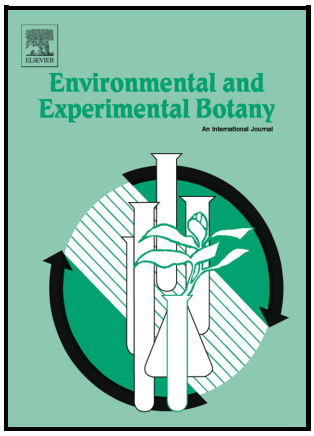

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Impacts of natural variations in the *TaLEA-1A* gene on seed dormancy and germination in wheat and transgenic Arabidopsis and rice

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Abstract: Late embryogenesis abundant (LEA) proteins play important roles in plant development and responses to diverse stresses. However, their specific roles in seed dormancy and germination remain unclear. In this study, we identified *TaLEA-1A*, an LEA gene highly expressed in wheat seeds and differentially expressed in imbibed seeds of weak-dormancy variety Jing 411 (J411) and strong-dormancy variety Hongmangchun 21 (HMC21). Sequence analyses revealed 14 variations in the promoter and coding regions of *TaLEA-1A* between J411 and HMC21. Subsequent validation established associations of a single nucleotide polymorphism (SNP) in the promoter (-1366 bp, G/A) and an insertion/deletion (Indel) variation in the coding region (+345 bp, -/C) with seed dormancy and germination across 192 wheat varieties. Furthermore, the effects of the strong-dormancy allele *TaLEA-1A-345-ins* and weak-dormancy allele *TaLEA-1A-345-del* on seed dormancy and germination were confirmed using transgenic Arabidopsis and rice lines as well as J411 mutant induced with ethyl methane sulphonate. Analyses of endogenous hormone contents and expression patterns suggested that *TaLEA-1A* may regulate seed dormancy and germination by mediating gibberellic acid and abscisic acid balance. These findings not only contribute to unraveling the intricate regulatory network governing seed dormancy and germination in wheat and other plants but also provide a favorable gene and molecular marker for genetic improvement of wheat PHS resistance.

Keywords: Wheat, late embryogenesis abundant protein, seed dormancy and germination, gibberellic acid, abscisic acid

1. Introduction

Wheat (*Triticum aestivum* L.), ranking among the world's top three grain crops (Brenchley et al., 2012), has a large (~16 Gb) and complex heterologous hexaploid genome (International Wheat Genome Sequencing Organization, 2018). As the global population continues to grow, the demand

for food escalates, emphasizing the importance of ensuring wheat production stability.

Pre-harvest sprouting (PHS) of wheat refers to the germination of grains on the spike in rainy days before harvest. This phenomenon threatens wheat grain yield, quality, and vigor due to the degradation of starch and proteins triggered by increased α-amylase and protease activities. Sprouted grains are discounted in the marketplace, making PHS a significant challenge for wheat production worldwide. Previous studies have indicated a close association between seed dormancy and PHS resistance, with wheat varieties exhibiting stronger seed dormancy displaying higher PHS resistance (Liu et al., 2008). Therefore, cultivating varieties with high dormancy levels can effectively reduce PHS damage. However, only a few genes related to seed dormancy and PHS resistance have been identified in wheat, including *TaVp-1*, *TaMFT-3A*, *TaMKK3*, *TaSdr*, *TaQsd1*, *Myb10-D*, and *TaPI4K-2A* (Yang et al., 2007; Nakamura et al., 2011; Torada et al., 2016; Zhang et al., 2017; Wei et al., 2019; Lang et al., 2021; Tai et al., 2023). Therefore, identifying additional dormancy-related genes is significant for improving wheat PHS resistance.

Late embryogenesis abundant (LEA) proteins are important proteins predominantly accumulated in the late stages of seed development (Battaglia et al., 2008) of various plants, such as *Oryza sativa* (Xiao et al., 2007), Arabidopsis (Hundertmark and Hincha 2008), *Zea mays* (Liu et al., 2013), wheat (Zan et al., 2020), and *Canavalia rosea* (Lin et al., 2021). Most LEA proteins are intrinsically disordered proteins characterized by a rich glycine content and a high proportion of hydrophilic amino acid residues (Hincha and Thalhammer, 2012). Based on the amino acid sequences and protein family domains, wheat LEA proteins are divided into eight groups: LEA_1, LEA_2, LEA_3, LEA_4, LEA_5, LEA_6, dehydrated protein, and seed maturation protein (Liu et al., 2019). Among them, the LEA_5 subfamily proteins, classified as group 1 in the early stage, are characterized by a conserved sequence of 20 amino acid residues (TRKEQ[L/M]G [T/E]EGY[Q/K]EMGRKGG[L/E]), a small molecular weight of 6.8-23.9 kD, and as high as 18% of glycine residues. Therefore, it has high hydrophilicity and strong hydration ability (Battaglia et al., 2008).

LEA proteins are recognized for their pivotal roles in regulating various stresses and contributing to plant development, particularly in conferring desiccation tolerance. For example, overexpressing barley LEA gene (*HVA1*) in rice enhances transgenic plants' drought resistance and salt tolerance (Xu et al., 1996), overexpressing wheat LEA genes *PMA80* and *PMA1959* improves the dehydration tolerance of transgenic rice (Cheng et al., 2002), overexpressing wheat LEA gene (*WCOR410*) significantly increases the cold acclimation of strawberry leaves (Houde et al., 2004), and overexpressing wheat LEA gene *WZY3-1* boosts the drought tolerance of transgenic Arabidopsis (Yu et al., 2019). Conversely, overexpressing *OsLEA9* significantly reduces the cold tolerance of rice at both the reproductive and seedling stages (Lou et al., 2022), and knocking out the *LEA3* gene renders cotton plants more susceptible to salinity and drought stresses (Shiraku et al., 2022). Furthermore, *SlLEA6* positively regulates the expression of downstream drought-responsive genes under drought stress, thereby improving the drought tolerance of tomato plants (Jia et al., 2022). In addition, the expression of maize *Em* gene (an LEA gene) in maize embryos relies strongly on the

transcriptional activator Viviparous-1 (Vpl), which is specifically required for the maturation program in seed development (McCarty et al., 1991). During Arabidopsis seed development, the expression of the LEA genes *AtEm7* and *AtEm6* is dramatically impaired in seeds with mutations in *abscisic acid* (*ABA*)-*insensitive 3* (*ABI3*) gene, a homolog to the maize *Vp1* (*abi3-4*) (Parcy et al., 1994). Notably, LEA proteins have also been proven to play a regulatory role in rice seed germination. For instance, Huang et al. (2017) reported that the rice *OsLEA5* gene regulates seed germination via reactive oxygen species (ROS) pathway. Li et al. (2020) showed that the rice *LEA33* gene facilitates seed germination by affecting the brassinosteroid and gibberellin (GA) biosynthesis pathways. However, the role of LEA proteins in wheat seed dormancy and germination remains unknown.

In this study, we identified an LEA_5 subfamily gene *TraesCS1A02G224600*, designated as *TaLEA-1A*, based on our previous transcriptome data. Our subsequent investigations confirmed that natural variations in the *TaLEA-1A* gene significantly correlated with wheat seed dormancy and germination phenotypes across 192 wheat varieties. By integrating expression analysis and physiological indicators, we proposed that *TaLEA-1A* may regulate seed dormancy and germination through ABA and GA metabolism and signaling pathways. Our findings not only provide a novel molecular marker for enhancing wheat PHS resistance through genetic improvement but also broaden our understanding of the functions of wheat LEA genes in seed dormancy and germination.

2. Materials and methods

2.1 Plant materials and growth conditions

Two wheat varieties, Jing 411 (J411, weak dormancy) and Hongmangchun 21 (HMC21, strong dormancy), were chosen for *TaLEA-1A* cloning and expression analysis. A total of 192 wheat varieties with different dormancy levels were used for validating the association of *TaLEA-1A* with seed dormancy and germination. Detailed information on these 192 varieties, including germination index (GI) and field sprouting (FS), could be found in a previous report (Zhu et al., 2019). All wheat materials were cultivated at the Dayangdian Experimental Station (31°58′N, 117°240′E) of Anhui Agricultural University in Hefei, China, during the 2014–2017 wheat growing seasons.

The ethyl methane sulfonate (EMS)-mutagenized wheat mutant *talea-1a-j411* (ID:310824) in J411 background was provided by Boredi Biotechnology Company (http://www.molbreeding.com) and used to validate the role of *TaLEA-1A* in seed dormancy and germination. The M_5 generation of *talea-1a*-j411 was cultivated in Dayangdian Experimental Station (Hefei, China) during the 2022-2023 cropping season.

Exons corresponding to *TaLEA-1A-345-del* from J411 and *TaLEA-1A-345-ins* from HMC21 were amplified and cloned into the pBWA(V)HS-GUS vector. The validated constructs were transformed into Arabidopsis (Col-0) using the Agrobacterium tumefaciens-mediated floral dip method (Clough and Bent 1998). The constructed *TaLEA-1A-345-ins* vector was also transformed into Nipponbare rice (Sahoo et al., 2011). Col-0 and transformed Arabidopsis plants were vernalized in Murashige and Skoog (MS) medium at 4℃ for three days. They were then transferred to an incubator with 8 h dark/16 h light cycle at 24±1℃ for 12 days before being transformed into a

square basin containing a mixture of black soil and vermiculite (1:3, v/v). Nipponbare and transgenic rice plants were grown naturally during the growing season after seedling cultivation at 28±1℃ in a greenhouse with 16 h light/8 h dark cycle. The Nipponbare and transgenic rice plants showed no significant differences in main agronomic traits during the growth and development period.

2.2 Seed dormancy assay

Physiologically matured wheat grains with seed moisture of approximately 12% were harvested. The intact spikes were submerged in deionized water for 3 h and then placed in a 90% moisture growth chamber in the dark for 3 days (Lang et al., 2021). The intact spikes and the seeds isolated from them were photographed. In addition, 40-50 selected seeds were sown on Petri dishes containing two layers of filter papers and 10 ml of distilled water at 20°C for 36 h with 18-25 seeds per Petri dish. The germinated seeds were counted at various intervals until all seeds had germinated. The germination index (GI) and germination percentage (GP) were calculated as described previously (Jiang et al., 2018) and used to assess seed dormancy. Similarly, harvested Arabidopsis and rice seeds (dehulled) were sown on germination papers in round glass Petri dishes with appropriate amounts of distilled water. These dishes were moved to a greenhouse with an 16 h light/ 8 h dark cycle at 24±1 ℃ and 28±1 ℃, respectively. The germinated seeds were counted at various intervals until all seeds had germinated, and the GP was calculated.

To test the germination phenotype of transgenic rice plants, freshly harvested panicles from the overexpression rice (*35S:TaLEA-1A-345-ins-1/2/3*) and wild-type Nipponbare cultivars (control) were immersed in sterile water for 3 h. After that, the panicles were placed vertically in an incubator with daily refreshed water under 8 h dark/16 h light cycle at 28±1℃ (Xu et al., 2022).

2.3 Amplification of *TaLEA-1A* gene and development of CAPS markers

To amplify the promoter (upstream 2000 bp) and exon sequences of *TaLEA-1A* gene in J411 and HMC21, various specific primer sets (Table S1) were designed using Primer Premier v.5.0 based on the *TaLEA-1A* gene sequence in the Chinese Spring genome. The amplified fragment sequences were analyzed using DNAMAN version 6. Two mutations occurred at -1633 bp in the promoter and +345 bp in the coding region of *TaLEA-1A* were identified using *cis*-element analysis (PlantCARE). Based on the obtained amino acid sequences, two corresponding cleaved amplified polymorphic sequences (CAPS) were developed as markers.

2.4 *TaLEA-1A* cloning and subcellular localization analysis

The *TaLEA-1A* cDNA was cloned into the vector pAN580 (from Wuhan BioRun Biosciences Co., Ltd.) with a GFP reporter gene for subcellular localization analysis. The obtained 35S::TaLEA-1A-GFP and vector control 35S::GFP were transformed into wheat protoplasts (Zhao et al., 2017), and GFP expression was screened using a confocal laser scanning microscope.

2.5 Determination of GA and ABA contents

A total of 0.1g *talea-1a-j411* and J411 seeds imbibed for 0, 6, 12, and 24 h with distilled water were used to determine GA and ABA contents with corresponding ELISA (enzyme-linked immunosorbent assay) kits (MM-013801 and MM-012501; Jiangsu Meimian Industrial Co., Ltd.,

China). The absorbance of each well was measured at 450 nm using a multifunctional enzyme labeling instrument (model SPARK, TECAN), and the ABA and GA contents were calculated following the instructions provided in the kits.

2.6 Total RNA extraction and qRT-PCR analysis

Total RNA was extracted using a TaKaRa RNA extraction kit. Reverse transcription was performed to synthesize cDNAs in a 40 μL reaction system using the Evo M-MLV RT Mix Tracking Kit (AG11706, Accurate Biotechnology (Hunan) Co., Ltd, ChangSha, China). The primers specific to *TaLEA-1A* and control *AtActin7, OsActin1* and *TaActin1* genes were designed using Primer Premier v.5.0 (Table S1) (Liu et al., 2017; Xu et al., 2022; Ji et al., 2011). qRT-PCR was performed on the Applied Biosystems 7500 Real-Time PCR System (Bio-Rad Laboratories, USA) under a condition consisting of a 30 second denaturation step at 90°C, followed by 30-40 cycles at 95°C for 30 seconds, 60°C for 15 seconds, and 72°C for 10 seconds. All experiments were performed in three biological replicates, and relative gene expression was determined using the $2^{-\Delta Ct}$ method (Schmittgen and Livak, 2008).

2.7 Statistical analysis

Statistical analysis was performed using SPSS version 25.0 software. Significant differences were examined using Student's t-test, U-test, and LSD (least significant difference) test at significance levels of $P < 0.05$ and $P < 0.01$. Graphs were generated using GraphPad Prism v8 Software. All assays were performed with at least three biological replicates.

3. Results

3.1 Identification and expression analysis of the *TaLEA-1A* gene

To unravel genes associated with seed dormancy and germination, we analyzed RNA-seq data from seeds collected from weak-dormancy J411 and strong-dormancy HMC21 at 4, 6, and 10 h imbibition stages in our previous study (Cheng et al., 2020). Notably, HMC21 seeds did not germinate at 4, 6, and 10 h imbibition stages. Although J411 seeds did not germinate at 4 h and 6 h stages, they did at 10 h stage. RNA-seq analysis revealed a significantly higher expression level of the *TraesCS1A02G224600* gene, designated *TaLEA-1A*, in HMC21 seeds than in J411 seeds at 10 h imbibition stage (Fig. S1A). This expression trend of *TaLEA-1A* was further validated using qRT-PCR (Fig. S1B). We also analyzed the expression patterns of *TaLEA-1A* in different wheat tissues using the public database (WheatOmics 1.0) and found that *TaLEA-1A* was highly expressed in grains in comparison to other tissues such as roots, stems, leaves, and spikes. These observations were further confirmed by qRT-PCR (Fig. S1C and 1D). Taking together the seed dormancy and germination phenotypes of J411 and HMC21 and the expression profiles of *TaLEA-1A*, we speculate that *TaLEA-1A* is involved in seed dormancy and germination processes.

3.2 Cloning and sequence analysis of *TaLEA-1A*

Based on the Chinese Spring draft genome sequence, we cloned the promoter (upstream 2000 bp) and full-length (355 bp) sequence of the *TaLEA-1A* gene in J411 and HMC21. The *TaLEA-1A* gene includes a 73-bp intron and two exons. Sequence analysis revealed 12 SNPs in the promoter and two variations in the coding region of *TaLEA-1A* between J411 and HMC21 (Fig. 1A). In the

promoter region, only the SNP at -1366 bp (G/A) resulted in a change to a *cis*-element AT~TATA-box, while other 11 did not. In the coding region, the 1-bp insertion/deletion (Indel, -/C) variation occurred at +345 bp in the second exon, causing a frameshift mutation. The other SNP (G/C) at +304 bp in the second exon was a synonymous mutation (Fig. 1A, Fig. S2A and B). In addition, the *cis*-elements in the promoter region (upstream 2000 bp) were related to responses to light (40.625%), abscisic acid (ABA, 25%), jasmonic acid (JA, 25%), anaerobic induction (6.25%), and circadian rhythm (3.125%) (Table S2).

To explore whether the 1-bp Indel (-/C, +345 bp) affects the protein properties and structures, we analyzed the physicochemical properties of amino acid residues and predicted protein secondary and tertiary structures of TaLEA-1A in J411 (J411-type TaLEA-1A) and HMC21 (HMC21-type TaLEA-1A). Compared with J411-type TaLEA-1A, HMC21-type TaLEA-1A had lower pI and instability index (II) and higher hydrophobic index and alpha-helix proportion (Table S3, Fig. S3).

To investigate the evolutionary relationship of LEA among wheat and other plant species, we constructed a phylogenetic tree using 21 homologous genes from the Ensembl Plants database. The results showed that TaLEA-1A shared 100% sequence identity with *Secale cereale*, *Triticum turgidum*, and *Triticum urartu* (Fig. S4A and B). The high homology of TaLEA-1A across different plants indicates that TaLEA-1A is highly conserved during evolution.

3.3. Association of *TaLEA-1A* with seed dormancy and germination

To investigate the association of *TaLEA-1A* with seed dormancy and germination, we developed two CAPS markers based on an SNP mutation (G/A, -1366 bp) in the promoter and a 1-bp Indel (-/C, +345 bp) in the second exon of *TaLEA-1A*, designated TaLEA-1A--1366 and TaLEA-1A-345, respectively (Fig. 1B). The two CAPS markers were further utilized to genotype 192 wheat varieties with distinct dormancy and germination phenotypes. The two allelic variations were identified as *TaLEA-1A--1366-G* and *TaLEA-1A--1366-A* for TaLEA-1A--1366 and *TaLEA-1A-345-ins* and *TaLEA-1A-345-del* for TaLEA-1A-345. U-test results revealed significant differences in GI values between cultivars carrying TaLEA-1A-1366 and TaLEA-1A-345 (Table S4). As depicted in Fig. 1C, for TaLEA-1A--1366, the allele *TaLEA-1A--1366-A* (15GI15, 0.58; 16GI5, 0.37; 16GI15, 0.45) was significantly associated with lower GI values compared to *TaLEA-1A--1366-G* (15GI15, 0.66; 16GI5, 0.48; 16GI15, 0.57), but no significant difference was observed in 15GI5, 15FS, and 16FS between varieties harboring the two alleles (15GI5, 16GI5, 15GI15, and 16GI15 represent the GI values measured at 5 days and 15 days after harvest in 2015 and 2016, respectively; 15FS and 16FS represent the FS values measured in 2015 and 2016, respectively). For TaLEA-1A-345, the allele *TaLEA-1A-345-ins* (15GI5, 0.31; 15GI15, 0.48; 16GI5, 0.31; 16GI15, 0.40; 15FS, 0.15; 16FS, 0.14) was significantly associated with lower GI and FS values compared to *TaLEA-1A-345-del* (15GI5, 0.55; 15GI15, 0.65; 16GI5, 0.47; 16GI15, 0.55; 15FS, 0.34; 16FS, 0.34) (Fig. 1D). These data indicate that the 1-bp Indel (-/C, +345 bp) in the second exon has a stronger association with seed dormancy and germination phenotypes compared to the SNP mutation (G/A, -1366 bp) in the promoter of *TaLEA-1A*.

The two CAPS markers formed three haplotypes, namely *Hap1*, *Hap2*, and *Hap3*, in the 192

wheat varieties (Fig. S5). Significant differences were observed in GI and FS values among varieties with *Hap1*, *Hap2*, and *Hap3*. The haplotype *Hap3* was significantly related to lower GI and FS values under all conditions compared to *Hap1* and *Hap2* (Fig. 1E). Based on these results, we propose that *Hap3* is a favorable haplotype for high seed dormancy and PHS resistance.

3.4. Effects of *TaLEA-1A-345-ins* and *TaLEA-1A-345-del* on Arabidopsis seed dormancy and germination

Arabidopsis is an excellent model plant for functional research due to the ease of generating transgenic lines. Previous studies have demonstrated that certain seed dormancy genes, such as *Vp-1*/*ABI3*, *MFT*, *Qsd1*, *Sdr*, and *MKK3*, exhibit conserved functions in Arabidopsis, wheat, rice, and barley (Yang et al., 2007; Nakamura et al., 2011; Wei et al., 2019; Zhang et al., 2017; Torada et al., 2016). To investigate the effect of the 1-bp Indel (-/C, +345 bp) in the second exon of *TaLEA-1A* on seed dormancy and germination in Arabidopsis, we cloned *TaLEA-1A-345-ins* and *TaLEA-1A-345-del* into the pBWA(V)HS-GUS vector and transformed the validated constructs into Arabidopsis (Col-0) through Agrobacterium tumefaciens-mediated floral dip. The obtained transgenic T_1 seeds were used for preliminary screening on the Murashige and Skoog (MS) medium containing 50 mg/L hygromycin, and T_1 leaves were used for GUS test. The genetically transformed lines were stained blue, while the parent Col-0 line was not stained blue (Fig. S6). After GUS test, DNA from the transgenic and wild-type plants was extracted and used for PCR amplification using specific primers (Table S1). The results revealed a 1003-bp band in transgenic lines (Fig. S7A and C) but not in the Col-0 line. We further detected *TaLEA-1A* expression levels in three randomly selected lines from the transgenic seedlings. The results revealed a significantly higher *TaLEA-1A* expression in the three lines (Fig. S8A and B).

Germination test of the freshly harvested T_3 generation of *35S:TaLEA-1A-345-ins* transgenic and wild-type seeds showed that the transgenic lines had significantly lower average GP on the 2nd and 3rd days compared to Col-0. The GP was close to 100% on the 4th and 5th days for both the *35S:TaLEA-1A-345-ins* transgenic and Col-0 lines (Fig. 2A and 2C). In addition, germination test of the freshly harvested T_3 generation of *35S:TaLEA-1A-345-del* transgenic and wild-type seeds showed that the transgenic lines had significantly higher average GP on the 2nd and 3rd days compared to Col-0 (Fig. 2B and D). These findings indicate that *35S:TaLEA-1A-345-ins* overexpression delays Arabidopsis seed germination and increases dormancy level, whereas *35S:TaLEA-1A-345-del* overexpression accelerates Arabidopsis seed germination and decreases dormancy level, supporting that *TaLEA-1A* has a significant effect on Arabidopsis seed dormancy and germination.

3.5. Effect of *TaLEA-1A-345-ins* on rice seed dormancy and germination

Rice is another excellent model plant for functional analysis of target genes due to the ease of obtaining transgenic lines. Given that *TaLEA-1A-345-ins* is associated with high dormancy and PHS resistance, we further investigated its effect on rice seed dormancy and germination by transforming *TaLEA-1A-345-ins* into Nipponbare rice. *TaLEA-1A-345-ins* transgenic T_1 seeds were obtained. After initial selection with 50 mg/L hygromycin, specific primers were designed for amplification

(Fig. S7B) and expression level verification (Fig. 3A). Germination tests of the freshly harvested T_3 transgenic and Nipponbare seeds revealed that the average GP of *TaLEA-1A-345-ins* transgenic seeds was significantly lower than that of Nipponbare seeds (Fig. 3B and 3C). In addition, the germination behavior of *35S:TaLEA-1A-345-ins1/2/3* panicles was obviously delayed compared to Nipponbare panicles (Fig. 3D). These observations indicate that *35S:TaLEA-1A-345-ins* overexpression inhibits rice seed germination but enhances dormancy and PHS resistance.

3.6. Effect of *TaLEA-1A-345-del* on wheat seed dormancy and germination

Considering that J411 carries the weak-dormancy allele *TaLEA-1A-345-del*, we generated a G to A mutation at the 206th position of the second exon of *talea-1a-j411* in the J411 background using the EMS method, designated *talea-1a-j411*. The mutation resulted in the substitution of the 45th amino acid in the conserved domain from non-polar glycine (Gly) to polar aspartate (Asp) of the TaLEA-1A protein (Fig. S9A and B). Subsequently, we tested the germination of the intact spikes and threshed J411 and *talea-1a-j411*mutant seeds. As depicted in Fig. 4A, the germination behavior of *talea-1a-j411* mutant spikes was obviously delayed compared to J411 spikes. The average GP of the *talea-1a-j411* seeds before 24 h imbibition was significantly lower than that of J411 seeds, especially at 12 h of imbibition (Fig. 4B and C). These results suggest that the mutation of the weak-dormancy allele *TaLEA-1A-345-del* may enhance wheat seed dormancy and PHS resistance. In addition, compared to the wild-type J411, the *talea-1a-j411* mutant exhibited longer grain length, shorter grain width, no significant difference in thousand-grain weight and decreased plant height (Fig. S9C and D).

3.7. Potential regulatory mechanism of *TaLEA-1A* in mediating seed dormancy and germination

It is well known that the antagonism of plant hormones GA and ABA regulates seed dormancy and germination. To elucidate whether GA and ABA pathways are involved in *TaLEA-1A*'s regulatory mechanism in seed dormancy and germination, we measured GA and ABA contents in *talea-1a-j411* mutant and J411 seeds at different imbibition stages (0, 6, 12, and 24 h) (Fig. S10). As shown in Fig. 5A, GA content was significantly reduced while ABA content was significantly increased in *talea-1a-j411* mutant seeds compared to J411 seeds, leading to a decreased GA/ABA ratio. This observation might partially account for the significant increase in seed dormancy of *talea-1a-j411* mutant seeds.

We further investigated the transcriptional changes of several key genes involved in GA and ABA biosynthesis, catabolism, and signal transduction pathways, including *TaGA3ox3*, *TaNCED2*, *TaGA2ox*, *TaABA8'OH1*, *TaAmy1*, *TaABI3*, *TaPYL5,* and *TaSnRK2*, at different imbibition stages (0, 6, 12, and 24 h) (Mieog et al., 2017; Son et al., 2016; Jacobsen et al., 2013; Izydorczyk et al., 2018). The results demonstrated no significant difference in the expression levels of GA and ABA synthesis genes (*TaGA3ox3* and *TaNCED2*) between the *talea-1a-j411* mutant and J411 seeds. However, in *talea-1a-j411* mutant seeds, the expression of GA catabolism gene *TaGA2ox1* was significantly increased, while the expression of ABA catabolism gene *TaABA8'OH1* was significantly decreased compared to J411 seeds, aligning with the changes in GA and ABA contents. In addition, the

expression of *TaAmy1*, which is involved in GA signal transduction pathway, was significantly decreased, while the expression levels of *TaABI3*, *TaPYL5*, and *TaSnRK2*, which are involved in ABA signal transduction pathway, were significantly increased in *talea-1a-j411* mutant seeds (Fig. 5B). Based on these findings, we speculate that *TaLEA-1A* regulates wheat seed dormancy and germination via affecting GA and ABA catabolism and signaling pathways.

3.8. Distribution frequency of *TaLEA-1A-345-ins* and *TaLEA-1A-345-del*

We analyzed the distribution frequency of the allelic variations *TaLEA-1A-345-ins* and *TaLEA-1A-345-del* in 578 wheat germplasms collected from China (404) and other countries (174) utilizing the SnpHub wheat resequencing database (http://wheat.cau.edu.cn/WheatUnion/). The results revealed a significantly higher proportion of the weak-dormancy allele *TaLEA-1A-345-del* (475, 82%) across all 578 wheat germplasms than the strong-dormancy allele *TaLEA-1A-345-ins* (103, 18%). Similarly, among the subset of 404 Chinese wheat germplasms, the proportion of *TaLEA-1A-345-del* (310, 77%) was also significantly higher than that of *TaLEA-1A-345-ins* (94, 23%) (Table S5). Furthermore, the allele *TaLEA-1A-345-ins* accounted for a large proportion of wheat germplasms from Southwestern China and Northwestern Africa (Fig. S11). These results indicate that the strong-dormancy allele *TaLEA-1A-345-ins* has yet to gain widespread utilization in wheat breeding.

3.9. Subcellular localization of the TaLEA-1A protein

Most LEA proteins are distributed in the nucleus and cytoplasm (Lou et al., 2022). To observe the subcellular localization of TaLEA-1A, the *TaLEA-1A-GFP* expression vectors were transiently expressed in wheat protoplasts. *TaLEA-1A-GFP* signaling was primarily detected in the nucleus, whereas plants transformed with the constitutively expressed GFP control vector showed GFP distributed throughout cells (Fig. 6). These findings demonstrate that TaLEA-1A accumulates mainly in the nucleus and cytoplasm.

4. Discussion

PHS often occurs in wheat seeds with weak dormancy and poses a significant threat to wheat yields and quality, resulting in substantial economic losses for farmers and flour processing enterprises. The estimated global economic impact of PHS is approximately $1 billion annually (Vetch et al., 2019). Identifying genes associated with seed dormancy and germination is crucial for minimizing PHS damage and breeding wheat varieties with enhanced PHS resistance via transgenic and gene editing approaches. Our research uncovered the involvement of the *TaLEA-1A* gene, a member of the LEA gene family, in seed dormancy and germination and validated its role using expression analysis, sequence analysis, CAPS marker development, haplotype analysis, wheat EMS mutagenesis, and transgenic Arabidopsis and rice experiments.

Studies showed that many LEA genes in different plant species, including *TaLEA-1A*, were involved in seed dormancy and germination. *ATEM6*, an Arabidopsis homolog of *TaLEA-1A*, is specifically expressed in embryos and plays a crucial role in water binding/loss during embryo maturation. Importantly, seeds from homozygous mutant *atem6* plants demonstrated increased germination ability under standard conditions, underscoring that *ATEM6* is associated with seed

germination (Manfre et al., 2006, 2009). In rice, overexpressing *OsEm1*, another *TaLEA-1A* homolog, increases ABA sensitivity and drought tolerance, indicating that *OsEm1* functions as a positive regulator in the ABA signaling pathway and drought tolerance (Yu et al., 2016). ABA sensitivity has been linked to dormancy acquisition (Kawakami et al., 1997). Wheat cultivars with enhanced dormancy and PHS resistance are more sensitive to exogenous ABA (Walker-Simmons, 1987; Morris et al., 1989; Corbineau et al., 2000). Our results revealed that *TaLEA-1A* regulated seed dormancy and germination by affecting ABA and GA pathways, aligning with the functions of *TaLEA-1A* with its homologs *ATEM6* and *OsEm1* in seed dormancy and germination. Hong et al. (1992) found that during barley seed imbibition, the mRNA transcription of the LEA gene *HVA1* in dormant embryos declined significantly slower than in non-dormant embryos, suggesting that *HVA1* may contribute to delayed barley seed germination. Huang et al. (2017) found that the rice OsLEA5 protein interacts with the zinc finger transcription factor ZFP36 to co-regulate ABA-inhibited seed germination through the reactive oxygen species (ROS) pathway. Li et al. (2020) demonstrated that a mutation in the rice *LEA33* gene inhibits GA biosynthesis and increases brassinosteroid accumulation, negatively impacting seed germination. These findings suggest that *TaLEA-1A* is involved in seed dormancy and germination processes. Currently, we are in the process of constructing overexpressed and gene-edited wheat materials to validate our present findings.

Our study identified a 1-bp Indel variation in the coding region (+345 bp, -/C) of *TaLEA-1A*, leading to a frameshift mutation and the generation of two proteins with distinct lengths in J411 and HMC21, namely J411-type TaLEA-1A with shorter sequence (corresponding to 1-bp deletion) and HMC21-type TaLEA-1A with normal sequence. We further analyzed their physicochemical properties and structures and revealed that HMC21-type TaLEA-1A had higher hydrophobicity, lower hydrophilicity, and increased α-helical content in the secondary and tertiary structures compared to J411-type TaLEA-1A. LEA proteins typically contain predominantly charged and uncharged polar amino acid residues, making them hydrophilic with water-binding capacity. Gilles et al. (2007) have shown that changes in the α-helical content of LEA proteins' N-terminal structure significantly affect their normal folding during dehydration and rehydration. Therefore, we speculate that the observed changes in physicochemical properties and protein structure of TaLEA-1A may partly lead to phenotypic differences in seed dormancy and germination between J411 and HMC21. However, the hypothesis needs to be elucidated with more experimental data in the future.

Developing PHS-resistant germplasm resources is crucial for breeding new wheat varieties with high PHS resistance. Our study found that compared to wild-type J411, the wheat EMS mutant *talea-1a-j411* plants exhibited not only lower germination rate and higher dormancy level but also longer grain length, shorter grain width, and decreased plant height. In general, proper reduction of plant height can improve the lodging resistance of wheat plants. These changes in plant height and germination behavior make the mutant *Talea-1a-j411* plants a valuable genetic resource for improving lodging resistance and PHS resistance in wheat. However, the potential breeding applications of *talea-1a-j411* plants need thorough evaluation across diverse environmental conditions, especially considering the observed slight shrinkage phenomenon in the seed coat of

talea-1a-j411 plants harvested in 2023.

5. Conclusion

We identified a group 5 LEA gene in wheat, designated *TaLEA-1A*, and demonstrated that it is highly expressed in seeds. Furthermore, we provided preliminary experimental data supporting the association of *TaLEA-1A* with seed dormancy and germination through expression analysis, sequence variation analysis, CAPS marker development, haplotype analysis, physicochemical properties and protein structure analysis. Additionally, we verified the role of *TaLEA-1A* in seed dormancy and germination using transgenic Arabidopsis and rice materials, along with a wheat EMS mutant. Our findings strongly suggest that *TaLEA-1A* may regulate seed dormancy and germination by affecting GA and ABA metabolism and signal transduction pathways. This study not only lays a theoretical basis for in-depth functional analysis of *TaLEA-1A* in wheat seed dormancy and germination but also provides useful gene-special markers for improving wheat PHS resistance.

Author contributions

SYL, JY, CC, HPZ, and CXM designed the study. SYL and JY carried out the experiments. SYL, JY, CC, HPZ, and CXM drafted the manuscript. CXL, QX, and BBT prepared the figures and tables. XRC, JJC and JL revised the manuscript. CC, HPZ, and CXM supervised the experiment and coordinated the project.

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Competing interests

The authors declare no competing interests.

References

Battaglia M, Olvera-Carrillo Y, Garciarrubio A, Campos F, Covarrubias AA. 2008. The enigmatic LEA proteins and other hydrophilins. Plant Physiol, 148, 6-24. doi:10.1104/pp.108.120725.

Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhornou A, Bolser D, Kay S, Waite D, Trick M, Bancroft I, Gu Y, Huo N, Luo MC, Sehgal S, Gill B, Kianian S, Anderson O, Kersey P, Dvorak J, McCombie WR, Hall A, Mayer KF, Edwards KJ, Bevan MW, Hall N. 2012. Analysis of the bread wheat genome using whole-genome shotgun

sequencing. Nature, 491, 705-10. doi:10.1038/nature11650.

Cheng X, Cao J, Gao C, Gao W, Yan S, Yao H, Xu K, Liu X, Xu D, Pan X, Lu J, Chang C, Zhang H, Ma C. 2020. Identification of the wheat C3H gene family and expression analysis of candidates associated with seed dormancy and germination. Plant Physiol Biochem, 156, 524-537. doi:10.1016/j.plaphy.2020.09.032.

Cheng Z, Targolli J, Huang X, Wu R. 2002. Wheat LEA genes, *PMA80* and *PMA1959*, enhance dehydration tolerance of transgenic rice (*Oryza sativa* L.). Molecular Breeding, 10, 71-82. doi:10.1023/A:1020329401191.

Clough SJ and Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J, 16, 735-743. doi:10.1046/j.1365-313x.1998.00343.x.

Corbineau F, Benamar A, Côme, D. 2000. Changes in sensitivity to abscisic acid of the developing and maturing embryo of two wheat cultivars with different sprouting susceptibility. Israel Journal of Plant Sciences, 48, 189-197. doi:10.1560/HG09-MCP0-GF6Y-3TD6.

Gilles GJ, Hines KM, Manfre AJ, Marcotte WR Jr. 2007. A predicted N-terminal helical domain of a Group 1 LEA protein is required for protection of enzyme activity from drying. Plant Physiol Biochem, 45, 389-399. doi:10.1016/j.plaphy.2007.03.027.

Hincha DK and Thalhammer A. 2012. LEA proteins: IDPs with versatile functions in cellular dehydration tolerance. Biochem Soc Trans, 40, 1000-3. doi:10.1042/BST20120109.

Hong B, Barg R, Ho TH. 1992. Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. Plant Mol Biol, 18, 663-674. doi:10.1007/BF00020009.

Houde M, Dallaire S, N'Dong D, Sarhan F. 2004. Overexpression of the acidic dehydrin *WCOR410* improves freezing tolerance in transgenic strawberry leaves. Plant Biotechnol J, 2, 381-7. doi:10.1111/j.1467-7652.2004.00082.x.

Huang L, Jia J, Zhao X, Zhang M, Huang X, E Ji, Ni L, Jiang M. 2017. The ascorbate peroxidase APX1 is a direct target of a zinc finger transcription factor *ZFP36* and a late embryogenesis abundant protein *OsLEA5* interacts with *ZFP36* to co-regulate *OsAPX1* in seed germination in rice. Biochem Biophys Res Commun, 495, 339-345. doi:10.1016/j.bbrc.2017.10.128.

Hundertmark M and Hincha DK. 2008. LEA (late embryogenesis abundant) proteins and their encoding genes in Arabidopsis thaliana. BMC Genomics, 9, 118. doi:10.1186/1471-2164-9-118.

Izydorczyk C, Nguyen TN, Jo S, Son S, Tuan PA, Ayele BT. 2018. Spatiotemporal modulation of abscisic acid and gibberellin metabolism and signalling mediates the effects of suboptimal and supraoptimal temperatures on seed germination in wheat (*Triticum aestivum* L.). Plant Cell Environ, 41, 1022-1037. doi:10.1111/pce.12949.

Jacobsen JV, Barrero JM, Hughes T, Julkowska M, Taylor JM, Xu Q, Gubler F. 2013. Roles for blue light, jasmonate and nitric oxide in the regulation of dormancy and germination in wheat grain (*Triticum aestivum* L.). Planta, 238, 121-38. doi:10.1007/s00425-013-1878-0.

Ji X, Dong B, Shiran B, Talbot MJ, Edlington JE, Hughes T, White RG, Gubler F, Dolferus R. 2011. Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. Plant Physiol, 156, 647-62.

doi:10.1104/pp.111.176164.

Jia C, Guo B, Wang B, Li X, Yang T, Li N, Wang J, Yu Q. 2022. The LEA gene family in tomato and its wild relatives: genome-wide identification, structural characterization, expression profiling, and role of *SlLEA6* in drought stress. BMC Plant Biol, 22, 596. doi:10.1186/s12870-022-03953-7.

Jiang H, Zhao LX, Chen XJ, Cao JJ, Wu ZY, Liu K, Zhang C, Wei WX, Xie HY, Li L, Gan YG, Lu J, Chang C, Zhang HP, Xia XC, Xiao SH, Ma CX. 2018. A novel 33-bp insertion in the promoter of TaMFT-3A is associated with pre-harvest sprouting resistance in common wheat. Mol Breeding, 38, 69. doi:10.1007/s11032-018-0830-1.

Kawakami N, Miyake Y, Noda K. 1997. ABA insensitivity and low ABA levels during seed development of non-dormant wheat mutants. Journal of Experimental Botany, 48, 1415-1421. doi:10.1093/jxb/48.7.1415.

Lang J, Fu Y, Zhou Y, Cheng M, Deng M, Li M, Zhu T, Yang J, Guo X, Gui L, Li L, Chen Z, Yi Y, Zhang L, Hao M, Huang L, Tan C, Chen G, Jiang Q, Qi P, Pu Z, Ma J, Liu Z, Liu Y, Luo MC, Wei Y, Zheng Y, Wu Y, Liu D, Wang J. 2021. *Myb10-D* confers PHS-3D resistance to pre-harvest sprouting by regulating NCED in ABA biosynthesis pathway of wheat. New Phytol, 230, 1940-1952. doi:10.1111/nph.17312.

Li QF, Zhou Y, Xiong M, Ren XY, Han L, Wang JD, Zhang CQ, Fan XL, Liu QQ. 2020. Gibberellin recovers seed germination in rice with impaired brassinosteroid signalling. Plant Sci, 293, 110435. doi:10.1016/j.plantsci.2020.110435.

Lin R, Zou T, Mei Q, Wang Z, Zhang M, Jian S. 2021. Genome-Wide Analysis of the Late Embryogenesis Abundant (LEA) and Abscisic Acid-, Stress-, and Ripening-Induced (ASR) Gene Superfamily from *Canavalia rosea* and Their Roles in Salinity/Alkaline and Drought Tolerance. Int J Mol Sci, 22, 4554. doi:10.3390/ijms22094554.

Liu H, Xing M, Yang W, Mu X, Wang X, Lu F, Wang Y, Zhang L. 2019. Genome-wide identification of and functional insights into the late embryogenesis abundant (LEA) gene family in bread wheat (*Triticum aestivum*). Sci Rep, 9, 13375. doi:10.1038/s41598-019-49759-w.

Liu K, Qi S, Li D, Jin C, Gao C, Duan S, Feng B, Chen M. 2017. TRANSPARENT TESTA GLABRA 1 ubiquitously regulates plant growth and development from Arabidopsis to foxtail millet (Setaria italica). Plant Sci, 254, 60-69. doi: 10.1016/j.plantsci.2016.10.010.

Liu S, Cai S, Graybosch R, Chen C, Bai G. 2008. Quantitative trait loci for resistance to pre-harvest sprouting in US hard white winter wheat Rio Blanco. Theor Appl Genet, 117, 691-9. doi:10.1007/s00122-008-0810-7.

Liu Y, Wang L, Xing X, Sun L, Pan J, Kong X, Zhang M, Li D. 2013. *ZmLEA3*, a multifunctional group 3 LEA protein from maize (*Zea mays* L.), is involved in biotic and abiotic stresses. Plant Cell Physiol, 54, 944-59. doi:10.1093/pcp/pct047.

Lou Q, Guo H, Li J, Han S, Khan NU, Gu Y, Zhao W, Zhang Z, Zhang H, Li Z, Li J. 2022. Cold-adaptive evolution at the reproductive stage in Geng/japonica subspecies reveals the role of *OsMAPK3* and O*sLEA9*. Plant J, 111, 1032-1051. doi:10.1111/tpj.15870.

Manfre AJ, LaHatte GA, Climer CR, Marcotte WR Jr. 2009. Seed dehydration and the establishment of desiccation tolerance during seed maturation is altered in the Arabidopsis thaliana mutant *atem6-1*. Plant Cell Physiol, 50, 243-53. doi:10.1093/pcp/pcn185.

Manfre AJ, Lanni LM, Marcotte WR Jr. 2006. The Arabidopsis group 1 LATE EMBRYOGENESIS ABUNDANT protein *ATEM6* is required for normal seed development. Plant Physiol, 140, 140-9. doi:10.1104/pp.105.072967.

McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M, Vasil IK. 1991. The Viviparous-1 developmental gene of maize encodes a novel transcriptional activator. Cell, 66, 895-905. doi:10.1016/0092-8674(91)90436-3.

Mieog J, Janeček Š, Ral J. 2017. New insight in cereal starch degradation: identification and structural characterization of four α-amylases in bread wheat. Amylase, 1, 35-49. doi:10.1515/amylase-2017-0004.

Morris CF, Moffatt JM, Sears RG, Paulsen GM. 1989. Seed dormancy and responses of caryopses, embryos, and calli to abscisic Acid in wheat. Plant Physiol, 90, 643-7. doi:10.1104/pp.90.2.643.

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura H. 2011. A wheat homolog of MOTHER OF FT AND TFL1 acts in the regulation of germination. Plant Cell, 23, 3215-29. doi:10.1105/tpc.111.088492.

Parcy F, Valon C, Raynal M, Gaubier-Comella P, Delseny M, Giraudat J. 1994. Regulation of gene expression programs during Arabidopsis seed development: roles of the ABI3 locus and of endogenous abscisic acid. Plant Cell, 6, 1567-82. doi:10.1105/tpc.6.11.1567.

Sahoo KK, Tripathi AK, Pareek A, Sopory SK, Singla-Pareek SL. 2011. An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. Plant Methods, 7, 49. doi:10.1186/1746-4811-7-49.

Schmittgen, TD and Livak, KJ. 2008. Analyzing real-time PCR data by the comparative CT method. Nat Protoc, 3, 1101-1108. doi:10.1038/nprot.2008.73.

Shiraku ML, Magwanga RO, Zhang Y, Hou Y, Kirungu JN, Mehari TG, Xu Y, Wang Y, Wang K, Cai X, Zhou Z, Liu F. 2022. Late embryogenesis abundant gene LEA3 (Gh_A08G0694) enhances drought and salt stress tolerance in cotton. Int J Biol Macromol, 207, 700-714. doi:10.1016/j.ijbiomac.2022.03.110.

Son S, Chitnis VR, Liu A, Gao F, Nguyen TN, Ayele BT. 2016. Abscisic acid metabolic genes of wheat (*Triticum aestivum* L.): identification and insights into their functionality in seed dormancy and dehydration tolerance. Planta, 244, 429-47. doi:10.1007/s00425-016-2518-2.

Tai L, Wu JH, Jing YX, Liu HZ, Zeng QD, Xu XJ, Shi S, Wang HJ, Liu WT, Sun JQ, Han DJ, Chen KM. 2023. A genome-wide association study uncovers that *TaPI4K-2A* regulates pre-harvest sprouting in wheat. Plant Comm, doi:10.1016/j.xplc.2023.100739.

Torada A, Koike M, Ogawa T, Takenouchi Y, Tadamura K, Wu J, Matsumoto T, Kawaura K, Ogihara Y. 2016. A causal gene for seed dormancy on wheat chromosome 4A encodes a MAP kinase

kinase. Curr Biol, 26, 782-7. doi:10.1016/j.cub.2016.01.063.

Vetch JM, Stougaard RN, Martin JM, Giroux JM. 2019. Review: Revealing the genetic mechanisms of pre-harvest sprouting in hexaploid wheat (*Triticum aestivum* L.). Plant Science, 281, 180-185. doi:10.1016/j.plantsci.2019.01.004.

Walker-Simmons M. 1987. ABA Levels and Sensitivity in Developing Wheat Embryos of Sprouting Resistant and Susceptible Cultivars. Plant Physiol, 84,61-6. doi:10.1104/pp.84.1.61.

Wei WX, Min XY, Shan SY, Jiang H, Cao JJ, Li L, Wang JF, Wang SX, Zhu YL, Lu J, Si HQ, Xia XC, Ma CX, Zhang HP, Chang C. 2019. Isolation and characterization of *TaQsd1* genes for period of dormancy in common wheat (*Triticum aestivum* L.). Molecular Breeding, 39, 1-15. doi:10.1007/s11032-019-1060-x.

Xiao B, Huang Y, Tang N, Xiong L. 2007. Over-expression of a LEA gene in rice improves drought resistance under the field conditions. Theor Appl Genet, 115, 35-46. doi:10.1007/s00122-007-0538-9.

Xu D, Duan X, Wang B, Hong B, Ho T, Wu R. 1996. Expression of a Late Embryogenesis Abundant Protein Gene, HVA1, from Barley Confers Tolerance to Water Deficit and Salt Stress in Transgenic Rice. Plant Physiol, 110, 249-257. doi:10.1104/pp.110.1.249.

Xu F, Tang JY, Wang SX, Cheng X, Wang HR, Ou SJ, Gao SP, Li BS, Qian YW, Gao CX, Chu CC. 2022. Antagonistic control of seed dormancy in rice by two bHLH transcription factors. Nat Genet, 54, 1972-1982. doi:10.1038/s41588-022-01240-7.

Yang Y, Zhao XL, Xia LQ, Chen XM, Xia XC, Yu Z, He ZH, Röder M. 2007. Development and validation of a Viviparous-1 STS marker for pre-harvest sprouting tolerance in Chinese wheats. Theor Appl Genet, 115, 971-80. doi:10.1007/s00122-007-0624-z.

Yu J, Lai Y, Wu X, Wu G, Guo C. 2016. Overexpression of *OsEm1* encoding a group I LEA protein confers enhanced drought tolerance in rice. Biochem Biophys Res Commun, 478, 703-9. doi:10.1016/j.bbrc.2016.08.010.

Yu Z, Wang X, Tian Y, Zhang D, Zhang L. 2019. The functional analysis of a wheat group 3 late embryogenesis abundant protein in Escherichia coli and Arabidopsis under abiotic stresses. Plant Signal Behav, 14, 1667207. doi:10.1080/15592324.2019.1667207.

Zan T, Li L, Li J, Zhang L, Li X. 2020. Genome-wide identification and characterization of late embryogenesis abundant protein-encoding gene family in wheat: Evolution and expression profiles during development and stress. Gene, 736, 144422. doi:10.1016/j.gene.2020.144422.

Zhang Y, Xia X, He Z. 2017. 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. doi:10.1007/s00122-016-2793-0.

Zhao F, Zhao T, Deng L, Lv D, Zhang X, Pan X, Xu J, Long G. 2017. Visualizing the essential role of complete virion assembly machinery in effificient hepatitis C virus cell-to-cell transmission by a viral infection-activated split-intein-mediated reporter system. J Virol, 91, e01720-16. doi:10.1128/ JVI.01720-16.

Zhu Y, Wang S, Wei W, Xie H, Liu K, Zhang C, Wu Z, Jiang H, Cao J, Zhao L, Lu J, Zhang H,

Chang C, Xia X, Xiao S, Ma C. 2019. 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. doi:10.1007/s00122-019-03398-x.

Figure legends

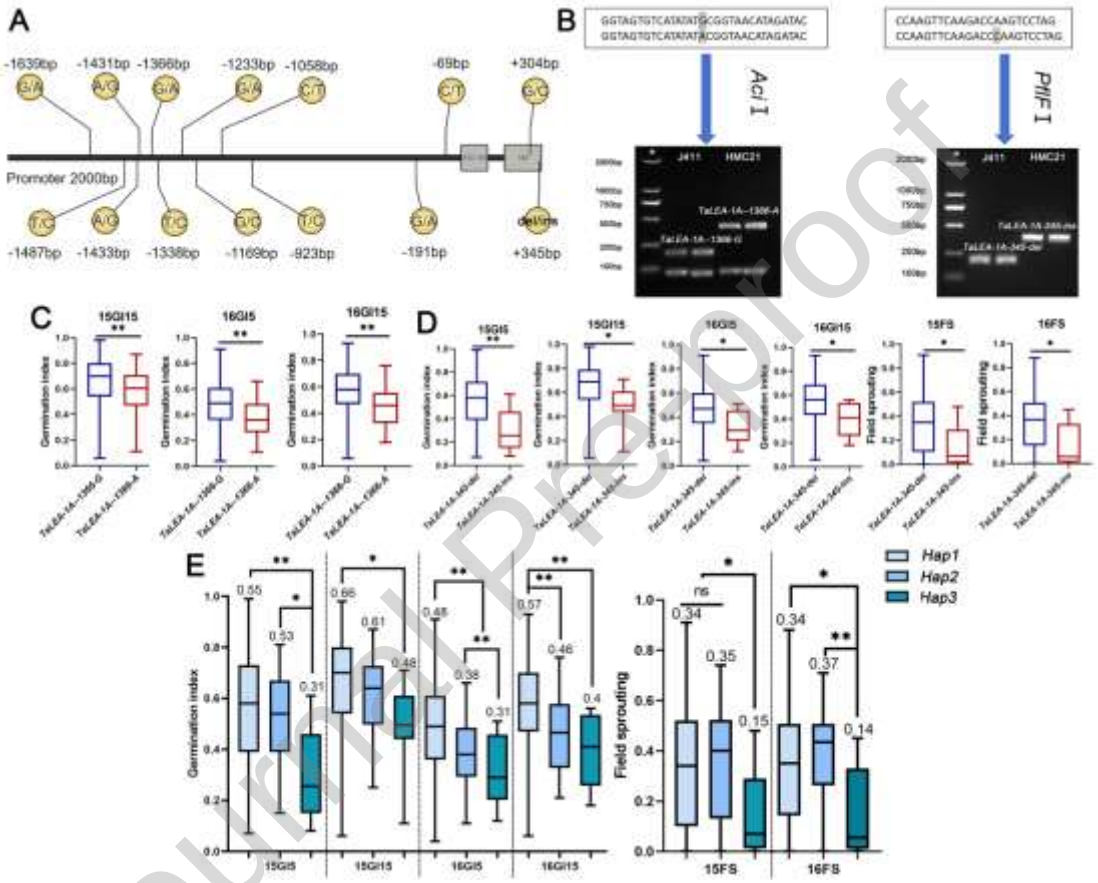

Fig. 1. Sequence variations of the *TaLEA-1A* gene and their association with seed dormancy and germination

A. Sequence analysis of the *TaLEA-1A* gene in weak-dormancy wheat variety Jing 411 (J411) and strong-dormancy Hongmangchun 21 (HMC21). **B.** Development of two CAPS markers and their application in detecting *TaLEA-1A--1366* and *TaLEA-1A-345* variants in *TaLEA-1A* in J411 and HMC21 using *Aci*I (C/CGC) and *Pfl*FI (GACN/NNGTC) on 1.5 % agarose gels, where M represents the 2K DNA marker. J411 and HMC21 indicate electrophoresis patterns of the J411 genotype (*TaLEA-1A--1366-G* and *TaLEA-1A-345-del*) and the HMC21 genotype (*TaLEA-1A--1366-A* and *TaLEA-1A-345-ins*), respectively. **C.** Significant differences observed in germination index (GI) values among the 192 wheat varieties with the two allelic variations (*TaLEA-1A--1366-G* and *TaLEA-1A--1366-A*) of *TaLEA-1A*. **D.** Significant differences observed in germination index (GI) and field sprouting (FS) values among the 192 wheat varieties with the two allelic variations (*TaLEA-1A-345-del* and *TaLEA-1A-345-ins*) of *TaLEA-1A*. **E.** Haplotype analysis of *TaLEA-1A* in

192 wheat varieties, with significant differences based on LSD test. 15GI5, 16GI5, 15GI15, and 16GI15 represent the GI values measured at 5 days and 15 days after harvest in 2015 and 2016, respectively. 15FS and 16FS represent the FS values measured in 2015 and 2016, respectively. *P < 0.05, **P < 0.01.

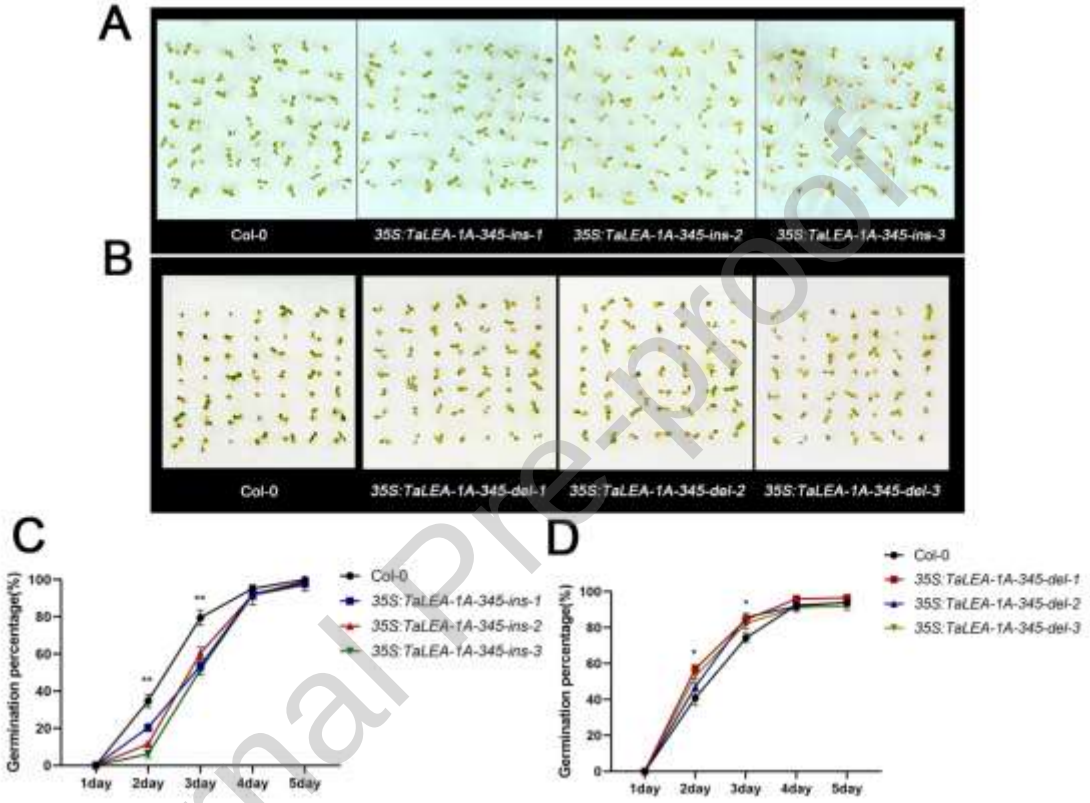

Fig. 2. Germination tests of Arabidopsis seeds with *TaLEA-1A-345-ins* and *TaLEA-1A-345-del* overexpression

A. Images depicting the germination phenotype of T_3 Arabidopsis seeds with *35S:TaLEA-1A-345-ins-1/2/3* overexpression and Col-0 seeds at 7 days of imbibition. **B.** Images depicting the germination phenotype of T_3 Arabidopsis seeds with *35S:TaLEA-1A-345-del-1/2/3* overexpression and Col-0 seeds at 5 days of imbibition. **C.** Germination percentages of T_3 Arabidopsis seeds with *35S:TaLEA-1A-345-ins-1/2/3* overexpression and Col-0 seeds at 5 days of imbibition. **D.** Germination percentages of T_3 Arabidopsis seeds with *35S:TaLEA-1A-345-del-1/2/3* overexpression and Col-0 seeds at 5 days of imbibition. *P < 0.05, **P < 0.01. Germination criteria refer to radicle emergence.

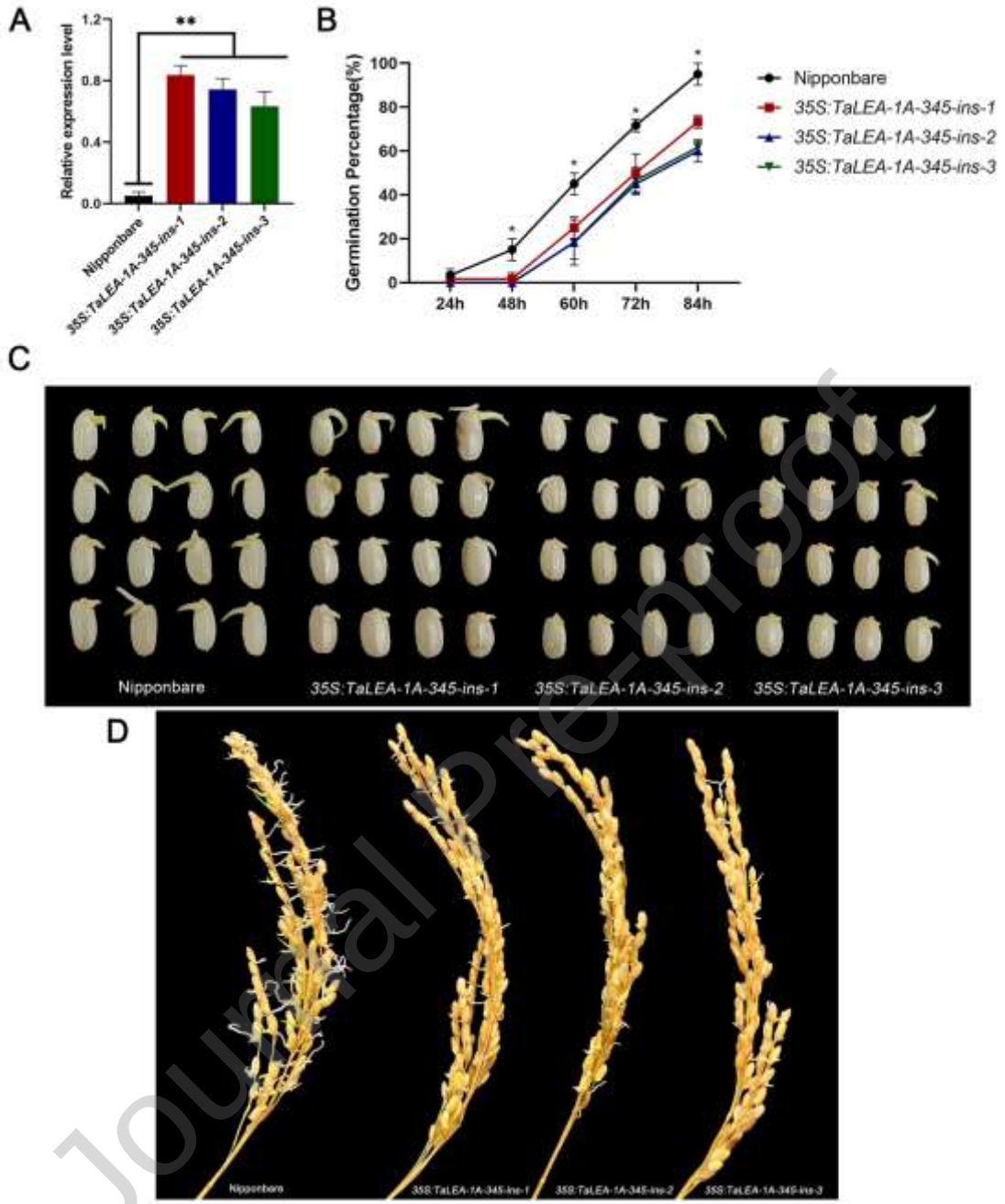

Fig. 3. Germination tests of *TaLEA-1A-345-ins* overexpression rice seeds and panicles

A. Relative expression of *TaLEA-1A* in leaves of wild-type Nipponbare and *35S:TaLEA-1A-345-ins-1/2/3* overexpression rice plants. **B.** Germination percentage of Nipponbare and *35S:TaLEA-1A-345-ins-1/2/3* overexpression rice seeds at 84 h of imbibition. *$P < 0.05$, **$P < 0.01$. **C.** Images of germination phenotype of Nipponbare and *35S:TaLEA-1A-345-ins-1/2/3* overexpression rice seeds at 84 h of imbibition. **D.** Germination phenotypes of the freshly harvested panicles from Nipponbare and *35S:TaLEA-1A-345-ins-1/2/3* overexpression rice plants at 7 days of imbibition. Rice seeds with radicle lengths of >1 mm were considered germinated.

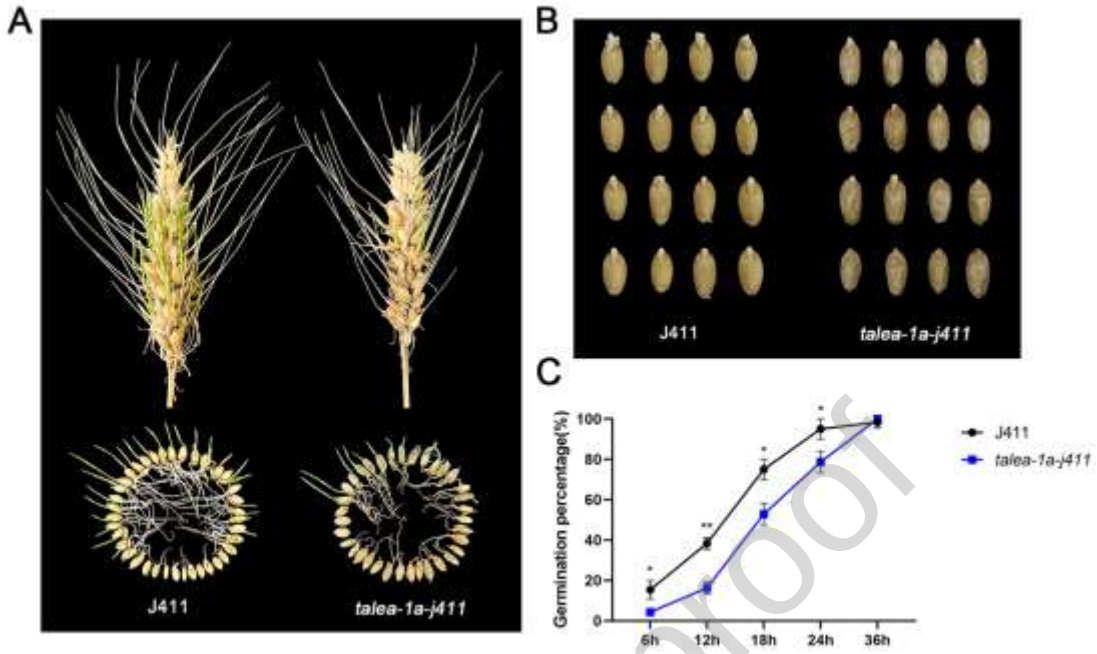

Fig. 4. Germination tests of wild-type J411 and EMS mutant *talea-1a*-j411 intact spikes and seeds **A.** Germination phenotypes of intact J411 and EMS mutant *talea-1a*-j411 spikes after 3 days of culture in a chamber with 90% relative humidity at 22°C. **B.** Germination phenotypes of J411 and EMS mutant *talea-1a*-j411 seeds at 18 h of imbibition. **C.** Germination percentages of J411 and EMS mutant *talea-1a*-j411 seeds at 36 h of imbibition. **P* < 0.05, ***P* < 0.01. The rupture of the pericarp over the embryo was considered germinated.

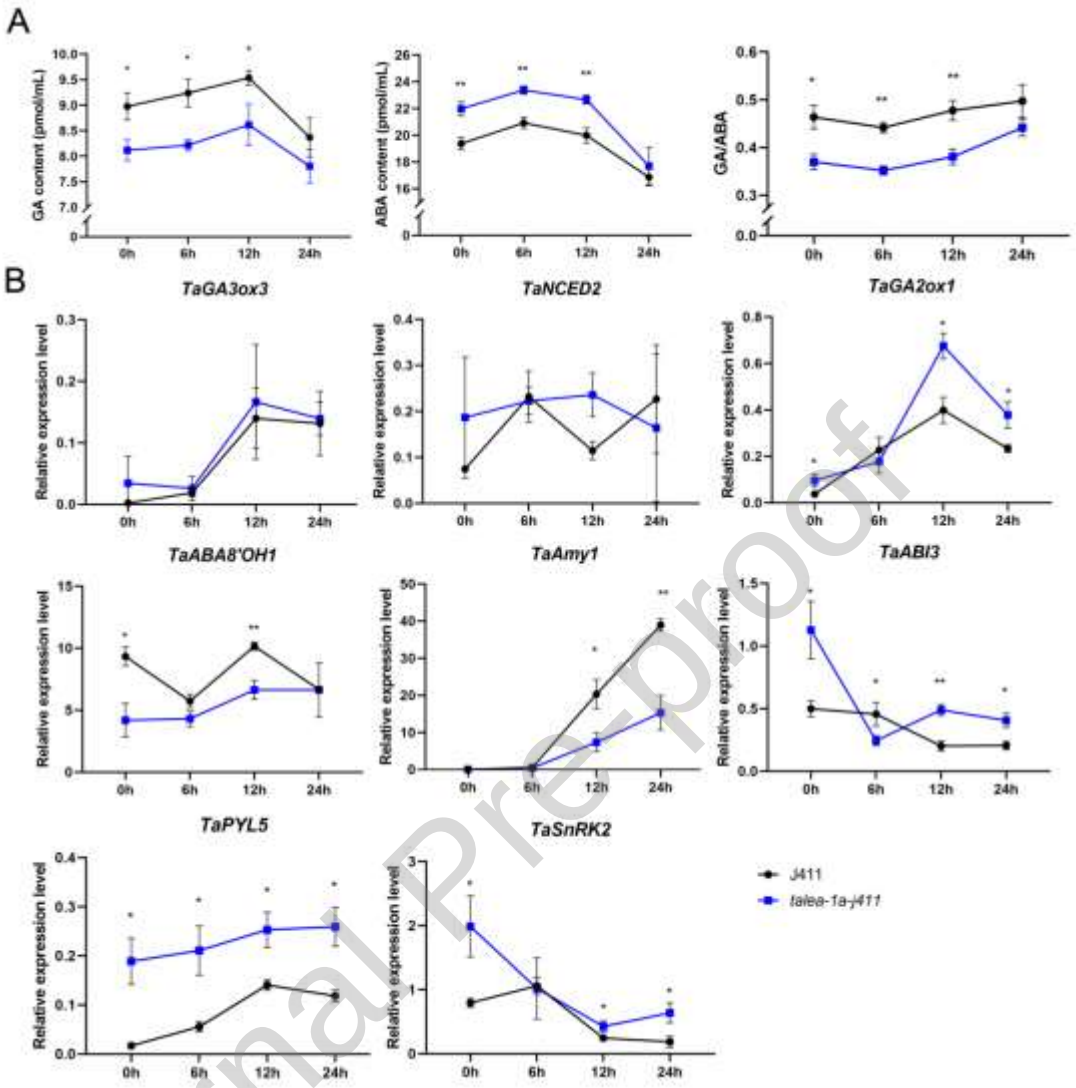

Fig. 5. Hormone GA and ABA contents and expression analysis of genes involved in GA and ABA pathways

A. GA and ABA contents and GA/ABA ratio in the seeds of J411 and EMS mutant *talea-1a*-j411 at 0, 6, 12, and 24 h of imbibition. **B.** Relative expression levels of key genes involved in GA and ABA biosynthesis, metabolism, and signal transduction pathways at 0, 6, 12, and 24 h of imbibition. **P* < 0.05, ***P* < 0.01.

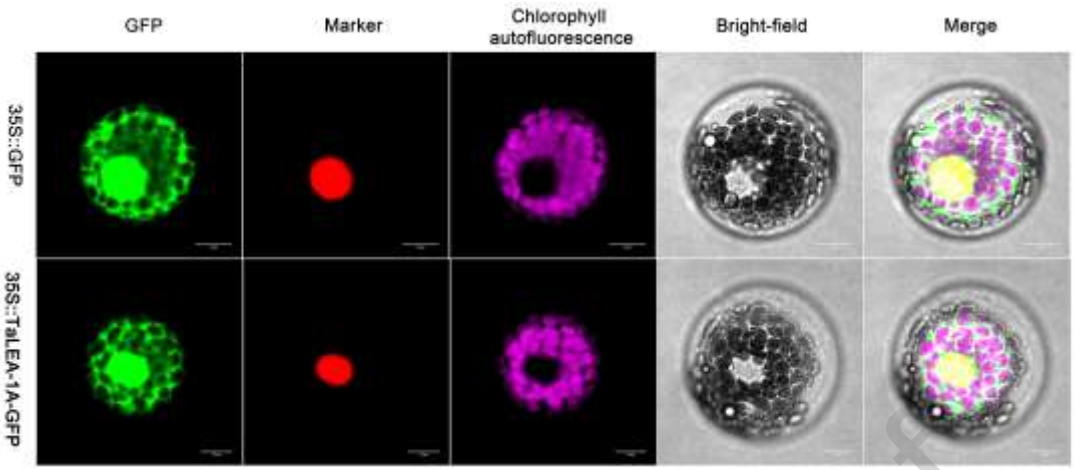

Fig. 6. Subcellular localization of TaLEA-1A in wheat protoplasts. GFP and TaLEA-1A-GFP were transiently expressed in wheat protoplasts under the control of the CaMV 35S promoter using a PEG-mediated approach. Scale bar = 10 μm. At least 5 independent protoplasts were imaged per experiment.

Author contributions

SYL, JY, CC, HPZ, and CXM designed the study. SYL and JY carried out the experiments. SYL, JY, CC, HPZ, and CXM drafted the manuscript. CXL, QX, and BBT prepared the figures and tables. XRC, JJC and JL revised the manuscript. CC, HPZ, and CXM supervised the experiment and coordinated the project.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Highlights

- Role of wheat Late embryogenesis abundant gene *TaLEA-1A* in seed germination and dormancy.
- Discovery of a superior haplotype of *TaLEA-1A* for PHS resistance in wheat.
- We verified the role of *TaLEA-1A* in seed dormancy and germination using transgenic Arabidopsis and rice materials, along with a wheat EMS mutant.
- *TaLEA-1A* may regulate seed dormancy and germination by affecting GA and ABA metabolism and signal transduction pathways.