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). 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. 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 agronomic 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 electrolyte 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 PSI. Moreover, these results are consistent with our observation that TaHAG1 enhanced maximal PSII quantum efficiency (Fv/Fm) in TaHAG1-OE lines under HS conditions.

**Keywords:** wheat, heat stress, genetic improvement, function analysis, regulatory mechanism.
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 homeolog 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–r) 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, 50 μm. (s) The ability of TaNACL and TaHAG1 to transactivate TaG1 and TaPSBR1 promoter expression. (t–u) EMSA analysis of TaNACL binding to TaG1 and TaPSBR1 promoters. (v) 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).

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 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.

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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. and Q.X.S. conceived the research; Z.R.H. analysed the data and drafted the manuscript.

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