

# *TaCNGC-2A* suppresses seed dormancy and activates pre-harvest sprouting through modulating calcium and hormonal signaling pathways

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Weak seed dormancy (SD) is prone to pre-harvest sprouting (PHS), which reduces cereal yield and quality. Here, through map-based analysis, we identify *TaCNGC-2A*, encoding a cyclic nucleotide-gated channel protein, as a negative regulator of wheat SD. Knocking out of *TaCNGC-2A* enhances SD and PHS resistance, with no yield penalty. Two transcription factors, TaMYB-5B and TaMYB-5D, directly bind to the T/A mutation site of *TaCNGC-2A* promoter to synergistically repress its expression. The calmodulin TaCaM-3A interacts with *TaCNGC-2A* to jointly modulate SD and PHS resistance through influencing calcium and multiple hormonal signaling pathways. Knocking out of *TaCaM-3A* not only enhances SD and PHS resistance, but also increases grain weight and per-plant yield. Finally, we identify allele combinations of *TaCNGC-2A* and other known dormancy genes associated with strong SD. This study uncovers a regulatory mechanism underlying SD and PHS resistance and provides gene targets for breeding wheat varieties with PHS resistance.

Pre-harvest sprouting (PHS) refers to the premature germination of physiologically mature grains, occurring in the spikes before harvest, typically under wet conditions in cereals including wheat, rice and maize, the most important food crops globally. PHS is a common problem in many major wheat-producing regions worldwide, such as the United States, Australia, Canada, and China, causing significant reductions in grain yield and flour quality, with the annual global losses

estimated to reach 1 billion US dollars<sup>1,2</sup>. In China, PHS poses a major challenge in the vast areas of Southwest, the Yangtze River Valley and the Yellow and Huai Valley wheat regions<sup>3</sup>. Noteworthy is that with the global climate change, PHS is occurring more frequently<sup>4,5</sup>. Hence, improving resistance to PHS, a long-lasting problem, has become even more urgent in cereal breeding. Seed dormancy (SD), defined as a temporary block to germination in mature viable seeds under

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favorable conditions, is considered as a major genetic component of PHS resistance<sup>6</sup>. Therefore, identifying genes that control SD can contribute to our efforts in enhancing PHS resistance in wheat as well as other cereals.

To date, nearly 200 quantitative trait loci (QTLs) have been associated with SD/PHS in wheat, especially on chromosomes 2A, 2B, 3A, 3B, 3D, 4A, and 6B<sup>7–12</sup>. However, only four candidate genes have been isolated at these loci by map-based cloning approach. *TaMFT-3A* (also known as *TaPHS1*), a wheat homolog of a *MOTHER OF FLOWERING TIME* (*MFT*)-like gene in *Arabidopsis* that encodes a phosphatidylethanolamine-binding protein, was identified as a causal gene underlying the major QTL *Qphs.pseru-3AS* (*QPhs.ocs-3A.1*) for SD/PHS resistance in wheat. The expression of *TaMFT-3A* is synergistically activated by *TaVp1* (homologous to *Arabidopsis* ABSCISIC ACID INSENSITIVE 3, *ABI3*) and *TaABI5*, two key transcription factors involved in ABA signaling pathway, thereby enhancing SD/PHS resistance, but its regulatory mechanism and downstream genes or pathways are unknown<sup>6,13–15</sup>. *TaMyb10-D*, which encodes a R2R3-MYB transcription factor, underlies the major locus *PHS-3D* for PHS resistance and grain color in wheat. It enhances PHS resistance by directly activating *9-cisepoxycarotenoid dioxygenase* (*NCED*) transcription and promoting ABA biosynthesis, but the upstream regulatory factors remain unknown<sup>16</sup>. *TaPI4K-2A*, encoding a phosphatidylinositol 4-kinase (PI4K), from the *Qphs2A.7\_nwafu* locus for wheat PHS resistance. It remains unclear as how *TaPI4K-2A* regulates PHS resistance<sup>17</sup>. In addition, several genes related to SD or PHS resistance have been identified by homologous-based cloning or other methods. *TaSdr*, orthologs of *Sdr4* (*Seed dormancy 4*) conferring rice SD by repressing active GA biosynthesis, is positively associated with wheat SD. However, the identity and function of the *TaSdr* protein remains unknown<sup>18–20</sup>. *DOG1* (*DELAY OF GERMINATION 1*), encoding a member of a plant-specific protein family that share a domain with the D class bZIP DNA-binding proteins, contributes to the major QTL for embryonic dormancy in *Arabidopsis*. It improves SD through enhancing seed responsiveness to ABA. *TaDOG1L1*, a wheat *DOG1*-like gene, has been shown to promote wheat SD<sup>21–23</sup>. Similarly, *TaGATA1*, a GATA transcription factor, contributes to wheat SD by directly binding to the GATA motif in the *TaABI5* promoter and inducing its expression<sup>24</sup>. *TaSRO1* (*SIMILAR TO RCD ONE*), belonging to a small plant-specific protein family, negatively modulates SD/PHS resistance by inhibiting the synergistic activation of *TaVp1* and *TaABI5* on the PHS resistance genes *TaPHS1* and *TaSdr*<sup>14</sup>.

The genes outlined above encode diverse protein products including transcription factors, kinases, enzymes and proteins with unknown functions, indicating the complex genetic basis of SD or PHS resistance in wheat. However, the direct up- and/or down-stream molecular components of these genes are still poorly understood, limiting our knowledge on the regulatory network of SD/PHS resistance. Importantly, it remains unclear regarding the breeding potentials of those genes for the improvement of wheat resistance to PHS. Indeed, there are few genes being directly utilized for breeding PHS resistant varieties. Therefore, identifying molecular components underlying SD/PHS resistance with breeding values will not only help clarify the complex molecular network of SD/PHS resistance, but could also provide gene targets and genetic resources for improving PHS resistance in wheat and other cereals through molecular breeding or genome editing and optimization.

Here, we show that *TaCNGC-2A*, encoding a CNGC protein (also named Ca<sup>2+</sup>-permeable channel protein) involved in Ca<sup>2+</sup> signaling, is the candidate gene underlying *Qsd.ahau-2AL*, a major locus, associated with wheat SD. The transcription factors *TaMYB-5B* and *TaMYB-5D* directly bind to the T/A mutation site of *TaCNGC-2A* promoter (–312 bp), which is the key mutation determining the phenotypic differences in SD, and synergistically repress its transcription, with mutation to A from T evoking stronger SD. *TaCNGC-2A* then interacts *TaCaM-3A* to

up- or down-regulate the expression of genes involved in Ca<sup>2+</sup> and multiple plant hormone signaling pathways and dormancy genes, thereby modulating SD. We also examine the combined effects of *TaCNGC-2A* and the known dormancy genes on SD. Together, our study discovers a negative regulator, *TaCNGC-2A*, for SD in wheat, and provides insights into the underlying mechanism through identifying its up- and down-stream molecular components. Our work further demonstrates the potential of exploiting *TaCNGC-2A* for breeding wheat varieties with enhanced PHS resistance.

## Results

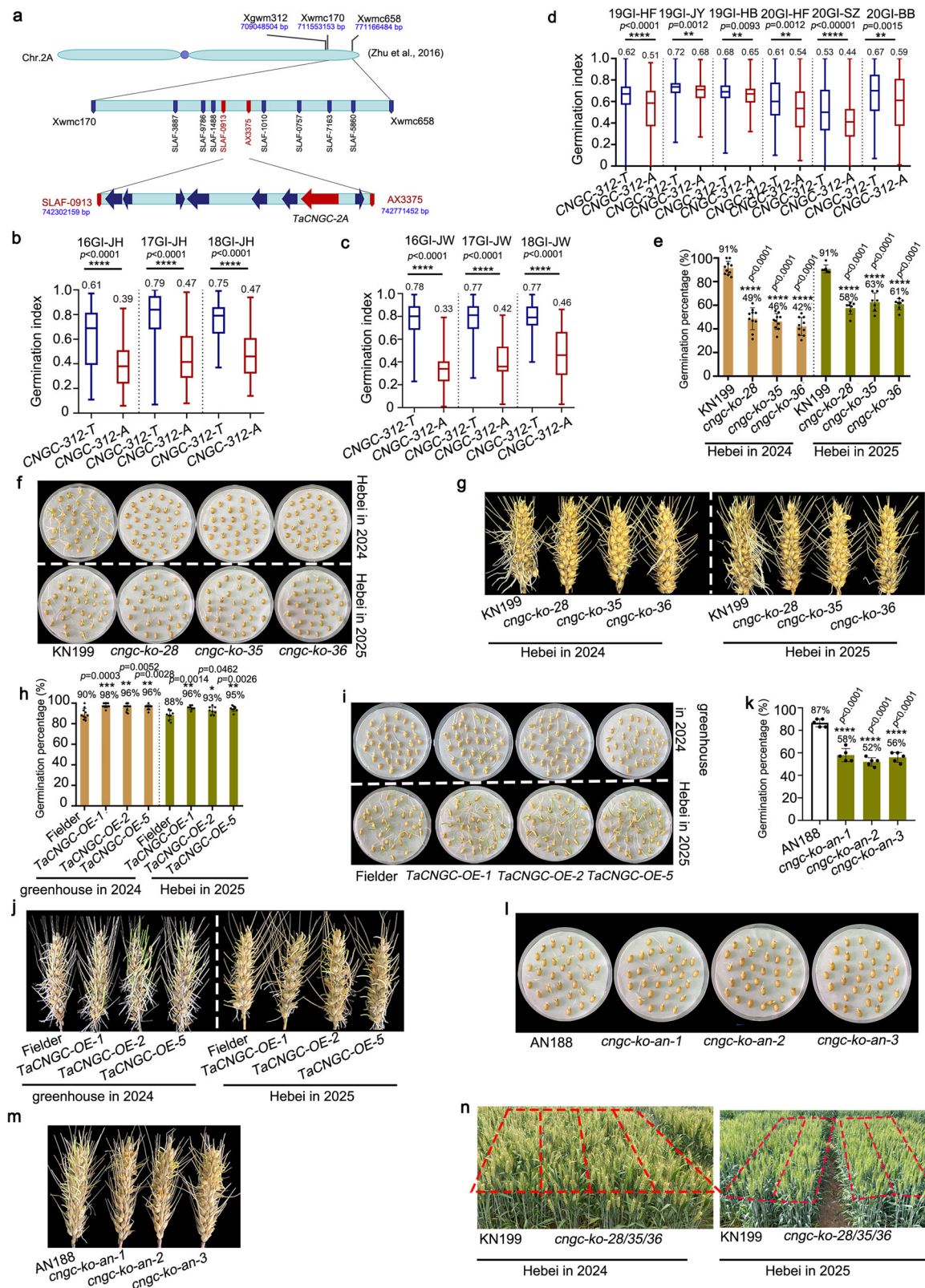
### Identification of candidate gene underlying *Qsd.ahau-2AL* for SD

Previously, we identified a major locus, *Qsd.ahau-2AL*, for SD in the recombinant inbred line (RIL) population from the cross of Jing 411 (J411, weak SD) × Wanxianbaimaizi (strong SD)<sup>10</sup>. Given that the strongly dormant variety Hongmangchun 21 (HMC21) also carried the same *Qsd.ahau-2AL* locus as Wanxianbaimaizi and the high-density genetic map of the J411 × HMC21 RIL population (JH-RILs) previously constructed by SLAF-seq (specific locus amplified fragment sequencing)<sup>25</sup>, we utilized this population to fine-map the target region. Within the *Qsd.ahau-2AL* interval, eight SNPs were converted into eight cleaved amplified polymorphic sequence (CAPS) markers. To identify additional molecular markers within the *Qsd.ahau-2AL* region, we used a wheat 660 K SNP array to genotype the two parents (J411 and HMC21) and the two extreme bulks (10 strong SD lines and 10 weak SD lines). A total of 53 SNPs showed consistent polymorphisms between the two parents and the two bulks. Among these, one SNP (AX\_109933375) was converted into a derived cleaved amplified polymorphic sequence (dCAPS) marker, designated AX3375. In total, 63 markers on chromosome 2A, including eight CAPS, one dCAPS, three SSR and 51 SLAF tags, were jointly used to fine map *Qsd.ahau-2AL* in the JH-RILs. Linkage mapping and QTL analysis revealed that *Qsd.ahau-2AL* was finely mapped to a 0.47 Mb interval flanked by SLAF0913 and AX3375, explaining 9.4% (17GI-JH) and 10.3% (18GI-JH) of the SD phenotypic variation (Fig. 1a, and Supplementary Data 1 and 2).

In the 0.47 Mb region, seven genes with high-confidence plus one with low-confidence were annotated according to IWGSC RefSeq v2.0. Based on our previous transcriptome sequencing data from the seeds of J411 and HMC21 sampled at 4, 6, and 10 h of imbibition (representing dormancy release process)<sup>26</sup>, we found that among these seven high-confidence genes, four were expressed. Among them, *TraesCS2A02G519600*, encoding a CNGC protein (named *TaCNGC-2A*), had the highest expression level, and the expression level of *TaCNGC-2A* in J411 seeds (all germinated) was significantly higher than that in HMC21 seeds (all ungerminated) after 10 h of seed imbibition (Supplementary Data 3). Subsequently, we investigated the expression profiles of *TaCNGC-2A* and the other six high-confidence genes by qRT-PCR, and found that the expression level of *TaCNGC-2A* was consistently the highest (Supplementary Fig. 1a). Additionally, we observed that among these seven genes, only *TaCNGC-2A* was highly expressed in different wheat tissues using public WheatOmics database (<http://202.194.139.32/>). qRT-PCR analysis further revealed that *TaCNGC-2A* was expressed much more in seeds than in roots, stems, and leaves (Supplementary Fig. 1b). These results suggest that *TaCNGC-2A* is a potential candidate gene underlying *Qsd.ahau-2AL*.

### Associations of *TaCNGC-2A* with wheat SD and PHS resistance

We then cloned the promoter (906 bp upstream of ATG) and the full-length (5786 bp) sequences of *TaCNGC-2A* in J411 and HMC21. Sequence alignment analysis revealed that there were multiple variations in *TaCNGC-2A* between J411 and HMC21. Next, we focused on two SNPs in the promoter (T/A, –312 bp; G/A, –514 bp) and two non-synonymous mutations in the coding region (G/C, +856 bp; T/A, +2467 bp), and developed four CAPS markers, named CNGC-312, CNGC-514, CNGC + 856, and CNGC + 2467. These four markers were



then used to genotype 174 lines derived from JH-RILs, 165 lines derived from J411 × Wanxianbaimaizi (JW-RILs), and 431 wheat varieties (431WVs) with different dormancy and PHS resistance levels. The U-test results revealed that significant differences were observed in germination index (GI) values between varieties (lines) carrying the two allelic variants of *CNGC-312* (*CNGC-312-T* and *CNGC-312-A*) in JH-RILs, JW-RILs, and 431WVs. The GI value of varieties (lines) carrying

*CNGC-312-A* was 4% to 45% lower than that of varieties carrying *CNGC-312-T* (Fig. 1b–d). However, no significant difference was observed in GI between varieties (lines) carrying the two allelic variants of *CNGC-514*, *CNGC + 856*, and *CNGC + 2467* in the above three populations (Supplementary Data 4). These results suggest that the T/A mutation is a key variation determining phenotypic differences in SD and PHS

**Fig. 1 | Identification of the candidate gene *TaCNGC-2A* underlying *Qsd.ahau-2AL*, and the role of *TaCNGC-2A* in wheat SD and PHS resistance. a** *Qsd.ahau-2AL* was narrowed down to the 0.47 Mb region containing eight genes. **b–d** Significant differences in germination index (GI) values between lines with *CNGC-312-T* and *CNGC-312-A* in JH-RILs, JW-RILs, and 431WVs. Box plots show the median (center line), interquartile range (box limits), and minimum to maximum values (whiskers). Mean values are indicated above each box (**b**)  $n = 101$  and 52 biologically independent plants for *CNGC-312-T* and *CNGC-312-A*, respectively. \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **c**  $n = 112$  and 53 biologically independent plants for *CNGC-312-T* and *CNGC-312-A*, respectively. \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **d**  $n = 276$  and 136 biologically independent plants for *CNGC-312-T* and *CNGC-312-A*, respectively. \*\* $P < 0.01$ , and \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **e–g** Germination percentages and phenotypes of the *TaCNGC* Cas9-edited lines and wild-type KN199 harvested in the field of Hebei Province (2024 and 2025). Seeds and spikes were imbibed for 3 days. **e** seed germination percentages. Data are

shown as means  $\pm$  SD (biologically independent plants were sampled in Hebei:  $n = 10$  in 2024;  $n = 7$  in 2025.). \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test) (**f**) seed germination phenotypes (**g**) spike germination phenotypes. **h–j** Germination percentages and phenotypes of the *TaCNGC-2A* overexpression lines and wild-type Fielder harvested in the greenhouse (2024) and in the field of Hebei Province (2025). Seeds and spikes were imbibed for 3 days. **h** seed germination percentages. Data are shown as means  $\pm$  SD (biologically independent plants were sampled in greenhouse and Hebei:  $n = 10$  in greenhouse;  $n = 8$  in Hebei). \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  (two-tailed Student's *t*-test). **k–m** Germination percentages and phenotypes of the *TaCNGC* Cas9-edited lines and wild-type AN188 harvested in the greenhouse (2025). Seeds and spikes were imbibed for 3 days. **k** seed germination percentages. Data are shown as means  $\pm$  SD ( $n = 5$  biologically independent plants). \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **n** Photographs of *TaCNGC* Cas9-edited lines and wild-type KN199 in the field of Hebei Province (2024 and 2025). Source data are provided as a Source Data file.

resistance, with the presence of A associated with stronger SD and resistance to PHS.

### *TaCNGC-2A* negatively regulates wheat SD and PHS resistance

Further efforts were made to assess the role of *TaCNGC-2A* in wheat SD and PHS resistance and to identify the downstream molecular components or pathways. We obtained an ethyl methane sulfonate (EMS) mutant with premature translation termination in the Jimai 22 (JM22) background, named *cngc-2a-jm22*. The germination percentage (GP) of *cngc-2a-jm22* seeds (53%) was significantly lower than that of JM22 seeds (93%) (Supplementary Fig. 1c, d). Additionally, seed germination in the *cngc-2a-jm22* spikes was significantly delayed compared to the JM22 spikes (Supplementary Fig. 1e). These results demonstrate that the loss-of-function of *TaCNGC-2A* promotes wheat SD and resistance to PHS. Furthermore, we investigated seed germination phenotypes of homologous genes *TaCNGC-2B* (*cngc-2b-j411*) and *TaCNGC-2D* (*cngc-2d-j411*) wheat EMS mutants. There was no significant difference in GP between *cngc-2b-j411*, *cngc-2d-j411* and J411 (Supplementary Fig. 1f–h). These observations suggest that *TaCNGC-2B* and *TaCNGC-2D* may have no effect on SD.

To further confirm the negative role of *TaCNGC-2A* in wheat SD and PHS resistance, we obtained the three independent triple mutants (*aabbdd*) of *TaCNGC*, the single mutants *TaCNGC-2B* (*AAbbDD*) and *TaCNGC-2D* (*AABBdd*) in Kenong199 (KN199, carrying the weak-dormancy allele) through CRISPR/Cas9 gene editing, as well as three *TaCNGC-2A* overexpression lines in Fielder. Field trials in Hebei province showed that the seed GP values were significantly lower in the three Cas9-edited mutants [*cngc-ko-28* (49% and 58% in 2024 and 2025, respectively), *cngc-ko-35* (46% and 63%, respectively), and *cngc-ko-36* (42% and 61%, respectively)] than in KN199 (91% and 91%, respectively) (Fig. 1e, f). Consistently, the germination of seeds in the spikes of the three Cas9-edited mutants was markedly delayed compared to the KN199 spikes (Fig. 1g). Similar results were observed in the field of Henan province (2024) (Supplementary Fig. 1i–k). Phenotypic data in the greenhouse showed that there was no significant difference in seed germination percentage between *cngc-2b-ko* (91%) and KN199 (94%), as well as between *cngc-2d-ko* (94%) and KN199 (94%) (Supplementary Fig. 1l, m). By contrast, the seed GP values of three *TaCNGC-2A* overexpression lines grown in the greenhouse (2024) and in the field of Hebei province (2025) were all higher than that of wild-type Fielder (Fig. 1h, i). Furthermore, seed germination started earlier in spikes of the three overexpression lines than in the Fielder spikes (Fig. 1j). These results further demonstrate the role of *TaCNGC-2A* in reducing wheat SD and promoting PHS.

To further explore the breeding value of *TaCNGC-2A*, we crossed the Cas9-edited line *cngc-ko-28* with modern variety Annong188 (AN188) and obtained an  $F_2$  population containing 2156 lines. The seed GP values of homozygous mutation-type lines (*aabbdd*, 59%) was

significantly lower than that of the AN188-type lines (92%) without affecting their eventual germination capacity (Supplementary Fig. 1n, o). In addition, three knockout lines (*cngc-ko-an-1/2/3*) were generated by CRISPR-Cas9 in AN188. The GP of *cngc-ko-an-1/2/3* (58%, 52%, and 56%, respectively) was significantly lower than that of AN188 (87%) (Fig. 1k, l). Consistently, the germination of seeds in the spikes of the three Cas9-edited mutants was markedly delayed compared to the AN188 spikes (Fig. 1m). These results demonstrate that the knockout of *TaCNGC-2A* can effectively enhance PHS resistance of modern varieties.

We further examined whether the *TaCNGC* Cas9-edited seeds exhibited phenotypes in seedling emergence rate and uniformity in the field. The germination test showed that all the Cas9-edited, KN199, and AN188 seeds reached nearly 100% germination rate when they were kept at room temperature at 25 °C for one month (representing full dormancy release). Field trials indicated that the loss-of-function of *TaCNGC-2A* in KN199 had no negative effects on yield-related traits such as plant height, tillering, spike architecture, and grain size and weight in the three field environments (Fig. 1n, and Supplementary Fig. 1p, q). These results indicate that *TaCNGC-2A* is useful for improving PHS resistance in wheat by gene editing.

### *TaCNGC-2A* ortholog similarly repressed SD and PHS resistance in rice

To test if the role of *TaCNGC-2A* is conserved in cereals, we overexpressed *TaCNGC-2A* in rice and knocked out the rice orthologous gene *OscNGC6* using genome-editing, which generated three overexpression and two mutant lines, respectively, in the rice Zhonghua 11 (ZH11) background. The three overexpression lines exhibited small but significant higher seed GP than in ZH11, whereas the seed GP values of two Cas9-edited mutant lines were reduced by 30–35% in comparison to that of ZH11 (Supplementary Fig. 1r). Further, the seeds from the panicles of the selected overexpression line, 35S:*TaCNGC-2A* #2 were germinated significantly faster than that in the ZH11 panicles, while those from the *oscngc6-1/2* panicles showed the opposite (Supplementary Fig. 1s). These results indicate that *TaCNGC-2A* shortens SD and diminishes resistance to PHS in rice, and the *OscNGC6* had a similar role as its counterpart in wheat.

### TaMYB-5B and TaMYB-5D directly bind to the *TaCNGC-2A* promoter and synergistically suppress *TaCNGC-2A* expression

Prediction analysis using PlantPAN (<https://plantpan.ips.ncku.edu.tw/>) on promoter *cis*-elements revealed that the T/A mutation (-312 bp) may affect the binding to GATA, TIFY, B3, and MYB transcription factors. To identify the upstream transcription factors that bind these motifs, we generated *TaCNGC-2A*<sup>312A</sup> (extending 10 bp before and after the A site at -312 bp) and *TaCNGC-2A*<sup>312T</sup> (extending 10 bp before and after the T site at -312 bp) DNA fragments for yeast one-hybrid (Y1H)

screening of the wheat seed cDNA library (Supplementary Fig. 2a). Self-activation experiments showed that TaCNGC-2A<sup>312A</sup> could inhibit the growth of background strain under the concentration of 900 ng/mL Aureobasidin A (AbA), while TaCNGC-2A<sup>312T</sup> did not. Therefore, TaCNGC-2A<sup>312A</sup> was selected for Y1H screening. A total of four transcription factors were identified, including *TraesCS5B02G407600* (TaMYB-5B), *TraesCS5D02G183000* (TaMYB-5D), *TraesCS4D02G071500* (TaB3-4D), and *TraesCS3B02G017400* (TaNF-YB2). Among them, TaNF-YB2 was excluded for further analysis because its expression levels had no significant difference between J411 and HMC21 seeds imbibed for 4, 6, and 10 h based on transcriptome data and qRT-PCR results (Supplementary Data 3 and Supplementary Fig. 2b).

Y1H assay showed that TaMYB-5B, TaMYB-5D, and TaB3-4D can bind to TaCNGC-2A<sup>312A</sup> (Fig. 2a). Subsequently, we performed the LUC reporter assay in which the LUC reporter cDNA was fused with the CNGC-312-T (906 bp promoter containing T at -312 bp) and CNGC-312-A (906 bp promoter containing A at -312 bp), respectively (Fig. 2b). In the presence of TaMYB-5B, the LUC activity driven by CNGC-312-T and CNGC-312-A was significantly reduced compared to the control group, with stronger effect on LUC activity driven by CNGC-312-A than that driven by CNGC-312-T (Fig. 2c). On the other hand, the presence of TaMYB-5D significantly reduced the LUC activity driven by CNGC-312-A but not that driven by CNGC-312-T (Fig. 2d). By contrast, the presence of TaB3-4D had no effect on the LUC activity driven by either CNGC-312-T or CNGC-312-A (Supplementary Fig. 2c). Finally, the electrophoretic mobility shift assay (EMSA) showed that TaMYB-5B can bind to the 5'-AGGCTTC-3' probe in TaCNGC-2A<sup>312T</sup> and the 5'-AGGCATC-3' probe in TaCNGC-2A<sup>312A</sup>, and TaMYB-5D can specifically bind to the 5'-AGGCATC-3' probe in TaCNGC-2A<sup>312A</sup> (Fig. 2e, f). Furthermore, chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) assay confirmed the binding of TaMYB-5B and TaMYB-5D to the TaCNGC-2A promoter in vivo (Fig. 2g).

Considering that TaMYB-5B and TaMYB-5D can bind the TaCNGC-2A promoter, we speculate that the two MYB transcription factors might interact with each other. This hypothesis was indeed verified by a set of experiments including bimolecular fluorescence complementation (BiFC), co-immunoprecipitation (Co-IP), and pull-down assays (Fig. 2h–j). We further found that the co-expression of TaMYB-5B and TaMYB-5D had a stronger transcriptional inhibitory effect on CNGC-312-A and CNGC-312-T than that of TaMYB-5B or TaMYB-5D alone, and that the LUC activity driven by CNGC-312-A was reduced much more than that driven by CNGC-312-T after co-expression (Fig. 2k). These results indicate that TaMYB-5B and TaMYB-5D synergistically suppress TaCNGC-2A expression.

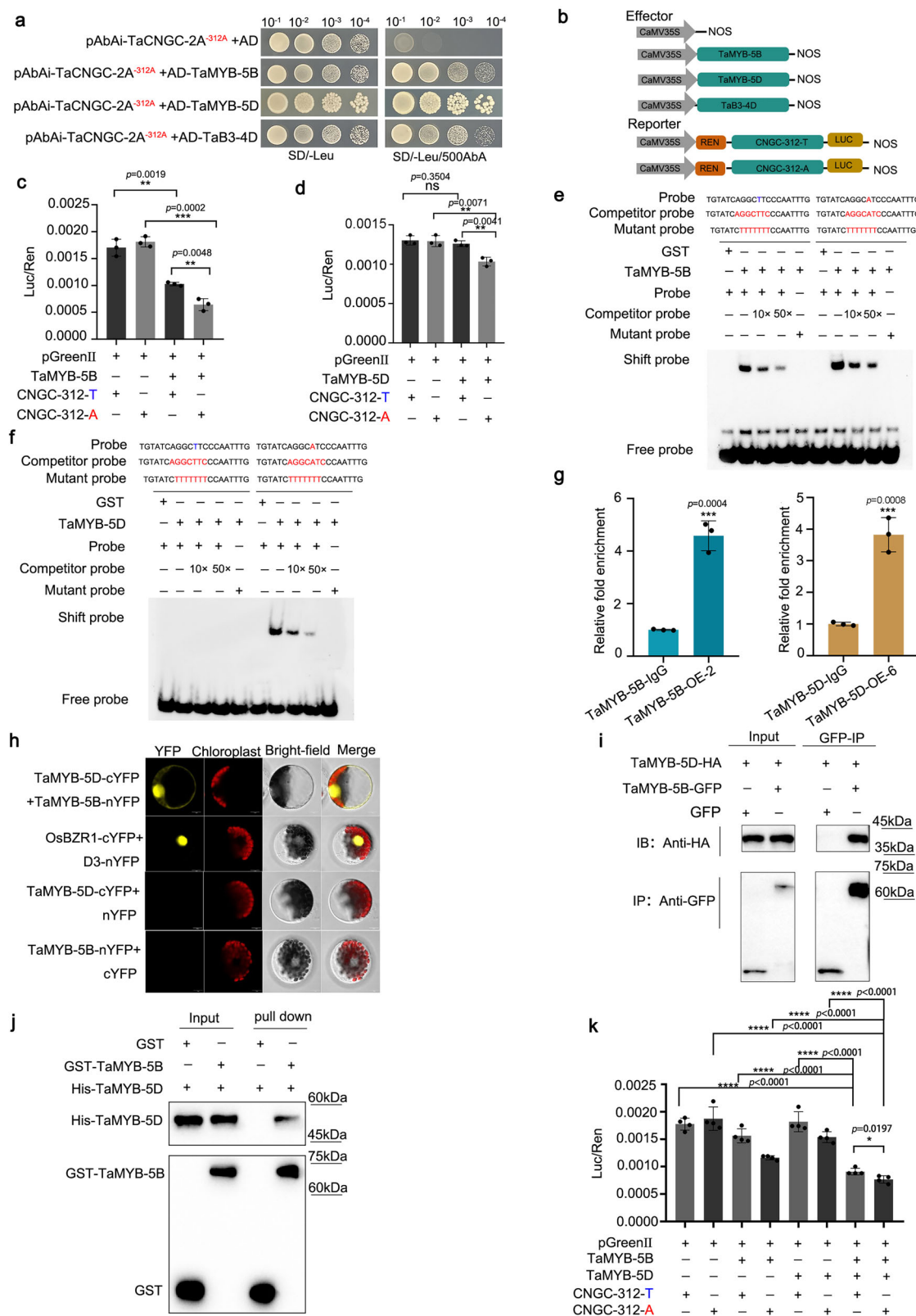
To assess the role of TaMYB-5B and TaMYB-5D in SD and PHS, we overexpressed TaMYB-5B and TaMYB-5D in Fielder (carrying A in -312 bp), respectively. The germination experiments indicated that this genetic intervention reduced the GP of the overexpressed seeds by approximately 30–40% compared to wild-type seeds in the greenhouse (2024) and in the field (2025) (Fig. 3a–d). Furthermore, seed germination on the spikes was significantly delayed in three overexpressed lines of TaMYB-5B and TaMYB-5D compared with wild-type Fielder spikes (Fig. 3e, f). Conversely, the EMS mutants of TaMYB-5B and TaMYB-5D displayed higher seed GP values than that of the background genotype, J411 (Supplementary Fig. 2d, e). To further investigate the genetic interaction between these two MYB transcription factors and TaCNGC-2A, we attempted to generate double overexpression lines via genetic crossing, named *5B-OE-2/OE-1#1/2* and *5D-OE-6/OE-1#1/2*. Overexpression of TaCNGC-2A partially restored the reduced germination phenotypes resulting from TaMYB-5B and TaMYB-5D overexpression (Fig. 3g, h). We further generated TaMYB-5B/5D dual overexpression lines (*OE-1#5B-5D\_OE1* and *OE-1#5B-5D\_OE2*) in the TaCNGC-OE-1 background via co-transformation. qRT-PCR analysis showed that the expression levels of TaCNGC-2A were

significantly reduced in the dual overexpression lines compared to TaCNGC-OE-1. Germination assays indicated that the overexpression of TaMYB-5B and TaMYB-5D decreased the germination rate of TaCNGC-OE-1 (Fig. 3i–k). These results, together with that in Fig. 2, indicate that TaMYB-5B and TaMYB-5D, as upstream regulatory factors, enhance SD and resistance to PHS in wheat through suppressing TaCNGC-2A expression.

### Calmodulin TaCaM-3A interacts with TaCNGC-2A

CNGCs typically possess multiple CaM-binding domains (CaMBD) at N- and C-terminus regions, with the conserved isoleucine–glutamine (IQ) motif within the CaMBD interacting with CaMs<sup>27,28</sup>. Prediction analysis on protein structure indicated that TaCNGC-2A is primarily composed of six ion transport transmembrane helices, a cyclic nucleotide-binding domain (CNBD), and a CaMBD encompassing  $\alpha$ C and IQ domains (Supplementary Fig. 3a). AtCNGC6, as the Arabidopsis homologs of TaCNGC-2A, was found to bind to calmodulin isoforms AtCaM1/2/3/4/5/6/7 to varying degrees, and this binding was dependent on the presence of the IQ motif<sup>29</sup>. Given these observations, we identified 10 wheat homologous genes (containing three sets of homologous genes located in the A/B/D sub-genome of wheat) of AtCaM1/2/3/4/5/6/7 based on sequence alignment. We then analyzed their expression patterns in our previous transcriptome sequencing data from the seeds of J411 and HMC21 after 4 h, 6 h, and 10 h of imbibition (Supplementary Fig. 3b). Among them, the expression levels of eight genes exhibited significant differences between J411 and HMC21 seeds at different imbibition stages. Subsequently, we selected three representative genes (*TraesCS1D02G308100*, TaCaM-1D; *TraesCS2D02G097500*, TaCaM-2D; *TraesCS3A02G184500*, TaCaM-3A) with relatively high expression levels from each of the three sets of homologous genes and *TraesCS4B02G178200* (TaCaM-4B) for further analysis. Luciferase complementation assays showed that TaCaM-1D, TaCaM-2D, TaCaM-3A, and TaCaM-4B all interacted with TaCNGC-2A to varying degrees. Particularly, the interaction between TaCaM-3A and TaCNGC-2A was obviously stronger than that between TaCaM-1D/2D/4B and TaCNGC-2A (Supplementary Fig. 3c). Based on these results, TaCaM-3A was selected for further analysis.

We then conducted the Y2H assay, which showed a physical interaction between TaCNGC-2A and TaCaM-3A (Fig. 4a), a finding verified by BiFC assay, revealing that TaCNGC-2A interacts with TaCaM-3A in the endoplasmic reticulum (ER) and cytoplasm based on the YFP fluorescence (yellow) of TaCNGC-2A-cYFP and TaCaM-3A-nYFP overlapped well with the red fluorescence of the ER marker (ER-MKate) and the cytoplasmic marker (Cytosol-MKate) (Fig. 4b). Co-IP assays confirmed the interaction of TaCNGC-2A with TaCaM-3A in vivo (Fig. 4c). We further observed that this interaction was enhanced with increasing Ca<sup>2+</sup> concentrations (Fig. 4d). In addition, using InterPro (<https://www.ebi.ac.uk/interpro/>), we predicted the key Ca<sup>2+</sup>-binding sites within the four EF-hands of TaCaM-3A. We then generated a Ca<sup>2+</sup>-binding-defective mutant (TaCaM-3A-mut) by site-directed mutagenesis of these key Ca<sup>2+</sup>-binding sites to alanine (Ala) (Supplementary Fig. 3d). Co-IP assays indicated that TaCaM-3A-mut exhibited a markedly reduced binding affinity for TaCNGC-2A compared to TaCaM-3A, suggesting that this interaction is Ca<sup>2+</sup> dependent (Fig. 4e). Subsequently, we also performed a truncation experiment, dividing TaCNGC-2A into the CNBD and CaMBD regions. Y2H assays showed that TaCaM-3A interacts with CaMBD, but not CNBD. Further division of CaMBD into  $\alpha$ C and IQ fragments revealed that TaCaM-3A interacts specifically with the IQ domain in Y2H assays (Fig. 4f), which was confirmed by pull-down assays (Fig. 4g). We further truncated each of the four EF-hands of TaCaM-3A, and then conducted the Y2H assays, respectively. The results showed that only TaCaM-3A containing all four EF-hands interacted with TaCNGC-2A-IQ. The truncation of any one of these four EF-hands resulted in the failure of the interaction between TaCNGC-2A-IQ and EF-hand 1/2/3/4 (Supplementary Fig. 3e).



These observations suggest that these four EF-hands, as a whole, are crucial for the interaction between TaCNGC-2A and TaCaM-3A.

### TaCaM-3A negatively regulates wheat SD and PHS resistance

To explore the role of *TaCaM-3A* in wheat SD and PHS resistance, we obtained two wheat EMS mutants with missense mutations in the EF-hand domain, including *cam-3a-jm22* in JM22 and *cam-3a-j411* in J411.

The seed GP values of *cam-3a-jm22* (59%) and *cam-3a-j411* (54%) were significantly lower than those of JM22 (93%) and J411 (91%), respectively (Supplementary Fig. 4a–d). Furthermore, the mutations significantly delayed seed germination from the spikes, compared to that of the JM22 and J411 (Supplementary Fig. 4e, f).

*TaCaM* was also knocked out in KN199 with Cas9-edited approach, knocked down in Fielder using RNAi method, and

**Fig. 2 | The MYB transcription factors TaMYB-5B and TaMYB-5D directly bind the *TaCNGC-2A* promoter and synergistically suppress *TaCNGC-2A* expression.**

**a** TaMYB-5B, TaMYB-5D, and TaB3-4D interact with the *TaCNGC-2A*<sup>-312A</sup> fragment (extending 10 bp before and after the A site at -312 bp) in the yeast one-hybrid assay. **b** Diagrams of the constructs used in dual luciferase reporter gene experiments. **c** TaMYB-5B binds to the CNGC-312-T (906 bp promoter, -312 bp, T) and CNGC-312-A (906 bp promoter, -312 bp, A) promoter in the LUC activity assay. Data are shown as means  $\pm$  SD ( $n = 3$  biologically independent experiments). \*\* $P < 0.01$  and \*\*\* $P < 0.001$  (two-tailed Student's *t*-test). **d** TaMYB-5D binds to the CNGC-312-A promoter in the LUC activity assay. Data are shown as means  $\pm$  SD ( $n = 3$  biologically independent experiments). \*\* $P < 0.01$ , and ns, not significant (two-tailed Student's *t*-test). **e** TaMYB-5B binds to the *TaCNGC-2A*<sup>-312T</sup> (extending 10 bp before and after the T site at -312 bp) and *TaCNGC-2A*<sup>-312A</sup> fragments in the EMSA assay. The experiment

was repeated 3 times with similar results. **f** TaMYB-5D binds to the *TaCNGC-2A*<sup>-312A</sup> fragment in the EMSA assay. The experiment was repeated 3 times with similar results. **g** ChIP-qPCR assays of TaMYB-5B and TaMYB-5D binding to the promoter of *TaCNGC-2A* in vivo. Immunoprecipitation was performed in the seedlings of *TaMYB-5B-OE-2* and *TaMYB-5D-OE-6* with anti-FLAG antibody. Data are shown as means  $\pm$  SD ( $n = 3$  biologically independent experiments). \*\*\* $P < 0.001$  (two-tailed Student's *t*-test). **h–j** Interaction between TaMYB-5B and TaMYB-5D in the BiFC, Co-IP, and GST pull-down assays. The experiments in **i** and **j** were repeated 3 times with similar results. **k** TaMYB-5B and TaMYB-5D synergistically suppress the transcription of CNGC-312-T and CNGC-312-A in the LUC activity assay. Data are shown as means  $\pm$  SD ( $n = 4$  biologically independent experiments). \* $P < 0.05$  and \*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). Source data are provided as a Source Data file.

overexpressed in Fielder. The germination experiments indicated that the seed GP values were significantly reduced in the three Cas9-edited mutant lines [*cam-ko-2* (64%), *cam-ko-3* (61%), and *cam-ko-6* (62%)] in comparison with KN199 (89%) in the field of Hebei Province (2025) (Fig. 5a, b). The three RNAi lines *cam-rnai-5* (63%), *cam-rnai-8* (57%), and *cam-rnai-9* (62%) had significantly lower seed GP values than Fielder (85%), while the three overexpression lines *CaM-OE-2* (95%), *CaM-OE-8* (96%), and *CaM-OE-9* (89%) had significantly higher seed GP values (Fig. 5c, d). Additionally, seed germination in the spikes of Cas9-edited and RNAi lines was significantly delayed compared to KN199 and Fielder spikes (Fig. 5e, f). In contrast, the seeds in the spikes of these overexpressed lines exhibited significantly earlier germination compared to Fielder (Fig. 5f). Similar results were observed in the field of Henan Province (2025) (Supplementary Fig. 4g–l). These findings indicate that *TaCaM-3A* shortens wheat SD and diminishes resistance to PHS, similar to *TaCNGC-2A*.

To further explore the genetic interaction of *TaCaM-3A* and *TaCNGC-2A*, we constructed the *cam-ko-3/TaCNGC-OE-1* mutant/overexpression lines (*ko-3/OE-1/#1* and *ko-3/OE-1/#2*) through hybridization, and conducted SD assessment. We found that overexpression of *TaCNGC-2A* did not restore the reduced germination phenotype of *cam-ko-3* (Fig. 5g, h). In addition, we obtained the *TaCaM-3A* overexpression lines in the *TaCNGC* Cas9-edited mutant background (*ko-28/CaM\_OE1* and *ko-28/CaM\_OE2*). Germination assays showed that *TaCaM-3A* overexpression could rescue the germination defect phenotype of the *cngc-ko-28* mutant (Fig. 5i, j). These findings indicate that *TaCaM-3A* functions downstream of *TaCNGC-2A* in repressing wheat SD.

We further examined whether the *TaCaM* Cas9-edited and RNAi seeds exhibited phenotypes in seedling emergence rate and uniformity in the field. The germination test showed that all the Cas9-edited, RNAi, KN199, and Fielder seeds reached nearly 100% germination rate when they were kept at room temperature for one month. Field trials conducted in Hebei showed that compared with wild-type, grain length (GL) of the *TaCaM* Cas9-edited and RNAi lines significantly increased, while there was no significant difference in grain width (GW), resulting in a significant increase in thousand-grain weight (TGW). The *TaCaM* Cas9-edited and RNAi lines showed enhanced yield performance, with per-plant yield increased by 12–20% and 5–13%, respectively (Fig. 5k–n). In contrast, *TaCaM-3A* overexpression lines produced smaller grains, with a significant decrease in GL, GW, and TGW. Furthermore, *TaCaM-3A* overexpression lines exhibited reduced grain yield, with per-plant yield decreasing by 2–8% compared with Fielder (Fig. 5m, n). In addition, *TaCaM-3A* had no negative effects on other agronomic traits such as plant height, tillering, and spike architecture related traits (Fig. 5o, p). Field trials in Henan also showed a similar trend (Supplementary Fig. 4m–q). These results suggest that *TaCaM-3A* not only enhances wheat PHS resistance through gene editing, but also increases grain yield.

**Pathways by which *TaCNGC-2A* mediates SD and resistance to PHS**

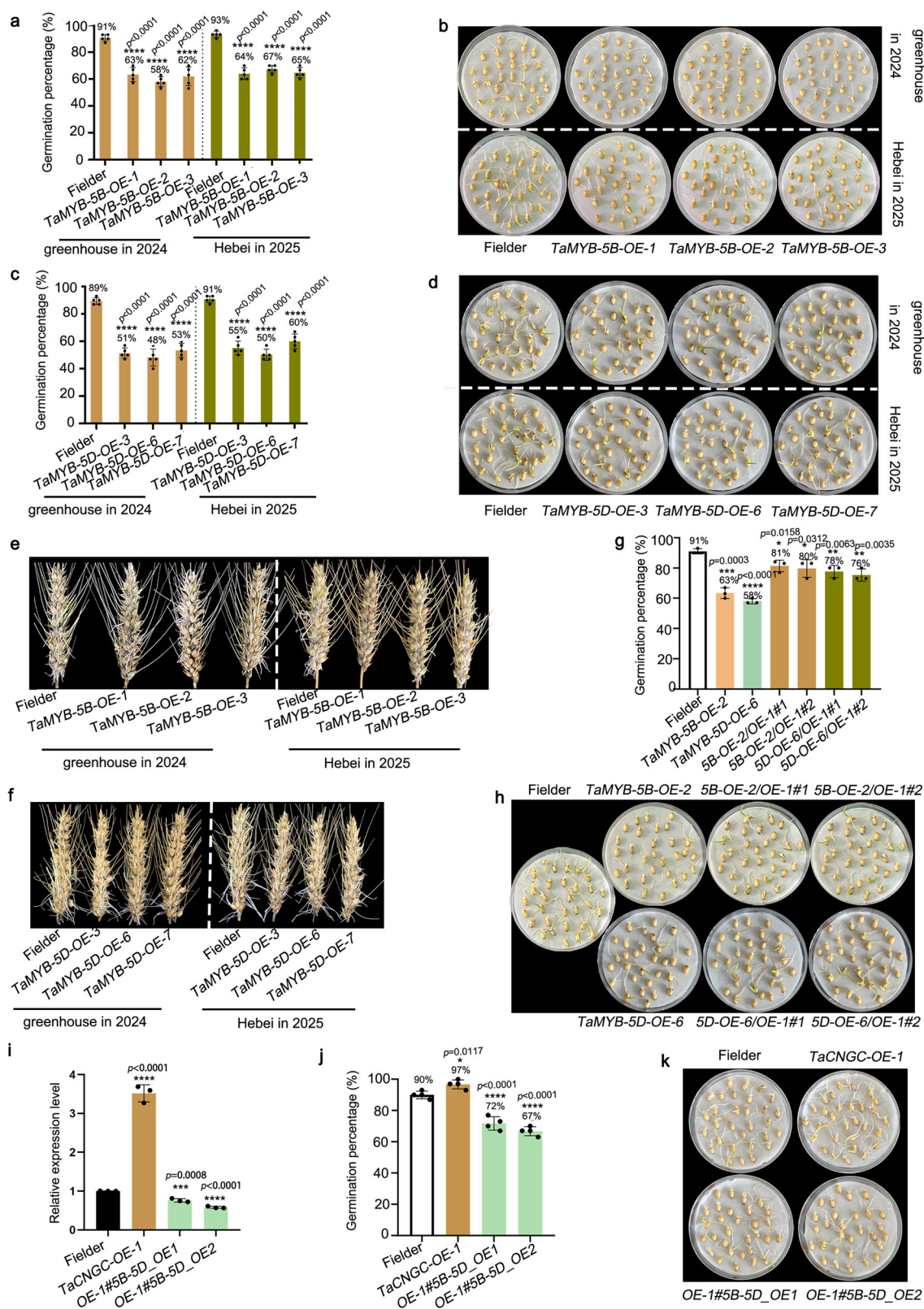
To explore the regulatory network mediated by *TaCNGC-2A*, we conducted a comparative transcriptomic analysis between *cngc-ko-28* and KN199 seeds imbibed for 30 min, 1 h, 18 h, and 36 h (Supplementary Fig. 5a). The gene ontology (GO) analysis revealed that differentially expressed genes (DEGs) were primarily enriched in the calcium ion binding pathway (GO:0005509) (Supplementary Data 5). Subsequently, we confirmed the expression patterns of seven DEGs (*TaCDPK40*, *TaCBL2*, *TaCIPK3*, etc.) involved in this pathway by performing qRT-PCR using the seeds imbibed for 18 h (Supplementary Fig. 5b, c). We further utilized ICP-MS to measure  $\text{Ca}^{2+}$  contents in the seeds of *TaCNGC* Cas9-edited line *cngc-ko-28* and KN199 after 18 h of imbibition. The  $\text{Ca}^{2+}$  contents in *cngc-ko-28* seeds was significantly lower than that in KN199 seeds (Supplementary Fig. 5d). These observations suggest that *TaCNGC-2A* regulates SD by modulating intracellular  $\text{Ca}^{2+}$  levels and the expression of genes involved in the  $\text{Ca}^{2+}$  signaling pathway.

In addition, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that DEGs were significantly enriched in plant hormone signal transductions (map04075) (Supplementary Fig. 5e–h and Supplementary Data 6). Subsequently, we verified the expression patterns of 11 key genes related to the abscisic acid (ABA), Jasmonates (JA), auxin (AUX), ethylene (ETH), gibberellin (GA), brassinolide (BR), and cytokinin (CTK) pathways, in *cngc-ko-28*, *TaCNGC-OE-1* and wild-type KN199 and Fielder seeds by qRT-PCR (Supplementary Fig. 5i, j). We further measured the contents of ABA, GA, Jasmonates, and AUX, as well as the activity of  $\alpha$ -amylase in *cngc-ko-28* and KN199 seeds imbibed for 18 h. The GA<sub>3</sub> and IAA contents and  $\alpha$ -amylase activity were significantly lower in *cngc-ko-28* seeds than in KN199 seeds. In contrast, the ABA and JA-ILE contents were significantly higher in *cngc-ko-28* seeds than in KN199 seeds (Supplementary Fig. 5k). These observations indicate that knockout of *TaCNGC-2A* reduced the level of growth hormones and potentially starch degradation, but increased those hormones repressing growth, thereby enhancing SD.

It is noteworthy that eight known dormancy-related genes, including *TaDOG1L1*<sup>30</sup>, *TaMFT-3A/TaPHS1*<sup>6,13</sup>, *TaSdr*<sup>19</sup>, *TaPM19*<sup>31</sup>, *TaMKK3-A*<sup>15</sup>, *TaQsd1-5B*<sup>32</sup>, *TaGATA124*, and *TaPI4K-2A*<sup>17</sup>, were also differentially expressed in *cngc-ko-28*, *TaCNGC-OE-1*, and wild-type KN199 and Fielder seeds. The expression trends of these genes were further confirmed by qRT-PCR (Supplementary Fig. 5l, m and Supplementary Data 7–9). These results reveal that the overexpression and knockout of *TaCNGC-2A* affected not only multiple hormonal pathways but also several dormancy-related genes.

**Nature variations and allele frequency analysis of *TaCNGC-2A***

To explore natural variations of *TaCNGC-2A*, we cloned *TaCNGC-2A* gene in 213 wheat genotypes with diverse genetic backgrounds. Sequence analysis revealed six SNPs in the coding region (two non-synonymous and four synonymous mutations), two in the promoter,

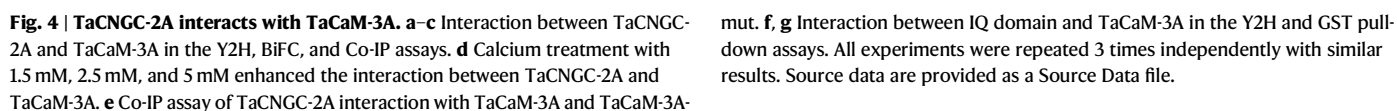


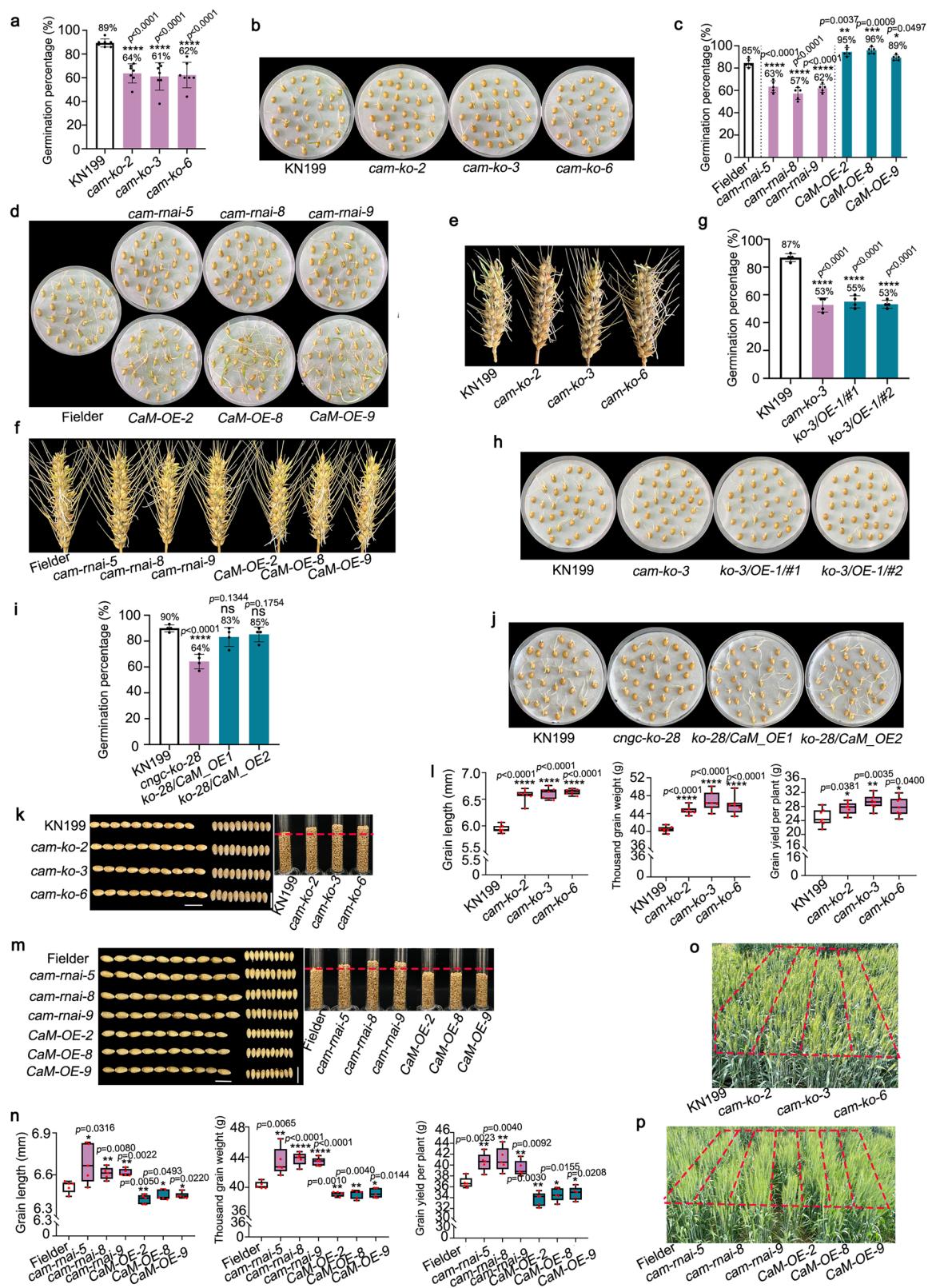
and 24 in the intron of *TaCNGC-2A*. Apart from the four variants mentioned above [two non-synonymous SNPs (G/C, +856 bp; T/A, +2467 bp) in the coding region and two (G/A, -514 bp; T/A, -312 bp) in the promoter], there were no mutations in the promoter and coding regions of *TaCNGC-2A*.

Among the 213 wheat genotypes, 107 (50.2%) carried the A allele (-312-A) associated with strong SD and resistance to PHS and 106

(49.8%) carried the T allele (-312-T) associated with weak SD and PHS resistance (Fig. 6a). The U-test results showed significant differences in GI values between genotypes carrying -312-A and -312-T in 213 wheat genotypes. However, no difference in GI was observed between genotypes carrying -514-A and -514-G, +856-C and +856-G, and +2467-A and +2467-T. These results confirm that the T/A mutation at -312 bp is the key variation determining SD and PHS resistance differences (Fig. 1 vs

phenotypes of *5B-OE-2/OE-1#1/2*, *5D-OE-6/OE-1#1/2*, *TaMYB-5B-OE-2*, *TaMYB-5D-OE-6*, and wild-type Fielder. Seeds were imbibed for 3 days. **g** seed germination percentages. Data are shown as means  $\pm$  SD ( $n = 3$  biologically independent plants). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **i** Expression analysis of *TaCNGC-2A* in overexpression line *TaCNGC-OE-1*, and dual overexpression lines *OE-1#5B-SD\_OE1* and *OE-1#5B-SD\_OE2*. Data are shown as means  $\pm$  SD ( $n = 3$  biologically independent experiments). \*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **j, k** Germination percentages and phenotypes of *OE-1#5B-SD\_OE1*, *OE-1#5B-SD\_OE2*, *TaCNGC-OE-1*, and Fielder. Seeds were imbibed for 3 days. **j** seed germination percentages. Data are shown as means  $\pm$  SD ( $n = 4$  biologically independent plants). \* $P < 0.05$  and \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). Source data are provided as a Source Data file.





6b and Supplementary Data 4). Furthermore, of the 107 genotypes carrying -312-A, 44 (41.1%) were improved varieties and 63 (58.9%) were landraces (Fig. 6a). These results indicate that the favorable A allele (-312 bp), associated with strong dormancy, is more frequent in landraces and has a potential value for breeding wheat varieties with high PHS resistance.

### Effect of combining *TaCNGC-2A* with known dormancy-related genes on SD and PHS resistance

Finally, we exploited the possibility of pyramiding *TaCNGC-2A* with other genes involved in SD for molecular breeding. We genotyped markers for six known dormancy-related genes in 177 of the 213 wheat genotypes described above: MFT-194 for *TaMFT-3A*, Vp1-b3 for *TaVp-1*,

**Fig. 5 | Role of *TaCaM-3A* in wheat SD.** **a, b** Germination percentages and phenotypes of the *TaCaM* Cas9-edited lines and wild-type KN199 harvested in Hebei (2025). Seeds were imbibed for 3 days. **a** seed germination percentages. Data are shown as means  $\pm$  SD ( $n = 7$  biologically independent plants). \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **c, d** Germination percentages and phenotypes of the *TaCaM* RNAi lines, *TaCaM-3A* overexpression lines, and wild-type Fielder harvested in Hebei (2025). Seeds were imbibed for 3 days. **c** seed germination percentages. Data are shown as means  $\pm$  SD ( $n = 5$  biologically independent plants). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **e, f** Germination images of the *TaCaM* Cas9-edited and RNAi lines, *TaCaM-3A* overexpression lines, and wild-type KN199 and Fielder harvested in the field of Hebei Province (2025). Spikes were imbibed for 3 days. **g, h** Germination percentages and phenotypes of *ko-3/OE-1#1/2*, *cam-ko-3*, and KN199 harvested in the greenhouse (2025). Seeds were imbibed for 3 days. **g** seed germination percentages. Data are shown as means  $\pm$  SD ( $n = 4$  biologically independent plants). \*\*\*\* $P < 0.0001$  (two-tailed

Student's *t*-test). **i, j** Germination percentages and phenotypes of *ko-28/CaM\_OEI/2*, *cngc-ko-28*, and KN199 harvested in the greenhouse (2025). Seeds were imbibed for 3 days. **(i)** seed germination percentages. Data are shown as means  $\pm$  SD ( $n = 4$  biologically independent plants). ns, not significant and \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **k–n** Grain length, thousand grain weight, and per-plant yield of the *TaCaM* Cas9-edited and RNAi lines, *TaCaM-3A* overexpression lines, and wild-type KN199 and Fielder. Five-hundred wheat seeds were placed in each measuring cylinder. Box plots show the median (center line), interquartile range (box limits), and minimum to maximum values (whiskers). Scale bars = 1 cm. **l** Data are shown as means  $\pm$  SD ( $n = 7$  biologically independent plants). \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **n** Data are shown as means  $\pm$  SD ( $n = 5$  biologically independent plants). \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\*\* $P < 0.0001$  (two-tailed Student's *t*-test). **o, p** Photographs of *TaCaM* Cas9-edited and RNAi lines, *TaCaM-3A* overexpression lines, and wild-type KN199 and Fielder in the field of Hebei Province (2025). Source data are provided as a Source Data file.

MYB10-D1 for *TaMyb10-D*, MKK3 for *TaMKK3-A*, Qsd1 for *TaQsd1-5B*, and PI4K for *TaPI4K-2A*. For each marker (including CNGC-312 for *TaCNGC-2A*), two alleles were identified: *R* (strong dormancy and PHS resistance) and *S* (weak dormancy and PHS susceptibility) (Supplementary Data 10). We particularly focused on the effect of the *R* allele of *TaCNGC-2A* combined with the *R* allele of the six known dormancy-related genes on SD. A total of 63 combined allele types were identified, including six *RR*, 15 *RRR*, 20 *RRRR*, 15 *RRRRR*, six *RRRRRR*, and one *RRRRRRR* (Supplementary Fig. 6a–f). Phenotypic analysis revealed six allele combinations had lower GI values: *CNGC-312/MKK3* of *RR*, *CNGC-312/MKK3/MFT-194* of *RRR*, *CNGC-312/MKK3/MFT-194/Qsd1* of *RRRR*, *CNGC-312/MKK3/MFT-194/Qsd1/Vp1-b3* of *RRRRR*, *CNGC-312/MKK3/MFT-194/Qsd1/Vp1-b3/PI4K* of *RRRRRR*, and the single *RRRRRRR* combination *CNGC-312/MKK3/MFT-194/Qsd1/Vp1-b3/PI4K/MYB10-D1*. We further found that among these allele combinations, GI was negatively correlated with the frequency of the *R* alleles, suggesting that pyramiding the *R* allele of *TaCNGC-2A* with more *R* alleles of other dormancy-related genes results in stronger SD and resistance to PHS (Fig. 6c).

## Discussion

SD is essential for the survival of plants and ensures that seeds germinate only at the right time for growth and reproduction. As such, it is an adaptive trait with far-reaching significance for the evolution of plant species<sup>33</sup>. For many important crops such as wheat, rice and maize, SD is a desired trait because it prevents PHS or vivipary<sup>12</sup>. Broadly, SD is affected by both external signals, mainly, light, temperature, water and internal factors, including hormones, small molecules such as NO, nitrate, ROS, and transcriptional and post-transcriptional regulators including transcriptional factors, microRNA, protein kinase, histone acetylase<sup>34–37</sup>. However, despite extensive research, the exact genetic and regulatory mechanisms underlying SD remain largely unknown, especially for heterohexaploid wheat with complex and large genomes, the most important crop as human food source. To this end, although at least 188 valid QTLs controlling SD or PHS resistance have been identified<sup>12</sup>, the causal genes of most of them have not been elucidated, and the up- and/or down-stream molecular components and their breeding potentials of a few identified causal genes remain unclear, hindering the development of PHS resistant wheat varieties.

Here, we identified a gene *TaCNGC-2A*, encoding a CNGC protein involved in  $Ca^{2+}$  signaling, underlying the major QTL *Qsd.ahau-2AL* for SD and resistance to PHS in wheat. Previously, *CNGC* genes, encoding  $Ca^{2+}$ -permeable channels, have been reported to participate in diverse aspects of plant development and response to biotic or abiotic stress<sup>38–44</sup>, but their possible roles in regulating cereal SD and PHS resistance remain unknown. In this study, using transgenic and gene-edited materials and EMS mutants, we discovered that *TaCNGC-2A*

negatively modulates wheat SD and resistance to PHS in both greenhouse and field environments.

SD could also be regulated by some MYB transcription factors<sup>45,46</sup>. For example, Lang<sup>46</sup> demonstrated that Myb10-D directly binds to the promoter of *TaNCD* gene involved in ABA biosynthesis to activate its expression, thereby enhancing SD and PHS resistance in wheat. In the present study, we found that two transcription factors, TaMYB-5B and TaMYB-5D, directly bind to the T/A mutation site of *TaCNGC-2A* promoter (-312 bp), which is the key mutation determining SD/PHS resistance differences, and synergistically repress its expression, with mutation to A from T evoking stronger SD and resistance to PHS.

CaMs, as  $Ca^{2+}$  sensors (also  $Ca^{2+}$ -binding proteins), decode, relay and amplify the  $Ca^{2+}$  signatures, and then regulate downstream signal transduction. They play diverse roles in plant development and stress responses<sup>47,48</sup>. However, no studies have reported that CaMs regulate SD or PHS resistance. In this study, we found that *TaCaM-3A* interacts with *TaCNGC-2A* and, similar to *TaCNGC-2A*, negatively modulates wheat SD and PHS resistance.

Our present transcriptome data revealed that many genes such as *TaCDPK40*, *TaCBL2*, and *TaCIPK3* involved in  $Ca^{2+}$  signaling pathway were differentially expressed in *TaCNGC-2A* overexpressed and *TaCNGC* Cas9-edited seeds as well as in *TaCaM-3A* overexpressed and *TaCaM* RNAi seeds. *TaCDPK40* (encoding a calcium-dependent protein kinase), *TaCBL2* (encoding a calcineurin B-like protein) and *TaCIPK3* (encoding a CBL-interacting protein kinase) belong to a group of calcium sensors that sense the  $Ca^{2+}$  flux changes and decode and transmit  $Ca^{2+}$  signature. Although these calcium sensors have been shown to play important roles in response to various developmental and environmental stimuli in plants<sup>49–51</sup>, there have been no reports about their potential functions in wheat SD or PHS resistance. In the current study, the expression levels of these calcium sensor genes were significantly down-regulated in *TaCNGC* Cas9-edited and *TaCaM* RNAi seeds, and significantly up-regulated in *TaCNGC-2A* and *TaCaM-3A* overexpression seeds (Supplementary Fig. 7a, b).

In addition to the calcium sensor genes, many genes involved in hormone signaling pathways and a cohort of known dormancy-related genes were differentially expressed in *TaCNGC-2A* overexpression and *TaCNGC* Cas9-edited seeds, as well as *TaCaM-3A* overexpression and *TaCaM* RNAi seeds (Supplementary Fig. 7c–f). For example, the expression levels of *TaABIS* related to ABA signaling, *TaCOI1* related to JA signaling, and dormancy-related genes *TaDOG1.1*, *TaSdr*, *TaGATA1*, and *TaPI4K-2A* were significantly up-regulated in *TaCNGC* Cas9-edited and *TaCaM* RNAi seeds with strong dormancy, but significantly down-regulated in *TaCNGC-2A* and *TaCaM-3A* overexpression seeds with weak dormancy. However, the expression levels of *TaSLR* related to Auxin signaling, *TaEIL2A* related to ETH signaling, *TaRR3* related to BR signaling, *TaGASR7B* related to GA signaling, and *TaAMY-6A* involved in starch degradation showed the opposite trends. Furthermore,

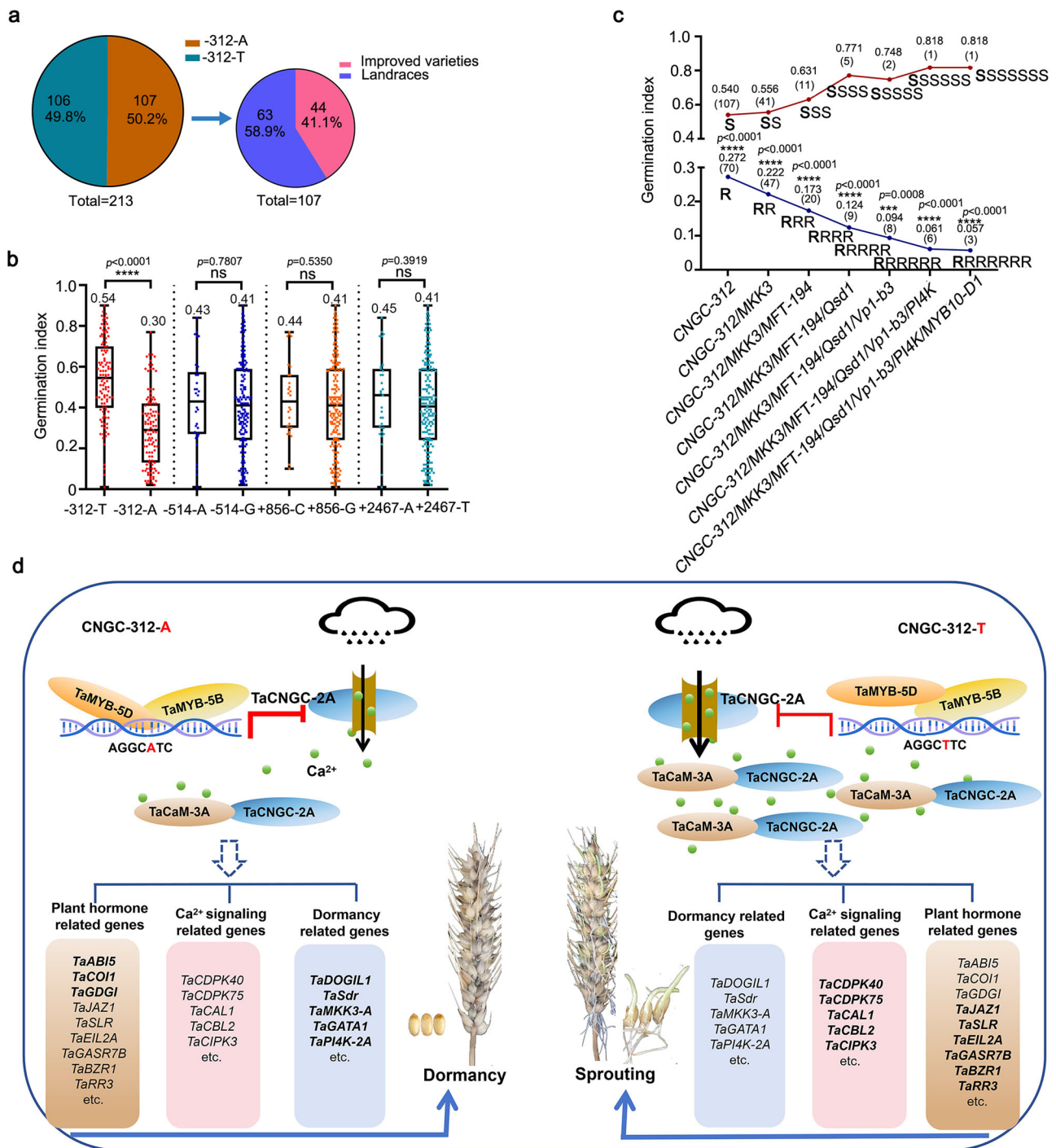


**Fig. 6 | Allele frequency analysis of *TaCNGC-2A*, effect of *TaCNGC-2A* combined with known dormancy genes on SD, A hypothesized working model of *TaCNGC-2A* regulating wheat SD and PHS resistance. **a**** Among 213 wheat genotypes, the proportion of genotypes carrying -312-A associated with strong SD/PHS resistance and -312-T associated with weak SD/PHS resistance, and the proportion of improved varieties and landraces carrying -312-A. **b** Phenotypic differences in SD between wheat genotypes carrying -312-A and -312-T, -514-A and -514-G, +856-C and +856-G, and +2467-A and +2467-T. Box plots show the median (center line), inter-quartile range (box limits), and minimum to maximum values (whiskers). Mean values are indicated above each box.  $n = 106, 107, 32, 181, 28, 185, 31$ , and 182 biologically independent plants for -312-T, -312-A, -514-A, -514-G, +856-C, +856-G, +2467-A, and +2467-T, respectively. ns, not significant, and \*\*\*\* $P < 0.0001$  (two-tailed Student's  $t$ -test). **c** Effect on SD of the *R* allele of *TaCNGC-2A* combined with the *R* allele of the six known dormancy genes. *R* and *S* indicate the strong- and weak-

dormancy allele, respectively. \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$  (two-tailed Student's  $t$ -test). **d** Following stimulation by rainfall, the upstream transcription factors TaMYB-5B and TaMYB-5D bind to the *TaCNGC-2A* promoter and synergistically regulate its expression. Compared with CNGC-312-T, they significantly inhibited the transcription of CNGC-312-A, leading to Ca<sup>2+</sup> channel blocking and reduced Ca<sup>2+</sup> flow from the endoplasmic reticulum into the cytoplasm, thus weakened the interaction between TaCNGC-2A and TaCaM-3A. As a result, the expression levels of genes involved in ABA, JA, and AUX pathways and known dormancy genes were up-regulated, and the expression levels of genes involved in Ca<sup>2+</sup>, JA, AUX, ETH, GA, and CTK pathways were down-regulated, which ultimately enhanced wheat SD and PHS resistance. The genes labeled in bold indicate that their expression levels are up-regulated in *cngc-ko-28*, and the genes not highlighted in bold indicate that their expression levels are down-regulated. Source data are provided as a Source Data file.

compared to the wild-type KN199 seeds with weak dormancy, the contents of GA<sub>1</sub>, GA<sub>3</sub>, IAA, and zeatin and  $\alpha$ -amylase activity were significantly decreased in *TaCNGC* Cas9-edited seeds with strong dormancy, while the contents of ABA and JA were significantly increased. These observations suggest that *TaCNGC-2A* modulates SD and PHS resistance through crosstalk with multiple plant hormone signaling pathways and known dormancy-related genes.

It has been reported that *TaJAZ1* modulated JA signaling to activate wheat PHS by interacting with *TaABI5* and inhibiting its transcriptional activation activity<sup>52</sup>. Wei et al.<sup>24</sup> demonstrated that *TaGATA1* positively regulates wheat SD and PHS resistance by directly binding to the GATA motifs in the *TaABI5* promoter and inducing its expression. Liu et al.<sup>14</sup> found that *TaVp1* and *TaABI5* specifically bind to the RY or ABRE motif in the *TaPHS1/TaMFT-3A* or *TaSdr* promoters to enhance wheat SD/PHS resistance. In this study, compared with wild-type KN199 and Fielder seeds, the expression levels of *TaGATA1*, *TaABI5*, *TaMFT-3A/TaPHS1*, and *TaSdr* were significantly increased in *TaCNGC* Cas9-edited and *TaCaM* RNAi seeds, while their expression levels were significantly decreased in *TaCNGC-2A* and *TaCaM-3A* overexpressed seeds. Combining the phenotypic data and expression trends, we speculate that *TaCNGC-2A* interacts with *TaCaM-3A* to regulate the expression of some downstream dormancy-related genes, thereby partially influencing wheat SD and PHS resistance (Fig. 6d). In addition, these observations indicate that plant hormones and dormancy genes do not regulate SD/PHS resistance independently, but interact with each other. Overall, our findings indicate that *TaCNGC-2A* interacts with *TaCaM-3A* to modulate multiple hormonal and metabolic pathways as well as some dormancy-related genes, thereby regulating SD and PHS resistance (Fig. 6d).

Based on what discussed above, we propose a hypothesized working model of *TaCNGC-2A* regulating wheat SD and PHS resistance. Following stimulation by rainfall, the upstream transcription factors *TaMYB-5B* and *TaMYB-5D* bind to the *TaCNGC-2A* promoter and synergistically suppress its expression. *TaCNGC-2A* interacts *TaCaM-3A* to alter the expression of multiple pathways, thereby reducing or enhancing SD and PHS resistance (Fig. 6d). As such, the findings provide insights into the up- and down-stream molecular components underlying the *TaCNGC-2A*-mediated control of SD and PHS in a major cereal crop, wheat.

It is worth noting that the breeding value of most previously identified genes involved in wheat SD or PHS resistance remains uncharacterized, largely due to the lack of field phenotypic evaluation in the transformed wheat lines or progenies obtained by introducing the target genes into modern varieties. Here, we found that knockout of *TaCNGC-2A* via gene editing significantly enhanced SD and resistance to PHS in wheat cultivar KN199 in field trials, with no adverse effects on yield-related traits. Consistently, the progenies obtained by crossing the Cas9-edited line with modern variety AN188 and the *TaCNGC* knockout mutants generated in the AN188 background exhibited significantly enhanced SD. Noteworthy is that knockout of *TaCaM* not only significantly enhanced SD and PHS resistance, but also increased grain yield. These observations demonstrate high breeding value of *TaCNGC-2A* and its interacting partner *TaCaM-3A* in improving PHS resistance of modern wheat varieties.

It is well known that PHS resistance is a complex quantitative trait controlled by multiple genes. Pyramiding different dormancy/PHS resistance genes is necessary for breeding wheat varieties with high PHS resistance, especially in regions with frequent rainfall during harvest. Through investigation of the effect of *TaCNGC-2A* in combination with six known dormancy-related genes (*TaMFT-3A*, *TaVp1*, *TaMyb10-D*, *TaMKK3-A*, *TaQsd1-5B*, and *TaPI4K-2A*) on SD, we identified six excellent allele combinations associated with strong dormancy and resistance to PHS, which represents valuable resources for efficiently improving wheat PHS resistance through gene pyramiding breeding.

In conclusion, we isolated a candidate gene *TaCNGC-2A* underlying *Qsd.ahau-2AL*, and revealed its negative roles in wheat SD and PHS resistance. We further identified its up- and down-stream molecular players and interacting protein involving calcium and various hormonal signaling and metabolic pathways. We also assessed the practical value of *TaCNGC-2A* in wheat breeding for stronger SD and resistance to PHS, which showed that the frequency distribution of the favorable A allele associated with high SD level and the combined effect of *TaCNGC-2A* with the known dormancy-related genes on SD and PHS resistance. Collectively, our findings (i) uncovered a negative regulator, *TaCNGC-2A*, of SD and resistance to PHS; (ii) dissected its up- and down-stream molecular components, and (iii) demonstrated the value of *TaCNGC-2A* and its interacting protein *TaCaM-3A* for breeding elite wheat germplasm with strong SD and PHS resistance through field trials and gene pyramiding.

## Method

### Plant materials and growth conditions

The RIL population derived from the cross of J411 and HMC21 (174 lines, JH-RILs, F<sub>9</sub>) was used for QTL analysis. The two parents (J411 and HMC21) and two bulk lines (10 lines with strong dormancy and 10 lines with weak dormancy) were used for Wheat 660 K SNP array genotyping. A total of 431 wheat varieties (431WVs) with different dormancy levels were used to determine the correlation between the two allelic variants of *TaCNGC-2A* and SD phenotypes.

JH-RILs and the two parents (J411 and HMC21) were grown in Hefei (HF) during the 2015–2016, 2016–2017, and 2017–2018 cropping seasons. JW-RILs and the two parents (J411 and Wanxianbaimaizi) were grown in HF during the 2015–2016, 2016–2017, and 2017–2018 cropping seasons. A total of 431WVs were grown in HF, Huaibei (HB), Jiyuan (JY), Bengbu (BB), and Suzhou (SZ) during the 2018–2019 and 2019–2020 cropping seasons. A total of 213 wheat varieties were planted in HF, SZ, and HB during the 2021–2022 cropping seasons.

The mutants mutagenized with EMS in wheat cultivars JM22 and J411 backgrounds were used to validate the roles of *TaCNGC-2A/2B/2D*, *TaCaM-3A*, *TaMYB-5B*, and *TaMYB-5D* in SD and PHS resistance, including *cngc-2a-jm22* (jm\_chr2A\_742721342), *cngc-2b-j411* (2B\_752482297\_E), *cngc-2d-j411* (2D\_612708049\_E), *cam-3a-jm22* (jm\_chr3A\_215482139), *cam-3a-j411* (3A\_227361535\_E), *myb-5b-j411-1* (5B\_586724814\_E), *myb-5b-j411-2* (5B\_586724855\_E), and *myb-5d-j411* (5D\_287637914\_E). The EMS mutants in the JM22 and J411 backgrounds were provided by Yantai Jien Biotechnology Company (<http://39.98.48.156:8822/#/>) and Boruidi Biotechnology Company (<http://www.molbreeding.com>), respectively. To minimize interference from genetic background mutations, these mutants were backcrossed three times with J411 and JM22, respectively, followed by self-pollination to obtain BC<sub>3</sub>F<sub>2</sub> progenies.

To perform genome editing using CRISPR/Cas9, we designed a sgRNA for *TaCNGC-2A* and two sgRNAs for *TaCaM-3A* in the second exon homologous regions using the CRISPR direct tool (<http://crispr.hzau.edu.cn/CRISPR2/>). The target areas of *TaCNGC-2A* (gRNA1: AGCCTGAGGTTTCAGCATGCCTGG) and *TaCaM-3A* (gRNA1: GAACGTGATGCGCTCGCTGGG and gRNA2: TCAGGATATGATCAATGAAGTGG) were introduced into the Cas9 vector and transformed into wheat cultivar KN199, 20 and 26 T<sub>0</sub> transgenic seedlings were obtained by sequencing, respectively. Gene fragments (ACTGAGGCA-GAGCTTCAGGATATGATCAATGAAGTGGATGCTGATGGCAATGAACGATTGACTTTCCTGAGTTCCTCAACCTGATGGCGCGTAAGATGAAGGCACTGATCCGAGGAGGAGCTTAAGGAGGCTTCCGCGTGTGACAGGACCAGAATGGTTTCATCTCGGCAGCTGAACCTCCGCCATGTCATGACCAACCTTGGTGAGAAGCTGACAGACGAGGAGGTGGATGAGATGATCCGTGAGGCTGATGTCGATGGTGACGGCCAGATCAATTACGAAGAGTTGTGAAGGTGATGATGGC) were inserted into each of the two polyclonal sites above and below the pWMB006 vector. After successful connection and sequencing verification, the large fragment was

recovered by double cutting with *Hind* III and *Eco*R I, and then connected to the *pCambia3301* vector with the same enzyme cutting (double cutting with *Hind* III and *Eco*R I) (*pWMB006* and *pCambia3301* vector from Xinxing Yunhe Agricultural Technology Co., Ltd.). Sequencing verified the correctness of the *TaCaM-3A*-RNAi vector. After successful construction, transformed into the wheat cultivar Fielder. The exon sequences of *TaCNGC-2A*, *TaCaM-3A*, *TaMYB-5B*, and *TaMYB-5D* were connected to the *UbiF1899* vector (from Spring Valley Agriscience Co., Ltd.) to obtain the correct recombinant vector *Ubi-TaCNGC-2A*, *Ubi-TaCaM-3A*, *Ubi-TaMYB-5B* and *Ubi-TaMYB-5D* which were then transformed into the wheat cultivar Fielder. Homozygous *T<sub>2</sub>* transgenic plants were used in this study.

The *TaCNGC* Cas9-edited lines in KN199 were planted in the field in Henan in 2024 and Hebei in 2024 and 2025. The *TaCNGC* Cas9-edited lines in AN188 were planted in a greenhouse at 23 °C ± 2 °C (16-h/8-h light/dark photoperiod, 60% relative humidity) in 2025. The *TaCNGC-2A* overexpression lines were planted in a greenhouse in 2024 and Hebei in 2025. *TaCaM-3A* overexpression, *TaCaM* Cas9-edited and RNAi lines were planted in the field in Hebei and Henan in 2025. *TaMYB-5B* and *TaMYB-5D* overexpression lines were grown in a greenhouse in 2024 and in Hebei in 2025. The EMS mutants in the JM22 and J411 backgrounds were cultivated at the Dayangdian Experimental Station of Anhui Agricultural University in Hefei, China, during the 2020–2024 wheat cropping seasons.

The *TaCNGC-2A* overexpression lines were used to investigate their roles in rice SD. The amplified *TaCNGC-2A* exon sequences were cloned into vector *pCambia1301* under the control of the 35S promoter and transformed into rice variety Zhonghua 11 (ZH11) by *Agrobacterium*-mediated transformation. The knockout mutants *osncg6-1* and *osncg6-2* were generated by CRISPR-Cas9 technology and provided by Jiangsu Biotechnology Company (Hefei, China). The target region is (gRNA: TCAGGGGTGAGAAAGGGTTC). All primer sequences used in this study are listed in Supplementary Data 11.

### SD and PHS resistance evaluation

The GI and GP of seeds and spikes were used to evaluate SD and PHS resistance. For each genotype (with two replicates for each genotype), about 30 intact and healthy seeds were placed in Petri dishes (90 mm diameter) on filter paper moistened with 5 mL of distilled water and incubated at 22 °C under a 14 h/10 h light/dark photoperiod and 80% relative humidity. Germination was defined as the visible rupture of the pericarp and testa. The number of germinated seeds in each culture dish was counted at the same time every day and removed, and the GI value was calculated using the following formula:

$$GI = (3 \times n1 + 2 \times n2 + 1 \times n3) / (3 \times N) \quad (1)$$

where *N* is the total number of seeds per plate, and *n1*, *n2*, and *n3* are the numbers of germinated seeds on the 1st, 2nd, and 3rd day, respectively. The GP value was calculated as the number of germinated seeds on the three days of the germination test divided by the total number of seeds. To investigate the germination behaviors of wheat spikes, we used artificial rainfall method to spray them for three days. At grain maturation (35 d after anthesis), the freshly harvested wheat spikes were immersed in sterile water for 6 h, then placed vertically on stainless steel racks and grown in a temperature-controlled greenhouse at 20 °C under a 16 h/8 h light/dark photoperiod and 100% relative humidity. They were sprayed for 30 min with sterile water every 18 h.

For JH-RILs, the GI values were measured at 5 days after harvest (DAH) in 2016, 2017, and 2018, named 16GI-JH, 17GI-JH, and 18GI-JH, respectively. For JW-RILs, the GI values were measured at 5 DAH in 2016, 2017, and 2018, designated 16GI-JW, 17GI-JW, and 18GI-JW, respectively. For the 431WVs, the GI values were determined at 5 DAH in 2019 (HF, JY and HB) and 2020 (HF, SZ, and BB), named 19GI-HF,

19GI-JY, 19GI-HB, 20GI-HF, 20GI-SZ, and 20GI-BB, respectively. For the 213WVs, the GI values were measured at 5 DAH in 2022 (HF, SZ, and HB), named 2022GI-HF, 2022GI-SZ, and 2022GI-HB.

Rice seeds with a radicle length of ≥ 1 mm were scored as germinated<sup>53</sup>.

### Genomic DNA and RNA extraction

Dry wheat seeds were ground to a powder using a FastPrep-96™ Homogenizer (MP Biomedicals, USA), and genomic DNA extraction was conducted using the MagCore Automated Nucleic Acid Extractor (RBC Bioscience Co., Ltd., Taiwan, China) following the manufacturer's instructions. Total RNA was extracted using a MiniBEST Plant RNA Extraction Kit (TakaRa, Japan). Genomic DNA and RNA quality was assessed by Nanodrop spectrophotometry (A260/A230 and A260/A280 ratio) and agarose gel electrophoresis (1.5%).

### Specific length amplified fragment sequencing (SLAF-seq) analysis

Genomic DNA of JH-RILs (174 lines) and the two parents (J411 and HMC21) was extracted for SLAF-seq. To ensure data accuracy, raw reads were filtered to remove those containing adapters, >10% unknown bases, or unaligned to the wheat reference genome (IWGSC RefSeq v1.0); single-end aligned reads were also discarded. Clean SLAF reads were aligned using BWA, and SNPs were called with GATK. SLAFs with ≥8 SNPs were excluded. SLAF markers were developed and polymorphic markers were classified into eight segregation types. For the RIL population, only aa × bb markers were retained. Final markers met these criteria: depth >6-fold in parents, missing data <25%, no significant segregation distortion (*P* < 0.001), and MLOD > 3.

### Wheat 660 K SNP assay

The two parents (J411 and HMC21) and two bulk lines (10 lines with strong dormancy and 10 lines with weak dormancy) were genotyped using the Wheat 660 K Affymetrix Axiom Array at CapitalBio Corporation (Beijing, China). SNP genotyping and clustering were performed using Genome Studio Polyploid Clustering v1.03. Consistent SNPs without missing data in J411 and the 10 weak dormancy lines were classified as group I, while those in HMC21 and the 10 strong dormancy lines were classified as group II. SNPs differing between groups I and II were selected for further analysis and the development of PCR-based molecular markers. Physical positions of these SNPs were identified through sequence BLAST with IWGSC RefSeq v2.0.

### CAPS and dCAPS marker development

PCR amplification was performed using 2 × Phanta Max Master Mix (Vazyme, Nanjing, China) in a 20 μL reaction mixture. The thermal cycling conditions were as follows: 95 °C for 5 min, followed by 38 cycles of 95 °C for 15 s and 60 °C for 30 s, and a final extension at 72 °C for 5 min. The products of eight CAPS markers SLAF-3887, SLAF-9786, SLAF-1488, SLAF-0913, SLAF-1010, SLAF-0757, SLAF-7163, and SLAF-5860 were digested using *Nla* III (N/GTAC), *Dde* I (C/TNAG), *Bsp*HI (AGTAC/T), *Nsi* I (ATGCA/T), *Hha* I (C/GCG), *Eae* I (GAGAAG(N)4/N), *Nla* III (N/GTAC), and *Aci* I (GGC/G) at 37 °C for 8 h, respectively. The products were then separated by 1.5% agarose gel electrophoresis.

The PCR reaction system and procedure for dCAPS labeling (AX3375) were the same as for CAPS labeling (SLAF-0913). The AX3375 PCR product was digested with *Eco*R I (G/AATTC) at 37 °C for 8 h. The *Eco*R I-digested products of AX3375 were separated via capillary electrophoresis with a Fragment Analyzer™ Automated CE System (Advanced Analytical Technologies, Inc.)<sup>10</sup>.

### qRT-PCR analysis

The seeds of *cnsg-ko-28*, *TaCNGC-OE-1*, *cam-rnai-5*, *TaCaM-OE-2*, KN199, and Fielder were imbibed in distilled water and sampled at 18 h following imbibition. At 18 h after imbibition, KN199, Fielder, *TaCNGC-*

*OE-1*, and *TaCaM-OE-2* seeds germinated, while *cngc-ko-28* and *cam-rnai-5* seeds did not. cDNA synthesis was conducted using *Evo M-MLV* RT Premix for qPCR (ACCURATE BIOTECHNOLOGY(HUNAN) CO., LTD, ChangSha, China). Real-time PCR was conducted using a Real-time PCR machine (I-Cycle, BioRad) following the manufacturer's instructions in a reaction mixture of 20  $\mu$ L of ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). The *TaActin* gene was used as an internal standard, and three biological replicates were performed. All the primer sequences used in this study are listed in Supplementary Data 11. The  $2^{-\Delta\Delta CT}$  method was used to determine the relative expression levels of genes.

### Cloning of *TaCNGC-2A* and CAPS marker development

The full-length sequence of *TaCNGC-2A* was obtained from the IWGSC RefSeq v2.0. Six sets of primers (CNGC2A1–CNGC2A6) (Supplementary Data 11) were designed using Primer Premier 5.0 (<http://www.premierbiosoft.com>). These primers were used to clone the full-length genomic sequence of *TaCNGC-2A* in J411 and HMC21. PCR amplification was performed using 2  $\times$  Phanta Max Master Mix (Vazyme, Nanjing, China) in a 20  $\mu$ L reaction mixture. The PCR products were separated using 1.5% agarose gel electrophoresis, and the target fragments were excised from the gel, purified using an EasyPure Quick Gel extraction kit, cloned into the *pEASY-Blunt* zero vector, and transformed into Trans-T1 chemically competent cells.

Sequence assembly and alignment of *TaCNGC-2A* revealed 16 sequence variants using DNAMAN 7 (<https://www.lynnnon.com>). The CAPS markers (CNGC-514, CNGC-312, CNGC + 856, and CNGC + 2467) were designed based on two SNPs (-514, G/A; -312, T/A) in the promoter and two missense mutations in the CDS (+856, G/C; +2467, T/A) (Supplementary Data 11). The PCR products were digested with *Ban* I (G/GYRCC), *Fok* I (GGATGN9), *Afl* III (A/CRYGT), and *Mlu* I (N/AATT) enzymes at 37 °C for 8 h. The digestion products were detected by 2.0% agarose gel electrophoresis.

### Yeast two-hybrid (Y2H) assay

The Y2H assay was conducted to assess protein-protein interactions using the Yeastmaker™ Yeast Transformation System 2 (Takara). Various combinations of *pGADT7* (AD) and *pGBKT7* (BD) vectors, each carrying target genes, were co-transfected into yeast strain AH109. The growth of yeast on SD/-Leu/-Trp plates followed by SD/-Leu/-Trp/-His/-Ade/X- $\alpha$ -gal plates at 28 °C determined interaction, with the presence of blue colonies indicating positive interactions.

The interaction between *TaCNGC-2A* and *TaCaM-3A* was assessed using the DUAL membrane yeast two-hybrid system (Dualsystems Biotech, Switzerland). The CDS sequences of *TaCNGC-2A* and *TaCaM-3A* were cloned into the *pBT3-STE* and *pPR3-N* vectors, respectively, generating the bait plasmid *pBT3-STE-TaCNGC-2A* and the prey plasmid *pPR3-N-TaCaM-3A*. The experimental pair (*pPR3-N-TaCaM-3A* + *pBT3-STE-TaCNGC-2A*), along with the positive control (*pNubG-Fe65* + *pTSU2-APP*) and negative controls (*pPR3-N* + *pTSU2-APP*, *pPR3-N* + *pBT3-STE-TaCNGC-2A*, and *pPR3-N-TaCaM-3A* + *pBT3-STE*), were co-transformed into the yeast reporter strain NMY51, grown at SD/-Trp-Leu and SD/-Trp-Leu-His-Ade media at 30 °C for 3–5 days to assess the interactions between the combinations. The appearance of blue colonies indicated positive interactions.

### Bimolecular fluorescence complementation (BiFC) assay

*TaCaM-3A* and *TaMYB-5B*, as well as *TaCNGC-2A* and *TaMYB-5D*, were cloned into bicellular fluorescence vectors *pUC-SPYNE* (n-YFP) and *pUC-SPYCE* (c-YFP), respectively (from Wuhan BioRun Biosciences Co., Ltd), creating *TaCaM-3A-nYFP* and *TaCNGC-2A-cYFP*, as well as *TaMYB-5B-nYFP* and *TaMYB-5D-cYFP* fusion constructs. These constructs were introduced into *Agrobacterium* GV3101. *TaCaM-3A-nYFP* and *TaCNGC-2A-cYFP* were used to transiently express the proteins in *N. benthamiana* protoplasts, *TaMYB-5B-nYFP* and *TaMYB-5D-cYFP*

were used to transiently express the proteins in *N. benthamiana* leaves. Fluorescence signals were observed using a Nikon C2-ER laser scanning confocal microscope.

### Co-immunoprecipitation (Co-IP) assay

The coding sequences of *TaCaM-3A* and *TaCNGC-2A* were fused with the GFP and MYC tags, and *TaMYB-5D* and *TaMYB-5B* were fused with the HA and GFP tags, respectively. Then they cloned into the *pBWA(V)* HS vector (from Wuhan BioRun Biosciences Co., Ltd). For the Co-IP assay, a suspension of *Agrobacterium tumefaciens* containing the *TaCNGC-2A-MYC* and *TaCaM-3A-GFP* constructs, as well as the constructs *TaMYB-5B-GFP* and *TaMYB-5D-HA*, were infiltrated into 3-week-old *N. benthamiana* leaves, respectively. Samples were taken after incubation for 48 h, and different concentrations of  $Ca^{2+}$  treatment solution were sprayed on the surface of tobacco leaves at 6 h before sampling. Total protein was extracted from *N. benthamiana* leaves. After protein extraction, 50  $\mu$ L agarose beads bound to the GFP antibody were added to each system, after re-suspension with PBS for 5 times, the target antibody and total protein were added, and incubated at 4 °C overnight. The immunoprecipitated complexes were subsequently analyzed by western blotting using anti-MYC (AE070, Abclonal; 1:10,000 dilution), anti-GFP (11814460001, Roche; 1:1,000 dilution), and anti-HA (Ab9110, Abcam; 1:4,000 dilution).

### Pull-down assay

The GST-*TaCaM-3A* and His-*TaCNGC-2A-IQ*, as well as GST-*TaMYB-5B* and His-*TaMYB-5D* fusion constructs, were transformed into *Escherichia coli* BL21(DE3), respectively, for protein expression. The purified fusion proteins GST-*TaCaM-3A* and His-*TaCNGC-2A-IQ*, as well as GST-*TaMYB-5B* and His-*TaMYB-5D*, were incubated with Glutathione Sepharose 4 Fast Flow, respectively, in GST pull-down buffer (50 mM Tris-HCl, pH 7.9, 5% glycerol, 1 mM EDTA, 1 mM DDT, 1 mM phenylmethylsulfonyl fluoride [PMSF], 0.01% Nonidet P-40, and 150 mM KCl) for 2 h. After washing five times with pull-down buffer, the beads were suspended in 30  $\mu$ L of 1 $\times$  protein loading buffer and boiled for 5 min. After brief centrifugation, the supernatants were collected and analyzed by immunoblotting using anti-His antibody (66005-1-Ig, Proteintech; 1:5,000 dilution) and anti-GST (30901ES50, YEASEN; 1:30,000 dilution).

### Luciferase (LUC) reporter assay

Fusion expression vectors were created by linking the promoter sequence of *TaCNGC-2A* to the *pGreenII0800* vector (from Wuhan BioRun Biosciences Co., Ltd). These vectors were introduced into *N. benthamiana* protoplasts along with a reporter gene encoding LUC using polyethylene glycol (PEG)-mediated transformation. The 35S promoter-driven Renilla luciferase (REN) gene was included as an internal reference. The firefly LUC and REN activities were quantified using the Dual Luciferase Reporter Assay Kit (DL101-01) from Vazyme Biotech. Measurements were taken with a multimode microplate reader (model: VICTOR Nivo™) from PerkinElmer.

### Yeast one-hybrid (Y1H) screening and assay

The cDNA of *TaMYB-5B*, *TaMYB-5D*, and *TaB3-4D* was inserted into the AD vector, while *TaCNGC-2A*<sup>-312T</sup> and *TaCNGC-2A*<sup>-312A</sup> were inserted into the pAbAi vector. The constructs were transformed into yeast strain Y1HGold and cultured on synthetic defined SD/-Leu/500AbAi medium to assess reporter gene expression.

### Electrophoretic mobility shift (EMSA) assay

The GST-*TaMYB-5B* and MBP-*TaMYB-5D* fusion construct was transformed into *Escherichia coli* BL21(DE3), and the recombinant GST-*TaMYB-5B* and MBP-*TaMYB-5D* protein was purified using a Glutathione Sepharose 4 Fast Flow kit. The single-stranded oligonucleotide fragments of probes were synthesized and then converted into

double-stranded DNA by heating the oligonucleotides at 90–95 °C in an annealing solution (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 100 mM NaCl) for 5 min, followed by slow cooling to room temperature. The interaction between the TaMYB-5B and TaMYB-5D protein and the corresponding probes was assessed following the protocol provided with the LightShift™ Chemiluminescent EMSA Kit (Thermo Fisher, Waltham, MA, USA).

### ChIP assay

The *TaMYB-5B* and *TaMYB-5D* overexpression lines (fused with 3×FLAG) were used for ChIP-qPCR analysis. Briefly, 4 grams of 10-day-old wheat seedlings were vacuum-infiltrated with 1% (v/v) formaldehyde cross-linking buffer for 30 minutes at 25 °C. The cross-linking reaction was stopped with 0.125 M glycine. The tissues were then ground into a fine powder in liquid nitrogen, followed by nuclei isolation and sonication. The resulting chromatin was incubated overnight at 4 °C with anti-FLAG antibody (M30971, Boster; 1:200 dilution). Immunoprecipitated DNA was purified, and the enrichment of target regions was analyzed by qPCR. Normal IgG (AC011, ABclonal, China; 1:200 dilution) was used as a negative control. Enrichment of immunoprecipitated DNA was calculated using the 2<sup>-ΔΔCT</sup> method with input DNA as the reference, and relative fold enrichment was normalized against the negative control. All primers used in the ChIP assay were listed in Supplementary Data 11.

### Measurement of Ca<sup>2+</sup> content

The seeds of *TaCNGC* Cas9-edited line *cngc-ko-28* and wild-type KN199 were imbibed for 18 h and then dried. Subsequently, 0.1 g of the dried samples were weighed and placed into a polytetrafluoroethylene (PTFE) digestion vessel. Then, 5 mL of nitric acid was added, and the mixture was soaked overnight. Maintain at 80 °C for 2 h, then at 120 °C for 2 h, and finally raising it to 160 °C for 4 h. Then let it cool naturally to room temperature. After opening the vessels, the solutions were heated to near dryness to remove excess acid. The residues were dissolved and transferred into a 25 mL volumetric flask. The PTFE vessel and its lid were rinsed three times with a small amount of nitric acid solution (1%), and the washings were combined into the same volumetric flask. The flask was then filled to the mark with 1% nitric acid, mixed thoroughly, and set aside for analysis. A reagent blank was prepared simultaneously following the same procedure. The Ca<sup>2+</sup> concentration was determined by inductively coupled plasma mass spectrometry, with three biological replicates for each sample.

### Transcriptome sequencing (RNA-seq) and data analysis

The freshly harvested *TaCNGC* Cas9-edited *cngc-ko-28* and KN199 seeds were imbibed in distilled water for 30 min, 1 h, 18 h, and 36 h. Transcriptome sequencing analysis was performed by Shanghai Personal Biotechnology (<http://www.personalbio.cn/>) with three biological replicates per sample. Differentially expressed genes (DEGs) were identified based on the following criteria:  $P \leq 0.05$  and expression level fold change > 2.

### Measurement of plant hormones and α-amylase activity

About 1.00 g of freshly harvested *TaCNGC* Cas9-edited line *cngc-ko-28* and wild-type KN199 wheat seeds imbibed for 18 h were ground into a fine powder in liquid nitrogen, and then ABA, GA, Jasmonates, and AUX was extracted three times with acetonitrile and purified with a Poroshell 120 SB-C18 column. The contents of these hormones were determined by LC-MS (Agilent 1290 Infinity-SCIEX 6500Qtrap, <https://www.agilent.com/>). These measurements were performed by RUIYUAN Biotechnology Co., Ltd (<http://www.bestofbest.top>).

α-amylase activity was measured using a commercial assay kit (BC0615; Beijing Solarbio Science & Technology Co., Ltd.) following

the manufacturer's instructions. Three biological replicates were included for each sample.

### Statistical analysis

Descriptive statistical analysis and the Mann-Whitney test (U-test) were conducted on GI values using SPSS 26.0 software (IBM, Armonk, NY, USA). Boxplots and bar charts were generated using Prism v8.0.1 (GraphPad Software).

### Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

### Data availability

The RNA-seq data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession [PRJNA1328368](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1328368). All the supporting data for this study are available in this article and its Supplementary Information files. Source Data are provided with this paper.

### References

- Ali, A. et al. Unraveling molecular and genetic studies of wheat (*Triticum aestivum* L.) resistance against factors causing pre-harvest sprouting. *Agronomy* **9**, 117 (2019).
- Vetch, J. M., Stougaard, R. N., Martin, J. M. & Giroux, M. J. Review: revealing the genetic mechanisms of pre-harvest sprouting in hexaploid wheat (*Triticum aestivum* L.). *Plant Sci.* **281**, 180–185 (2019).
- Xiao, S. H., Zhang, X. Y., Yan, C. S. & Lin, H. Germplasm improvement for preharvest sprouting resistance in Chinese white-grained wheat: an overview of the current strategy. *Euphytica* **126**, 35–38 (2002).
- Zhang, Y. J., Xia, X. C. & He, Z. H. The seed dormancy allele *TaSdr-A1a* associated with pre-harvest sprouting tolerance is mainly present in Chinese wheat landraces. *Theor. Appl. Genet.* **130**, 81–89 (2017).
- Mao, X. X. et al. MKK3 cascade regulates seed dormancy through a negative feedback loop modulating ABA signal in rice. *Rice* **17**, 2 (2024).
- Liu, S. B. et al. Cloning and characterization of a critical regulator for preharvest sprouting in wheat. *Genetics* **195**, 263–273 (2013).
- Osa, M. et al. Mapping QTLs for seed dormancy and *Vp1* homologue on chromosome 3A of wheat. *Theor. Appl. Genet.* **106**, 1491–1496 (2003).
- Chen, C. X., Cai, S. B. & Bai, G. H. A major QTL controlling seed dormancy and pre-harvest sprouting resistance on chromosome 4A in a Chinese wheat landrace. *Mol. Breed.* **21**, 351–358 (2008).
- Munkvold, J. D., Tanaka, J., Bensch, D. & Sorrells, M. E. Mapping quantitative trait loci for preharvest sprouting resistance in white wheat. *Theor. Appl. Genet.* **119**, 1223–1235 (2009).
- Zhu, Y. L. et al. Identification of major loci for seed dormancy at different post-ripening stages after harvest and validation of a novel locus on chromosome 2AL in common wheat. *Mol. Breed.* **36**, 1–12 (2016).
- Zhu, Y. L. et al. Genome-wide association study of pre-harvest sprouting tolerance using a 90K SNP array in common wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* **132**, 2947–2963 (2019).
- Tai, L. et al. Pre-harvest sprouting in cereals: genetic and biochemical mechanisms. *J. EXP BOT* **72**, 2857–2876 (2021).
- Nakamura, S. et al. A wheat homolog of *MOTHER OF FT AND TFL1* acts in the regulation of germination. *Plant Cell* **23**, 3215–3229 (2011).
- Liu, S., Li, L., Wang, W., Xia, G. X. & Liu, S. W. TaSRO1 interacts with TaVP1 to modulate seed dormancy and pre-harvest sprouting resistance in wheat. *J. Integr. Plant Biol.* **66**, 36–53 (2024).

15. Torada, A. et al. A causal gene for seed dormancy on wheat chromosome 4A encodes a MAP Kinase Kinase. *Curr. Biol.* **26**, 782–787 (2016).
16. Lang, J. et al. *Myb10-D* confers *PHS-3D* resistance to pre-harvest sprouting by regulating *NCED* in ABA biosynthesis pathway of wheat. *N. Phytol.* **230**, 1940–1952 (2021).
17. Tai, L. et al. A genome-wide association study uncovers that *TaPI4K-2A* regulates pre-harvest sprouting in wheat. *Plant Commun.* **13**, 5 (2024).
18. Sugimoto, K. et al. Molecular cloning of *Sdr4*, a regulator involved in seed dormancy and domestication of rice. *Proc. Natl. Acad. Sci. USA* **107**, 5792–5797 (2010).
19. Zhang, Y. J., Miao, X. L., Xia, X. C. & He, Z. H. Cloning of seed dormancy genes (*TaSdr*) associated with tolerance to pre-harvest sprouting in common wheat and development of a functional marker. *Theor. Appl. Genet.* **127**, 855–866 (2014).
20. Zhao, B. et al. *Sdr4* dominates pre-harvest sprouting and facilitates adaptation to local climatic condition in Asian cultivated rice. *J. Integr. Plant Biol.* **64**, 1246–1263 (2022).
21. Bentsink, L., Jowett, J., Hanhart, C. J. & Koornneef, M. Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **103**, 17042–17047 (2006).
22. Teng, S., Rognoni, S., Bentsink, L. & Smeekens, S. The *Arabidopsis* *GSQ5/DOG1* Cvi allele is induced by the ABA-mediated sugar signalling pathway, and enhances sugar sensitivity by stimulating *ABI4* expression. *Plant J.* **55**, 372–381 (2008).
23. Ashikawa, I., Mori, M., Nakamura, S. & Abe, F. A transgenic approach to controlling wheat seed dormancy level by using Triticeae *DOG1*-like genes. *Transgenic Res.* **23**, 621–629 (2014).
24. Wei, X. N. et al. The GATA transcription factor *TaGATA1* recruits demethylase *TaELF6-A1* and enhances seed dormancy in wheat by directly regulating *TaABI5*. *J. Integr. Plant Biol.* **65**, 1262–1276 (2023).
25. Cao, J. J. et al. Identification and validation of new stable QTLs for grain weight and size by multiple mapping models in common wheat. *Front. Genet.* **11**, 584859 (2020).
26. Cheng, X. R. et al. Identification of the wheat *C3H* gene family and expression analysis of candidates associated with seed dormancy and germination. *Plant Physiol. Biochem.* **156**, 524–537 (2020).
27. Fischer, C., Kugler, A., Hoth, S. & Dietrich, P. An IQ domain mediates the interaction with calmodulin in a plant cyclic nucleotide-gated channel. *Plant Cell Physiol.* **54**, 573–584 (2013).
28. DeFalco, T. A. et al. Multiple calmodulin-binding sites positively and negatively regulate *Arabidopsis* CYCLIC NUCLEOTIDE-GATED CHANNEL12. *Plant Cell* **28**, 1738–1751 (2016).
29. Niu, W. T. et al. *Arabidopsis* cyclic nucleotide-gated channel 6 is negatively modulated by multiple calmodulin isoforms during heat shock. *J. Exp. Bot.* **71**, 90–104 (2020).
30. Ashikawa, I., Abe, F. & Nakamura, S. Ectopic expression of wheat and barley *DOG1*-like genes promotes seed dormancy in *Arabidopsis*. *Plant Sci.* **179**, 536–542 (2010).
31. Barrero, J. M. et al. Transcriptomic analysis of wheat near-isogenic lines identifies *PM19-A1* and *A2* as candidates for a major dormancy QTL. *Genome Biol.* **16**, 93 (2015).
32. Wei, W. X. et al. Isolation and characterization of *TaQsd1* genes for period of dormancy in common wheat (*Triticum aestivum* L.). *Mol. Breed.* **39**, 150 (2019).
33. Finkelstein, R., Reeves, W., Ariizumi, T. & Steber, C. Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* **59**, 387–415 (2008).
34. Shu, K., Liu, X. D., Xie, Q. & He, Z. H. Two faces of one seed: hormonal regulation of dormancy and germination. *Mol. Plant* **9**, 34–45 (2016).
35. Penfield, S. Seed dormancy and germination. *Curr. Biol.* **27**, 874–878 (2017).
36. Tognacca, R. S. & Botto, J. F. Post-transcriptional regulation of seed dormancy and germination: current understanding and future directions. *Plant Commun.* **2**, 100169 (2021).
37. Sajeev, N., Koornneef, M. & Bentsink, L. A commitment for life: decades of unraveling the molecular mechanisms behind seed dormancy and germination. *Plant Cell* **36**, 1358–1376 (2024).
38. Xu, Y. et al. *OsCNGC13* promotes seed-setting rate by facilitating pollen tube growth in stylar tissues. *PLoS Genet.* **13**, e1006906 (2017).
39. Zeb, Q. S. et al. The interaction of *CaM7* and *CNGC14* regulates root hair growth in *Arabidopsis*. *J. Integr. Plant Biol.* **62**, 887–896 (2019).
40. Cui, Y. M. et al. CYCLIC NUCLEOTIDE-GATED ION CHANNELS 14 and 16 promote tolerance to heat and chilling in rice. *Plant Physiol.* **183**, 1794–1808 (2020).
41. Luan, S. & Wang, C. Calcium signaling mechanisms across kingdoms. *Annu. Rev. Cell Dev. Bi.* **37**, 311–340 (2021).
42. Zhao, C. H. et al. A mis-regulated cyclic nucleotide-gated channel mediates cytosolic calcium elevation and activates immunity in *Arabidopsis*. *N. Phytol.* **230**, 1078–1094 (2021).
43. Yang, Y. et al. OPEN STOMATA 1 phosphorylates CYCLIC NUCLEOTIDE-GATED CHANNELS to trigger  $\text{Ca}^{2+}$  signaling for acid-induced stomatal closure in *Arabidopsis*. *Plant Cell* **36**, 2328–2358 (2024).
44. Peng, Y. et al. Differential phosphorylation of  $\text{Ca}^{2+}$ -permeable channel CYCLIC NUCLEOTIDE-GATED CHANNEL 20 modulates calcium-mediated freezing tolerance in *Arabidopsis*. *Plant Cell* **36**, 4356–4371 (2024).
45. Yu, H. H. et al. Transcription factor *OsMYB30* increases trehalose content to inhibit  $\alpha$ -amylase and seed germination at low temperature. *Plant Physiol.* **194**, 1815–1833 (2024).
46. Yuan, Y. P. et al. Absciscic acid-induced transcription factor *PsMYB306* negatively regulates tree peony bud dormancy release. *Plant Physiol.* **194**, 2449–2471 (2024).
47. Li, T. et al. Phosphorylation of *MdCYTOKININ RESPONSE FACTOR4* suppresses ethylene biosynthesis during apple fruit ripening. *Plant Physiol.* **191**, 694–714 (2023).
48. Liu, S. et al. Calmodulins and calmodulin-like proteins-mediated plant organellar calcium signaling networks under abiotic stress. *Crop J.* **12**, 1321–1332 (2024).
49. Li, M. D. et al. *CBL1/CIPK23* phosphorylates tonoplast sugar transporter *TST2* to enhance sugar accumulation in sweet orange (*Citrus sinensis*). *J. Integr. Plant Biol.* **67**, 327–344 (2024).
50. Wang, B. F. et al. *OsCPK12* phosphorylates *OsCATA* and *OsCATC* to regulate  $\text{H}_2\text{O}_2$  homeostasis and improve oxidative stress tolerance in rice. *Plant Commun.* **5**, 3 (2024).
51. Yu, Q. H. et al. Phosphorylation-dependent *VaMYB4a* regulates cold stress in grapevine by inhibiting *VaPIF3* and activating *VaCBF4*. *Plant Physiol.* **197**, 2 (2025).
52. Ju, L. et al. JAZ proteins modulate seed germination through interaction with *ABI5* in bread wheat and *Arabidopsis*. *N. Phytol.* **223**, 246–260 (2019).
53. Li, Q. F. et al. Gibberellin recovers seed germination in rice with impaired brassinosteroid signalling. *Plant Sci.* **293**, 110435 (2020).

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## Author contributions

H.Z., Y.R., C.M., C.Chang., H.S., J.L., C.Chen. and W.G. conceived and guided the experiments, and helped in coordinating the project; H.Z. and Y.R. analyzed the data and write the manuscript. All authors read and approved of the manuscript. B.T., X.C., J.C., W.L., Z.Y., J.C., B.L., H.W., S.L., W.G., L.Z., Y.L. and S.W. performed the experiments, analyzed the data and participated in manuscript writing; Y.F., Y.Z., and C.K. participated in the cultivation and management of transgenic and edited materials in the field.

## Competing interests

The authors declare no competing interests.

## Additional information

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