-silenced wheat plants. However, the contents of hydrogen peroxide (H2O2), malondialdehyde (MDA), and Cu were significantly increased in these silenced wheat plants. Further investigation found that transiently ectopic overexpression of TaWRKY74 increased GSH content, whereas decreased MDA content during Cu stress. Notably, exogenous application of GSH could reversed the adverse effects of transiently TaWRKY74-silenced wheat plants during Cu stress. Taken together, our results suggested that TaWRKY74 regulated TaGST1 expression and affected GSH accumulation under Cu stress, and could be useful to ameliorate Cu toxicity for crop food safety.

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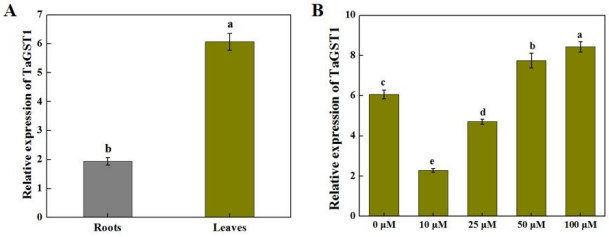

Fig. 1 Expression patterns of *TaGST1* gene in wheat. A, The expression levels of *TaGST1* were analyzed by using qPCR in roots and leaves in wheat plants. B, The expression levels of *TaGST1* were analyzed in wheat leaves under Cu stress. Two-week-old wheat seedlings were treated with different concentrations of Cu (0-100 μM) for 3 days. *Glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) was used as the internal control. Data present means ± SE (n = 3). Different letters represent significant difference at $P < 0.05$.

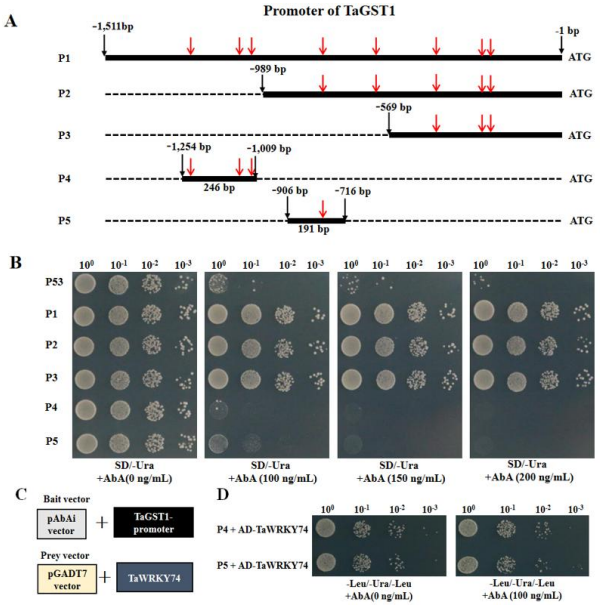

Fig. 2 Y1H assays between TaWRKY74 and *TaGST1* promoter. A, The different fragments of *TaGST1* promoter. Red arrows represent the TaWRKY74 *cis*-acting element [(T/C)TGAC(T/C)], which contains P1-P5 (P1, 1,511 bp; P2, 989 bp; P3, 569 bp; P4, 246 bp; P5, 191 bp) in the upstream regions from *TaGST1* start codon (ATG), respectively. B, Self-activation assay of *TaGST1* promoter. The five different fragments P1 (-1,511 bp ~ -1 bp), P2 (-989 bp ~ -1 bp), P3 (-569 bp ~ -1 bp), P4 (-1,009 bp ~ -1,254 bp), and P5 (-716 bp ~ -906 bp) of *TaGST1* promoter region, which were divided according to W-boxes in Fig.2A, were separately transformed into yeast cells and grown on media selecting for plasmids (SD/-Ura), supplemented with 100, 150, and 200 ng/mL AbA, for 3 days at 30 °C, respectively. C, The TaWRKY74 prey vector and *TaGST1* promoter bait vector. D, Y1H assay of TaWRKY74 and *TaGST1* promoter. The two promoter fragments P4 (-1,009 bp ~ -1,254 bp) and P5 (-716 bp ~ -906 bp) of TaGST1 promoter region, whose self-activations have been inhibited by AbA in Fig. 2B, were separately combined with TaWRKY74 via Y1H Gold. Yeast cells were grown in liquid media selecting for plasmids (SD/-Ura/-Leu) to an OD_{600} of 0.1 (10-fold) and diluted in a 10 × dilution series (from 10^0 to 10^{-2}). From each dilution 5 µL was spotted on media selecting for plasmids (SD/-Ura/-Leu), supplemented with 100 ng/mL AbA.

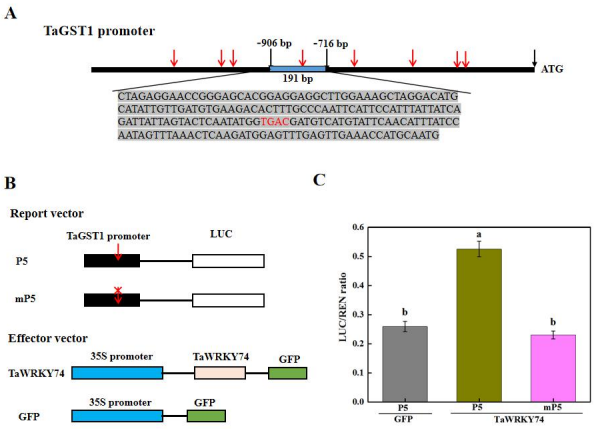

Fig. 3 Transient assay analysis of the interaction between TaWRKY74 and the *TaGST1* promoter. A, Sequences of *TaGST1* promoter. B, Vector constructions of transient expression assay. The normal and mutant W-boxes of P5 (-716 bp ~ -906 bp) fragments in *TaGST1* promoter were inserted into the reporter vector pGreenII0800-LUC, which carries both LUC and REN ORFs (Hellens et al., 2005), and TaWRKY74 was cloned into the effector vector (pCambia1300); LUC, Firefly luciferase activity; REN, Renilla luciferase activity (control). Red arrows and cross, which were labelled in the promoter of *TaGST1*, represent W-box elements or mutant W-box element, respectively. C, Ratios of LUC to REN in normal or mutant W-box in P5 fragments, respectively. Data are shown as means ± SD of three biological replicates. Different letters represent significant difference at $P < 0.05$.

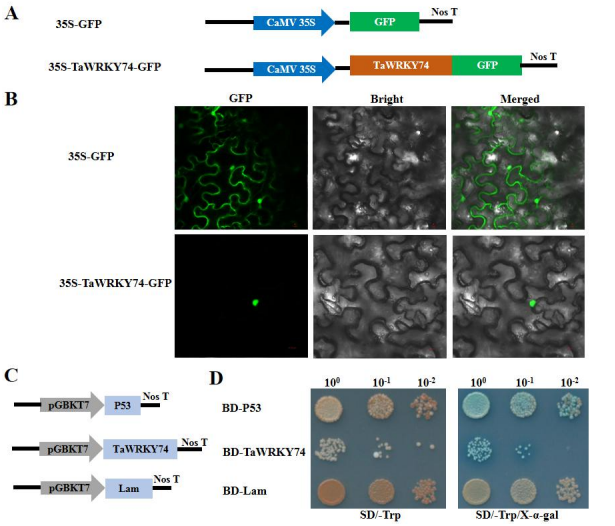

Fig. 4 Subcellular localization and transcriptional activity of TaWRKY74. A, Vector construction of 35S-TaWRKY74-GFP fusion protein; 35S-GFP as the control. B, Subcellular localization of TaWRKY74. 35S-GFP and 35S-TaWRKY74-GFP fusion proteins is determined in infiltrated tobacco leaves. Bar = 20 μm. C, Vector construction of pGBKT7-TaWRKY74 fusion protein; BD-P53 and BD-Lam are used as the positive and negative controls, respectively. D, Transcriptional activity of TaWRKY74 by Y2HGold. Yeast cells carrying different fusion proteins are grown on media selecting for plasmids (SD/-Trp), supplemented with X-α-gal, for 3 days at 30 °C, respectively.

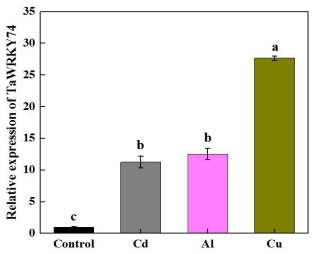

Fig. 5 Expression patterns of *TaWRKY74* under Cd, Al, and Cu stresses. Two-week-old wheat seedlings were treated with Cd, Al, or Cu stress for 3 d. And the *GAPDH* gene has been used for the internal control gene. Data present means ± SE (n = 3). Different letters represent significant difference at $P < 0.05$.

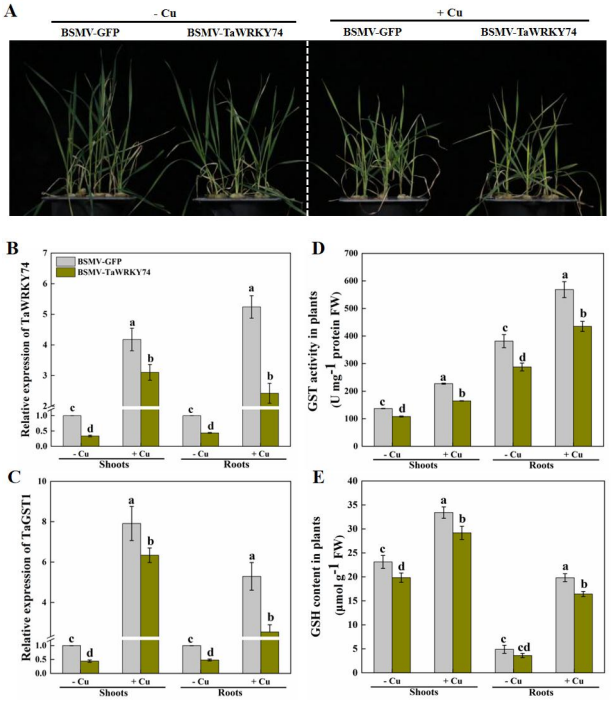

Fig. 6 Phenotype of BSMV-TaWRKY74-inoculated wheat plants and their GSH synthesis under Cu stress. A, Phenotype of BSMV-GFP- and BSMV-TaWRKY74-inoculated wheat plants suffering from Cu stress; B and C, Relative expression levels of TaWRKY74 and TaGST1 in wheat plants; D, Activity of GST; E, Content of GSH. BSMV-GFP- and BSMV-TaWRKY74-inoculated wheat plants grown in Hoagland solution are exposed to 100 μM or 0 Cu for 10 d, and their shoots and roots were used to measure the above indexes, respectively. Data are given as means ± SD of three biological replicates. Different letters represent significant difference at $P < 0.05$.

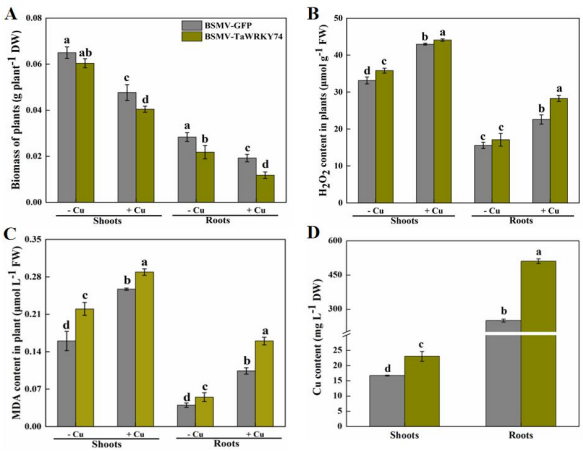

Fig. 7 Characteristics of BSMV-TaWRKY74-inoculated wheat plants suffering from Cu stress. A, The biomass of wheat plants in inoculated wheat plants after Cu stress. B and C, The contents of H_2O_2 and MDA in BSMV-TaWRKY74-inoculated wheat plants. D, The content of Cu in shoots and roots of BSMV-TaWRKY74-inoculated wheat plants after Cu stress. BSMV-GFP- and BSMV-TaWRKY74-inoculated wheat plants were exposed to Hoagland solution with or without 100 μM Cu for 10 d. Data are given as means ± SD of three biological replicates. Different letters represent significant difference at $P < 0.05$.

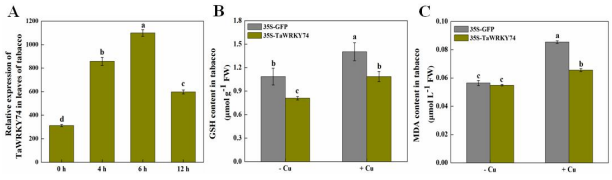

Fig. 8 Expression of *TaWRKY74* and contents of MDA and GSH in leaves of its transient-overexpression tobacco plants suffering from Cu stress. A, Expression of TaWRKY74 in leaves of 35S-TaWRKY74 vector-infected tobacco plants suffering from 50 μM Cu stress for 4 h, 6 h, and 12 h. B and C, Contents of GSH and MDA in leaves of 35S-TaWRKY74 vector-infected tobacco plants suffering from Cu stress for 6 h. Data are given as means ± SD of three biological replicates. Different letters represent significant difference at $P < 0.05$.

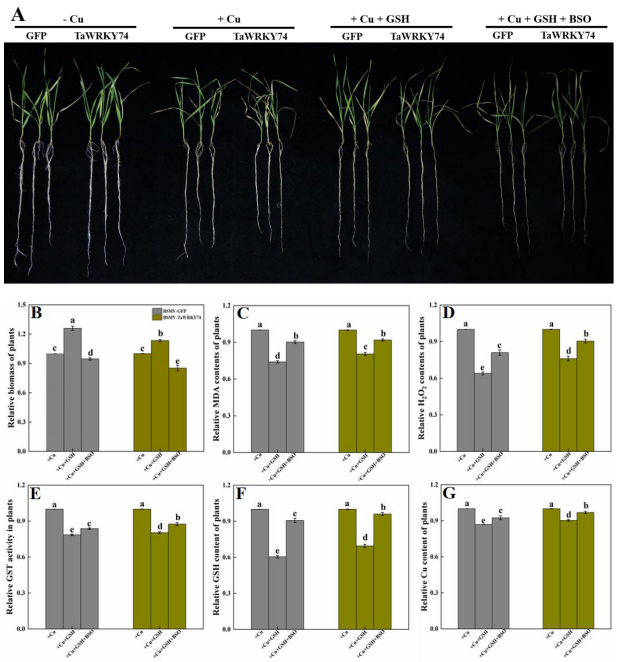

Fig. 9 Effect of Cu and GSH supplementation on the growth indexes of BSMV-TaWRKY74-inoculated wheat plants. A, Phenotype of BSMV-GFP- and BSMV-TaWRKY74-inoculated wheat plants under -Cu, +Cu, +Cu+GSH, and +Cu+GSH+BSO stresses for 10 days; B-G, Relative change of biomass, MDA content, H_2O_2 content, GST activity, GSH contents, and Cu content under these two inoculated wheat plants. The relative values of +Cu+GSH and +Cu+GSH+BSO were calculated compare with +Cu treatment, respectively. Data are given as means ± SD of three biological replicates. Different letters represent significant difference at $P < 0.05$.

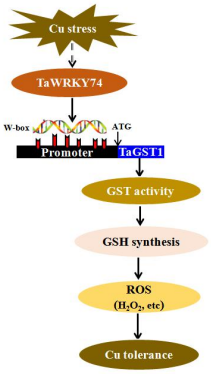

Fig. 10 Model of TaWRKY74 participates Cu tolerance in wheat plant. Cu stress induced the expression of TaWRKY74, and then TaWRKY74 positively regulated the expression of *TaGST1* by binding to W-boxes of *TaGST1* promoter and improved GSH synthesis, resulting in the decreased ROS accumulation and enhanced Cu tolerance.