Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2022) **20**, pp. 1645–1647 doi: 10.1111/pbi.13881

Brief Communication

Histone acetyltransferase TaHAG1 interacts with TaNACL to promote heat stress tolerance in wheat

Jingchen Lin†, Na Song†, Debiao Liu, Xingbei Liu, Wei Chu, Jinpeng Li, Shumin Chang, Zehui Liu, Yongming Chen, Qun Yang, Xiaoyu Liu, Yingyin Yao, Weilong Guo, Mingming Xin, Huiru Peng, Zhongfu Ni, Qixin Sun and Zhaorong Hu*

Frontiers Science Center for Molecular Design Breeding, State Key Laboratory for Agrobiotechnology, Key Laboratory of Crop Heterosis and Utilization (MOE), Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, Beijing, China

Received 11 April 2022;
revised 11 May 2022;
accepted 18 June 2022.
**Correspondence* (Tel +86-10-62733044; fax +86-10-62733044; email zrhu@cau.edu.cn)
†These authors contributed equally to this work.

Keywords: wheat, heat stress, genetic improvement, function analysis, regulatory mechanism.

Heat stress (HS) is becoming a major and constant threat to crop production and food security as global warming progresses. Consequently, understanding and improving crop tolerance to HS are currently among the most important targets in plant biology and breeding research (Langridge and Reynolds, 2021). Recent evidences suggest epigenetic mechanisms act as new layer of regulation to cope with HS (Ohama *et al*., 2017; Song *et al*., 2021). However, the specific regulatory module composed of epigenetic factor and transcription factor in establishing thermotolerance remains unclear. As a typical cool-season crop, wheat is vulnerable to HS, especially at the flowering and grain-filling stages (Kaur *et al*., 2019). Here, we report that TaHAG1 plays a pivotal role in thermotolerance by maintaining the photosynthetic stability in wheat.

TaHAG1 encodes a histone acetyltransferase, orthologous with *Arabidopsis* AtHAG1/GCN5 and rice OsHAG702 (Zheng *et al*., 2021). There are three *TaHAG1* homoeologs in hexaploid wheat, whose deduced proteins contain conserved N-terminal HAT domain and C-terminal bromodomain (Figure 1a). TaHAG1 localizes in the nucleus (Figure 1b). Transcript of three TaHAG1 homoeologs was induced quickly under HS and then gradually increased in a similar pattern with the time of stress prolonging (Figure 1c).

To explore the function of *TaHAG1* in the regulation of thermotolerance, we obtained transgenic wheat plants that either had *TaHAG1* overexpressed (OE) or silenced via RNA interference (RNAi). The OE and RNAi plants showed similar phenotypes with wild-type Fielder under normal conditions (Figure 1d). Upon HS treatments, both Fielder and transgenic lines displayed a certain degree of wilting and growth inhibition. However, after recovery, the OE lines presented an obvious increase in fresh weight than Fielder, whereas the RNAi lines showed a pronounced wilting phenotype than Fielder and could not recover growth (Figure 1d,e). In addition, the OE lines kept more chlorophyll content and lower membrane damage than wild-types and RNAi lines after HS (Figure 1f,g).

To further validate the function of *TaHAG1* in thermotolerance, the knockout lines of *TaHAG1* were generated using CRISPR/Cas9 system. The mutation simultaneously in three homoeologs of *TaHAG1* was lethal for wheat. Thus, the lines with simultaneous mutations at the two homoeologs *TaHAG1-A* and *TaHAG1-B* (1 bp insertion in *TaHAG1-A* and 25 bp deletion in *TaHAG1-B*, respectively) were selected for analysis. As expected, the KO lines exhibited stronger defects in thermotolerance as compared with Fielder plants under HS condition, including reduced fresh weight, more wilted leaves, lower chlorophyll content and severe membrane damage (Figure 1h–j).

We further examined the thermotolerance of transgenic lines with wild-types under field conditions. The plants were covered with manually constructed thermo-stress tents in grain-filling stage for 30 days, with uncovered individuals grown alongside as controls (Figure 1k,l). Under HS conditions, all OE lines exhibited much better fitness than the Fielder, including higher thousand kernel weight and grain width. In contrast, RNAi and KO lines exhibited severe inhibition compared with Fielder in term of these traits (Figure 1m,n). Meanwhile, no significant differences were found in agronomic phenotypes between OE, RNAi, KO and Fielder under control conditions, suggesting TaHAG1 contributes to thermotolerance without negative consequences for other developmental traits.

To explore the molecular basis of TaHAG1 in the regulation of thermotolerance, we performed RNA-sequencing in seedlings of Fielder and *TaHAG1*-OE after HS treatment. We reasoned that TaHAG1-regulated genes involved in thermotolerance would be enriched in the clusters where their expressions were up-regulated in OE lines compared with wild-type and induced by HS. Based on this, 663 genes were identified and considered as TaHAG1-regulated genes in response to HS. The most significantly enriched classes of these genes were those responsible for nucleosome organization, which was consistent with the role of TaHAG1 in histone modification (Figure 1o). Moreover, cellular component of plasma membrane and thylakoid was greatly enriched, indicating the membrane and photosynthetic system of *TaHAG1*-OE might be better adapted to HS treatment, which was further supported by lower electrolytic leakage and higher chlorophyll content in *TaHAG1*-OE plants. Notably, a series of typical genes reported to be involved in the regulation of photosynthetic apparatus were detected, such as *TaG1* and *TaPSBR1* that involved in stable assembly of PSII were up-regulated significantly in *TaHAG1*-OE lines than wild-type plants under HS (Figure 1p). These results are consistent with our observation that TaHAG1 enhanced maximal PSII quantum efficiency (Fv/Fm) in *TaHAG1*-OE lines under HS conditions

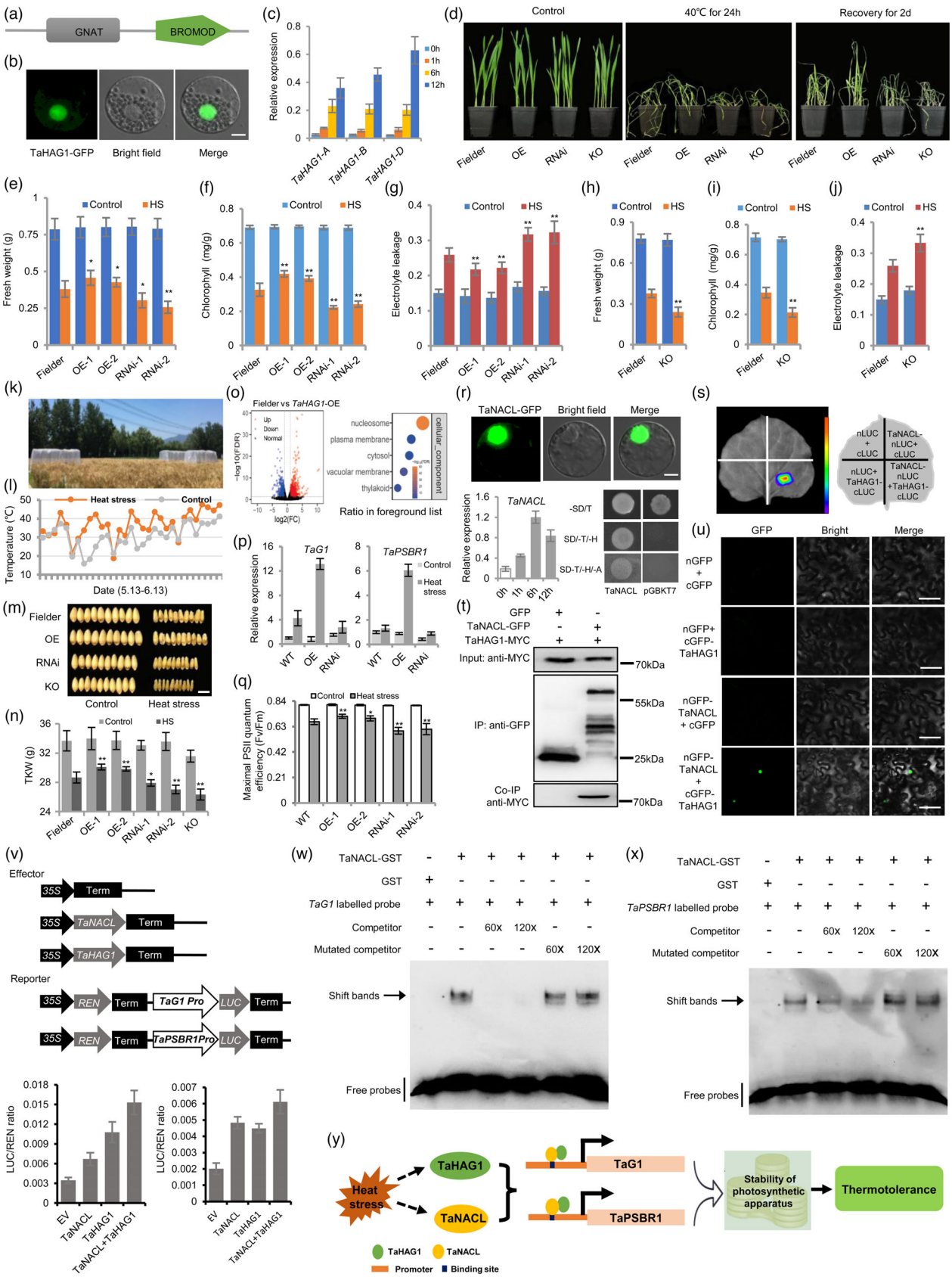

Figure 1 Functional characterization of TaHAG1 on wheat thermotolerance. (a) Schematic diagram showing TaHAG1 conserved domain. (b) Subcellular localization of TaHAG1 in wheat leaf protoplasts. Scale bar, 20 μm. (c) *TaHAG1* homoeologs expression in leaves after HS at the seedling stage. (d) The thermotolerance of different *TaHAG1* transgenic lines under HS treatment. (e–j) The aboveground fresh weight (e and h), chlorophyll content (f and i) and electrolyte leakage (g and j) of seedlings under normal and HS conditions. (k and l) The heat treatment facility (k) and daily temperature in the fields and sheds (l). (m–n) The kernel phenotypes and TKW of Fielder and *TaHAG1* transgenic plants under normal and HS conditions. Bars, 5 mm. (o) DEGs between Fielder and *TaHAG1*-OE plants at 6 h after HS treatment, as shown by volcano plots; and GO enrichment of TaHAG1-regulated genes in response to HS. (p) *TaG1* and *TaPSBR1* expression pattern in leaves of different lines after HS. (q) The maximal PSII quantum efficiency of flag leaves in different *TaHAG1* transgenic lines under normal and HS condition. (r) Subcellular localization, expression and transcriptional activity of TaNACL. Bars, 20 μm. (s–u) LCI (s), Co-IP (t) and BiFC (u) assays confirming the TaHAG1–TaNACL interaction. Bars, 50 μm. (v) The ability of TaNACL and TaHAG1 to transactivate *TaG1* and *TaPSBR1* promoter expression. (w,x) EMSA analysis of TaNACL binding to *TaG1* and *TaPSBR1* promoters. (y) Schematic model. Asterisks indicate significant differences between *TaHAG1* transgenic lines with Fielder plants under the same condition (*$P < 0.05$, **$P < 0.01$ by two-sided *t*-test).

(Figure 1q). Moreover, this suggested that elevated Fv/Fm may be part of the thermotolerance mechanism mediated by *TaHAG1* overexpression.

As coactivators, the TaHAG1 is likely to be recruited to target promoters by direct or indirect interaction with DNA-binding regulators. To further explore regulatory mechanisms of TaHAG1, we performed yeast two-hybrid screening and identified one of TaHAG1 interactors as TaNACL, a NAC domain-containing protein. *TaNACL* is up-regulated after HS and encodes a nuclear protein with transcriptional activation activity (Figure 1r). We then corroborated the TaHAG1–TaNACL interaction using luciferase complementation assays, where coexpression of TaHAG1 with TaNACL generated strong luminescence signals that were not detected in the control pairs (Figure 1s). We also confirmed their interaction using Co-IP and BiFC assays (Figure 1t,u). The above findings that TaHAG1 facilitates *TaG1* and *TaPSBR1* expressions after HS, together with the interaction of TaHAG1 and TaNACL, promoted us to investigate whether *TaG1* and *TaPSBR1* are direct target of TaNACL. Transient transactivation assay showed that TaNACL was able to activate the expression of *TaG1* and *TaPSBR1* promoter-driven luciferase (LUC) reporters. Moreover, coexpression TaHAG1 with TaNACL led to a significant increase in *TaG1* and *TaPSBR1* promoter activation compared with the expression of each single effector (Figure 1v). We also conducted electrophoretic mobility shift assays to confirm whether TaNACL directly binds to *TaG1* and *TaPSBR1* regulatory regions using recombinant protein TaNACL-GST. It was shown that TaNACL physically bound to the biotin-labelled *TaG1* and *TaPSBR1* promoters in a CACG motif-dependent manner, and the binding was competed by unlabelled wild-type probes but not mutated probes (Figure 1w,x).

Together, our results demonstrate that TaHAG1 regulates the transcription of *TaG1* and *TaPSBR1* through interacting with TaNACL to enhance thermotolerance in wheat (Figure 1y). This study provides a potential approach to improve wheat thermotolerance by increasing *TaHAG1* expression, without observable penalty on plant growth. The regulatory factors involved in thermotolerance identified in this study could also be of great value for genetic improvement in wheat and in other crops.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (32130078, 32072001) and Frontiers Science Center for Molecular Design Breeding (2022TC145) and the 2115 Talent Development Program of China Agricultural University.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

J.C.L., N.S., D.B.L., X.B.L., W.C., J.P.L., S.M.C., Z.H.L., Y.M.C., Q.Y. and X.Y.L performed the experiments; Y.Y.Y., W.L.G., M.M.X., H.R.P. and Z.F.N. contributed to materials; Z.R.H. and Q.X.S. conceived the research; Z.R.H. analysed the data and drafted the manuscript.

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