Isolation and characterization of kelch repeat-containing F-box proteins from colored wheat

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Abstract
F-box proteins play important roles in the regulation of various developmental processes in plants. Approximately 1796 F-box genes have been identified in the wheat genome, but details of their functions remain unknown. Moreover, not much was known about the roles of kelch repeat domain-containing F-box genes (TaKFBs) in wheat. In the present study, we isolated five TaKFBs to investigate the roles of KFBs at different stages of colored wheat grain development. The cDNAs encoding TaKFB1, TaKFB2, TaKFB3, TaKFB4, and TaKFB5 contained 363, 449, 353, 382, and 456 bp open reading frames, respectively. All deduced TaKFBs contained an F-box domain (IPR001810) and a kelch repeat type 1 domain (IPR006652), except TaKFB2. Expression of TaKFBs was elevated during the pigmentation stages of grain development. To clarify how TaKFB and SKP interact in wheat, we investigated whether five TaKFB proteins showed specificity for six SKP proteins using a yeast two-hybrid (Y2H) assay. An Y2H screen was performed to search for proteins capable of binding the TaKFBs and interaction was identified between TaKFB1 and aquaporin PIP1. To examine the subcellular localization of TaKFBs, we transiently expressed TaKFB-green fluorescent protein (GFP) fusions in tobacco leaves; the TaKFB-GFP fusions were detected in the nucleus and the cytoplasm. Y2H and bimolecular fluorescence complementation (BiFC) assays revealed that TaKFB1 specifically interacts with aquaporin PIP1. These results will provide useful information for further functional studies on wheat F-box proteins and their possible roles.

Keywords Wheat · F-box protein · Kelch repeat · Grain development · Yeast two-hybrid · TaKFB

Introduction
Protein degradation via ubiquitin attachment occurs through the following sequential three-step reaction involving ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes [1]. An E3 ligase recognizes target proteins and transfers ubiquitin from the E2 to a specific substrate via the 26S proteasome pathway. E3 ligases are capable of recognizing and binding specifically to target proteins to recruit them for protein degradation, thus ensuring that development and growth are timed accurately. The specificity of protein degradation is mainly determined by E3 ligases, so there are many more different kinds of E3 ligase than E1 or E2 ligase [2]. E3 ligases are highly diverse and important enzymes; it is estimated that there are over 1000 in plants [2]. They may be categorized into three subclasses according to their subunit composition and mechanism of action: CRLs (cullin–RING ligases), RING (really interesting new gene)/U-box ligases, and APC/C (anaphase-promoting complex/cyclosome) ligases [3]. RING/U-box E3 ligases are single-subunit complexes that contain both an active site and binding pocket for the E2 intermediate, while CRLs are multi-subunit complexes that consist of a cullin, a RING H2 finger protein that binds to ubiquitin, and variable substrate adaptors [4]. The SCF [S-phase kinase-associated protein 1 (SKP1)/CULLIN1 (CUL1)/F-Box] complex is the largest in the CRL E3 ligase family in plants. The APC/C is a multi-subunit E3 ligase that plays an important role in the progression of mitotic cell cycle [5].

Electronic supplementary material
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F-box proteins are one of the components of the SCF complex that function in substrate recognition and substrate recruitment for degradation by the 26S proteasome. The N-terminal of F-box proteins is composed of a well-conserved F-box domain (35–60 amino acids) that directly interacts with SKP1. The C-terminal region contains one or several functional domains such as kelch repeats, leucine-rich repeats, WD40 repeats, F-box-associated domains, and tubby domains, which bind specifically to different substrates [6] via protein–protein interaction to recruit target proteins. F-box proteins serve as specificity determinants due to the extensive variability of these C-terminal target-binding domains. In a previous report, 897, 971, and 425 F-box–coding genes were identified in Arabidopsis, rice, and poplar [7], respectively, and categorized into 10 subfamilies based on the type of motif existing at the C-terminal [6].

In plants, F-box proteins are regarded as one of the largest protein superfamilies, which reflect the crucial roles that they play in the modulation of various developmental and physiological processes. Functions of F-box proteins have mainly been reported from Arabidopsis; LKP1/ZTL, LKP2/FKL, and FKF1 are involved in photomorphogenesis, the circadian clock, and flowering time [8–11], while EBF1 and EBF2 regulate ethylene signaling [12] and SLY1 and SNE control gibberellic acid signaling [13]. The role of F-box proteins in jasmonate (COI1) and auxin (TIR1) signaling has also been documented [14, 15]. Additionally, OsFBKJ2 reportedly regulates leaf senescence and grain size through the degradation of target substrates in rice [16]. Few of the functions of F-box proteins have been studied in plant species other than the model plant Arabidopsis, despite the fact that they play important roles in many aspects of plant growth, development, and other physiological processes.

One region of F-box proteins, the kelch repeat domain, was first identified in Drosophila melanogaster [17]. Kelch repeat domain-containing F-box proteins (KFBs) appear more frequently in rice and Arabidopsis than in mammals and yeast [6]. In addition to the functions listed above, KFBs are involved in the regulation of pigmentation. The degradation of Arabidopsis phenylalanine-lyase (PAL) isozymes via the 26S proteasome system is regulated by KFBs, which act as repressors in the phenylpropanoid pathway [18, 19]. Shao et al. demonstrated that KFBs regulate pigment synthesis and deposition in rice hulls. However, the molecular functions of KFBs in wheat are largely unknown [20].

Previous studies in Arabidopsis reported that up or down-regulation of Arabidopsis KFB expression conversely affected the accumulation/deposition of a set of phenylpropanoids, including flavonols, anthocyanins, and condensed tannins [18]. However, not much is known about the roles of KFB in the development of colored wheat grain. Therefore, we selected and isolated wheat KFB genes that showed high sequence similarity to Arabidopsis KFBs. We used colored wheat seeds in the present study; therefore, we tried to search KFB that prompted change in grain color, related to the flavonoid biosynthesis pathway. We isolated kelch repeat domain-containing F-box genes (KFBs) from wheat spike cDNA and examined the expression patterns of TaKFB (Triticum aestivum kelch repeat domain-containing F-box) genes in grains at different developmental stages by qRT-PCR. Green fluorescent protein (GFP) was used to examine the subcellular localization of TaKFB proteins. We explored proteins that may potentially interact with TaKFBs through yeast two-hybrid (Y2H) and bimolecular fluorescence complementation (BiFC) assays. Also, we confirmed that TaKFBs are F-box proteins that can interact with SKPs (a component of the SCF complex). Our results suggest that interaction between TaKFBs and TaSKPs may produce SCF complexes and that TaKFBs have a minor influence on pigmentation in wheat grains.

Materials and methods

Plant materials

A line of common hexaploid wheat with a deep purple grain color (accession no. K4191), developed by Korea University (Republic of Korea), was used in this study [21]. Wheat seeds were grown on a radiation breeding research farm located at 35.5699° N and 126.9722° E (Jeongeup-si, Jeollabuk-do, Korea). After flowering, we tagged wheat spikes to confirm the stage of grain development. Then, we collected wheat grain samples according to their developmental stage (days after fertilization: DAF, DAF9, DAF12, DAF16, DAF20, DAF25, DAF29, and DAF35). Samples were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

Cloning of TaKFBs and respective interacting genes

In order to isolate the full sequences of five TaKFBs and respective interacting genes, primer sets were designed based on sequences downloaded from wheat IWGSC Reference Sequence v1.0 and the NCBI database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Table 1). Then, PCR was performed under the following cycling conditions: initial denaturation at 94 °C for 10 min, 31 cycles of denaturation at 94 °C for 1 min, annealing at a gene-specific temperature for 2 min, elongation at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products were cloned into a pCR8/GW/TOPO cloning vector (Thermo

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Fisher Scientific, USA) and sequenced. Sequencing results were analyzed using the NCBI database and InterProScan (https://www.ebi.ac.uk/interpro/search/sequence-search).

**RNA extraction and qRT-PCR analysis**

Total RNA was extracted from wheat seeds according to the protocol described by Meng and Feldman [22]. For qRT-PCR analysis, 1 μg RNA was converted to cDNA using a Power cDNA Synthesis kit (iNtRON Biotechnology, Korea) according to the manufacturer’s protocol. Then, qRT-PCR was performed in a 48-well plate (Eco Real-Time PCR System, USA) using 1 μL of reverse-transcribed cDNA and 2 × SYBR premix Ex Taq II (Tli RNaseH plus, Takara, Japan). The qRT-PCR thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 10 s, and annealing and extension at 65 °C for 30 s. Three replicates were performed for all qRT-PCR experiments.

**RNA sequencing analysis**

Recombinant inbred lines (RILs) of common wheat used in this study were developed as according to previous reports [23]. Seeds of colored and yellow wheat were grown in controlled environments at 23–26 °C under long-day conditions (16 h/8 h, day/night). We collected plant samples at different developmental stages, including DAF10, DAF20, and DAF30. Total RNA was extracted from each sample using TRIzol reagent (Invitrogen, Waltham, MA, USA). Total RNA (10 μg) from sample was used for constructing RNA-Seq paired-end libraries by TruSeq RNA Sample Preparation Kit (catalog #RS-122-2001; Illumina, CA, USA), and they were sequenced using Illumina Hiseq2000 platform. For reads mapping to the wheat reference genome sequence, an index of the genome sequence was constructed and all reads were aligned to the wheat genome sequence using HISAT2 with default values [24]. HTSeq v0.6.1 counted the number of reads mapped to the exons of each gene [25]. Read count normalization and calculation of log2 fold change were conducted using DESeq2 [26]. Read count and log2 fold changes were used to construct heatmaps of TaFBX genes expression profiling at different seed developmental stages using R package.

**Y2H cDNA library construction and screening assay**

A wheat (Triticum aestivum L.) cDNA library was constructed for Y2H screening from deep purple color wheat seeds (accession no. K4191). First, total RNA was extracted from wheat seeds (DAF20) using Trizol reagent. Next, 2 μg total RNA was used to construct a wheat cDNA library with the Make Your Own Mate & Plant™ Library System (Clontech, Takara, Japan) as per the manufacturer’s protocol.
colonies were pooled with freezing medium (YPDA medium with 25% glycerol), and 1 mL aliquots of the cDNA library were stored at – 80 °C. For the Y2H screen, full-length TaKFB1, TaKFB2, TaKFB3, TaKFB4, and TaKFB5 coding sequences were transferred into a pGBK77-GW bait vector using a Gateway LR recombination reaction (Thermo Fisher Scientific, USA) and used as bait protein for screening. The bait constructs (pGBK77-TaKFBs) were transformed into the yeast strain Y2HGold using the Matchmaker Gold Y2H System (Clontech, Takara, Japan) according to the manufacturer’s protocol. Y2HGold strains harboring bait vectors were maintained in minimal SD medium with tryptophan dropout supplement (SD/-Trp). Then, bait strains and cDNA library (prey) were mixed and incubated at 30 °C with shaking at 30–50 rpm for 20–24 h. The mating cultures were spread onto SD/-Leu/-Trp/X-α-Gal/AbA plates (DDO/X/A) until colonies appeared (after approximately 5–7 days). Colonies on DDO/X/A were transferred to SD/-Ade/-His/-Leu/-Trp/X-α-Gal/AbA (QDO/X/A) plates for further selection.

**Rescue of prey plasmids**

Yeast colonies grown on QDO/X/A plates were colonies with potential positive interactions between bait and prey proteins. Yeast colony PCR using Matchmaker Insert Check PCR Mix 2 (Clontech, Takara, Japan) was performed to select positive clones. Prey plasmids were rescued from the yeast using Easy Yeast Plasmid Isolation kits (Clontech, Takara, Japan). After plasmid DNA extraction, the prey inserts were identified by sequencing, the results of which were analyzed using NCBI/GenBank databases.

**Pairwise Y2H assay**

cDNAs encoding TaKFBs and PIP1 were amplified by PCR using the gene-specific primers listed in Table 1. Amplification products were cloned into the Gateway-compatible shuttle vector pCR8/GW/Topo (Thermo Fisher Scientific, USA) and subcloned into the Gateway-compatible pGBK77 (bait) and pGADT7 (prey) vectors to generate bait DNA-binding and activation domain fusion proteins. For pairwise verification between TaKFBs and PIP1, AH109 yeast cells (Clontech, Takara, Japan) were used according to the manufacturer’s instructions.

**β-galactosidase activity assay**

To confirm the strength of a protein interaction, quantitative β-galactosidase activity was assayed with 2-nitrophenyl β-D-galactopyranoside (ONPG, Sigma) using the Clontech yeast protocols handbook (protocol no. PT3024-1). The yeast cells were grown overnight at 30 °C in SD selective medium; and 2 mL of culture cells were then transferred to 8 mL of YPD media, and the culture was incubated until the OD600 reached 0.8. Thereafter, yeast cells were centrifuged and resuspended in Z-buffer (60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, pH 7.0). The yeast cell pellets were lysed via freeze–thaw cycles. All reactions were initiated by the addition of Z-buffer containing β-mercaptoethanol as well as 4 mg/mL ONPG solution to the samples. The reactions were incubated at 30 °C and stopped after 30 min for the development of yellow color by adding chilled 1 M Na2CO3. Absorbance was measured at 420 nm [27], and β-galactosidase activity was calculated according to the following formula: β-galactosidase activity = 1000 × A420/(T × V × A600), where T = time of reaction (min), and V = volume of culture used in the assay (mL).

**Subcellular localization analysis and BiFC assay**

For subcellular localization analysis, the open reading frames (ORFs) of TaKFBs and PIP1 were cloned into a pMDC43 vector [28] to be fused to the N-terminus of an enhanced GFP gene with LR clonase (Thermo Fisher Scientific, USA). The plasmids, 35S:GFP-TaKFBs and 35S:GFP-PIP1, were introduced into Agrobacterium tumefaciens GV3101 using the freeze–thaw method. Agrobacterium GV3101 harboring 35S:GFP-TaKFBs and 35S:GFP-PIP1 was grown in LB medium until the optical density of the culture reached 1.0 at 600 nm. The Agrobacterium cells were pelleted at 5000 g for 10 min at room temperature and the pellets were resuspended in infiltration buffer (pH 5.6; 10 mM MES, 10 mM MgCl2, 200 mM acetosyringone). Then, tobacco leaves were injected with Agrobacterium solution using a syringe. For the BiFC assay, GFP fusion proteins were produced by the recombination (LR clonase reaction) of the entry vectors pCR/GW/Topo-TaKFBs, and pPI with the destination vectors pGTQL1211-YN (GFP-N) or pGTQL1221-YC (GFP-C) using the Gateway system [29]. All constructs were then introduced into Agrobacterium GV3101 and infiltrated into tobacco leaves based on the methods detailed in a previous report [30]. Tobacco leaves were assayed for fluorescence 72 h after infiltration for both analyses of subcellular localization and the BiFC assay using a confocal laser-scanning microscope (Zeiss LSM800, Germany).

**MG132 treatment and western blot**

Four-week-old N. benthamiana (tobacco) plants were used for infiltration. Agrobacterium tumefaciens GV3101 carrying the 35S-GFP-PIP1 constructs were infiltrated into tobacco leaves and incubated for 48 h at 22 °C. For proteasome inhibitor treatments, tobacco leaves were injected with 50 μM MG132 24 h after Agrobacterium infiltration, then collected after a further 12 h. Total protein was extracted from the tissues in protein extraction buffer (Elpis Biotech,
Korea. The concentration of total protein was determined using a Bradford assay kit (Takara, Japan). For western blotting, total proteins were separated on 12% SDS-PAGE gel and transferred to nitrocellulose membranes using the iBlot 2 Dry Blotting system. Membranes were incubated with primary antibody (rabbit anti-GFP antibody, abcam, UK). Then, HRP-conjugated goat anti-rabbit (Thermo Fisher Scientific, USA) was used as secondary antibody to generate the light signal. The membrane was visualized using Amersham ECL western blotting detection reagents (GE Healthcare, USA) as primary antibody (rabbit anti-GFP antibody, abcam, UK).

Results

Isolation and sequence analysis of KFBs in wheat

Previous reports demonstrate that a group of KFBs interacts with flavonoid biosynthesis proteins to regulate phenylpropanoid biosynthesis in Arabidopsis [18, 19, 31]. In the present study, four Arabidopsis KFBs were used to isolate wheat KFBs: AtKFB1 (AT1G15670.1), AtKFB20 (AT1G80440.1), AtKFB39 (AT2G44130.1), and AtKFB50 (AT3G59940.1). These were BLAST searched against a wheat reference genome sequence (wheat IWGSC Reference Sequence v1.0) provided by URGI (https://urgi.versailles.inra.fr/) with an E-value threshold < 1e−5. We investigated the F-box proteins that regulate anthocyanin biosynthesis during wheat grain development using colored wheat, the seed coat of which starts to change color from green to red at DAF20 [21]. We collected KFBs from a wheat database (Triticum aestivum IWGSC1.0), and putative wheat KFBs were confirmed by the presence of F-box domains in the N-terminus and the kelch repeat domain of the C-terminus using the InterProScan database (https://www.ebi.ac.uk/interpro/search/sequence-search). We performed BLAST-search of the protein sequences of TaKFBs and only selected the sequences with maximum number of hits with quality score (Supplementary Table 1). Finally, KFB1 (TraesCS6A02G037900.1), KFB2 (TraesCS2B02G484200.1), KFB3 (TraeS-C6D02G195800.1), KFB4 (TraesCS6D02G043400.1), and KFB5 (TraesCS7D02G016400.1) were selected five complete TaKFB ORFs (TaKFB1, TaKFB2, TaKFB3, TaKFB4, and TaKFB5) were isolated from wheat spike cDNA. These ORFs were 1092, 1350, 1062, 1149, and 1371 bp in size, respectively, and encoded 363, 449, 353, 382, and 456 amino acids, respectively (Table 2). The molecular weights of the TaKFBs had a pI range between 38.80 and 49.98 kDa (Table 2). According to the blast results, TaKFB were likely to be located on chromosomes 2, 6, and 7. TaKFB1, TaKFB3, and TaKFB4 produced significant hits to sequences located on wheat chromosome 6A, 6B, and 6D (Supplementary Table 1). Especially, TaKFB1 and TaKFB4 represented hits of same region in wheat chromosome 6. Moreover, TaKFB1 and TaKFB4 shared higher levels of amino acid sequence similarity (Supplementary Fig. 1). The domain annotation of TaKFB genes was conducted using DoMosaics software based on a local installation of HMMscan against Pfam-defined models (Table 1). Sequence analysis indicated that all deduced TaKFBs contain an F-box domain (IPR001810), except TaKFB2 (Fig. 1). TaKFB2 encoded a truncated form of F-box protein in wheat (Supplementary Fig. 2). The C-terminus region of the TaKFBs consists of approximately 1–3 kelch repeat type 1 domains (IPR006652), as predicted (Fig. 1).

TaKFBs interact with TaSKPs

F-box protein, a component of the E3 ubiquitin ligase SCF complex, interacts with SKP1 [32]. To confirm the interaction between TaKFBs and TaSKPs, we investigated whether five TaKFB proteins exhibited specificity for six TaSKP proteins using Y2H assay. The TaKFB sequences were fused to the DNA-binding domain of GAL4 in a pGBKTK7 vector to serve as bait, and six TaSKP sequences were fused to the DNA activation domain of GAL4 in a pGADT7 vector [33]. After mating, all combinations grew well on DDO/X/A (Fig. 2a), whereas several combinations showed no interaction on QDO/X/A. We found that TaKFB1, TaKFB3, and TaKFB4 could interact with TaSKP1 and TaSKP6 on QDO/X/A (Fig. 2b). In addition, TaKFB4 interacted with TaSKP3. However, there was no interaction between TaKFB2 and TaKFB5 and any of the TaSKPs examined. Results of the Y2H screening for the quantification of the relative interaction strength between TaKFBs and TaSKPs were measured by performing β-galactosidase assay (Fig. 2c). β-galactosidase activities for the interaction between TaKFBs and TaSKPs ranged from 18.6 to 33.9 units. Although all constructs showed low activities compared with the positive control, the relative strength of interaction between TaKFB1, TaKFB3, and TaKFB4 as well as between TaSKP1, TaSKP4, and TaSKP6 was at remarkable levels. As expected, β-galactosidase activity was very low in the negative control.

Screening of proteins interacting with TaKFBs

To identify potential TaKFB-binding partners, each TaKFB fragment fused with the GAL4 DNA-binding domain was used as bait to screen the wheat grain development cDNA library fused to a synthetic activation domain in yeast. From a total of approximately 2 × 10⁸ transformants, the first screening, involving blue/white selection on DDO/X/A, identified 61 (TaKFB1-interacting partners) and 74...
| Gene   | CDS (bp) | Total length (aa) | MW (kDa) | pI   | InterProScan F-box domain (IPR001810) | Kelch repeats (IPR006652) | HMMscan results | Pfam ID | Position | E-value |
|--------|----------|-------------------|----------|------|--------------------------------------|---------------------------|------------------|---------|----------|---------|
| TaFBX1 | 1092     | 363               | 41.10    | 6.51 | 22–92                                | 128–175                   | F-box            | PF00646 | 28–68    | 1.60E-07 |
|        |          |                   |          |      | Kelch_1                              |                           |                  | PF01344 | 117–162  | 6.60E-08 |
|        |          |                   |          |      | Kelch_4                              |                           |                  | PF13418 | 125–163  | 9.30E-07 |
|        |          |                   |          |      | Kelch_1                              | 234–280, 294–327         |                  | PF01344 | 234–280  | 6.20E-09 |
|        |          |                   |          |      | Kelch_4                              |                           |                  | PF13418 | 234–279  | 2.30E-04 |
|        |          |                   |          |      | Kelch_4                              |                           |                  | PF134144 | 283–327  | 2.30E-07 |
| TaFBX2 | 1350     | 449               | 49.98    | 6.10 | –                                    | 234–280, 294–327         |                  | PF01344 | 117–162  | 6.60E-08 |
|        |          |                   |          |      | Kelch_1                              | 234–279                 |                  | PF13414 | 234–279  | 2.30E-04 |
|        |          |                   |          |      | Kelch_1                              | 283–327                 |                  | PF13414 | 283–327  | 2.30E-07 |
|        |          |                   |          |      | Kelch_1                              | 283–327                 |                  | PF13414 | 283–327  | 2.30E-07 |
| TaFBX3 | 1062     | 353               | 38.80    | 6.39 | 9–52                                 | 109–166, 167–215, 297–352 |                  | PF00646 | 11–46    | 2.00E-06 |
|        |          |                   |          |      | Kelch_1                              | 99–152                  |                  | PF01344 | 99–152   | 1.40E-07 |
|        |          |                   |          |      | Kelch_1                              | 155–201                 |                  | PF01344 | 155–201  | 2.70E-12 |
|        |          |                   |          |      | Kelch_4                              | 155–200                 |                  | PF13418 | 155–200  | 2.40E-04 |
|        |          |                   |          |      | Kelch_6                              | 156–201                 |                  | PF13418 | 156–201  | 3.60E-06 |
|        |          |                   |          |      | Kelch_2                              | 157–199                 |                  | PF07646 | 157–199  | 2.50E-05 |
| TaFBX4 | 1149     | 382               | 43.25    | 6.51 | 41–112                               | 136–194                 |                  | PF00646 | 47–89    | 3.80E-07 |
|        |          |                   |          |      | Kelch_1                              | 136–181                 |                  | PF01344 | 136–181  | 7.10E-08 |
|        |          |                   |          |      | Kelch_4                              | 144–182                 |                  | PF13418 | 144–182  | 1.00E-06 |
| TaFBX5 | 1371     | 456               | 49.72    | 7.53 | 36–82                                | 171–217, 218–268         |                  | PF00646 | 39–80    | 4.20E-05 |
|        |          |                   |          |      | Kelch_1                              | 159–204                 |                  | PF01344 | 159–204  | 3.60E-12 |
|        |          |                   |          |      | Kelch_1                              | 212–255                 |                  | PF01344 | 212–255  | 1.70E-08 |
(TaKFB3-interacting partners) clones that consistently activated LacZ expression. TaKFB2, TaKFB4, and TaKFB5 showed no interaction with any transformants. To avoid false positive clones, we performed a secondary screening on QDO/X/A plates, which identified six and five blue colony clones from TaKFB1 and TaKFB3, respectively. After the exclusion of false positive and out-of-frame clones, we obtained four and three clones that potentially interacted with TaKFB1 and TaKFB3, respectively (Table 3). Finally, aquaporin1 (PIP1) was identified as a TaKFB1-interacting protein.

**Subcellular localization of TaKFB proteins**

The subcellular localization of TaKFBs was predicted using the WoLF PSORT II program, the results of which are shown in Table 4. Subcellular localization of TaKFBs was different according to the amino acid composition. To confirm the results of the bioinformatic predictions, we identified the subcellular localization of TaKFB proteins in plant cells. We constructed a vector expressing translational GFP-TaKFB fusion proteins and then infiltrated tobacco leaves with *Agrobacterium tumefacies* strain Lamin-C (pGBK7-Lam) were used as positive (P) and negative controls (N), respectively. The strength of interactions of the TaKFBs and SKPs was measured by performing ß-galactosidase assay using ONPG as a substrate.

| Table 3 TaKFB interaction partners identified by Y2H screening |
|---|
| **Bait** | **Putative identification** | **Organism** |
| TaKFB1 | Chlorophyll a-b binding protein CP26 | *Aegilops tauschii* |
| | NAD(P)H-quinone oxidoreductase subunit 1 | *Aegilops tauschii* |
| | Aquaporin PIP1 (PIP1) | *Triticum aestivum* |
| TaKFB3 | Nonspecific lipid transfer protein 5 (LTP5) | *Triticum aestivum* |
| | Alpha-gliadin storage protein | *Triticum aestivum* |
| | Serine carboxypeptidase-like 18 | *Brachypodium distachyon* |
| | Heat shock protein 90 | *Triticum aestivum* |

Clones used for pairwise Y2H and BiFC assays are indicated in bold.
GV3101, pMDC43 (GFP alone) was used as a control. Tobacco leaves transformed with pMDC43 showed bright fluorescence localized to the nucleus and the cytoplasm. The result of subcellular localization of TaKFBs by GFP in vivo assay in tobacco cell showed different results from the prediction by WoLF PSORT II program. The GFP signals of TaKFB1, TaKFB3, TaKFB4, and TaKFB5 were detected in the nucleus (Fig. 3a), but the fluorescence signal of the GFP-TaKFB2 fusion protein was detected in the plasma membrane or the cytoplasm (Fig. 3a). PIP1, a TaKFB1-interacting partner, was mainly observed in the plasma membrane and plasmodesmata, and PIP GFP signals were also detected in the cytoplasm or cytoskeleton (Fig. 3b). The bioinformatic prediction for the PIP1 protein was therefore similar to the actual experimental results. However, the experimental results and bioinformatic predictions conflicted for TaKFBs (Table 4).

**TaKFB1 protein interacts with aquaporin PIP1**

To confirm the specificity of the interaction between TaKFBs and candidate proteins, we amplified the full-length cDNAs of the TaKFBs and their respective candidate interacting genes and revalidated their pairwise interactions by yeast co-transformation. The experiment showed that all five TaKFB proteins interact with the PIP1 protein, as

![Subcellular localization of GFP-TaKFBs and PIP1-fusion proteins visualized by confocal microscopy.](image)

**Table 4** Prediction of the subcellular localization of TaKFBs using WoLF PSORT II

| Name  | PSORT predictions | Localization in plant cell |
|-------|-------------------|---------------------------|
| TaKFB1 | cyto: 10, mito: 2, chlo: 1 | Nucleus |
| TaKFB2 | nucl: 9, cyto: 2, mito: 1, chlo:1 | Plasma membrane or cytoplasm |
| TaKFB3 | nucl: 3, cyto: 5, chlo:3, cysk: 2 | Nucleus |
| TaKFB4 | chlo: 9, extr: 2, ER: 2 | Nucleus |
| TaKFB5 | nucl: 1.5, nucl_plas: 2, cyto: 6, chlo: 3, plas: 1.5, | Nucleus |
|        | pero: 1           |              |
| PIP1   | plas: 7, cyto: 3, cysk: 2.5, cysk_nucl: 2 | Plasma membrane or plasmodesmata |

nucl nuclear, cyto cytosol, mito mitochondria, chlo chloroplast, plas plasma membrane, cysk cytoskeleton, extr extracellular, pero peroxisome

![Subcellular localization of GFP-TaKFBs and PIP1-fusion proteins visualized by confocal microscopy.](image)
evaluated by growth on QDO/X/A plates. However, the strength of these interactions was lower than the positive control, but the β-galactosidase activity between TaKFB1 and PIP1 was strong enough (Fig. 4b). To examine the interactions between TaKFB1 and PIP1 protein in planta, we performed a BiFC assay using tobacco leaves. TaKFB1 was fused to the N-terminal fragment of GFP (EGFP-N) to generate pGTQL1211-TaKFB1, and PIP1 was fused to the C-terminal fragment of GFP (EGFP-C), generating pGTQL1221-PIP1. GFP signals of pairwise expression of TaKFB1 and PIP1 were detected in the nucleus (Fig. 4c).

Expression of TaKFB genes and their respective interacting partners during grain development

To explore the functions of the TaKFB genes and their respective interacting genes, we examined gene expression patterns by qRT-PCR. The wheat grain sample used in this

Fig. 5 Relative expression of TaKFB genes at different time points during grain development. a Different stages of wheat grain development. Wheat seed coats started to change color from green to purple at DAF20. DAF day after fertilization. b TaKFBs and PIP1 transcripts quantified by qRT-PCR in wheat grains at various developmental stages. Each bar represents the mean ± SD of three independent experiments. (Color figure online)
study started to change color from green to purple at DAF20 (Fig. 5a). The pattern of expression of TaKFB1 and TaKFB4 was similar. TaKFB1 and TaKFB4 transcripts were detected in high numbers at DAF20, but their expression levels were similarly low at all other developmental stages (Fig. 5b). TaKFB2 expression was highest at DAF9, then decreased at DAF12 and DAF16, and slightly increased at DAF20. TaKFB3 showed the highest expression at DAF20, followed by a sharp decline at DAF24 and DAF29 before increasing again at DAF35. The TaKFB5 transcripts were continuously induced up to DAF24, then decreased thereafter. The expression of the TaKFB1-interacting protein aquaporin PIP1 (PIP1) was continuously induced up to DAF16, then sharply decreased at DAF20, and increased again at DAF25 (Fig. 5b). After that, a strong induction of PIP1 transcripts was observed at DAF35. In addition, we used RNA-seq analysis to compare the expression of TaKFB genes between colored and yellow wheat during different seed developmental stages to determine whether their expressions are specific to colored seed pericarp. Corresponding with the results of qRT-PCR, heatmap analysis of TaKFB3 showed a high expression pattern at DAF20 and DAF30, and the expression levels of other TaKFB genes were increased at DAF20, with the pericarp color turning purple because of the accumulation of pigments, such as anthocyanin (Supplementary Fig. 3).

TaKFB1-interacting protein is degraded by the 26S proteasome

In order to investigate PIP1 protein degradation by the 26S proteasome, we analyzed a cell-free extract in either the presence or absence of the proteasome inhibitor MG132 (Sigma, USA) using the transient expression in tobacco. Western blotting with GFP antibodies was used to validate the expression of the transgene. As shown in Fig. 6, the GFP-PIP1 signal was increased in extracts treated with MG132 at the same point (Fig. 6). These results indicate that the TaKFB1 target substrate protein (aquaporin PIP1) is regulated by 26S proteasome-mediated proteolysis.

Discussion

F-box proteins are involved in controlling various processes in plants, including photomorphogenesis, the circadian clock, flower development, hormone signaling, self-incompatibility, and senescence [34]. F-box proteins, a component of the SCF complex and a large protein superfamily in plants, contain an F-box motif in the N-terminal region and various types of protein–protein interaction domains in the C-terminal region, such as leucine-rich repeats, kelch repeats, tetratricopeptide repeats, and WD40 repeats [6] that bind target substrates and can be used to categorize the F-box protein. The diversity of the C-terminal domain confers specificity to the F-box proteins, which bind specific interacting substrates. In plants, the kelch domain is one of the most frequently occurring subfamilies among the many C-terminal domains of F-box proteins [35]. Previous reports indicate that a few characterized wheat KFBs are involved in inflorescence development or tolerance to heat stress [36, 37]. However, little information is available about KFBs in wheat. The present study was carried out to elucidate the functions of KFBs in wheat grain color changes during grain development.

The conserved F-box domain interacts with SKP1-related proteins to form the SCF complex and F-box proteins recruit specific substrates via a protein–protein interaction domain in the C-terminal region to degrade target substrates [7]. Previous reports demonstrate that Arabidopsis KFBs function as regulators controlling phenylpropanoid biosynthesis via the mediation of PAL ubiquitination and subsequent degradation, affecting seed coloration [18, 19]. Furthermore, Yu et al. demonstrated that Arabidopsis SMALL AND GLOSSY LEAVES1 (SAGL1), a Kelch repeat F-box protein, regulates phenylpropanoid biosynthesis post-translationally at PAL1 and transcriptionally at anthocyanin synthase (ANS) [38]. However, the functions of wheat KFBs in grain development are not known. To investigate this topic, we isolated five TaKFBs from colored wheat grains by PCR-based cloning. Although the sequence compositions of the five respective TaKFB proteins are different, we confirmed that all contained a conserved F-box domain at their N-terminal and one to three kelch repeats at the C-terminal (Fig. 1). Except for TaKFB2, all TaKFBs had one F-box domain in their N-terminal region. The F-box motif is a well-conserved N-terminally located sequence of about 70 amino acids that mediates protein–protein interaction [39]. F-box proteins can interact with SKP1-related proteins via an N-terminal F-box.

![Fig. 6](https://example.com/fig6.png) Inhibition of protein degradation by 26S proteasome in planta. Western blot analysis using protein extracts prepared from transiently transformed tobacco leaves expressing GFP-PIP1 treated with (+) or without (−) 50 µM MG132. The GFP-PIP1 was monitored by western blot using anti-GFP antibody. CBB staining served as the control for the amount of protein loading.
domain to form the SCF complex for ubiquitination by the 26S proteasome [6]. In the present study, we confirmed the interactions between TaKFBs and TaSKPs (Fig. 2). Our data showed that TaKFB1, TaKFB3, and TaKFB4 can interact with TaSKP1 and TaSKP6, and that TaKFB4 can interact with TaSKP3, whereas TaKFB2 and TaKFB5 did not show any significant interaction with any of the tested TaSKPs. No interactions between TaKFB2, which lacks an F-box domain, and TaSKPs were found. These results indicate that F-box domains at the N-terminus end of F-box proteins are essential for interaction with SKP1 proteins in wheat. Also, the GFP signals of TaKFB2 were detected in the plasma membrane, but all other TaKFB-GFP signals were localized to the nucleus (Fig. 3). These data indicate that the F-box domain may be involved in the translocation of TaKFBs to the nucleus. Y2H assay showed that TaKFB5 did not interact with any TaSKPs, even though TaKFB5 contains an F-box domain. It is possible that not all F-box domains participate in the formation of an SCF complex, and some F-box proteins seem to function without interacting with SKP1.

TaKFB1 and TaKFB4 contain one conserved domain, kelch repeat type 1 (IPR006652), at the C-terminal. TaKFB2 and TaKFB5 have two kelch repeats and TaKFB3 has three kelch repeats in the C-terminal region. The kelch motif is composed of about 44–56 amino acid residues, which constitute five to seven repeats that form a β-propeller tertiary structure [40]. Kelch β-propellers have the ability to serve as scaffolds for protein–protein interaction [41]. F-box and kelch domains are the most commonly discovered protein domains in the living organism; however, the co-existence of F-box and kelch domains is an interesting feature of plant F-box proteins [35]. TaKFBs contain F-box domain and one to three kelch repeats like plant feature. We hypothesized that TaKFBs recognize specific proteins via the kelch repeat domain at C-terminus. Also, sequence differences in the kelch repeat suggest that TaKFBs may be able to interact with multiple substrates. In this study, we carried out a yeast library screening analysis in order to elucidate the roles of TaKFBs and their interacting proteins in wheat grain development. The Y2H assay showed that TaKFB1 physically interacts with aquaporin PIP1 (Table 3, Fig. 4).

Aquaporins are thought to play an important role in increasing membrane permeability to facilitate the mobilization of water [42]. The roles of Arabidopsis aquaporin (TIP1:1, TIP1:2) in anthocyanin content changes and catalase activity reduction have been reported [43]. Also, aquaporin is known as a regulator, which is involved in various environmental stress responses and is generally located in the plasma membrane [44, 45]. As in previous reports, aquaporin PIP1-GFP signals were mainly detected in the plasma membrane and plasmodesmata in the present study (Fig. 3b). The BiFC assay revealed that an interaction occurs between TaKFB1 and PIP1 in the nucleus (Fig. 4c); it is, therefore, possible that PIP1 protein is regulated by TaKFB1 in the nucleus. Using a Y2H assay and BiFC analysis, we confirmed that TaKFB1 could interact with aquaporin PIP1 (Fig. 4). Our results suggest that TaKFB proteins are involved in the formation of the SCF complex and act as a connector between the SCF complex and target substrates.

We used qRT-PCR analysis to investigate the functions of TaKFBs and their respective interacting proteins (Fig. 5) and confirmed that TaKFB genes were upregulated during the pigmentation stage of grain development in wheat (Fig. 5b). The result of phylogenetic tree showed that TaKFB1 and TaKFB4 belong to same clade (Supplementary Fig. 1). Moreover, high levels of sequence similarity between TaKFB1 and TaKFB4 showed similar expression patterns. These results indicated that sequence similarity between TaKFB1 and TaKFB4 may influence similar functions of gene expression at grain development stages. Although we only performed expression studies of TaKFBs at the grain development stage, the expression of TaKFB genes might be associated with the characteristics of organ- or tissue-specific expression partitioning in different tissues and under various environmental conditions. For example, the expression profiles of TaGFs, one of the wheat gene families, were diverse in different tissues and grain developmental periods, which could be inferred from the analysis of WheatExp in public transcriptome data [46]. Therefore, further work is still needed to demonstrate expression patterns of TaKFBs in different tissues under diverse conditions.

PIP1 transcripts showed the lowest level at the seed pigmentation stage (Fig. 5b). The Arabidopsis double mutant tip1;1-1 tip1;2-1 exhibited a slightly increased anthocyanin content when compared with that in wild type [43]. Similar to TIP1;1-1 TIP1;2-1, the change in anthocyanin content at grain development in colored wheat might be associated with the action of PIP1. According to our analysis of gene expression and co-localization, we hypothesized that an increase in anthocyanin content due to increased TaKFB gene expression might be associated with decreased PIP1 expression at the functions of F-box proteins. Using the Y2H assay and BiFC analysis, we confirmed that TaKFB1 is an F-box protein and could interact with SKP1 in an F-box domain-dependent manner, and it acts as a connector between aquaporin PIP1 and SKP1. Moreover, we confirmed that PIP1 protein is regulated by the 26S proteasome by using tobacco leaves treated with MG132, a proteasome inhibitor that prevents protein degradation by the 26S proteasome. GFP-PIP1 was highly detectable in MG132-treated samples (Fig. 6), indicating that MG132 treatment greatly blocked the degradation of PIP1 protein.

According to the present study, it is possible that TaKFB1 acts as a connector between PIP1 and the SCF complex and provides SCF with substrate specificity for ubiquitin ligase complexes. Also, we found that PIP1 protein is targeted by...
TaKFB1 for degradation by the 26S proteasome. Here, we report that TaKFB1, a kelch repeat F-box protein, interacts with PIPI protein for proteasome-mediated degradation to regulate anthocyanin content in colored wheat. Although further studies are needed to reveal the functions of TaKFBs and their respective interacting proteins, identification and characterization of KFBs in the present study will help to facilitate future research into the molecular basis of F-box proteins in wheat.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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References

1. Hillmann H, Estelle M (2002) Plant development: regulation by protein degradation. Science 297:793–797
2. Chen L, Hellmann H (2013) Plant E3 ligases: flexible enzymes in a sessile world. Mol Plant 6:1388–1404
3. Zeng LR, Park CH, Venu RC, Gough J, Wang GL (2008) Classification, expression pattern, and E3 ligase activity assay of rice U-box-containing proteins. Mol Plant 1:800–815
4. Hua Z, Vierstra RD (2011) The cullin-RING ubiquitin-protein ligases. Annu Rev Plant Biol 62:299–334
5. Eloy NB, Lima MF, Ferreira PCG, Inzé D (2015) The role of the anaphase-promoting complex/cyclosome in plant growth. Crit Rev Plant Sci 34:487–505
6. Jain M, Nijhawan A, Arora R, Agarwal P, Ray S, Sharma P, Kapoor S, Tyagi AK, Khurana JP (2007) F-box proteins in rice. Genomes-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. Plant Physiol 143:1467–1483
7. Hua Z, Zou C, Shiu SH, Vierstra RD (2011) Phylogenetic comparison of F-Box (FBX) gene superfamilies within the plant kingdom reveals divergent evolutionary histories indicative of genomic drift. PLoS ONE 6:e16219
8. Nelson DC, Lasswell J, Rogg LE, Cohen MA, Bartel B (2000) FKFI, a clock controlled gene that regulates the transition to flowering in Arabidopsis. Cell 101:331–340
9. Schulman BA, Carrano AC, Jeffrey PD, Bowen Z, Kinningan ER, Fimm MA, Elledge SJ, Harper JW, Pagano M, Schultz TF, Kiyosue T, Yanovsky M, Wada M, Kay SA (2001) A role for LKP2 in the circadian clock of Arabidopsis. Plant Cell 13:2659–2670
10. Imaizumi T, Tran HG, Swartz TE, Briggs WR, Kay SA (2003) FKFI is essential for photoperiodic-specific light signaling in Arabidopsis. Nature 426:302–306
11. Han L, Mason M, Risseeuw EP, Crosby WL, Somers DE (2004) Formation of an SCF (ZTL) complex is required for proper regulation of circadian timing. Plant J 40:291–301
12. Guo H, Ecker JR (2003) Plant responses to ethylene gas are mediated by SCF (EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. Cell 115:667–677
13. McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM (2003) The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15:1120–1130
14. Devoto A, Ellis C, Magusin A, Chang HS, Chilcott C, Zhu T, Turner JG (2005) Expression profiling reveals COII to be a key regulator of genes involved in wound and jasmonate-induced secondary metabolism, defence, and hormone interactions. Plant Mol Biol 58:497–513
15. Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCF (TIR1)-dependent degradation of AUX/IAA proteins. Nature 414:271–276
16. Chen Y, Xu Y, Luo W, Li W, Chen N, Zhang D, Chong K (2013) The F-box protein OsFBK12 targets OsSAM1 for degradation and affects pleiotropic phenotypes, including leaf senescence in rice. Plant Physiol 163:1673–1685
17. Bork P, Doolittle R (1994) Drosophilia kelch motif is derived from a common enzyme fold. J Mol Biol 236:1277–1282
18. Zhang X, Gou M, Liu CJ (2013) Arabidopsis Kelch repeat F-box proteins regulate phenylpropanoid biosynthesis via controlling the turnover of phenylalanine ammonia-lyase. Plant Cell 25:4994–5010
19. Zhang X, Gou M, Guo C, Yang H, Liu CJ (2015) Down-regulation of Kelch domain-containing F-box protein in Arabidopsis enhances the production of (poly)phenols and tolerance to ultraviolet radiation. Plant Physiol 167:337–350
20. Shao T, Qian Q, Tang D, Chen J, Li M, Cheng Z, Luo Q (2012) A novel gene IBF1 is required for the inhibition of brown pigment deposition in rice hull furrows. Theor Appl Genet 125:381–390
21. Hong MJ, Kim DY, Nam BM, Ahn JW, Kwon SJ, seo YW, Kim JB (2019) Characterization of novel mutants of hexaploid wheat (Triticum aestivum L.) with various depths of purple grain color and antioxidant capacity. J Sci Food Agric 99:5544–5010
22. Meng L, Feldman L (2010) A rapid TRIZol-based two-step method for DNA-free RNA extraction from Arabidopsis siliques and dry seeds. Biotechnol J 5:183–186
23. Shin OH, Kim DY, Seo YW (2017) Effects of different depth of grain colour on antioxidant capacity during water imbibition in wheat (Triticum aestivum L.). J Sci Food Agric 97:2750–2758
24. Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12:357–360
25. Anders S, Pyl PT, Huber W (2015) HTSeq- Python framework to work with high-throughput sequencing data. Bioinformatics 31:166–169
26. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550
27. Millar JH (1972) Experiments in molecular genetics: assay of 8-galactosidase. CSH laboratory press, Cold Spring Harbor, New York, pp 352–355
28. Curtis MD, Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in plants. Plant Physiol 133:462–469
29. Lu Q, Tang X, Tian G, Wang F, Liu K, Nguyen V, Kohalmi SE, Keller WA, Tsang EW, Harada JI, Rothstein SJ (2010)
Arabidopsis homolog of the yeast TREX-2 mRNA export complex: components and anchoring nucleoporin. Plant J 61:259–270
30. Walter M, Chaban C, Schutze K, Batistic O, Weckermann K, Nake C, Blazevic D, Grefen C, Schumacher K, Oecking C (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. Plant J 40:428–438
31. Zhang X, Abraham C, Colquhoun TA, Liu CJ (2017) A proteolytic regulator controlling chalcone synthase stability and flavonoid biosynthesis in Arabidopsis. Plant Cell 29:1157–1171
32. Bai C, Sen P, Hofmann K, Ma L, Harper JW, Elledge SJ (1996) SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell 86:263–274
33. Hong MJ, Kim DY, Seo YW (2013) SKP1-like-related genes interact with various F-box proteins and may form SCF complexes with Cullin-F-box proteins in wheat. Mol Biol Rep 40:969–981
34. Lechner E, Achard P, Vansiri A, Potushak T, Genschik P (2006) F-box proteins everywhere. Curr Opin Plant Biol 9:631–638
35. Hassan MN, Zainal Z, Ismail I (2015) Plant kelch containing F-box proteins: structure, evolution and functions. RSC Adv 5:42808–42814
36. Hong MJ, Kim DY, Kang SY, Kim DS, Kim JB, Seo YW (2012) Wheat F-box protein recruits proteins and regulates their abundance during wheat spike development. Mol Biol Rep 39:9681–9696
37. Li Q, Wang W, Wang W, Zhang G, Liu Y, Wang Y, Wang W (2018) Wheat F-box protein gene TaFBA1 is involved in plant tolerance to heat stress. Front Plant Sci 9:521
38. Yu SI, Kim HJ, Yun DJ, Suh MC, Lee BH (2019) Post-translational and transcriptional regulation of phenylpropanoid biosynthesis pathway by kelch repeat F-box protein SAGL1. Plant Mol Biol 99:135–148
39. Schulman BA, Carrano AC, Jeffrey PD, Bowen Z, Kinnucan ERE, Finnin MS, Elledge SJ, Harper W, Pagano M, Pavletich NP (2000) Insights into SCF ubiquitin ligases from the structure of the Skp1–Skp2 complex. Nature 408:381–386
40. Adams J, Kelso R, Cooley L (2000) The kelch repeat superfamily of proteins: propellers of cell function. Trends Cell Biol 10:17–24
41. Ito N, Phillips SE, Stevens C, Ogil ZB, McPherson MJ, Keen JN, Yadav KD, Knowles PF (1991) Novel thioether bond revealed by a 1.7 A crystal structure of galactose oxidase. Nature 350:87–90
42. Chaumont F, Moshelion M, Daniels MJ (2005) Regulation of plant aquaporin activity. Biol Cell 97:749–764
43. Alexander Is E, Bientert GP, Kichey T, Laursen KH, Johanson U, Kjellhom P, Schjoerring JK, Jahn TP (2008) The effects of the loss of TIP1;1 and TIP1;2 aquaporins in Arabidopsis thaliana. Plant J 56:756–767
44. Afzal Z, Howton TC, Sun Y, Mukhtar MS (2016) The roles of aquaporins in plant stress responses. J Dev Biol 4:9
45. Selazny E, Borst JW, Muylaert M, Batoko H, Hemminga MA, Chaumont F (2007) FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. Proc Nat Acad Sci 104:12359–12364
46. Guo J, Dai S, Li H, Liu A, Liu C, Cheng D, Cao X, Chu X, Zhai S, Liu J, Zhao Z, Song J (2018) Identification and expression analysis of wheat TaGF14 Genes. Front Genet 9:12

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