

Genome-wide evolutionary analysis and the expression patterns of RLK gene family in wheat and other plants

Jun Yan (✉ xinsinian2006@163.com)

Shandong Agricultural University

Peisen Su

Liaocheng University

Xianyong Meng

Shandong Agricultural University

Pingzeng Liu

Shandong Agricultural University

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Abstract

Background: Receptor-like kinases (RLKs) gene family contains huge number of members in plants. They are membrane proteins with an extracellular receptor domain, and participate into abiotic and biotic stress responses.

Results: In this study, we identified RLKs in 15 representative plants genomes including wheat, and classified them into 64 subfamilies by using four kinds of phylogenetic trees and HMM models. Conserved exon-intron structures with conserved exon phase in the kinase domain were found in many RLK subfamilies from *Physcomitrella patens* to *Triticum aestivum*. Domain distributions of RLKs were also diagrammed. Collinearity events and tandem gene clusters suggested that polyploidizations and tandem duplication events contributed to the member expansions of *T. aestivum* RLKs. Global expression pattern analysis of *T. aestivum*, *Aegilops tauschii* and *Brachypodium distachyon* RLKs under abiotic and biotic stresses were performed by using public transcriptome data. We also selected 9 RLKs to validate the prediction of transcriptome by using qRT-PCR under drought treatment and with infection of *Fusarium graminearum*.

Conclusion: In this study, we performed the identification, classification, evolution, and expression patterns of RLKs in wheat and relative plants. Thus, our results will help researchers study evolutionary history and molecular mechanisms of wheat RLKs.

Introduction

Receptor-like kinases (RLKs) are membrane proteins with an extracellular receptor domain, such as leucine rich repeats (LRRs), lectin (Lec), lysine motif (LysM) or wall associated kinases (WAK) [1]. RLK gene families from various plants have been identified in a large number of articles. We summarized these findings in the following.

(1) Whole RLKs: In 2003 and 2004, Shiu et al. identified more than 600 in *Arabidopsis thaliana* and more than 1200 in *Oryza sativa* [2–3]. They play important roles in the plant growth, development, defense response to stresses. More than 440 RLKs from *O. sativa* might have originated from the domain fusion events after the split of rice and *Arabidopsis* in evolution. In 2009, Lehti-Shiu et al. found that the expansion of RLK members coincided with the establishment of land plants [4]. In 2018, Lin et al. identified 563 RLK genes in Jilin ginseng (*Panax ginseng* C.A. Meyer), and analyzed their evolution, functional diversity and co-expression networks [5].

(2) LRR-RLKs (subfamily of RLK): In 2013, Zan et al. identified 379 LRR-type RLK genes in *Populus trichocarpa*. 312 *Pt*LRR-RLK genes out of 379 are located in segmental duplication blocks. Genome-wide analysis of microarray data showed that some *Pt*LRR-RLKs responded to shoot organogenesis, low ammonium feeding, wounding, hypoxia and seasonal dormancy [6]. In 2017, Liu et al. studied the origin and diversity of LRR-RLKs in plants, and found that most LRR-RLKs were established in early land plants [7]. In 2018, Sun et al. identified a total of 1641 LRR-RLK genes in four *Gossypium* species (*Gossypium*

arboreum, *Gossypium barbadense*, *Gossypium hirsutum*, and *Gossypium raimondii*). Tandem duplication played an important role in the expansion of *Gossypium* LRR-RLK gene family. Expression pattern analysis showed that *Gossypium* LRR-RLKs were widely involved in various stress defenses and diverse developmental processes [8]. In 2020, Meng et al. identified 329 LRR-RLK genes in *Medicago truncatula*. Analysis of classification, duplication events, exon/intron organization, expression profiling were performed in *M. truncatula* LRR-RLKs [9]. In 2021, 437 LRR-RLK genes were identified in *Saccharum spontaneum*, and categorized into 14 groups. Analysis of promoter sequences and expression profiles showed that SsLRR-RLKs were strongly regulated by various environmental stimuli, transcription factors and phytohormonal factors, suggesting that they responses to various diverse abiotic and biotic stresses [10]. In 2022, Song et al. identified 444 *Bn*LRR-RLKs in *Brassica napus* cultivar “Zhongshuang 11” and classified them into 22 subfamilies. Based on CRISPR/Cas9 technology, they obtained six partial knockouts of *BnBRI1* homologs to generate semi-dwarf lines without decreased yield compared with controls [11]. In 2022, 15 *Ta*RPK1 (Receptor-like protein kinase 1, a calcium independent Serine-Threonine kinase that belongs to the subfamily LRR-RLK) genes were identified in *Triticum aestivum*. 18 putative miRNAs targeting and *Cis*-Regulatory elements (light-related, hormone responsiveness, and stress elements) were identified in *Ta*RPK1 genes. *In silico* expression analysis and qRT-PCR validated that *Ta*RPK1 genes exhibited higher expressions in roots of drought-tolerant varieties than drought-susceptible variety [12].

(3) LecRLKs (subfamily of RLK): In 2020, 46 putative Lectin receptor-like kinases were identified in the cucumber (*Cucumis sativus* L.) genome, and were classified into three groups including 23 G-type, 22 L-type, and one C-type *Cs*LecRLK gene. Analysis of promoter regulatory elements and expression patterns revealed that some *Cs*LecRLKs were associated with phytohormones and stress responses [13]. In 2020, Singh et al. identified 73 putative *Vr*LecRLKs in mungbean (*Vigna radiata* L. Wilczek), and classified them into three families, such as G-type, L-type, and C-type *Vr*LecRLKs [14]. In 2021, 1311 *Ah*RLKs including *Ah*LRR-RLKs and *Ah*Lec-RLKs were identified from the peanut (*Arachis hypogaea*) genome. The result of mining transcriptome data showed that 14 of 90 Al-responsive *Ah*RLKs expressed specifically in root tissue [15].

(4) LysM-RLKs (subfamily of RLK): In 2020, Yang et al. identified 493 RLKs (LysM-RLKs and LRR-RLKs) and 228 RLPs (LysM-RLPs and LRR-RLPs) in the genome of *Brassica juncea*. The majority of RLKs (90.17%) and RLPs (52.83%) of *B. juncea* are from duplication events, indicating that duplication events significantly contributed to the expansion of RLK and RLP gene families [16]. In 2021, Abedi et al. identified 33 LysM-RLK genes (subfamily of RLK) in three *Brassica* species (17 in *Brassica napus*, 8 in *Brassica rapa* and 8 in *Brassica oleracea*). RNA-seq expression analysis revealed that *BnLYP6* exhibited high expression in response to various biotic stresses. Structural modeling and docking simulation revealed that several residues in the active sites of *BnLYP6* could directly contact with the chitin [17].

(5) CRKs (Cysteine-rich receptor-like kinases, subfamily of RLK): In 2019, Quezada et al. identified 46 CRKs in *Phaseolus Vulgaris*, and performed the comprehensive analysis, including identification, chromosomal localization, gene structures, transcript expression profiles, and in silico promoter analysis

[18]. In 2019, Shumayla et al. identified 43, 37, 36, 38 and 170 CRK genes in the genome of *Brachypodium distachyon*, *Hordeum vulgare*, *O. sativa*, *Sorghum bicolor* and *T. aestivum*, respectively. These CRKs were tightly clustered into four phylogenetic groups, and were variably conserved in exon/intron, domains and motifs. Tissue-specific expression analysis suggested that some CRK genes are involved in plant development [19].

(6) PERKs (Proline-rich extensin-like receptor kinases, subfamily of RLK): In 2004, 15 predicted *At*PERKs in *A. thaliana*, and some *At*PERK members were identified as tissue-specific genes [20]. In 2022, 37 *Ta*PERKs were identified in wheat (*T. aestivum* L.), and were classified into eight well-defined groups. Analysis of *cis*-acting regulatory elements and expression profile revealed that some *Ta*PERKs are in response to phytohormones, various biotic and abiotic stresses [21].

Some articles had reported that RLKs play important roles in response to abiotic and biotic stresses, such as drought, heat, salinity and cold. Transgenic *Arabidopsis* plant (35S:PdERECTA) of over-expression *PdERECTA* (transformed with *Populus deltoides* *PdERECTA*, an LRR-type RLK) improves the water use efficiency and enhances the drought resistance [22]. Rice (*O. sativa*) *OsSIK1* (LRRs RLK) improves the tolerance of drought and salt stress. Transgenic rice plants with overexpression of *OsSIK1* enhance the tolerance to salt and drought stresses, while the knock-out and RNA interference plants exhibit sensitive to drought and salt stresses [23]. *OsLecRLK* overexpression and downregulation (through artificial miRNA) transgenic lines proved that rice *OsLecRLK* enhances salinity tolerance through ion homeostasis [24]. The interaction network of 255 *Arabidopsis* LRR-RLKs (567 pairs interaction relationships) was established by using a sensitized high-throughput interaction assay. Plants have evolved LRR-RLK networks to process extracellular signals into cell, functioning in plant growth and immunity [25]. MtDMI2 (a Leu rich repeat-type receptor kinase) and MtPUB2 (a novel plant U-box (PUB)-type E3 ligase) interact to form a negative feedback loop, playing an important role in nodulation homeostasis [26]. The systemin receptor SYR1 (an LRR-RLK) of tomato is not decisive for local and systemic wound responses, but important for defense against insect herbivory [27]. The *A. thaliana* lectin RLK *AtLecRK-IX.2* can modify pathogen effector AvrPtoB to dampen their virulence in *Arabidopsis* [28]. By using a map-based cloning strategy, Duriez et al. identified a sunflower protein HaOr7 (LRR-RLK) as a gene that confers resistance to *Orobanche cumana* [29]. Rao et al. constructed nine higher order mutants of *A. thaliana* receptor-like cytoplasmic kinase (RLCK) subfamily VII, revealing that numerous RLCK VII members are involved in plant development and pattern-triggered immune signaling [30]. *A. thaliana* *AtRIPK* (RPM1-INDUCED PROTEIN KINASE), an RLCK VII subfamily member, contributes to ROS (reactive oxygen species) production in plant immune system [31].

In recent years, biological functions of wheat RLKs mediating the response to biotic stress have been reported. In 2016, Rajaraman et al. found that a barley LRR-type RLK gene *HvLEMK1* was a factor mediating non-host resistance in barley and quantitative host resistance in wheat to the wheat powdery mildew fungus [32]. In 2018, Saintenac et al. discovered that the RLK gene *Stb6* (a conserved wall-associated receptor kinase (WAK)-like protein, subfamily of RLK) in wheat is a natural resistance gene to fungal pathogen *Zymoseptoria tritici* [33]. In 2019, Wang et al. found an LRR-type RLK gene *TaXa21* in

wheat is highly homologous to the rice bacterial blight resistance gene Xa21. They also found that *TaXa21* is a positive regulator of wheat high-temperature seedling plant (HTSP) resistance to *Puccinia striiformis* f. sp. tritici. This process is mediated by the H₂O₂ and ethylene (ET) signaling pathway, and associating with the transcription factor *TaWRKY76* and *TaWRKY62* [34]. In 2020, Gu et al. discovered a novel cysteine-rich RLK gene *TaCRK2* which positively regulates the leaf rust resistance in wheat [35]. Using comparative genomics, mutagenesis and complementation, Saintenac et al. identified a wheat cysteine-rich RLK gene Stb16q, which exhibited resistance against Septoria tritici blotch (Stb, pathogen *Zymoseptoria tritici*), and localized at the plasma membrane in infection cycle [36]. In 2021, Guo et al. identified a novel CRK RLK gene *TaCRK3* which could defend against *Rhizoctonia cerealis* in wheat. *TaCRK3* protein contains two DUF26 (DOMAIN OF UNKNOWN FUNCTION 26) domains which could inhibit the growth of *R. cerealis* mycelia [37]. In 2021, the Wheat Wall-Associated Receptor-Like Kinase (WAK, subfamily of RLK) *TaWAK-6D* and *TaWAK7D* were identified to mediate broad resistance to Fungal pathogens (*Fusarium pseudograminearum* and *R. cerealis*) [38–39]. In 2022, *TaPsIPK1* (a wheat receptor-like cytoplasmic kinase gene) is identified to be a susceptibility gene as the target of stripe rust (caused by *P. striiformis* f. sp. tritici) effectors [40].

In this study, we performed genome-wide identification, classification, evolutionary analysis of RLK gene family in 15 representative plants, including four wheat and *Aegilops tauschii*. Several conserved intron–exon structures within conserved kinase domain were found in 15 representative plants, suggesting that these RLKs might play important roles in plant developmental and evolutionary processes. Chromosome locations and collinearity events among *T. aestivum*, *B. distachyon* and *O. sativa* RLKs were determined to study the expansion of wheat RLKs. Some tandem gene clusters of wheat RLKs were found on 21 chromosomes. Global expression analysis of stresses were performed in individual *T. aestivum* RLKs. QRT-PCR of 9 selected RLKs were performed to validate the prediction of transcriptome under drought condition and *Fusarium graminearum* infection. Our results will help researchers study evolutionary history and molecular mechanisms of wheat RLKs.

Result

Genome-wide Identification and Classification of RLK Gene Family in Wheat, *Ae. tauschii* and Other Plants

We identified RLK genes with typical kinase domain and corresponding HMM model (see Method) in 15 representative plants, including four wheat and *Ae. tauschii* (Table 1, Table S1). Other PKs (protein kinase) with typical kinase domain were also identified and classified in 15 representative plants (Table S1). The result showed that most proportion of RLKs in PKs were 75%-78% in 15 representative plants (Table 1).

We classified the RLKs into 64 subfamilies by HMM models (Table S2). We only selected 1-3 members from every subfamily as the representative sequences to construct phylogenetic trees. In order to confirm the classification form HMM models, four kinds of phylogenetic trees (including Bayesian tree, Maximum

likelihood (ML) tree, neighbour-joining (NJ) tree with JTT model, and NJ tree with p-distance model) were constructed based on the truncated kinase domain sequences (Fig. 1, Fig. S1A-D and Table S3). The result showed that almost all the classifications of RLK subfamilies from HMM models and four phylogenetic trees were the same (Table S3). Among 64 RLK subfamilies, we noticed that all RLK-Pelle_RLCK-IXb clades from Bayesian and NJ phylogenetic trees contained a green algae sequence (PNW75571), suggesting that the RLK-Pelle_RLCK-IXb clade might be the ancestral subfamily of 64 RLK subfamilies. The HMM scan results showed that all of the two green algae sequences (PNW75571 and PNW75214) belonged to the RLK-Pelle_RLCK-IXb clade.

Evolution and Conserved Exon-intron Structures of RLK Gene Subfamilies

To obtain further insight of RLK evolution, we diagrammed the exon-intron structures within the kinase domain in the 15 investigated plants (Fig. S2). The results showed that some conserved exon-intron structures were present in the same RLK subfamilies across the investigated plants, especially in the kinase domain. We summarized these conserved exon-intron structures in six representative plants, including *T. aestivum*, *B. distachyon*, *Vitis vinifera*, *Amborella trichopoda*, *Selaginella moellendorffii*, and *Physcomitrella patens* (Fig. S3). For instance, a conserved exon-intron structure with exon phase “0112-0” was existed in RLK-Pelle_LRR-I-1 subfamily from *P. patens* to *T. aestivum* (Fig. 2). The similar example within “0112-0” exon-intron structure could also be found in RLK-Pelle_RLCK-IXa subfamily (Fig. 2).

In order to study the domain distributions, all RLK genes of 15 investigated plants were scanned against the Pfam 34 in batch and diagrammed in our perl and R script (Fig. S4). The result showed that some special domains were existed across multiple RLK subfamilies. For example, LRR_8 Repeat domains (Pfam profile: PF13855) and LRRNT_2 Family domain (Pfam profile: PF08263) were existed in almost all RLK-Pelle_LRR (Leucine-rich repeat) subfamilies. We also noticed that some RLK subfamilies contained only kinase domain. For example, some RLK-Pelle_DLSV members from *P. patens* (Pp3c22_10300V3.1), *S. moellendorffii* (EFJ35044), *A. trichopoda* (ERN15963), *B. distachyon* (PNT75271) and *T. aestivum* (TraesCS7B02G494200.1) contained only kinase domain.

Chromosome Location and Duplication Events of Wheat RLKs

The chromosome locations of *T. aestivum* RLKs were mapped on 21 chromosomes (Fig. S5 and Table S4). The distribution of *T. aestivum* RLKs was among the A, B and D subgenomes.

In order to study the whole genome duplication (WGD) events of wheat RLKs, we identified 2114 RLK gene pairs relating to collinearity events by MCscanX (Table S5). Most *Ks* values of these wheat RLK collinearity events ranged from 0-0.35, and formed a peak of *Ks* 0-0.15 (Fig. S6). These collinearity events of wheat RLKs were visualized on 21 chromosomes (Fig. 3A-C). The results showed that collinearity events within *Ks* 0-0.35 were mainly located among the corresponding subgenomes, such as between 1A, 1B and 1D, suggesting that these collinearity events occurred along the polyploidization of *T. aestivum*. Moreover, collinearity events within *Ks* >0.35 were mainly located across the corresponding subgenomes.

232 clusters of tandem duplication (TD) were identified in *T. aestivum* RLKs (Fig. S7 and Table 2). Chromosome 2D, 3B and 5D contained more than 15 clusters, which were 19, 17 and 18, respectively (Table S6). We noticed that some of these clusters belonged to the RLK-Pelle_DLSV subfamily. For example, a cluster on chromosome 5D within 4 genes (TraesCS5D02G357700.1, TraesCS5D02G357800.1, TraesCS5D02G358600.1 and TraesCS5D02G358700.1) belonged to the RLK-Pelle_DLSV subfamily. The largest cluster within 14 members was a RLK-Pelle_DLSV cluster, which was at the end of chromosome 2A. The second largest cluster within 13 members was also a RLK-Pelle_DLSV cluster, which was at the end of chromosome 7B.

To further study the mechanism of RLK duplication events, comparative syntenic maps of *T. aestivum*, *B. distachyon* and *O. sativa* RLKs were constructed (Fig. 4A-D). 1081 RLK syntenic gene pairs were detected between *T. aestivum* and *B. distachyon*. Similarly, 961 RLK syntenic gene pairs were detected between *T. aestivum* and *O. sativa* (Table S7). We noticed that some RLK syntenic gene pairs shared the same *T. aestivum* RLK member associated with *B. distachyon* and *O. sativa*, suggesting that they might descended from a single common ancestral sequence before the *Graminaceae* split in evolution. For instance, *Tae-Bid* gene pair (TraesCS7D02G241500.1 and KQJ98873) and *Tae-Osa* gene pair (TraesCS7D02G241500.1 and Os08t0501200-00) shared the *T. aestivum* RLK gene (TraesCS7D02G241500.1), and all of these three genes belonged to the RLK-Pelle_WAK subfamily. We also calculated the *Ks* values between *Tae-Bid* and *Tae-Osa* syntenic gene pairs (Table S7 and Fig. S8A-B). The result showed that the *Ks* values of *Tae-Bid* syntenic RLK gene pairs ranged from 0-0.95, and formed a peak within *Ks* 0.4-0.45. However, *Tae-Bid* syntenic gene pairs formed a perk within *Ks* 0.3-0.35. Similarly, the *Ks* values of *Tae-Osa* syntenic RLK gene pairs ranged from 0-1.35, and formed a peak within *Ks* 0.5-0.55.

Expression Patterns of *T. aestivum*, *Ae. tauschii* and *B. distachyon* RLKs under Drought Stress

We studied the expression patterns of *T. aestivum*, *Ae. tauschii* and *B. distachyon* RLKs under abiotic and biotic stresses by using public transcriptome data at NCBI. According to the quality control performed by software FastQC, two transcriptome samples were excluded in the following analysis (Table S8). In order to search the expression pattern of wheat RLKs under drought stress, three public transcriptome data of wheat was selected to study (Fig. S9A-C and Table S9). (1) "TAM 111" and "TAM 112" (Bioproject: 659916): Wheat cultivars "TAM 111" and "TAM 112" (grown in the southern of America) have excellent drought tolerance. This RNA-seq analysis was conducted to compare gene expression difference in flag leaves of "TAM 111" and "TAM 112" under wet and dry conditions. We extracted expression patterns of RLKs from this RNA-seq analysis. Our result showed that some RLKs exhibited different expression patterns between "TAM 111" and "TAM 112", suggesting that different drought-tolerance mechanisms were existed in "TAM 111" and "TAM 112". For example, TraesCS5B02G059000 (RLK-Pelle_DLSV) from TAM112_Heading and TAM112_GrainFilling sample exhibited down-regulation ($\log_2$ Fold Change, -1.71 and -2.07), while TraesCS5B02G059000 (RLK-Pelle_DLSV) from TAM111_Heading and TAM111_GrainFilling sample exhibited up-regulation (1.35 and 1.52). (2) "Svevo" and "IL20-2" (Bioproject: 686121): Two wheat genotypes, "Svevo" and "IL20-2", were treated under well watered and water limited

conditions. Then the wheat seedling root tissues of “Svevo” and “IL20-2” were extracted RNA to perform transcriptome. We studied the expression patterns of RLKs from this RNA-seq analysis. We noticed that some RLKs exhibited the same expression trends between “Svevo” and “IL20-2”. For instance, the expression trends of TraesCS2B02G008400 (RLK-Pelle_L-LEC) were down-regulation with $\log_2$ FC values -1.57 and -1.99 in “IL20-2” and “Svevo”, respectively. (3) “L-82” and “Marvdasht” (Bioproject: 450487): Two wheat genotypes, “L-82” (drought-tolerant) and “Marvdasht” (drought-sensitive), were treated under well watered and drought conditions. Then the wheat root tissues of “L-82” and “Marvdasht” were extracted RNA to perform transcriptome. Our result showed that some RLKs exhibited different expression patterns between “L-82” and “Marvdasht”, suggesting that different molecular mechanisms of drought tolerance were existed in “L-82” (drought-tolerant) and “Marvdasht” (drought-sensitive). For instance, the expression trends of TraesCS5D02G374300 (RLK-Pelle_WAK) were down- and up-regulation with $\log_2$ FC values -3.47 and 1.58 in “Marvdasht” and “L-82”, respectively.

In order to further determine the expression patterns of public transcriptome data under drought stress, we selected six wheat RLKs to examine their expression patterns under PEG (drought) treatment by using qRT-PCR (Fig. 5A-B). We only compared the expression patterns between the public transcriptome (“TAM 111” and “TAM 112”, Bioproject: 659916) and qRT-PCR, because all of their sample tissues were from wheat leaves (the other two data were from root tissues). The result showed that the expression trends were almost the same between public transcriptome and qRT-PCR. For example, the $\log_2$ values of TraesCS7D02G355800 (RLK-Pelle_LRR-XII-1) were 2.93, 1.50, 1.50 and 3.97 in TAM112_Heading, TAM112_GrainFilling, TAM111_Heading and TAM111_GrainFilling samples, respectively. Our qRT-PCR results validated that the expression trend of TraesCS7D02G355800 was the same as transcriptome (Bioproject: 659916), exhibiting a peak within more than 1000-fold up-regulation at 24 h (hours).

The expression patterns of *Ae. tauschii* and *B. distachyon* RLKs under drought stress were also studied by using public transcriptome data at NCBI (Fig. S10A-B and Table S10). (1) *Ae. tauschii* cultivars “XJ98” and “XJ2” (Bioproject: 482066): We noticed that some *Ae. tauschii* RLKs exhibited the similar expression trends between “XJ98” and “XJ2”. For example, the $\log_2$ values of AET5Gv20565600 (RLK-Pelle_SD-2b) were 2.30 and 3.33 in “XJ98” and “XJ2”. (2) *B. distachyon* cultivars “ABR4”, “ABR8” and “KOZ1” (Bioproject: 524106): Most *B. distachyon* RLKs exhibited different expression trends among “ABR4”, “ABR8” and “KOZ1”. For instance, the $\log_2$ values of BRADI_3g13827v3 (KQJ94884, RLK-Pelle_LRR-IV) were -1.05, 0.35, -2.0, -2.0, 2.15 and 2.09 in “ABR4” t1, “ABR4” t2, “ABR8” t1, “ABR8” t2, “KOZ1” t1 and “KOZ1” t2 samples.

Expression Patterns of *T. aestivum* RLKs under Other Abiotic Stresses

We also studied the expression patterns of *T. aestivum* RLKs under other abiotic stresses by using public transcriptome data at NCBI. (1) Heat stress (Bioproject: 598150): We noticed that the expression patterns of some *T. aestivum* RLKs exhibited a consecutive rise or decline at 10 days (d) and 14 d under heat stress treatment. For instance, the $\log_2$ values of TraesCS2D02G297100 (RLK-Pelle_SD-2b) were 2.66 and 5.55 at 10 d and 14 d, exhibiting the expression trend of a consecutive rise. Similarly, the $\log_2$ values of

TraesCS7D02G386000 (RLK-Pelle_LRR-V) were -1.03 and -2.53 at 10 d and 14 d, exhibiting the expression trend of a consecutive decline. (2) Salinity stress (Bioproject: 573996): Under salinity stress, some *T. aestivum* RLKs exhibited different expression patterns between leaf and root tissue. For instance, the log₂ values of TraesCS6B02G182900 (RLK-Pelle_LRR-III) was -4.06 in root tissue, but 1.51 in leaf tissue. (3) Waterlogging stress (Bioproject: 604012): There were differences in waterlogging tolerance among different wheat varieties. Among the three investigated wheat varieties, the seeds of “Bainong 607” germinated earlier than “Bainong 207” and “Zhoumai 22” under waterlogging stress. We noticed that some *T. aestivum* RLKs exhibited up-regulation in “Bainong 607”, but down-regulation in “Bainong 207” and “Zhoumai 22”, suggesting that different molecular mechanisms of waterlogging tolerance were existed in different wheat varieties. For instance, the log₂ values of TraesCS2D02G388000 (RLK-Pelle_RLCK-VIIa-2) was -1.47, -2.55 and 3.70 in “Zhoumai 22”, “Bainong 207” and “Bainong 607”.

Expression Patterns of *T. aestivum* RLKs under Biotic Stresses

We studied the expression patterns of *T. aestivum* RLKs under various abiotic stresses by using public transcriptome data at NCBI. (1) *Fusarium graminearum* (EBI study accession: PRJEB12358): Near isogenic wheat lines (NILs) “NIL38” and “NIL51”, which were different in the presence of both or none of the FHB-resistance QTL Fhb1 and Qfhs.ifa-5A, have been sequenced with *F. graminearum* treatments (3, 6, 12, 24, 36 and 48 h). Some *T. aestivum* RLKs exhibited the similar expression patterns between “NIL38” and “NIL51”. For example, almost all the log₂ values of TraesCS3A02G007200 (RLK-Pelle_LRR-XII-1) were up-regulations between “NIL38” (4.23, 1.08, 3.45, 6.52, 4.00 and 4.56 at 3, 6, 12, 24, 36 and 48 h) and “NIL51” (5.66, 0.56, 3.22, 4.84, 2.56 and 5.89 at 3, 6, 12, 24, 36 and 48 h). However, there were also the other *T. aestivum* RLKs whose expression patterns were different between NIL38 and NIL51. For example, almost all the log₂ values of TraesCS7A02G435000 (RLK-Pelle_LRR-Xb-1) were opposite (up-regulations or down-regulations) between “NIL38” (-0.02, -2.03, -0.32, -1.31, 0.30 and 0.45 at 3, 6, 12, 24, 36 and 48 h) and “NIL51” (-1.04, 0.38, 3.16, 1.49, -0.76 and 2.13 at 3, 6, 12, 24, 36 and 48 h). (2) Stripe rust (Bioproject: 613349): NILs “FLW29” (resistant), and cv. “PBW343” (susceptible) were performed transcriptome in response to *P. striiformis* f. sp. *tritici* (*Pst*). We noticed that some *T. aestivum* RLKs exhibited up-regulation in “FLW29” (resistant) after *Pst* treatment, but down-regulation in “PBW343” (susceptible), suggesting that these *T. aestivum* RLKs might participate the signal pathways in response to *Pst*. For instance, the log₂ values of TraesCS7D02G486900 (RLK-Pelle_DLSV) were opposite (up-regulations or down-regulations) between “FLW29” (2.63, 1.74 and 1.82 at 3, 6 and 12 h) and “PBW343” (-0.94, -1.84 and -1.73 at 3, 6 and 12 h). (3) *Xanthomonas translucens* with *Funneliformis mossae* (Bioproject: 474303): RNAseq of wheat cultivar “Chinese Spring” were performed in roots and leaves tissues during a long term interaction with *F. mossae* (2 months) with or without a pathogen infection by *X. translucens* CFBP 2054. Some *T. aestivum* RLKs exhibited the opposite expression patterns between root and leaf tissues with the infection of *Xanthomonas*. For example the log₂ values of TraesCS5B02G208600 (RLK-Pelle_RLCK-VIIa-2) were -1.42 and 2.14 in roots and leaves, respectively. (4) *X. translucens* infection (Bioproject: 401247): The aim of this transcriptome was to detect the response of wheat cultivar “Chinese Spring” in the infection of *X. translucens* pathogen. We noticed that some *T. aestivum* RLKs exhibited the opposite

expression pattern between roots and leaves tissues with the infection of *Xanthomonas*. For example the $\log_2$ values of TraesCS3B02G043700 (RLK-Pelle_DLSV) were -1.96 and 4.37 in roots and leaves, respectively.

In order to further determine the expression patterns of public transcriptome data under Fusarium head blight (FHB) stress, we selected three wheat RLKs to examine their expression patterns with *F. graminearum* treatments by using qRT-PCR (Fig. 6A-B). The expression trends of three wheat RLKs from public transcriptome data were consistent with the results of qRT-PCR. For instance, the $\log_2$ values of TraesCS1B02G454000 (RLK-Pelle_DLSV) in public transcriptome data were almost all up-regulations to form a peak in NIL38 (0.48, -0.38, 0.50, 1.62, 1.00 and 1.89 at 3, 6, 12, 24, 36 and 48 h) and NIL51 (-1.36, 0.17, -0.92, 3.53, 1.89 and 4.28 at 3, 6, 12, 24, 36 and 48 h). Similarly, our qRT-PCR result showed that TraesCS1B02G454000 (RLK-Pelle_DLSV) exhibited a peak of up-regulation at 96 h after *F. graminearum* treatment.

Discussion

Evolution and duplication events of RLK gene family in wheat and other plants

In this study, we identified RLKs in 15 representative plants including green algae (*Chlamydomonas reinhardtii*) and moss (*P. patens*). Our result showed that there are only 4 RLKs (1 RLK-Pelle_C-LEC, 1 RLK-Pelle_L-LEC and 2 RLK-Pelle_RLCK-IXb) in *C. reinhardtii*, while the members have expanded into 298 RLKs (almost 64 RLK subfamilies) in *P. patens*. In 2021, Gong et al. also studied the early evolution and diversification of RLKs in 36 representative plants, including 5 rhodophytes, 1 glaucophyte, 1 prasinodermophyte, 18 chlorophytes, 6 charophytes, 2 bryophytes, and 3 vascular plants. Their result showed that RLKs have extensively diversified in charophytes, and charophyte RLKs mainly contribute to the diversity of land plant RLKs [41]. This was consistent with our results that moss RLKs had expanded into 298 members and almost 64 subfamilies. In 2021, the rice *OsARK1* (ARBUSCULAR RECEPTOR-LIKE KINASE 1) was reported to have an ancient paralogue in spermatophytes, ARK2 [42]. Their result showed that *OsARK1* belongs to an URK-2 (Unknown Receptor Kinase-2) subfamily, and a new domain SPARK (Pfam ID: PF19160) was found in URK-2 orthologs. In our result, we also identified two RLK genes (Os07t0227300-00 and Os04t0465900-00) in *O. sa* RLK-Pelle_URK-2 subfamily. Interestingly, the rice sequence (Os07t0227300-00) also contained the SPARK domain (Fig. S4).

Among 64 RLK subfamilies, we noticed that the members of some *T. aestivum* RLK subfamilies, such as RLK-Pelle_DLSV (829), RLK-Pelle_L-LEC (320), RLK-Pelle_LRR-XII-1 (337), RLK-Pelle_SD-2b (331), RLK-Pelle_WAK (385) and RLK-Pelle_LRR-XI-1 (237), were extremely huger than others, suggesting that certain RLK subfamilies had experienced the expansion during the evolution (Table S2). This was consistent with the result of 2009 article that RLKs had the extensively expanding subfamilies, including DUF26, LRR10L-2, LRR-I, LRR-XII, SD1, SD-2b, and WAK [4]. The allohexaploid bread wheat (*T. aestivum*) genome contained three closely related subgenomes (A, B, and D). The A and B genomes diverged from a common ancestor about 7 million years ago, and D genome through homoploid hybrid speciation 5-6

million years ago. Bread wheat genome had experienced multiple rounds of hybrid speciation [43]. Our result showed that almost all RLKs were homologous sequences among the subgenome A, B and D chromosomes (Fig. 3). The *Ks* values of these RLK collinearity events were 0-0.35, suggesting that they were associated with the polyploidization events among wheat A, B and D subgenomes (Table S5 and Fig. S6). It was reported that polyploidy, tandem duplications, segmental duplications and transposition events are the main mechanisms for the expansion of wheat expansin gene family [44]. Consistent with their results, we also found some tandem RLK clusters on wheat chromosomes, suggesting that tandem duplication events also contributed to the expansion of RLK members during evolution (Fig. S7).

Expression pattern of wheat RLKs under drought and *F. graminearum* stresses

We studied the RLK expression patterns under drought stress by using public transcriptome data and qRT-PCR. (1) DLSV-RLK: In 2021 *Arabidopsis* cysteine-rich receptor-like protein kinase *AtCRK33* was identified to affect drought tolerance and stomatal density. CRKs contain the DUF26 (Domain of Unknown Function 26) domain [45]. The DUF26 domain, also known as stress-antifungal domain (PF01657, Pfam domain name, Stress-antifung Family) in pfam website [46], could be found in RLK-Pelle_DLSV subfamily of our result (Fig. S4). We also detected the expression pattern of a RLK-Pelle_DLSV member (TraesCS6A02G390300) under drought stress by using public transcriptome data and qRT-PCR (Fig. 5). The qRT-PCR result showed that it exhibited a peak of up-regulation at 3 h. The log₂ value of TraesCS6A02G390300 in TAM111_GrainFilling c-w sample was 4.29. (2) LRR-RLK: In 2011, it was reported that over-expression of *PdERECTA* (*Populus deltoides* LRR-RLK gene) in *Arabidopsis* enhances the drought resistance [22]. In 2021, Li et al. discovered that overexpression of the *PdERECTA* gene in *Poplar* improved water use efficiency and enhanced the drought tolerance by reducing stomatal density and restricting water consumption [47]. Similar result was also reported that overexpression of *Sorghum bicolor* gene *SbERECTA* in *Arabidopsis* and maize enhanced their drought tolerance [48]. In our result, we also checked the expression pattern of two LRR-type RLKs (RLK-Pelle_LRR-VI-1 TraesCS5D02G437000, and RLK-Pelle_LRR-XII-1 TraesCS7D02G355800) under drought stress by using public transcriptome data and qRT-PCR (Fig. 5). The qRT-PCR result showed that they all exhibited a peak of up-regulation at 24 h. The log₂ values of them in four samples were all up-regulations. (3) SD-2b: In 2020, an S-domain RLK gene *OsESG1* (LOC_Os01g12410) in rice (*O. sativa*) was identified in early crown root development and drought response by controlling auxin response and distribution [49]. We got the protein sequence (LOC_Os01g12410, Os01t0223800-01) and converted the ID in website (<https://rapdb.dna.affrc.go.jp/>). In our result, it belonged to the RLK subfamily RLK-Pelle_SD-2b (Table S1).

Some articles studied the relationship between *F. graminearum* infection and RLKs in wheat and other plants. After *F. graminearum* infection, Manes et al. screened 227 RLKs and innate immune response genes in *Arabidopsis*, and identified nine genes (including RLK7) which play roles in *F. graminearum* resistance [50]. An LRR-RLK gene (GRMZM2G132212) in maize was identified as a defense or recognition gene in the response to fungal pathogens (*Cochliobolus heterostrophus* and *F. graminearum*). However, *F. graminearum* might be able to exploit this LRR-RLK gene (GRMZM2G132212) function to increase its virulence [51]. We converted ID (GRMZM2G132212, Zm00001eb293660) in NCBI and Ensembl plant, and

found that it belonged to RLK subfamily RLK-Pelle_LRR-XI-1 in our result (Table S1). Two LRR-RLK genes (*HvLRRK-6H* and *TaLRRK-6D*) were identified to contribute to *Fusarium* resistance in cereals (*Hordeum vulgare* and *T. aestivum*) [52]. In our result, we also checked the expression pattern of two DLSV-type RLKs (RLK-Pelle_DLSV TraesCS1B02G454000, and RLK-Pelle_DLSV TraesCS3D02G097000) with *F. graminearum* infection by using public transcriptome data and qRT-PCR (Fig. 6). The qRT-PCR showed that they all exhibited a peak of up-regulation at 48 and 96 h. The log₂ values of them in some NIL38 and NIL51 samples (24, 36 and 48 h) were all up-regulations. Maybe they are new defense genes to contribute to *Fusarium* resistance. Indeed, it was reported that a novel CRK RLK (DLSV-RLK subfamily in our result) gene *TaCRK3* which could defend against other fungal pathogen *R. cerealis* in wheat [37].

Materials And Methods

Identification and Classification of RLKs in Plants

The genomes and proteomes of *T. aestivum*, *Triticum spelta*, *Triticum turgidum*, *Triticum dicoccoides*, *Triticum urartu*, *Ae. tauschii*, *B. distachyon*, *Zea mays*, *O. sativa*, *A. thaliana*, *V. vinifera*, *A. trichopoda*, *S. moellendorffii*, *P. patens* and *C. reinhardtii* were downloaded from Ensembl Plant release-51 (<http://plants.ensembl.org/>). To identify the PKs (protein kinase), all the proteomes of the fifteen plants were scanned by our local server HMMER3.1 (PK_Tyr_Ser-Thr.hmm pfam profile PF07714.19, Pkinase.hmm PF00069.27) and website pfam 34.0 (<http://pfam.xfam.org/>) in batch mode with an E value of 0.01. Atypical PKs with kinase (PK_Tyr_Ser-Thr or Pkinase) domain covering less than 50% alignment were excluded in the following analysis. Classifications of “typical” sequences of PK subfamilies were performed by HMMER 3.1 with HMM models developed by Legti-Shiu and Shui [53].

We selected 1-3 members as the representative sequences from every RLK subfamily to construct the phylogenetic trees. Each RLK subfamily was selected by the following criteria: members ≤ 6, 1 RLK; 6 < members ≤ 30, 2 RLKs; members > 30, 3 RLKs. The alignment of truncated RLK sequences in kinase (PK_Tyr_Ser-Thr or Pkinase) domain was performed by ClustalW v2.0 [54]. Bayesian phylogenetic tree was performed using MrBayes v3.2.7 [55] with the mixed amino acid substitution model, MCMC chain with 10,000,000 generations was used. Markov chains were sampled every 100 generations, and the first 25% of the trees was discarded as burn-in. The result of MrBayes v3.2.7 was analyzed by TreeGraph v2.14 [56] and our Perl scripts. The ML phylogenetic tree was performed using PhyML v3.1 [57] with 100 bootstrap replicates. The appropriate model of ML method including model parameters was calculated using the Akaike Information Criterion (AIC) with ProtTest v3.4 [58]. The NJ phylogenetic trees were constructed by Megacc 7.0 [59] with a model (p-distance or JTT) and 1000 bootstrap repetitions. The four kinds of phylogenetic trees were constructed by above descriptions in our local server.

Domain and Intron–exon Structure Diagram of RLKs

The domain and intron–exon structures of RLK sequences in these fifteen plants were generated by our Perl and R scripts based on the corresponding GFF file information from Ensembl Plant release-51

(<http://plants.ensembl.org/>). The domain information of pfam-A models was downloaded from pfam 34.0 (<http://pfam.xfam.org/>) , and then scanned in our local server.

Chromosome Locations, Duplication Events and Synthetic Analysis of Wheat RLKs

Based on the extracted information in GFF files from Ensembl Plants release-51 (<http://plants.ensembl.org/>), the chromosome locations of *T. aestivum* RLKs were diagrammed using software GenomePixelizer [60]. BLASTP was performed against RLKs of *T. aestivum*, *B. distachyon* and *O. sativa* with an E value of e-100. Based on the GFF files and BLAST results, tandem duplication and segmental duplication were searched using MCScanX [61]. The *Ka* and *Ks* values were calculated by “add_ka_and_ks_to_collinearity.pl” from MCScanX. Based on the GFF files and MCScanX results, synthetic diagrams among *T. aestivum*, *B. distachyon* and *O. sativa* were generated by using our perl scripts and software Circos (<http://circos.ca/>). The chromosome locations of tandem duplication RLKs were mapped on each chromosome by Mapchart v2.3 (<http://www.wageningenur.nl/en/show/mapchart.htm>).

Bioinformatics Analysis of Public Transcriptome Expression Data

Public wheat (*T. aestivum*), *Ae. tauschii* and *B. distachyon* transcriptome expression datasets were retrieved from the Sequence Read Archive (SRA) of NCBI. (1) Drought stress: Three drought RNA-seq data of wheat were about three groups of wheat cultivars, which were “TAM 111” and “TAM 112” (Bioproject: 659916), “Svevo” and “IL20-2” (Bioproject: 686121), “L-82” and “Marvdasht” (Bioproject: 450487), respectively. Two drought RNA-seq data of *Ae. tauschii* and *B. distachyon* were about *Ae. tauschii* cultivars “XJ98” and “XJ2” (Bioproject: 482066), *B. distachyon* cultivars “ABR4”, “ABR8” and “KOZ1” (Bioproject: 524106), respectively. (2) Other abiotic stresses: Three wheat RNA-seq data of abiotic stresses were about heat stress (Bioproject: 598150), salinity stress (Bioproject: 573996), and waterlogging stress (Bioproject: 604012). (3) Biotic stresses: Four wheat RNA-seq data of biotic stresses were about *F. graminearum* infection (EBI study accession: PRJEB12358), stripe rust (Bioproject: 613349), interactions with mycorrhizal fungi (*F. mossae*) with and without a pathogen attack by *X. translucens* (Bioproject: 474303), *X. translucens* infection (Bioproject: 401247).

Quality control assessment of raw data was performed using FastQC v0.11.7 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). High-quality RNA-seq reads were aligned to reference wheat (*T. aestivum*), *Ae. tauschii* and *B. distachyon* genomes of Ensembl Plants release-51 by software Hisat2 v2.2 [62], respectively. The counts of expression genes were performed using Samtools v1.10 [63] and HTseq v0.11.3 [64] software. The expression levels of transcriptome (log₂ value) were calculated by using R software and R package DESeq2. Heat maps of wheat RLK expression levels were generated using Mev4.9 [65].

Plant Materials and Stress Treatments

The wheat (*Triticum aestivum* L.) cultivar “SuMai 3” was used in this study. The wheat seedlings were planted into pots for growth at 22–25 °C with a photo-period of 16 h/8 h. the seedlings of wheat (*T. aestivum* L.) cultivar “SuMai3” at the three-leaf and one-heart stage were treated with drought stress including 20% (m/V) PEG-6000 for 0, 3, 6, 12 and 24 h. The wheat leaves were harvested and immediately frozen in liquid nitrogen for expression analysis. At least 15 samples of each experimental replicate were analyzed.

RNA Extraction and qRT-PCR

The total RNA of all samples was extracted using the RNAprep Pure Plant Kit (Tiangen) and was reversed into cDNA using HiScript III 1st strand cDNA synthesis kit (Vazyme). The cDNA was used as samples of qRT-PCR analysis. The qRT-PCR analysis was performed through the Roche LightCycler® 480 (Roche Diagnostics GmbH, Mannheim, Germany). The wheat gene *β-Actin* was used as an endogenous control. Relative expression levels of genes were calculated using the formula $2^{-\Delta\Delta CT}$. All the qRT-PCR primers in this study are supplied in Table S13.

Declarations

Supplementary Information

The online version contains supplementary material available at: XXX

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Statement of plant material collection and experimental methods

We have the formal permissions to collect all the materials used in this manuscript. All experimental methods were performed in accordance with the relevant guidelines and regulations.

Authors' contributions

JY and PSS conceived and designed the experiments. JY performed the identification and evolutionary analysis of RLK gene family in wheat and other plants, wrote the corresponding descriptions in manuscript, and revised all the manuscript. PSS performed the qRT-PCR experiments. PSS, XYM and PZL revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The genomes, proteomes and GFF files of the investigated plants are available in Ensembl Plants 51-release (<http://plants.ensembl.org/>). The accession numbers of plants are *T. aestivum* (IWGSC), *T. Spelta* (PGSBv2.0), *T. Turgidum* (Svevo.v1), *T. dicoccoides* (WEWSeq_v.1.0), *T. urartu* (ASM34745v1), *Ae. tauschii* (Aet_v4.0), *B. Distachyon* (v3.0), *Z. Mays* (Zm-B73-REFERENCE-NAM-5.0), *O. Sativa* (IRGSP-1.0), *A. Thaliana* (TAIR10), *V. Vinifera* (12X), *A. Trichopoda* (AMTR1.0), *S. Moellendorffii* (v1.0), *P. Patens* (Phypa_V3) and *C. Reinhardtii* (v5.5). Public wheat (*T. aestivum*), *Ae. tauschii* and *B. distachyon* transcriptome expression datasets were retrieved from the SRA database of NCBI and EBI. The SRA accession numbers of transcriptomes are 659916, 482066, 524106, 598150, 573996, 604012, 613349, 474303 and 401247. EBI study accession of *F. graminearum* infection is PRJEB12358.

Ethics approval and consent to participate

The wheat material (cultivar "Sumai 3") used in this study was obtained from the College of Agronomy, Liaocheng University, Liaocheng 252059, P.R. China and is publicly available for non-commercial purposes.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. The numbers of PK gene superfamily and RLK gene family in 15 plants.

Species	PK	RLK	RLK/PK
<i>Chlamydomonas reinhardtii</i>	497	4	0.80%
<i>Physcomitrella patens</i>	637	298	46.78%
<i>Selaginella moellendorffii</i>	975	532	54.56%
<i>Amborella trichopoda</i>	583	375	64.32%
<i>Vitis vinifera</i>	1141	864	75.72%
<i>Arabidopsis thaliana</i>	1004	615	61.25%
<i>Oryza sativa</i>	1134	810	71.43%
<i>Zea mays</i>	1292	787	60.91%
<i>Brachypodium distachyon</i>	1200	839	69.92%
<i>Aegilops tauschii</i>	1552	1183	76.22%
<i>Triticum urartu</i>	1223	958	78.33%
<i>Triticum dicoccoides</i>	2834	2153	75.97%
<i>Triticum turgidum</i>	3209	2510	78.22%
<i>Triticum spelta</i>	4660	3551	76.20%
<i>Triticum aestivum</i>	5015	3889	77.55%

Table 2 The tandem duplication clusters of RLKs in *T. aestivum* 21 chromosomes.

Chromosome	Clusters of tandem duplication
1A	6
1B	9
1D	9
2A	13
2B	16
2D	19
3A	9
3B	17
3D	10
4A	9
4B	5
4D	4
5A	9
5B	15
5D	18
6A	10
6B	13
6D	10
7A	15
7B	8
7D	8

Figures

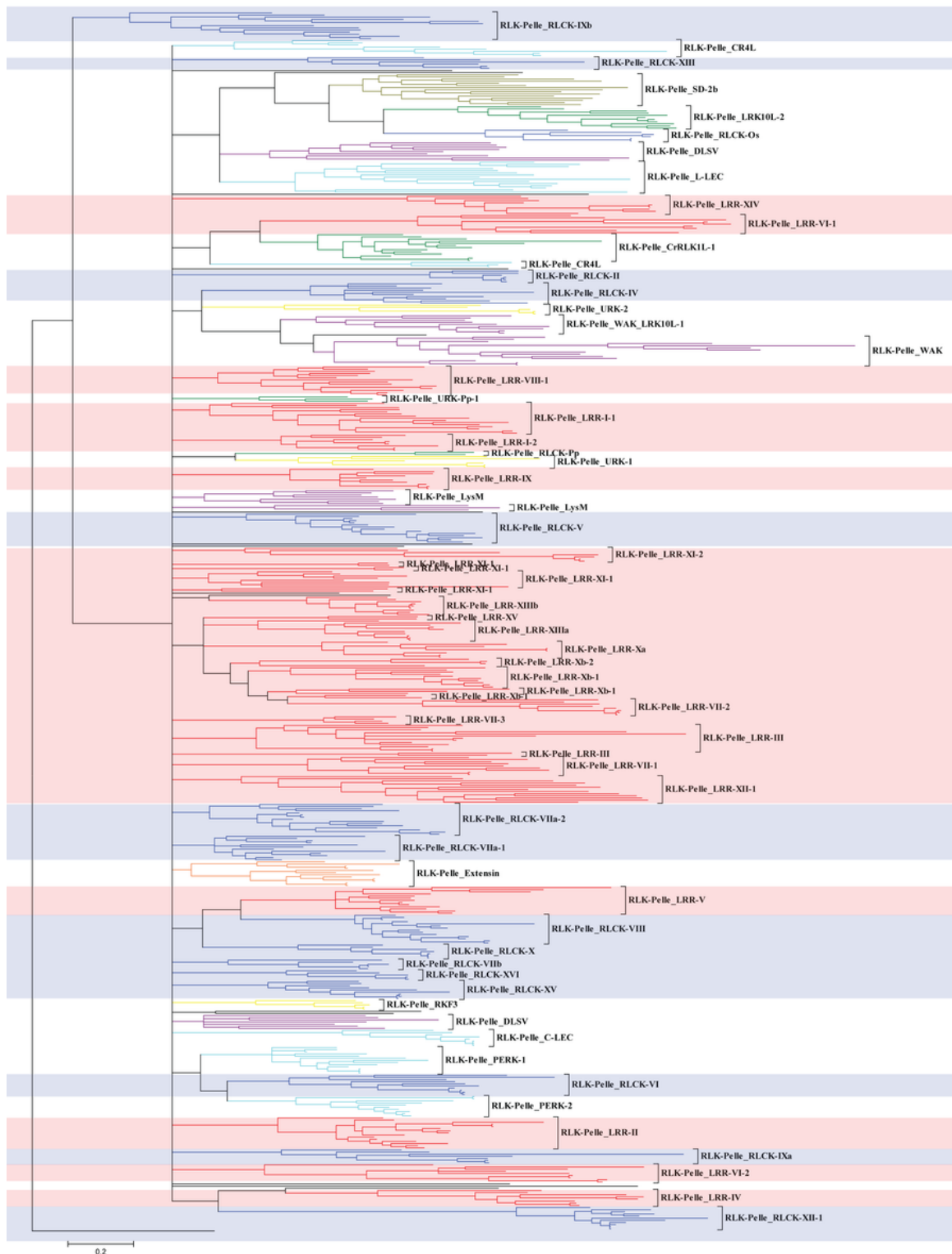


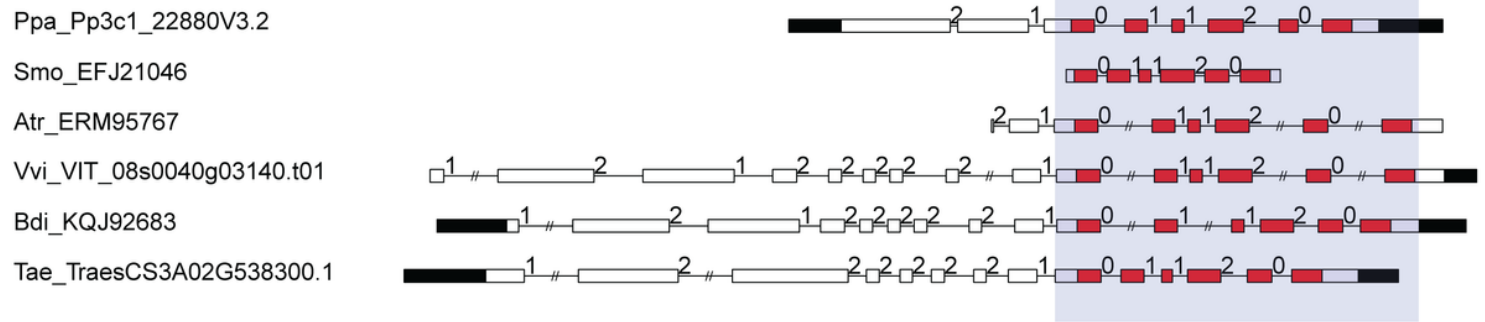
Figure 1

Classification and phylogenetic relationships of RLKs in wheat and other 8 representative plants.

The Bayesian phylogenetic tree was built by the kinase domain amino acid sequences from 9 representative plants (*C. reinhardtii*, *P. patens*, *S. moellendorffii*, *A. trichopoda*, *A. thaliana*, *B. distachyon*, *Ae. tauschii*, *T. urartu* and *T. aestivum*) using the MrBayes v3.2.7 . Random representatives of each

subfamily are selected by the following criteria: members<=6, 1 RLK; 6<members<=30, 2 RLKs; members>30, 3 RLKs. Detailed information is provided in Fig. S1A.

RLK-Pelle_LRR-I-1



RLK-Pelle_RLCK-IXa

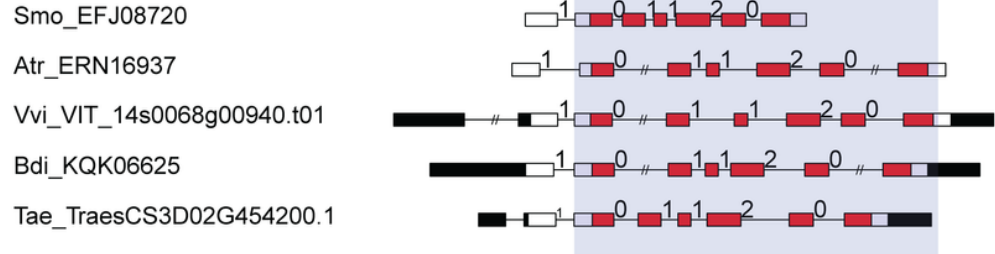
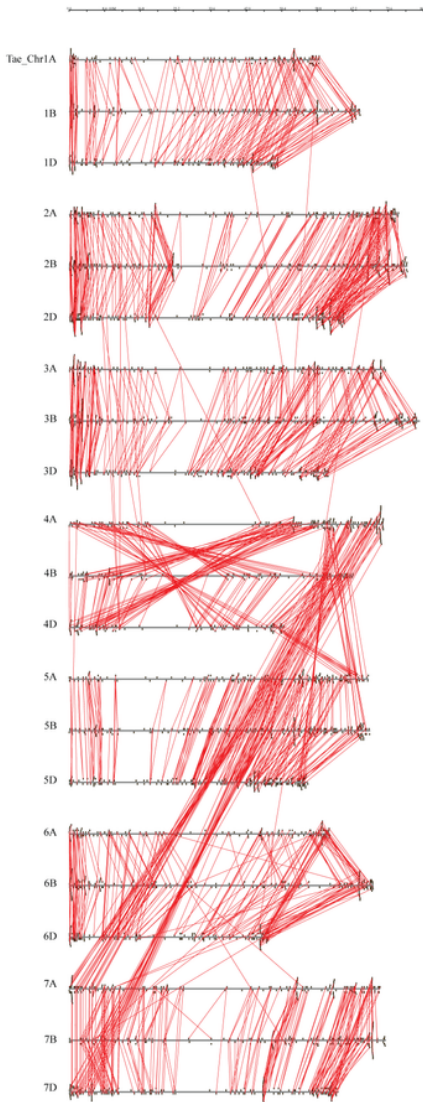


Figure 2

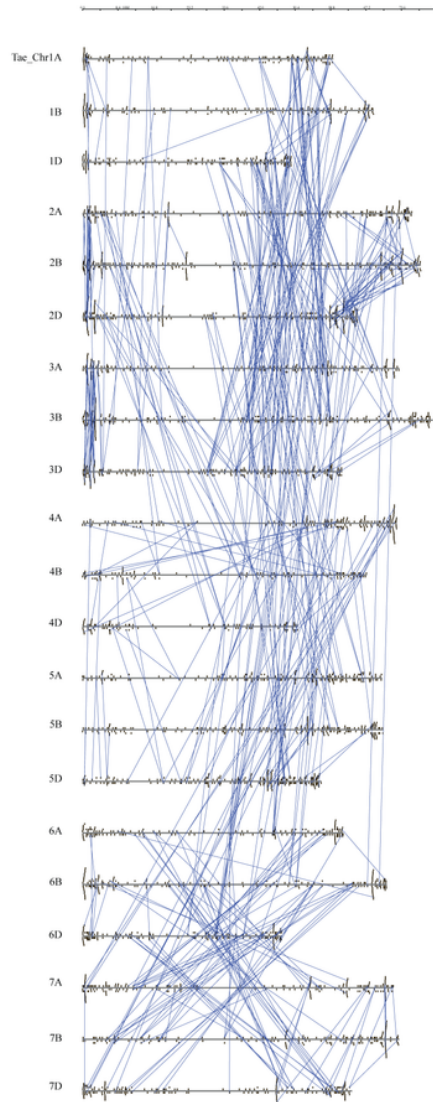
Two examples of conserved exon-intron structure in RLKs.

This diagram indicates that conserved exon-intron structure with conserved exon phases was existent in kinase domain. Filled boxes: red represents kinase (PK_Tyr_Ser-Thr or Pkinase) domain; black boxes: untranslated regions (UTRs); white boxes: other exon regions; lines: introns. Numbers 0, 1, and 2: exon phases. The lengths of the boxes and lines are scaled based on the length of genes. The long introns were shorted by “//”. (A) RLK-Pelle_LRR-I-1; (B) RLK-Pelle_RLCK-IXa.

A



B



C

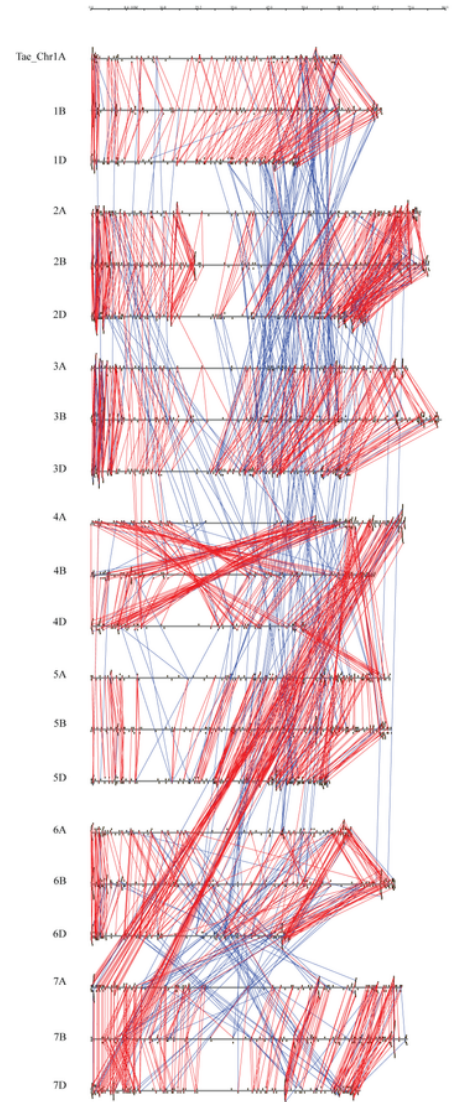


Figure 3

Collinearity events of *T. aestivum* RLK genes.

(A) The collinearity events with the *Ks* values 0-0.35. (B) The other collinearity events. (C) All collinearity events. Red lines denote the collinearity events with the *Ks* values 0-0.35. Blue lines denote the other collinearity events.

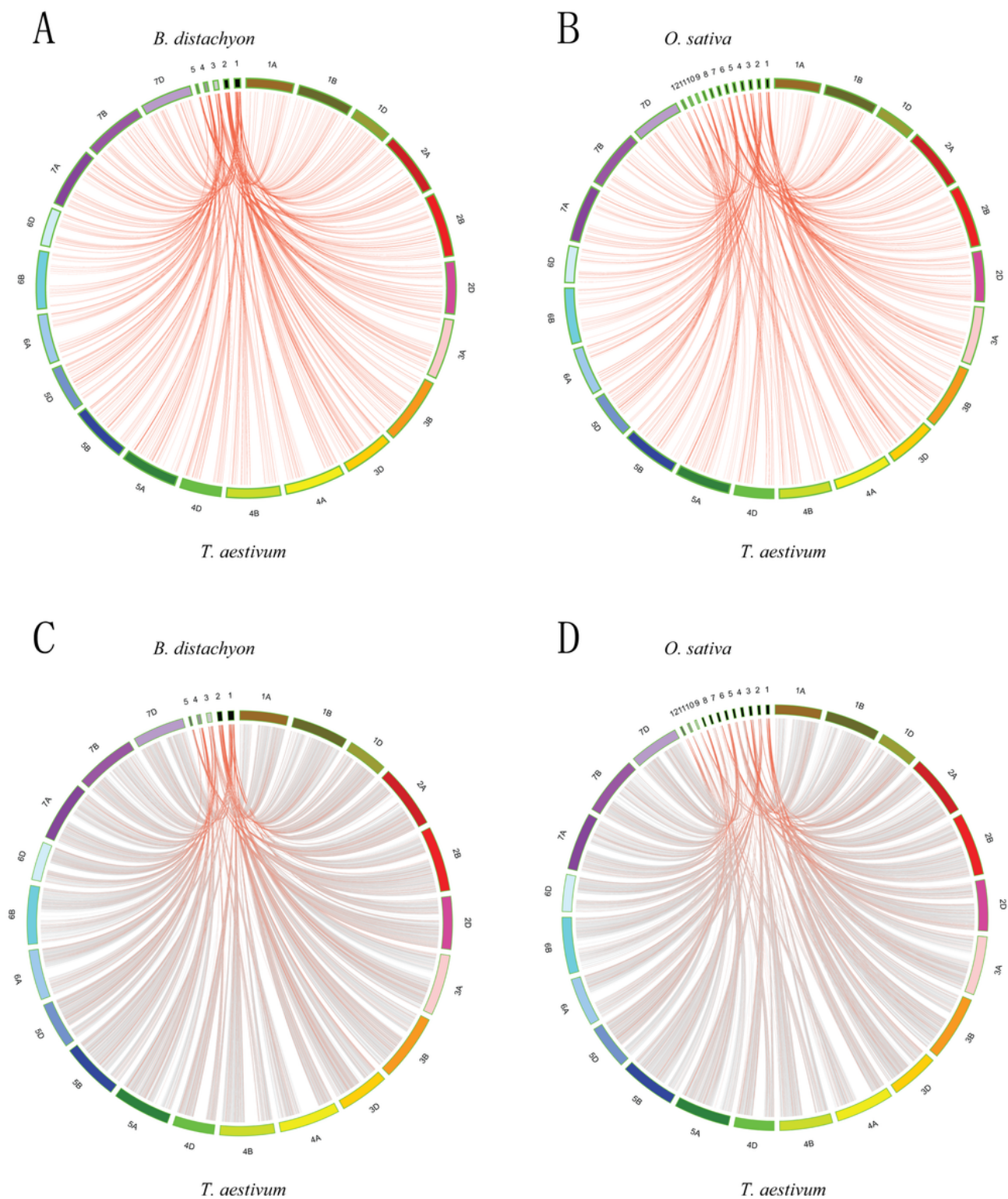


Figure 4

Synteny analysis of RLK genes.

This graph displays syntenic maps among *T. aestivum*, *B. distachyon* and *O. sativa*. Red curves represent syntenic gene pairs between the RLKs, and grey curves represent other genes. (A) Syntenicity of RLKs between *T. aestivum* and *B. distachyon*; (B) Syntenicity of RLKs and other genes between *T. aestivum* and *B.*

distachyon; (C) Synteny of RLKs between *T. aestivum* and *O. sativa*; (D) Synteny of RLKs and other genes between *T. aestivum* and *O. sativa*.

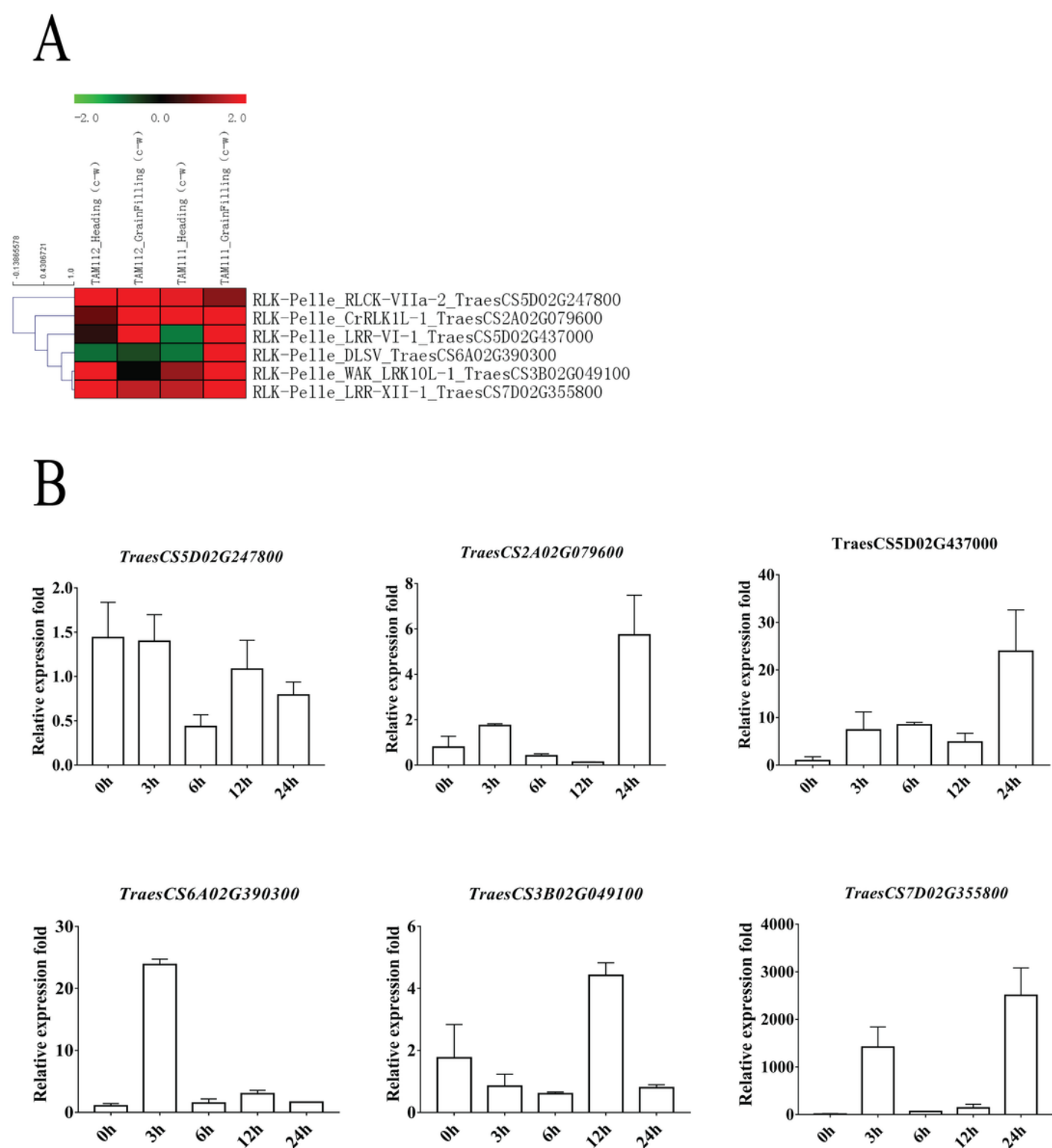


Figure 5

Heatmap of 6 selected wheat RLKs and their qRT-PCR under drought condition.

(A) Heatmap of transcriptome; (B) qRT-PCR under PEG-600 treatment.

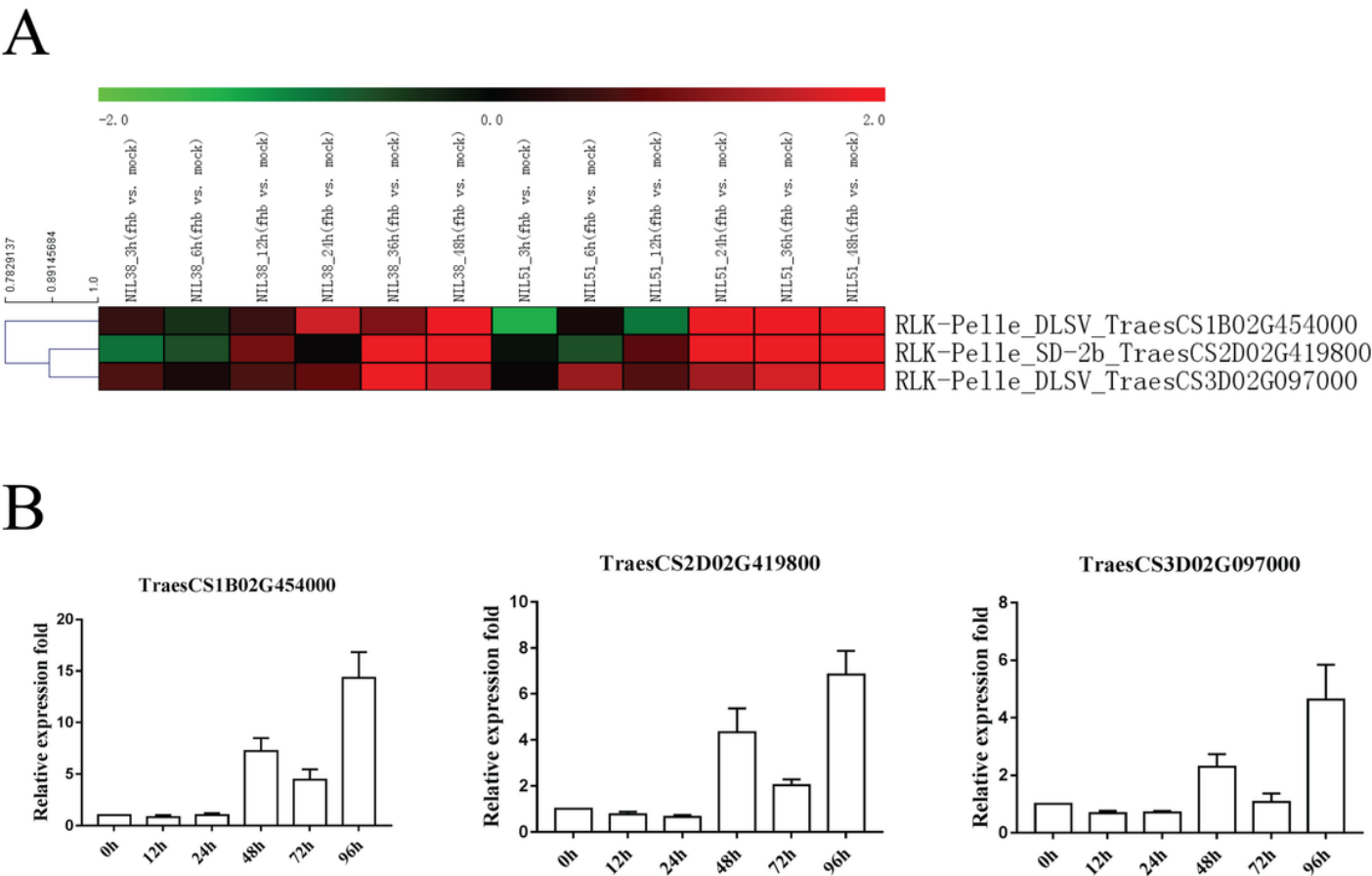


Figure 6

Heatmap of 3 selected wheat RLKs and their qRT-PCR with *Fusarium graminearum* infection.

(A) Heatmap of transcriptome; (B) qRT-PCR with *Fusarium graminearum* infection.

Supplementary Files

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