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ZmSCE1a positively regulates drought tolerance by enhancing the stability of ZmGCN5

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SUMMARY

Drought stress impairs plant growth and poses a serious threat to maize (*Zea mays*) production and yield. Nevertheless, the elucidation of the molecular basis of drought resistance in maize is still uncertain. In this study, we identified ZmSCE1a, a SUMO E2-conjugating enzyme, as a positive regulator of drought tolerance in maize. Molecular and biochemical assays indicated that E3 SUMO ligase ZmMMS21 acts together with ZmSCE1a to SUMOylate histone acetyltransferase complexes (ZmGCN5-ZmADA2b). SUMOylation of ZmGCN5 enhances its stability through the 26S proteasome pathway. Furthermore, *ZmGCN5*-overexpressing plants showed drought tolerance performance. It alleviated O_2^- accumulation, malondialdehyde content, and ion permeability. What's more, the transcripts of stress-responsive genes and abscisic acid (ABA)-dependent genes were also significantly upregulated in *ZmGCN5*-overexpressing plants under drought stress. Overexpression of *ZmGCN5* enhanced drought-induced ABA production in seedlings. Taken together, our results indicate that ZmSCE1a enhances the stability of ZmGCN5, thereby alleviating drought-induced oxidative damage and enhancing drought stress response in maize.

Keywords: maize, *ZmGCN5*, *ZmSCE1a*, SUMOylation, drought tolerance.

INTRODUCTION

Due to global climate change, drought severely affects crop production and yields worldwide. It is reported that global scale losses in crop yield due to drought stress estimated 1820 million Mg over the past four decades (Lesk et al., 2016). Maize (*Zea mays* L.) is a major crop widely cultivated worldwide, serving as a major resource for food, livestock feed, and fuel (Dietz et al., 2021; Tian et al., 2023). However, maize is extremely sensitive to water scarcity. Drought stress severely curtails seedling development, photosynthesis, root growth, and grain filling, leading to widespread yield reduction in maize (Gupta et al., 2020; Singh et al., 2023). Previous research has shown that drought was responsible for a reduction at approximately 40% in the yield of maize and only 20% in wheat (*Triticum aestivum* L.) yield between 1980 and 2015 (Daryanto et al., 2016). A recent analysis evidence suggests that extreme drought has reduced maize yield by 37% in the United States (Li, Guan, et al., 2019). Thus, it is imperative to elucidate the response mechanism of maize to adapt to drought stress.

Histone acetylation is a reversible process of transcription catalyzed by histone acetyltransferases (HATs) (Kumar et al., 2021). As a major histone acetyltransferase, GCN5 is not only involved in regulating plant growth and development (Cohen et al., 2009; Kim et al., 2018; Kotak et al., 2018; Zhou et al., 2017), but also participated in modulating abiotic environmental stimuli in different species. For instance, the *gcn5* loss-of-function mutants showed sensitivity to salt stress due to the disruption of cell wall integrity in Arabidopsis (*Arabidopsis thaliana*) (Zheng et al., 2019). Moreover, TaHAG1 (TaGCN5) serves as a critical controller in enhancing salt tolerance by modulating reactive oxygen species (ROS) homeostasis and signal specificity in wheat (Zheng et al., 2021). In *Populus trichocarpa*, AREB1-ADA2b-GCN5 complexes are recruited to the ABRE motifs in promoters of *PtrNAC* genes to elevate their H3K9ac levels and expression activity, thus enhancing drought tolerance (Li, Lin, et al., 2019). The process of GCN5-mediated histone acetylation is essential for responding to phosphate starvation response via At4–miR399–PHO2 pathway in Arabidopsis (Wang, Xing, et al., 2019). GCN5 plays a crucial role in

the preservation of thermotolerance by regulating the transcription of *HSFA3* and *UVH6* genes through H3K9 and H3K14 hyperacetylation (Hu et al., 2015). Previous findings demonstrated that TaHAG1 modulates transcription of the *TaG1* and *TaPSBR1* by interacting with TaNACL, thereby enhancing thermotolerance in wheat (Lin et al., 2022). In maize, *ZmGCN5* and *ZmHATB* rapidly regulate the expression of cell wall-related genes by facilitating H3K9 acetylation in the promoter region under salt stress (Li et al., 2014). Nevertheless, the precise mechanism of GCN5 in response to drought stress remains unclear.

SUMOylation is a dynamic post-translational modification (PTM) that covalently transfers a small ubiquitin-like modifier (SUMO) to the lysine residues of protein substrates through action of E1–E2–E3 enzymes, thereby altering their subcellular localization, activity, and stability (Augustine & Vierstra, 2018; Rosa & Abreu, 2019; Roy & Sadanandom, 2021). Conversely, SUMOylation can be reversed by deSUMOylation proteases (DSPs) (Castro et al., 2018; Liebelt et al., 2019; Yates et al., 2016). In plants, SUMOylation responses to a variety of adverse environmental stresses, including drought, extreme temperature, salt, and nutrient homeostasis (Ghimire et al., 2020; Joo et al., 2022; Miura et al., 2007; Srivastava et al., 2016; Wang et al., 2020; Zhang et al., 2019). SCEs act as important regulators in drought stress. For instance, heterologous expression of SUMO-conjugating enzyme (E2) genes, *IbSCE1a/b/c* (*Ipomoea batatas*), enhanced drought tolerance in Arabidopsis (Zhang et al., 2023). What's more, heterologous overexpression of *SaSce9* (*Spartina alterniflora*) from a grass halophyte conferred salinity and drought tolerance by scavenging ROS in Arabidopsis (Karan & Subudhi, 2012). In this study, we revealed the regulatory mechanism of ZmSCE1a-mediated SUMOylation of ZmGCN5 in response to drought stress.

RESULTS

ZmSCE1a positively regulates drought tolerance

In maize, seven SCE1 genes (SCE1a–g) were clustered into two distinct subfamilies. Subtypes of maize class-I include SCE1a to SCE1d, while subtypes of maize class-II include SCE1e to SCE1g (Figure S1a). In addition, amino acid sequence alignments showed that these class-I ZmSCE1s had higher identity (>90%) (Figure S1b).

We firstly generated transgenic maize plants that overexpressed *ZmSCE1a* with a GFP tag (*ZmSCE1a*-GFP-OE2 and *ZmSCE1a*-GFP-OE3) (Figure S2a,b). Western blot analysis showed that protein abundance of ZmSCE1a increased gradually after 3 h of 18% PEG treatment in *ZmSCE1a*-GFP-OE plants (Figure 1A). Subcellular localization analysis in *Nicotiana benthamiana* leaves showed that ZmSCE1a is a nuclear-localized protein (Figure S3). We also generated *sce1a* loss-of-function mutant via CRISPR/Cas9 genome editing system. The *Zmsce1a*-411 mutant harbored a 5-bp deletion (from 120 to 124 bp downstream of ATG), resulting in frameshifts in the ORF and premature termination of translation (Figure S2c). *ZmSCE1a*-OE lines displayed a drought-tolerance phenotype compared with the wild-type (WT) ND101 inbred line after being subjected to drought stress. The *Zmsce1a* mutant was more sensitive to drought stress, as expected (Figure 1B). Moreover, the percentage of electrolyte leakage was higher in *Zmsce1a* mutant plants than those in WT plants and lower in *ZmSCE1a*-OE plants under drought stress (Figure 1C). After 3 days of rewatering, *ZmSCE1a*-OE lines had higher survival rates, while *Zmsce1a* mutant had lower survival rates than WT plants (Figure 1D,E). Moreover, the water loss rate of detached leaves was higher in the *Zmsce1a* mutant lines, whereas it was lower in the *ZmSCE1a*-OE lines than WT (Figure 1F). The stomatal apertures were significantly smaller in *ZmSCE1a*-OE seedlings than in WT seedlings under drought stress conditions. Consistently, the stomatal apertures of the *Zmsce1a* mutant lines were larger than that of the WT lines under drought stress (Figure 1G,H). These results demonstrated that ZmSCE1a positively regulates drought tolerance in maize.

ZmSCE1a contributes to SUMOylation and stabilization of ZmGCN5 protein

In Arabidopsis, histone acetyltransferase GCN5 has been identified by a mass spectrometry (MS) approach as a potential substrate for SUMOylation modification (Miller et al., 2010). We thus verify the relationship between ZmSCE1a and ZmGCN5. We performed a firefly luciferase complementation assay (LCI) in *N. benthamiana*. We observed strong LUC signals can only be detected in the *N. benthamiana* leaves when both GCN5-CLUC and SCE1a-NLUC were expressed (Figure 2A,B). What's more, the GCN5-SCE1a interaction was also validated in bimolecular fluorescence complementation (BiFC) experiments. Fluorescence signals were only observed when ZmGCN5 and ZmSCE1a are co-expressed in the *N. benthamiana* leaves (Figure 2C). However, we did not find an interaction between ZmSCE1a and ZmGCN5 through Y2H assay (Figure S4a). These results confirmed that ZmSCE1a physically interacts with ZmGCN5 *in vivo*. In addition, we found that ZmSCE1a interacts with a E3 SUMO ligase ZmMMS21 to form E2-E3 complex via LCI assay (Figure 2D,E). What's more, our findings implied a direct interaction between ZmGCN5 and ZmMMS21 (Figure S4b–d), suggesting that ZmMMS21 may play a role in mediating ZmGCN5 SUMOylation.

To investigate whether the interaction of ZmSCE1a-ZmGCN5 is based on covalent conjugation, SUMOylation assays in a reconstituted *Escherichia coli* BL21 (DE3) system were performed (Okada et al., 2009). The molecular

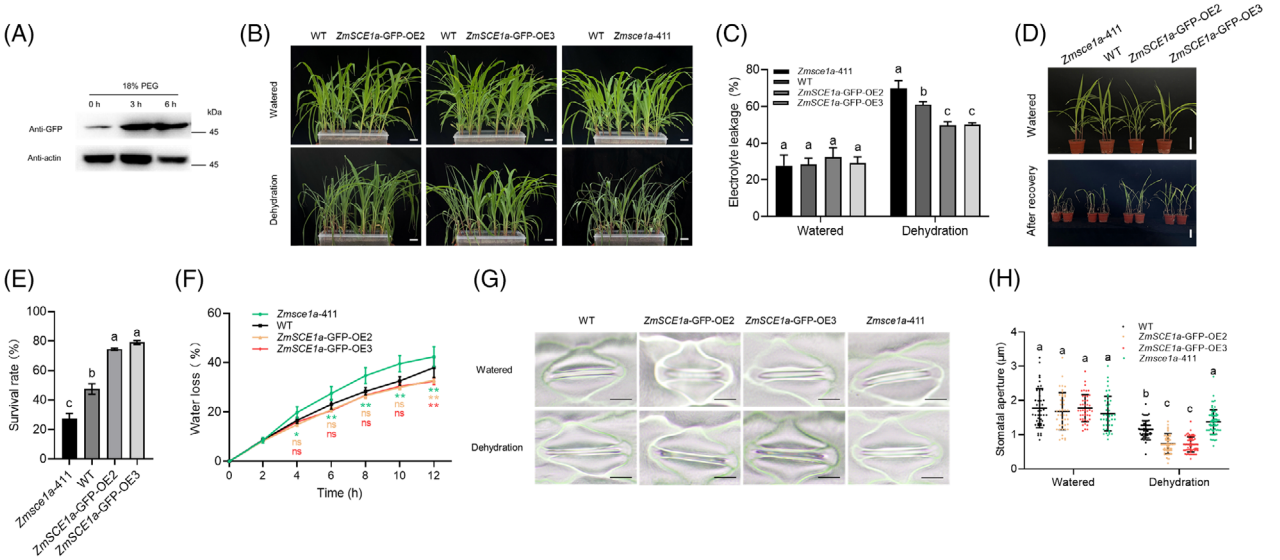

Figure 1. ZmSCE1a positively regulates drought tolerance in maize.
(A) ZmSCE1a protein abundance under 18% PEG6000 treatment. Fourteen-day-old ZmSCE1a-GFP seedlings were treated with 18% PEG for the indicated time. ZmSCE1a protein was detected with anti-GFP antibody.
(B) Phenotype of wild-type (WT), *ZmSCE1a*-OE, and *Zmsce1a*-411 seedlings under water and drought treatment. Scale bars = 5 cm.
(C) Leaf electrolyte leakage assays of WT, *ZmSCE1a*-OE, and *Zmsce1a*-411 plants under drought treatment as shown in (B).
(D) Phenotype of WT, *ZmSCE1a*-OE, and *Zmsce1a*-411 seedlings after rehydration. The 14-day-old seedlings were treated with drought by withholding water for 15 days and then rehydrated for 3 days before being photographed. Scale bars = 5 cm.
(E) Statistical analysis of survival rates after rehydration as shown in (D).
(F) Water loss of detached leaves from WT, *ZmSCE1a*-OE, and *Zmsce1a-411* seedlings. Fourteen-day-old seedlings were used. Threesecond fully expanded leaves were measured in each replicate. Data are means ± SD of three biological replicates. Three independent experiments were performed with similar results. Asterisks represent significant differences based on Student's *t*-test (*$P < 0.05$, **$P < 0.01$). ns, not significant.
(G) Representative images of stomatal aperture from WT, *ZmSCE1a*-OE, and *Zmsce1a*-411 leaves under water and drought treatment. Scale bars = 20 μm.
(H) Quantification of stomatal aperture under water and drought treatment. Sixty stomata of three leaves from three seedlings were measured. In (C, E, H), different letters represent significant differences ($P < 0.05$, one-way ANOVA).

weight shift of MBP-ZmGCN5 was exclusively observed in sample expressing ZmGCN5–SUMO1$_{GG}$ but not in ZmGCN5–SUMO1$_{AA}$ negative control sample (Figure 3A), suggesting that ZmGCN5 is SUMOylated. ADA2b as an adaptor protein that affects the HAT activity of GCN5 (Vlachonasios et al., 2003). Intriguingly, the molecular weight shift of MBP-ZmADA2b was also observed in sample using AtSUMO$_{GG}$, but not in sample using AtSUMO$_{AA}$, revealing that ZmADA2b is also covalently modified by SUMO1 as a chaperone protein for ZmGCN5 (Figure 3B).

To further explore whether ZmSCE1a influences the stability of ZmGCN5 in plants, a cell-free degradation assay was carried out. The degradation rate of MBP-ZmGCN5 proteins did not show much difference after incubation with the total proteins from WT and *Zmsce1a*-411 plants, whereas the MBP-ZmGCN5 proteins incubated with the total proteins of *ZmSCE1a*-OE plants exhibited a slower degradation rate (Figure 3C). In addition, MG132 inhibited the degradation of ZmGCN5 protein in all plants, indicating that ZmGCN5 protein was degraded via the 26S proteasome pathway. We then analyzed whether SCE1a-mediated SUMOylation of ADA2b affects the stability of ADA2b. The results showed that ZmSCE1a has no significant effect on the stability of ZmADA2b (Figure S5). Moreover, we also further investigated whether ADA2b affects the stability of GCN5 protein. Intriguingly, the results demonstrated that ADA2b had no significant impact on the stability of GCN5 (Figure S6). Taken together, the results indicated an important role of ZmSCE1a-mediated ZmGCN5 SUMOylation in regulating ZmGCN5 stability.

Overexpression of *ZmGCN5* enhances drought tolerance in maize seedlings

The *ZmGCN5* gene was cloned from maize inbred line B73. The full-length coding sequence (CDS) of *ZmGCN5* was 1548 bp and encoded a 515 aa protein contain conserved N-terminal HAT domain and C-terminal bromodomain (Figure S7a,b). Phylogenetic analysis showed that GCN5 proteins from dicotyledonous and monocotyledonous plant species are divided into different clusters (Figure S7c). Under 18% PEG6000 treatment, the expression of *ZmGCN5* was initially rapidly induced at 3 h (Figure S7d), suggesting that ZmGCN5 may function in drought response.

Previous studies have shown that histone acetyltransferases are involved in plant responses to different environmental stresses (Li, Lin, et al., 2019; Lin et al., 2022;

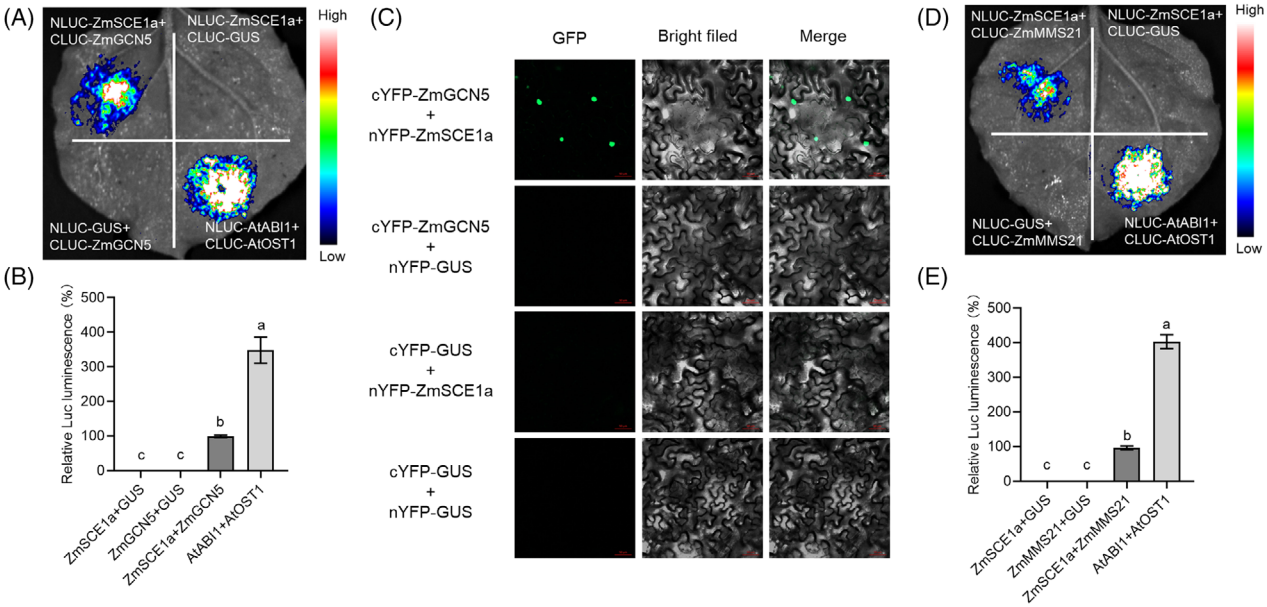

Figure 2. ZmSCE1a interacts with ZmGCN5.
(A) Luciferase complementation imaging (LCI) assay showed that ZmSCE1a interacts with ZmGCN5 in *Nicotiana benthamiana*. NLUC/CLUC-GUS were used as negative controls. NLUC-AtABI1 and CLUC-AtOST1 were used as positive controls.
(B) Quantification of luminescence signals shown in (A). Data are means ± SD of three biological replicates. Different letters represent significant differences ($P < 0.05$, one-way ANOVA).
(C) Bimolecular fluorescence complementation (BiFC) assay showed that ZmSCE1a interacts with ZmGCN5 in *N. benthamiana* leaf epidermal cells. cYFP/nYFP-GUS were used as negative controls. Bars = 50 μm.
(D) LCI assay showed that ZmSCE1a interacts with ZmMMS21 in *N. benthamiana*. NLUC/CLUC-GUS were used as negative controls. NLUC-AtABI1 and CLUC-AtOST1 were used as positive controls.
(E) Quantification of luminescence signals shown in (D). Data are means ± SD of three biological replicates. Different letters represent significant differences ($P < 0.05$, one-way ANOVA).

Zheng et al., 2021). To investigate the effect of *ZmGCN5* on drought tolerance in maize, we generated and analyzed two *ZmGCN5* overexpression transgenic maize plants by PCR analysis (Figure 4A). Quantitative reverse transcription PCR (qRT-PCR) detection demonstrated that transcription of *ZmGCN5* in overexpression seedlings were markedly increased compared with that of WT (Figure 4B).

We performed the drought treatment to evaluate their drought-resistance phenotypes. We found no difference in the growth of maize seedlings between WT and transgenic lines under fully watered conditions. However, *ZmGCN5*-OE lines exhibited a weaker wilting phenotype than WT plants under water deficit condition (Figure 4C). After rehydration, *ZmGCN5*-OE plants had higher survival rates than WT plants (Figure 4D). The water loss of detached leaves of *ZmGCN5*-OE plants was slightly slower than in WT plants (Figure 4E). What's more, the stomatal apertures were slightly smaller in leaves of *ZmGCN5*-OE seedlings than in those of WT seedlings under drought stress (Figure 4F,G). These results demonstrated that ZmGCN5 positively affects the tolerance to drought stress in maize.

To evaluate whether the stability of ZmGCN5 might be modulated by drought stress in maize, we conducted a cell-free degradation assay using crude leaf extracts obtained from maize seedlings. Compared to the control (without drought-treated) seedlings, we found that the degradation of MBP-ZmGCN5 protein was delayed in the crude extracts of drought-treated maize seedlings (Figure S8a). This finding suggested that the stability of ZmGCN5 is enhanced by drought stress.

To further investigate whether ZmSCE1a influences the stability of ZmGCN5 under drought stress, a cell-free degradation assay was carried out. The degradation rate of MBP-ZmGCN5 proteins did not show much difference after incubation for 20 min with the total proteins from WT and *Zmsce1a*-411 plants, whereas the MBP-ZmGCN5 proteins incubated with the total proteins of *ZmSCE1a*-OE plants exhibited a slightly slower degradation (Figure S8b). This finding suggested that the stability of ZmGCN5 is also enhanced by ZmSCE1a under drought stress.

ZmGCN5 could modulate abscisic acid levels and ROS accumulation under drought stress

To explore the potential role of *ZmGCN5* in stress responses, the transcript levels of abscisic acid (ABA)-responsive genes were examined by RT-qPCR. We calculated the ratio of the transcriptional levels under drought conditions to that under water conditions to

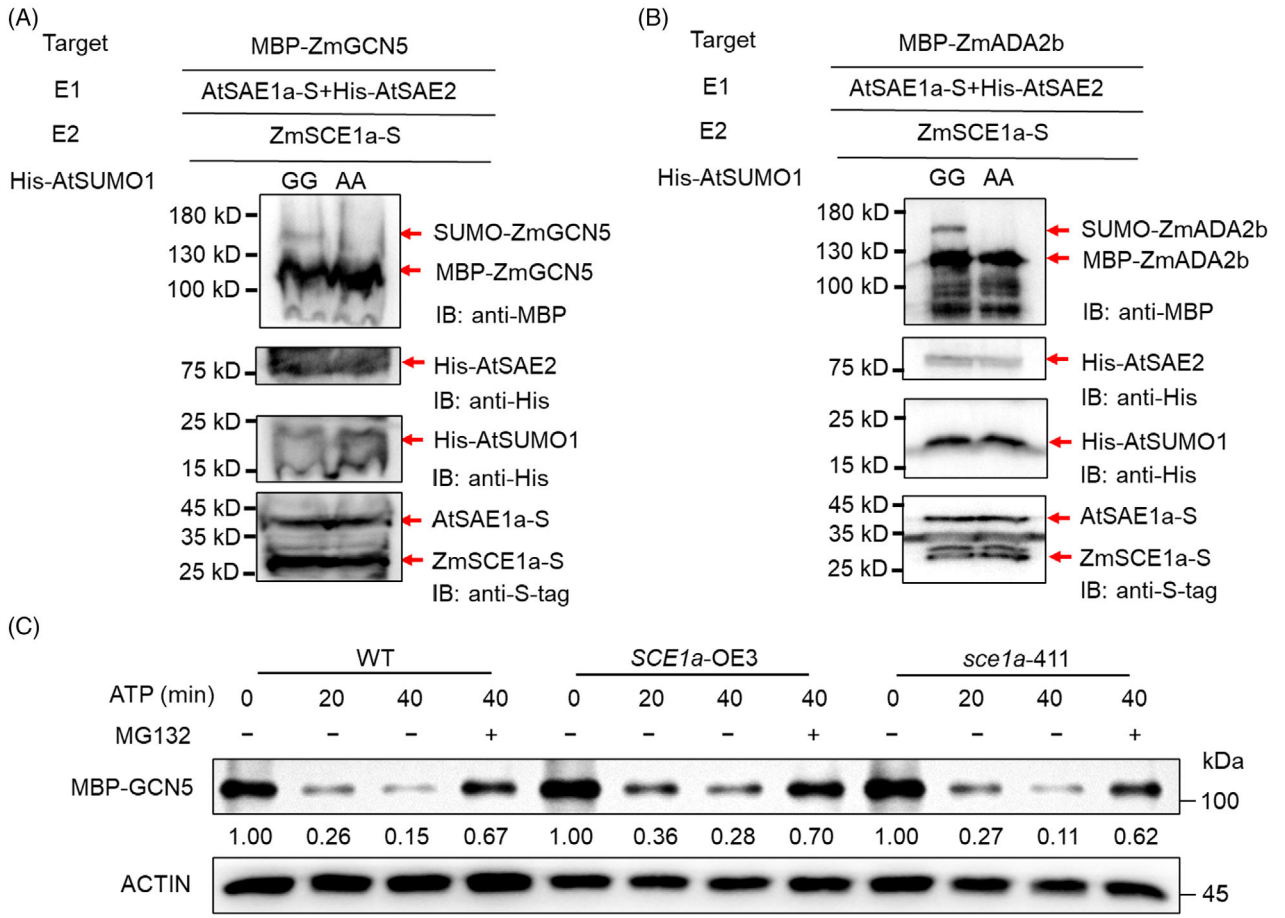

Figure 3. ZmSCE1a Contributes to SUMOylation and stabilization of ZmGCN5 protein.
(A) *In vitro* reconstructed SUMOylation assay in *Escherichia coli* cells showed the SUMOylation of ZmGCN5. GG indicated that the C-terminal Gly-Gly sequence of AtSUMO1 was exposed and covalently attached to the target protein; AA indicated that C-terminal Gly-Gly sequence of AtSUMO1 was mutated to Ala-Ala, a form that cannot covalently attached to the target protein.
(B) *In vitro* reconstructed SUMOylation assay in *E. coli* cells showed the SUMOylation of ZmADA2b. GG indicated that the C-terminal Gly-Gly sequence of AtSUMO1 was exposed and covalently attached to the target protein; AA indicated that C-terminal Gly-Gly sequence of AtSUMO1 was mutated to Ala-Ala, a form that cannot covalently attached to the target protein.
(C) *In vitro* cell-free degradation assay revealed that ZmSCE1a regulates the stability of ZmGCN5 under normal conditions. MBP-GCN5 abundance was detected with anti-MBP antibody. β-Actin served as a control. Protein levels at initial time 0 were set to 1.0. The immunoblot results were quantified using ImageJ software.

represent the level of each gene expression. After drought stress, the transcript levels of stress-responsive genes (*NCED3*, *RD29B*, *DREB2.1*, and *ERD*) and ABA-related genes (*ZEP1*, *ABA2*, *ABA3*, *NCED3*, and *ABF2*) (Zhu et al., 2020) in *ZmGCN5*-OE plants were higher than in WT plants (Figure 5A–H). Taken together, these data suggested that ZmGCN5 may enhance drought resistance by activating the expression of ABA-responsive genes.

To determine whether ZmGCN5 regulates ABA synthesis, we measured ABA content. We found no significant difference in ABA content between the *ZmGCN5*-OE and WT plants under water conditions. Intriguingly, *ZmGCN5*-OE seedlings had slightly lower ABA content than WT seedlings under drought treatment (Figure 5I). To explain this result, we measured the fresh weight of the plants. We found that the fresh weight of *ZmGCN5*-OE plants was significantly higher than that of WT plants under drought stress (Figure 5J). Therefore, the *ZmGCN5*-OE1 and *ZmGCN5*-OE2 seedlings accumulated more ABA content per plant than WT seedlings under drought conditions, reaching 35.9% and 27.2%, respectively (Figure 5K). These results suggested that overexpression of *ZmGCN5* increased drought-induced ABA accumulation in seedlings and subsequently enhanced drought tolerance in maize.

Drought stress can rapidly induce the accumulation of reactive oxygen species (ROS) in plants, leading to oxidative damage. We further explored whether ZmGCN5 regulates drought-induced ROS accumulation. Therefore, ROS levels in WT and *ZmGCN5*-OE plants were identified by

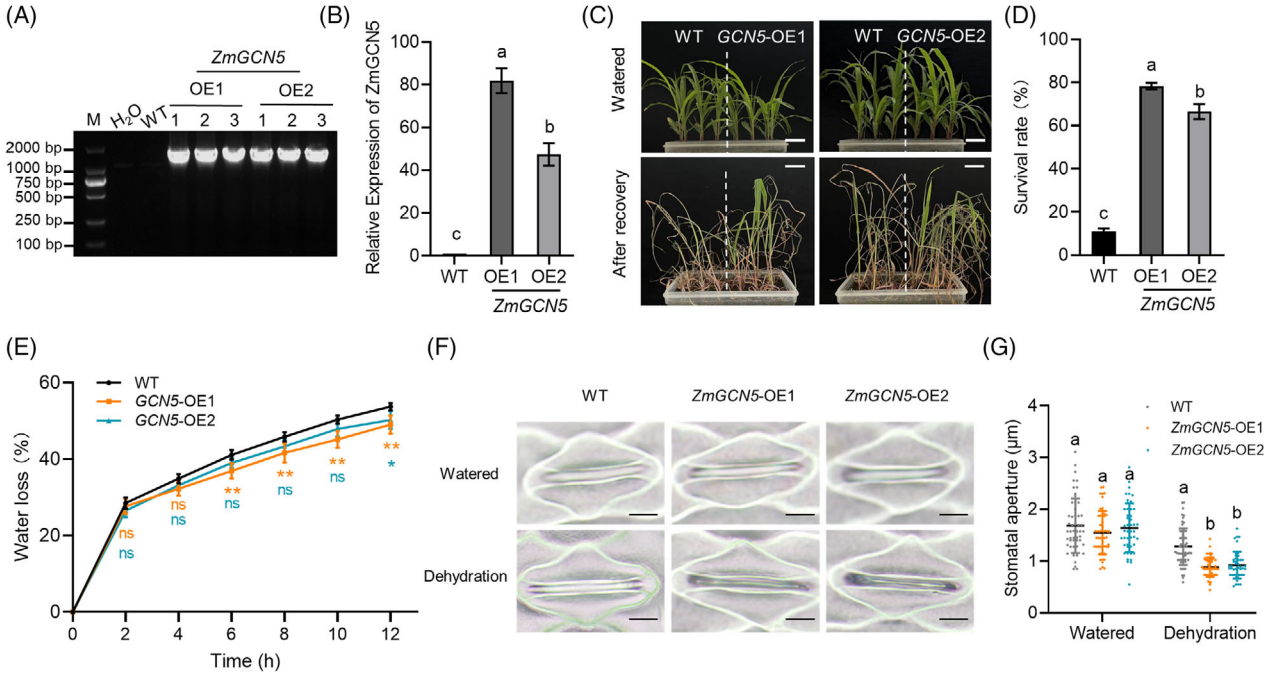

Figure 4. Overexpression of *ZmGCN5* enhances drought tolerance in maize.
(A) Two transgenic lines were identified at DNA level. M: marker; WT: wild-type maize plants (ND101); OE1 and OE2: *ZmGCN5*-OE transgenic lines 1, 2, three replicates per transgenic line; and H_2O: negative control. We used H_2O as a negative control for the PCR template.
(B) qRT-PCR analysis of the two transgenic lines.
(C) Comparison of drought-tolerant phenotypes of two independent *ZmGCN5* transgenic plants with WT plants growing in same tray. Bars = 5 cm.
(D) Statistical analysis of survival rates after rehydration as shown in (C).
(E) Water loss of detached leaves from WT and *ZmGCN5*-OE seedlings. Fourteen-day-old seedlings were used. Three-second fully expanded leaves were measured in each replicate. Data are means ± SD of three biological replicates. Three independent experiments were performed with similar results. Asterisks represent significant differences based on Student's *t*-test (*$P < 0.05$, **$P < 0.01$). ns, not significant.
(F) Representative images of stomatal aperture from WT and *ZmGCN5*-OE leaves under water and drought treatment. Scale bars = 20 μm.
(G) Quantification of stomatal aperture under water and drought treatment. Sixty stomata of three leaves from three seedlings were measured. In (B, D, G), different letters represent significant differences ($P < 0.05$, one-way ANOVA).

evaluating O_2^- accumulation using nitro-blue tetrazolium (NBT) staining under normal and drought stress conditions. Under normal growth conditions, the accumulation of O_2^- was almost similar in all plants. However, the accumulation of O_2^- was substantially lower in *ZmGCN5*-OE plants than those in WT plants under drought conditions (Figure 6A). Consistently, the accumulation of malondialdehyde (MDA) content in *ZmGCN5*-OE plants was markedly lower than WT plants under drought conditions (Figure 6B). Correspondingly, the electrolyte leakage of *ZmGCN5*-OE was significantly lower than that of WT plants (Figure 6C). Taken together, these results indicated that the cell membrane of *ZmGCN5*-OE plants is less damaged under water deficit.

DISCUSSION

ZmSCE1a conferred drought tolerance in maize

Drought is one of the major abiotic stresses that threatens crop production globally. Thus, it is an urgent and indispensable task to elucidate the regulatory mechanism of maize response to drought stress. SUMOylation is an essential PTM involved in the regulation of plant development and stress responses (Augustine & Vierstra, 2018; Ghimire et al., 2020). To date, several protein components of the SUMOylation pathway have been functionally characterized in abiotic stress responses in different species, such as SAE, SCE1, and SIZ1 (Joo et al., 2022; Srivastava et al., 2016; Wang et al., 2020; Zhang et al., 2019). In maize, seven SCE1 homologs have been identified by genome-wide informatics and responded to abiotic stress (Augustine et al., 2016). For example, ZmSCE1d and ZmSCE1e have positive regulatory effects on drought stress (Wang et al., 2019a, 2019b), while ZmSCE1b contributes to glyphosate resistance in maize (Wang et al., 2021). In the study, our findings revealed that overexpression of the *ZmSCE1a* enhances drought resistance in maize (Figure 1). In addition, OsSCE1 and OsSCE3 play opposite roles in drought response in rice (Joo et al., 2019), indicating that SCE1 isoforms may have significant functional divergence.

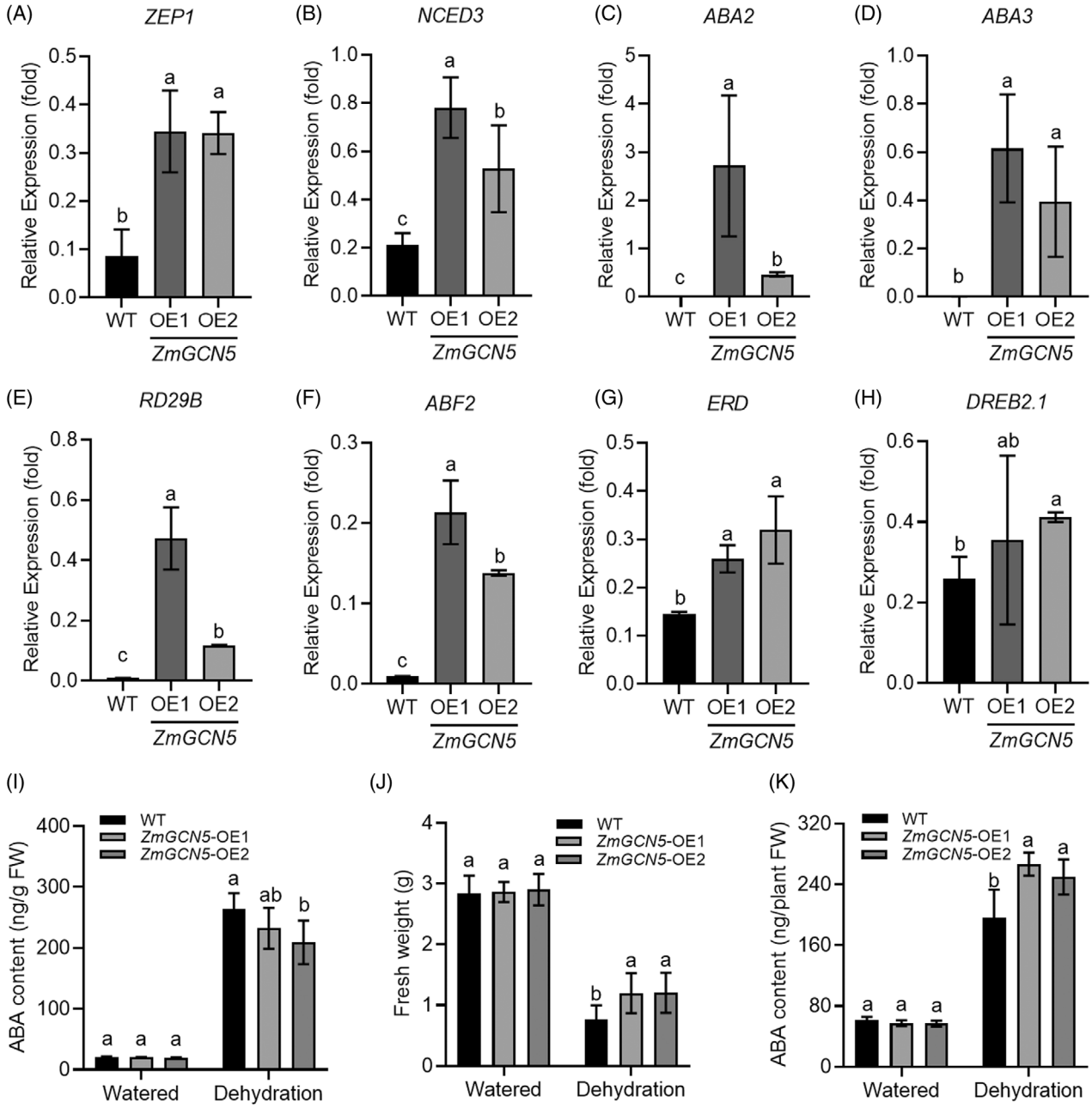

Figure 5. ZmGCN5 positively regulates drought-induced genes and abscisic acid (ABA) accumulation under drought stress.
(A–H) The expression of drought-induced genes in WT and *ZmGCN5*-overexpressing plants. Transcription of each gene was normalized to that in WT and *ZmGCN5*-overexpressing seedlings under water conditions.
(I) ABA content of *ZmGCN5*-OE and WT seedlings under water and drought conditions.
(J) Fresh weight in *ZmGCN5*-OE and WT seedlings under water and drought conditions.
(K) ABA content per plant of *ZmGCN5*-OE and WT seedlings under water and drought conditions. Data are means ± SD of three biological replicates. Different letters represent significant differences ($P < 0.05$, one-way ANOVA).

ZmSCE1a SUMOylated ZmGCN5 to enhance the stability of ZmGCN5

In Arabidopsis, hundreds of SUMO-related proteins, including histone modified proteins, have been identified by MS approach (Miller et al., 2010). What's more, GCN5 as a SUMOylation substrate in yeast participates in transcriptional regulation (Sterner et al., 2006). In general, important components of the SUMOylation pathway are enriched in the nucleus and conjugate with substrate proteins (Saracco et al., 2007). As expected, transient gene

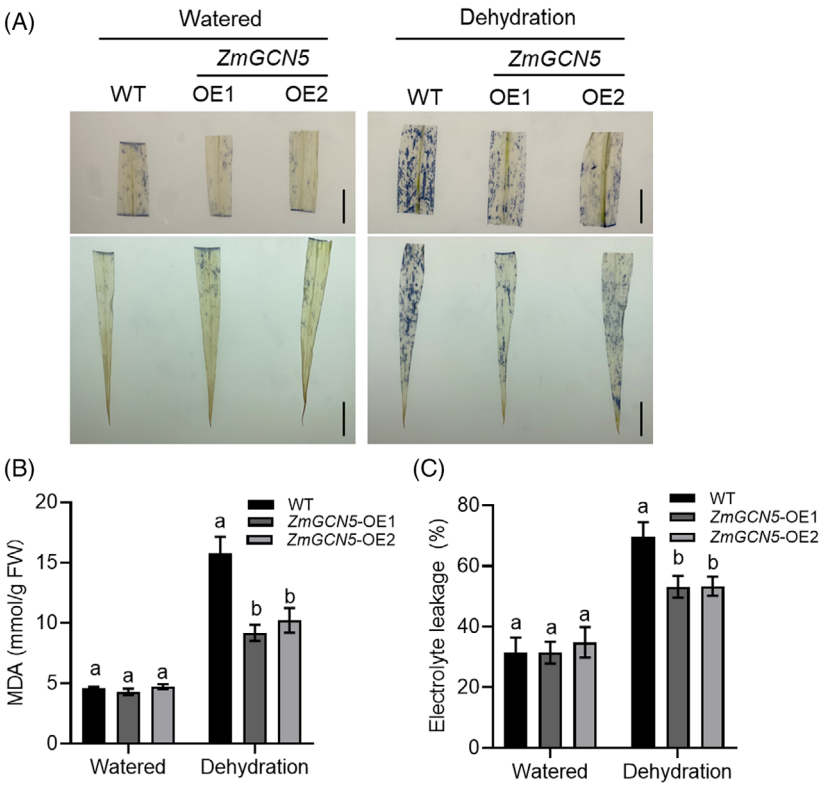

Figure 6. Effect of ZmGCN5 on lipid peroxidation and O_2^- accumulation under drought stress.
(A) O_2^- accumulation in leaves of WT and *ZmGCN5*-OE plants detected by nitro-blue tetrazolium (NBT) staining under normal and drought conditions. Scale bars = 1 cm.
(B) Detection of MDA contents in WT and *ZmGCN5*-OE plants under drought treatment.
(C) Leaf electrolyte leakage assays of WT and *ZmGCN5*-OE plants under drought treatment. In (B, C), data are means ± SD of three biological replicates. Different letters represent significant differences ($P < 0.05$, one-way ANOVA).

expression and BiFC assays in the leaves of *N. benthamiana* revealed that ZmSCE1a interacts with ZmGCN5 in the nucleus (Figure 2A–C). What's more, our data indicated that ZmSCE1a mediates the SUMOylation of ZmGCN5 (Figure 3A). Interestingly, we found that the SUMO ligase ZmMMS21 co-regulates the SUMOylation of ZmGCN5 (Figure S4). Previous studies have shown that AtADA2b, as a potential SUMO substrate, was SUMOylated in Arabidopsis and yeast (Elrouby & Coupland, 2010; Wohlschlegel et al., 2004). Similarly, we found that ZmADA2b was SUMOylated in maize (Figure 3B). Moreover, previous study has shown interaction between ZmGCN5 and ZmADA2b (Bhat et al., 2003). Intriguingly, our further results suggested that ZmADA2b may not have a significant impact on the stability of ZmGCN5 (Figure S6). The ADA2b can stimulate acetylation activity of GCN5 by enhancing the binding of its cofactor acetyl-CoA in human (*Homo sapiens*) (Sun et al., 2018). Therefore, the effect of ADA2b on the HAT activity of GCN5 in maize should be further explored.

SUMO can be covalently attached to target proteins to alter their activity, localization, or stability (Augustine & Vierstra, 2018). In this study, we demonstrated that ZmSCE1a enhances the stability of ZmGCN5 protein by inhibiting 26S proteasome degradation (Figure 3C). Interestingly, we observed that knockout of *ZmSCE1a* did not have a corresponding reduced effect on ZmGCN5 protein abundance (Figure 3C). The maize genome contains seven *SCE1* genes (Augustine et al., 2016), while only one *SCE1* gene in Arabidopsis (Saracco et al., 2007), implying that there may be gene functional redundancy and compensation in maize.

ZmGCN5 contributes to drought tolerance of maize

Increasing evidence suggests that GCN5 is associated with plant growth, development, and abiotic stress (Kim et al., 2018; Kotak et al., 2018; Lin et al., 2022; Zheng et al., 2021), but its regulation mechanism in drought tolerance is still unclear. Here, we found that its expression was induced by 18% PEG and enhanced drought tolerance in maize seedlings (Figure 4; Figure S7).

In plants, water deficit accumulates excessive ROS, which leads to serious oxidative damage and disrupts the integrity of cell membrane (Choudhury et al., 2017). Therefore, the ROS homeostasis is crucial for plant to adapt to adverse environment. TaHAG1 (TaGCN5) alleviated salt damage by modulating ROS production and homeostasis in wheat (Zheng et al., 2021). In our study, O_2^- accumulation in *ZmGCN5*-OE plants was remarkably lower than in WT using NBT staining (Figure 6A). What's more, *ZmGCN5*-OE plants contained less MDA content and ion leakage under water deficit conditions (Figure 6B,C), indicating that overexpression of *ZmGCN5* is beneficial for alleviating stress-induced plasma membrane damage.

GCN5, as a critical component of the SAGA (Spt-Ada-Gcn5 acetyltransferase) complex, participates in the

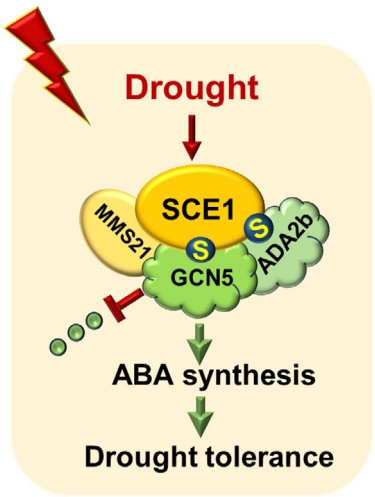

Figure 7. A working model for the role of *ZmSCE1a* in response to drought stress.
When challenged with drought, the protein abundance of ZmSCE1a accumulates rapidly and increases the drought tolerance in maize. Subsequently, ZmSCE1a acts on the SUMOylation of ZmGCN5-ZmADA2b complex and enhances the stability of ZmGCN5. The accumulation of ZmGCN5 can increase ABA level, thus leading to improve drought tolerance of maize.

transcription of almost all active genes in yeast and plants (Robert et al., 2004; Wu et al., 2021). In poplar, an important factor AREB1 (ABF2) was discovered to recruit GCN5-ADA2b to form the AREB1-ADA2b-GCN5 complex and regulate the transcriptional activation of downstream drought-responsive genes *PtrNAC006*, *PtrNAC007*, and *PtrNAC120*, thereby regulating the response to drought stress (Li, Lin, et al., 2019). In our study, we found that the transcription level of *ZmABF2* in *ZmGCN5*-OE plants significantly increased by qRT-PCR under drought stress (Figure 5). We speculated that GCN5 may regulate the transcription of downstream drought-responsive genes by affecting ABF2 activity. In future work, we should explore the transcriptional activation of drought-responsive genes by the transcription factors influenced by GCN5.

In conclusion, based on the current research, we propose a working model of drought tolerance. When challenged with drought, the protein abundance of ZmSCE1a accumulates rapidly and increases the drought tolerance in maize. ZmSCE1a acts on the SUMOylation of ZmGCN5-ZmADA2b complex and enhances the stability of ZmGCN5. The accumulation of ZmGCN5 can increase ABA level, thus leading to improve drought tolerance of maize (Figure 7).

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

All maize plants were obtained from the Center for Crop Functional Genomics and Molecular Breeding, China Agricultural University. The mutant of *ZmSCE1a* was generated using CRISPR-Cas9 genome editing and cloned into *pBUE411* according to previous study (Xing et al., 2014). The full-length coding sequences (CDSs) of *ZmGCN5* and *ZmSCE1a* were cloned into *pBCXUN* or *pCUN(m)-GFP* vectors to generate overexpressing plants. The constructs were transformed into immature embryos of the inbred line ND101 by *Agrobacterium*-mediated transformation (Ishida et al., 2007).

Maize plants were cultured in enriched soil mixture (soil and vermiculite in a ratio of 1:1) in a growth chamber at 25°C with a 14-h light/10-h dark photoperiod and 60% relative humidity. At the three-leaf stage (14-day-old), drought treatment was carried out by withholding water. Approximately 8 days later, drought phenotypes were observed and physiological indexes were measured. When the wilting phenotype appeared (15–20 days), the plants resumed watering and the survival rate of plants was recorded after 3 days.

For PEG treatment, 14-day-old seedlings were transferred to modified Hoagland's hydroponic nutrient solution containing 18% (w/v) PEG6000 in a chamber. Samples were harvested at indicated intervals to analyze gene expression and protein level.

Physiological analyses

To assess oxidative damage, we conducted a drought stress experiment in which 14-day-old maize seedlings were deprived of water for 8 days. The malondialdehyde (MDA) content and the percentage of electrolyte leakage were performed as described previously (Li et al., 2010).

To stain O_2^-, the second maize leaf was stained with NBT staining as described previously (Yan et al., 2023). In brief, 14-day-old maize plants were subjected to drought by withholding water for 8 days. The leaves were immersed in 0.3 mg ml^{-1} of NBT solution (Coolaber, Beijing, China) for a duration of 12 hours in darkness at room temperature. The leaves were washed and decolorized with 95% ethanol and then photographed to record the staining.

Leaf water loss measurement

Fully expanded leaves were detached from 14-day-old seedlings, and the fresh weights of leaves were measured at the indicated time.

Measurements of stomatal aperture

For stomatal aperture assay, 14-day-old maize seedlings were treated after 5 days of water deprivation. The abaxial epidermis of leaves was coated with nail polish, dried, and placed on a glass slide, and the stomata were observed under a 40-fold objective lens. The stomatal apertures were analyzed using ImageJ software. More than 20 stomatal apertures per sample were measured, and each treatment included three replicates.

Determination of ABA contents

Sixty milligrams of each leaf sample was ground into powder in liquid nitrogen. Five hundred microlitre extraction buffer (2:1:0.002, isopropanol/H_2O/HCl, v/v/v) and 50 µl internal standard solution (D_2-ABA) were added and vortexed vigorously for 10 sec. The mixture was shaken at 900 rpm for 30 min at 4°C. Then, add 1 ml chloroform to the mixture and swirl for another 30 min. After centrifugation at 15000 ***g*** for 10 min at 4°C, the lower phase was transferred into a fresh clean tube and dried with nitrogen at room temperature. The enriched extracts were re-dissolved in 100 µl methanol and filtered through a 0.45-µm membrane filter for HPLC-MS analyses (Zhu et al., 2020).

Bioinformatics analysis of the ZmSCE1a and ZmGCN5

The amino acid sequences of SCE1 and GCN5 homologs were obtained from National Center for Biotechnology Information database (http://blast.ncbi.nlm.nih.gov). Multiple sequences were aligned using ESPript 3.0 (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi) and MEGA-X software. The phylogenetic tree was constructed by Neighbor-Joining method in MEGA-X software.

RNA extraction and qRT-PCR

Total RNA extraction was conducted by TRIzol reagent (Sigma, St. Louis, MO, USA) from the maize leaves. One microgram of total RNA was utilized in the synthesis of cDNAs with the RevertAid First Strand cDNA Synthesis Kit (Invitrogen, Cartshad, CA, USA) following the manufacturer's recommendations. qRT-PCR assays were performed using SYBR Green reagent (Takara, Otsu, Shiga, Japan) in a 7500 Real-Time PCR System (Applied Biosystem, Foster City, CA, USA). The maize Ubiquitin 1 gene served as the internal control. Primer sequences are listed in Table S1.

Protein subcellular localization

.The CDS of *ZmSCE1a* was cloned into *pCUN(m)-GFP* vector. The recombinant construct was introduced into *Agrobacterium* strain GV3101 and then injected into *N. benthamiana* to express about 48 h. The fluorescence signals of GFP were observed at 488 nm by confocal laser-scanning microscope (Zeiss 880, Oberkochen, Germany).

BiFC assays

BiFC assays were performed as described previously (Chu et al., 2024). Briefly, the CDS of *ZmGCN5* was cloned into cYFP vectors. The CDS of *ZmSCE1a* was cloned into nYFP vectors. What's more, the cYFP-GUS and nYFP-GUS constructs used in the study were described previously (Guo et al., 2023). The constructs were individually transformed into *Agrobacterium* strain GV3101 and co-expressed in *N. benthamiana* leaves for 72 h. The fluorescence signal of GFP was detected by a confocal laser-scanning microscope (Zeiss 880, Oberkochen, Germany).

LCI assays

Transient LCI assays were performed as reported (Chen et al., 2008). Briefly, the CDS of *ZmSCE1a* and *ZmMMS21* was cloned into pCAMBIA1300-nLUC. The CDS of *ZmGCN5* and *ZmMMS21* was cloned into pCAMBIA1300-cLUC. What's more, the cLUC-GUS, nLUC-GUS, cLUC-AtOST1, and nLUC-AtABI1 constructs used in the study were described previously (Guo et al., 2023). All constructs were co-transformed into *Agrobacterium* strain GV3101 and then co-injected into *N. benthamiana* leave for 48–72 h. Before imaging, the abaxial sides of leaves were sprayed with 1 mM luciferin, and LUC signal was observed by a CCD camera (Tanon 5200, Shanghai, China) at −20°C with 10-min exposures. Quantification of signals from three independent experiments was calculated by the ImageJ software.

Yeast-two-hybrid (Y2H) assays

Y2H assays were performed according to the manufacturer's protocols. The CDS of *ZmSCE1a* and *ZmMMS21* was amplified and cloned into pGBKT7 vector. The CDS of *ZmGCN5* was cloned into the pGADT7 vector. The empty vectors pGADT7 and pGBKT7 were co-transformed as negative controls. The plasmids were transformed into AH109 yeast cells and cultured in SD–L/T medium and SD–L/T/H medium for 3 days to verify the interaction.

Protein purification

The CDS of *ZmGCN5* and *ZmADA2b* was cloned into pMAL-c2X to obtain the MBP-GCN5 and MBP-ADA2b proteins. Constructs were transformed into *Escherichia coli* BL21 (DE3) strain, and the fusion proteins were induced by 0.5 mM IPTG for 3 h at 37°C. Protein extracts were purified in extraction buffer (20 mM Tris–HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl, 1 mM PMSF, and 1 mM DTT) and incubated with amylose resin (New England Biolabs, Ipswich, MA, USA) at 4°C for 3 h. Proteins were eluted by maltose buffer.

Protein extraction and immunoblot analysis

Total proteins were extracted from maize leaves with protein extraction buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 2.5 mM $MgCl_2$, 0.5 mM EDTA, 10% glycerin, 0.2% NP-40, 1 mM DTT, 1 mM PMSF and 1 × protease inhibitor cocktail). 4 × SDS loading buffer was added into samples and boiled at 95°C for 10 min. After separation on SDS-PAGE gel, ZmSCE1a protein was detected with anti-GFP (BioDragon, Suzhou, China) antibody. β-Actin (Lablead, Beijing, China) antibody was used as control.

SUMOylation assays

The SUMOylation assay was conducted as described previously with some minor modifications (Okada et al., 2009). In brief, *E. coli* BL21(DE3) cells were transformed by pACYCDuet-AtSAE1a-AtSAE2 to preparate competent cells. For SUMOylation reactions, pMAL-c2X-ZmGCN5/ZmADA2b and pCDFDuet-AtSUMO1(AA or GG)-ZmSCE1a vectors were transferred into competent cells. The combined cells were induced in 5 ml LB medium at 37°C until the OD_{600} reached 1.0, and 1 mM IPTG was added. After induction at 37°C for 6 h, 1 ml cell culture was harvested and 100 μl 1 × SDS loading buffer was added, followed by boiled at 95°C for 10 min. The SUMOylation analysis of ZmGCN5 or ZmADA2b was detected by immunoblotting incubated with anti-MBP (Lablead, Beijing, China), anti-His (Lablead, Beijing, China), and anti-S-tag (BioDragon, Suzhou, China).

Cell-free protein degradation assay

The cell-free protein degradation assay was conducted as described previously (Chen et al., 2018). Briefly, total proteins were extracted from 14-day-old maize leaves with or without drought stress for 8 days in degradation buffer (25 mM Tris–HCl, pH 7.5, 10 mM NaCl, 10 mM $MgCl_2$, 5 mM DTT, 4 mM PMSF and 10 mM ATP) and collect supernatants by two centrifugation steps (15 000 ***g***, 10 min, 4°C). Equal amounts of recombinant MBP-ZmGCN5 or MBP-ZmADA2b protein and total proteins extracted from WT, *ZmSCE1a*-OE, and *Zmsce1a*-411 plants were incubated at 25°C for the indicated intervals. Protein abundance was determined using anti-MBP antibody (Lablead, Beijing, China) by immunoblotting analysis. β-Actin (Lablead, Beijing, China) antibody was used as control.

Cell-free degradation assay with drought treatment was conducted as described previously (Joo et al., 2022). Briefly, crude leaf protein extracts were obtained from 14-day-old maize leaves treated with or without drought stress for 8 days using an extraction buffer (25 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM DTT, 0.1% Triton X-100, 10 mM ATP, and 10 mM NaCl). The MBP-ZmGCN5 protein was incubated with prepared crude leaf protein extracts at 25°C for the time indicated using dimethyl sulfoxide or 50 μM of the MG132 treatment. Protein abundance was determined using anti-MBP antibody (Lablead, Beijing, China) by immunoblotting analysis. β-Actin (Lablead, Beijing, China) antibody was used as control.

Statistical analysis

IBM SPSS software (version 26, Chicago, IL, USA) was used for statistical analysis. Data are mean ± SD ($n = 3$). Chats were drawn with GraphPad Prism software (version 8.3.0, San Diego, CA, USA). Protein quantification analysis was performed with ImageJ software.

ACCESSION NUMBERS

Sequence data from this study can be found in Maize Genetics and Genomics Database (https://www.maizegdb.org/) under the accession numbers: *ZmGCN5* (GRMZM2G046021), *ZmUbi1* (GRMZM2G409726), *ZmSCE1a* (GRMZM2G063931), *ZmADA2b* (GRMZM2G177974), and *ZmMMS21* (GRMZM2G022065).

AUTHOR CONTRIBUTIONS

YZ and LD designed the experiments. TF, YW, and JZ performed the experiments and collected the data. MZ analyzed the data. TF wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

All relevant data in the article can be found in the manuscript and supporting materials.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Sequence alignment and phylogenetic analysis of ZmSCE1s proteins.

Figure S2. Detection the *ZmSCE1a* transgenic plants.

Figure S3. Subcellular localization of ZmSCE1a-GFP in *N. benthamiana* leaves.

Figure S4. ZmGCN5 interacts with ZmMMS21.

Figure S5. ZmSCE1a has no effect on the stability of ZmADA2b.

Figure S6. ZmADA2b has no effect on the stability of ZmGCN5.

Figure S7. Phylogenetic relationship and expression pattern of *ZmGCN5*.

Figure S8. Enhanced stability of ZmGCN5 in response to drought stress.

Table S1. Primers used in this study.

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