Phosphorylation and dephosphorylation of the receptor-like kinase domain by various kinases and protein phosphatases, respectively, are crucial for regulating receptor function.

The receptor-like protein kinase domain typically consists of a variable N-terminal region, a kinase domain, a transmembrane region, and a C-terminal region. The kinase domain is responsible for catalyzing the phosphorylation of a substrate, typically a serine/threonine residue in the extracellular or intracellular domain of the receptor.

The kinase domain is typically highly conserved among different receptors, and its structure is often used to predict the function of different receptors. The kinase domain is classified into several subfamilies, such as the tyrosine kinase (TK) domain, the serine/threonine kinase (STK) domain, and the receptor-like kinase (RLK) domain.

Phosphorylation at the kinase domain can regulate the activity of the receptor, either by activating or inactivating it. The phosphorylation status of the receptor can be regulated by various kinases and protein phosphatases, which can be activated or inhibited by different stimuli.

In summary, the receptor-like protein kinase domain plays a crucial role in regulating the function of receptor-like kinases, and its phosphorylation status can be regulated by various kinases and protein phosphatases.

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INTRODUCTION

Globally, wheat is a staple food and a source of nutrition. In the last 2 decades, the production of wheat increased by up to 1% annually (Manès et al., 2012), but this increase is not enough to meet the demand of the population, which will increased from 7.8 billion to 9.7 billion in 2050 (Roser and Ortiz-Ospina, 2013). The climatic changes including abiotic and biotic stresses are the main causes which extremely effect the quality and yield of the crops. To face these challenges, it is vital to explore the crop genotypes that can stand up to all of these hurdles. Plants are immobile in nature; they don’t move here and there in search of food, but their roots do. The root is the major organ that has a crucial role in the adaptation of the plant to its unfavorable environment. Root systems captivate the water and nutrients essential for the growth and maintenance of plant (Alahmad et al., 2019; Grzesiak et al., 2019). Hence, improved root system overcomes the challenges of the harsh environment and might enhance crop production (Djanaguiraman et al., 2019; Danakumara et al., 2021; Rasool et al., 2021).

Drought is one of the major abiotic stresses caused due to scarce rainfall that affects productivity. An increase in drought in the coming 30 years will have adverse effects on crop yield with 6–12 bushels/acre (Zargar et al., 2017). Creation of drought tolerance is a very complicated because many genes such as TaERI, 2, and 3, TaZFP34, TaWRKY11, 10, 33, 44, and 93, TaDR O 1, and TaRAP2.1 directly or indirectly involved. In animals, receptor protein kinases (RPKs) are the genes which play a significant role in the stimulation of hormones and other growth factors (Fantl et al., 1993). In plants, similar to animals’ RPKs, there is a receptor-like protein kinase (RLK) gene family. The RLK family is a huge family of genes found in many plants. The typical RLK structure comprises an extracellular domain at the N-terminal, a membrane helix, and an intracellular conserved kinase domain (KD) at the C-terminal. The extracellular domains of the RLK family are highly diverged, which results in the differentiation of RLKs into 17 distinct subfamilies, including the receptor-like kinases (Mishra et al., 2021). The LRRKs (leucine-rich repeat kinase) represents biggest subfamily of RLK with 531 TaLRRK genes in wheat (Sharma et al., 2016), comprising of ECD (extracellular domain) to receive signals, TM (transmembrane) region to bound it to cell membrane and cytoplasmic kinase domain for phosphorylation of substrate (Gou et al., 2010; Dievart et al., 2020). The LRRKs has numerous roles in plants as it is involved in initiating innate defense at front-line against microbial pathogens (Nejat and Mantri, 2017), morphogenesis, organogenesis, hormone signaling, abiotic, and biotic stress regulation in plants (Dièvart and Clark, 2003; Li and Tax, 2013; Dufayard et al., 2017). Later on the role of LRR-RLKs in pathogen sensing and activation of downstream defense response has been reviewed deeply (Nejat and Mantri, 2017). Due to the indispensable roles of LRR-RLKs in plants, they have been classified into two main classes (Dièvart and Clark, 2003). First, the LRR-RLK is crucial for morphogenesis, organogenesis, hormone signaling, signifying development, and growth regulation. Secondly, numerous LRR-RLK members respond to biotic and abiotic stresses like Fusarium wilt, drought, salt, and cold, and hence are associated with defense (Afzal et al., 2008; Cao et al., 2020). Some of the LRR-RLKs have dual roles that might be because of the cross-talk among development and defense cascades or due to the binding of several ligands to a receptor (Afzal et al., 2008).

The RPK1 gene is a calcium independent Serine-Threonine (Ser-Thr) kinase that belongs to the subfamily of leucine-rich receptor kinases (LRR kinases) and family of Receptor-Like Kinases (RLK) (Zou et al., 2014). RPK1 is one of the short subfamilies with few genes that regulates abiotic stresses and root system architecture. The RPK1 comprises of extracellular six LRR motifs, a transmembrane domain, extracellular ligand-binding domain, and single cytoplasmic kinase conserved domain in rice (Hong et al., 1997; Cheng et al., 2009; Motte et al., 2014). Studies in rice have shown that RPK1 is involved in root system architecture (RSA) via regulating negatively polar auxin transport (PAT) and accumulation of auxin in roots (Zou et al., 2014). In other studies of rice, it was also reported that auxin defective mutants showed stunted growth and shorter roots (Uzair et al., 2021). Down-regulation of RPK1 endorsed the growth and enhanced the height of the plant and number of tillers, whereas up-regulation resulted in immature lateral roots, adventitious roots, and a decreased apical meristem of roots (Zou et al., 2014). In Arabidopsis, the inhibition of AtRPK1 displayed greater salt tolerance than normal plants, while overexpressed plants exhibited lesser salt tolerance degrees (Shi et al., 2014). The levels of AtRPK1 were enhanced ominously under less water, abscisic acid (ABA), high salt and lower temperature (Hong et al., 1997). In Arabidopsis thaliana, inhibition of RPK1 delayed ABA-induced senescence significantly (Lee et al., 2011). AtRPK1 is also prerequisite for cotyledon primordial initiation of cotyledons during embryogenesis in Arabidopsis thaliana (Nodine et al., 2007; Nodine and Tax, 2008). AtRPK1 positively regulates CaM1 gene expression, which in turn regulates ROS (reactive oxygen species) production, leaf senescence, and ABA response (Dai et al., 2018).

Triticum aestivum L., commonly known as “bread wheat”, is a cereal and staple food grain all over the globe. Being a most consumed cereal crop, it was grown on a large scale of 240 million hectares in 2016 (Milner et al., 2018). However, due to water scarcity, nutrient deficiency, and abiotic stresses, wheat yield is curtailed (Mondal et al., 2015; Abbas et al., 2022). Wheat is a drought sensitive crop. Therefore, in order to meet the global demand, that is 50% of the grain in 20 years approximately, the varieties of wheat with effective utilization of minerals and water are requisite (Odegard and Van der Voet, 2014). Since roots are the main structures for the minerals and water uptake and decipher stress stimuli from soil (Fang et al., 2017). Hence, identification of stress-tolerant genes within the root system could be propitious.

Since the genome of T. aestivum has been sequenced, it is feasible to carry out a genome-wide analysis of different genes. In this study, 15 TaRPK1 genes were analyzed for their structure, chromosomal location within the genome, phylogenetic relationships, conserved motifs, synteny, and cis-regulatory elements. Additionally, the patterns of expression of all
15 TaRPK1 members were also studied in silico. RT-PCR expression analysis of TaRPK1 members was also performed in Pakistan-13, Galaxy (drought tolerant), and Shafaq (drought susceptible) wheat varieties under normal and drought conditions. The current study enlightens the role of TaRPK1 genes in plant developmental processes under drought conditions and provides a solid foundation for the functional characterization of the wheat RPK1 gene family.

MATERIALS AND METHODS

Identification of RPK Gene Family Members in T. aestivum

The sequence IDs of Arabidopsis and rice RPK1 genes were acquired from the available literature (Shi et al., 2014; Zou et al., 2014). These sequences were retrieved from Ensembl plants and NCBI, which were then used as queries for the Basic-Local Alignment Search tool (BlastP and BlastN) against IWGSC (INSDC Assembly GCA_900519105.1 July 2018 database version 106.4), NCBI (https://www.ncbi.nlm.nih.gov) and Ensembl plants (plantshttp://plants.ensembl.org/index.html) for T. aestivum. For all of the candidate RPK1 genes, the kinase domain presence was substantiated with Pfam (http://pfam.sanger.ac.uk), and by SMART (http://smart.embl-heidelberg.de/) (Letunic and Bork, 2018) databases. The sequences in which the kinase domain was absent were removed (Supplementary Table S1). In silico based putative protein information of RPK1 genes (physio-chemical) was analyzed through the Protparam (https://web.expasy.org/protparam/) tool. The subcellular localization of RPK1 proteins was predicted via Plant-mSubP and pLoc-mPlant (http://bioinfo.usu.edu/Plant-mSubP/; http://www.jci-bioinfo.cn/pLoc-mPlant) (Cheng et al., 2017; Sahu et al., 2020).

Chromosomal Location of TaRPK1 Genes

The chromosomal locations of all candidate RPK1 genes in T. aestivum were acquired from Ensembl (http://plants.ensembl.org/Triticum_aestivum/Info/Index). The gene map of TaRPK1 genes was drawn with the help of MapChart and confirmed through TBtools.

Phylogenetic Analysis of RPK1 Proteins

To retrieve the RPK1 protein sequences, the amino acid sequences of 15 TaRPK1 members were used as queries to blast (BLASTP) against the Triticum turgidum, Triticum dicoccoides, Triticum urartu, Triticum speltaeoides, Aegilops tauschii, Hordeum vulgare, Arabidopsis thaliana, and different species of Oryza (rufipogon, japonica, indica, and glaberrima). The sequences with > 60% identities were retrieved from Ensembl (http://plants.ensembl.org). The phylogenetic trees were made by means of MEGA-X software with NJ (neighbor-joining method) (Kumar et al., 1994). The parameter Poisson model and pairwise deletion were used with replicates of 1,000 bootstraps for assessment of node significance.

Prediction of Gene Structure and Conserved Motifs in TaRPK1 Proteins

The number of exons and introns was predicted by the gene structure display server (GSDS, http://gsdscbi.pku.edu.cn/) and the genomic sequences and coding sequences were aligned using ClustalW. Conserved motifs in RPK1 proteins of T. aestivum were analyzed using MEME, a multiple-EM for motif elicitation program (http://meme-suite.org/tools/meme) (Bailey et al., 2009). The execution of MEME search was done with default parameters apart from motif maximum number, which was set to 10, and optimum motif width of ≥6 and ≤200 was selected.

Gene Ontology Enrichment Analysis

The analysis of TaRPK1 gene ontology was performed by TaRPK1 protein sequences via the online gProfiler tool (https://biit.cs.ut.ee/gprofiler/gost) with default parameters (Raudvere et al., 2019).

miRNA Prediction in Wheat RPK1 Family Genes

The miRNA prediction was performed as mentioned formerly (Yan et al., 2019). The TaRPK1 sequences were submitted for potential miRNA prediction through a search against the available wheat miRNA reference by means of the psRNATarget Server (https://www.zhaolab.org/psRNATarget/), using default settings (Dai and Zhao, 2011). The visualization of the interaction network of the predicted miRNA with their corresponding TaRPK1 target genes was done by Cytoscape software (https://cytoscape.org/) with default settings (Shannon et al., 2003).

Interpretation of Putative Regulatory Cis-Acting Elements

The sequence size of 2 kb in the upstream region were dug out from all TaRPK1 genes of T. aestivum that acted as promoters for the regulatory cis acting elements prediction through the PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) database (Lescot et al., 2002).

Collinearity Prediction and Synteny Analysis

The GFF3 files and proteomes of Triticum aestivum and its ancestors, including Aegilopes tauschii, Triticum spelta, Triticum dicoccum, and Triticum dicoccoides, were used from the Ensembl Plants database for collinearity prediction via the MCScanX algorithm (Wang et al., 2012). Synteny scrutiny of RPK1 family members was performed via TBtools (Chen et al., 2018).

Three-Dimensional Protein Structure Prediction

The TaRPK1 protein structures were modeled via amino acid sequence using the SWISS-MODEL database (https://www.swissmodel.expasy.org) (Biasini et al., 2014), and for
visualization of 3D structure Pymol software (https://pymol.org/2/) was applied. The verification and validation of the predicted 3D structures of TaRPK1 proteins were assessed using the Ramachandran Plot—Zlab, (https://zlab.umassmed.edu/bu/rama/)(Anderson et al., 2005).

**In Silico** Differential Expression Patterns of RPK Genes

In *silico* expression analysis was performed using the wheat-expression browser (www.wheat-expression.com) at different wheat stages (Kaur et al., 2017). The data were unruffled in the course of developing seedling, vegetative, and reproductive stages from different organs of wheat such as roots, leaf sheath, leaf blade, shoot, spike, and grain. The heatmap was then created from the composed data, based on the expression values of genes (in TPM) by means of Tbtool.

**Interaction Network and Co-Expression Analysis**

For interaction network studies, String (https://string-db.org/) was used by selecting *Triticum aestivum* as a platform species. For visualization of the molecular library, Cytoscape was used.

### TABLE 1 | In silico prediction of identified RPK1 genes in wheat and sequence characteristics.

| Sr. no. | New name | Gene ID | Chr No. | Chr location | Orientation | CDS (bp) | No. of exons | Coding exons | No. of introns |
|---------|-----------|---------|---------|--------------|-------------|----------|-------------|--------------|---------------|
| 1       | TaRPK1.1 (TaRPK1) | TraesCS1A02G304200 | 1A | 497,503,763–497,509,507 | R | 2,895 | 19 | 19 | 18 |
| 2       | TaRPK1.2 (TaRPK2) | TraesCS1B02G314700 | 1B | 539,546,762–539,552,423 | R | 2,895 | 19 | 19 | 18 |
| 3       | TaRPK1.3 (TaRPK3) | TraesCS1D02G303700 | 1D | 401,666,525–401,672,077 | R | 2,895 | 19 | 19 | 18 |
| 4       | TaRPK1.4 (TaRPK4) | TraesCS3A02G340100 | 3A | 587,403,291–587,406,585 | F | 2,676 | 17 | 17 | 16 |
| 5       | TaRPK1.5 (TaRPK5) | TraesCS3B02G371700 | 3B | 584,546,469–584,551,744 | F | 2,676 | 17 | 17 | 16 |
| 6       | TaRPK1.6 (TaRPK6) | TraesCS3D02G333600 | 3D | 445,633,883–445,639,177 | F | 2,676 | 17 | 17 | 16 |
| 7       | TaRPK1.10 (TaRPK10) | TraesCS2A02G176500 | 2A | 136,053,228–136,056,887 | R | 3,372 | 2 | 1 | 0 |
| 8       | TaRPK1.11 (TaRPK11) | TraesCS2B02G202900 | 2B | 182,708,242–182,711,907 | R | 3,372 | 2 | 1 | 0 |
| 9       | TaRPK1.12 (TaRPK12) | TraesCS2A02G183900 | 2D | 129,186,794–129,190,494 | R | 2,949 | 2 | 2 | 1 |
| 10      | TaRPK1.13 (TaRPK13) | TraesCS2A02G260600 | 2A | 410,851,518–410,855,096 | F | 2,187 | 2 | 2 | 1 |
| 11      | TaRPK1.14 (TaRPK14) | TraesCS2B02G281400 | 2B | 388,595,342–388,597,540 | R | 1830 | 2 | 2 | 1 |
| 12      | TaRPK1.15 (TaRPK15) | TraesCS2D02G263100 | 2D | 320,280,150–320,283,723 | R | 2,199 | 2 | 1 | 0 |
| 13      | TaRPK1.17 (TaRPK17) | TraesCS3A02G340000 | 3A | 587,396,690–587,401,627 | F | 2,772 | 18 | 18 | 17 |
| 14      | TaRPK1.18 (TaRPK18) | TraesCS3B02G371600 | 3B | 584,539,043–584,544,883 | F | 2,874 | 19 | 19 | 18 |
| 15      | TaRPK1.19 (TaRPK19) | TraesCS3D02G333500 | 3D | 445,627,122–445,632,371 | F | 2,775 | 18 | 18 | 17 |

**TABLE 2 | In silico-based putative protein information of RPK1 genes identified in T. aestivum.**

| Sr. no. | New name | Sequence ID | PL (Aa) | Domain loc | Mol. wt. (Kda) | pl | II | AI | GRAVY | SCL |
|---------|-----------|-------------|---------|-----------|----------------|----|----|----|-------|-----|
| 1       | TaRPK1.1 (TaRPK1) | TraesCS1A02G304200 | 964 | 632–901 | 104 | 28.37 | 91.76 | -0.032 | Cell membrane |
| 2       | TaRPK1.2 (TaRPK2) | TraesCS1B02G314700 | 964 | 632–901 | 104 | 29.41 | 91.05 | -0.046 | Cell membrane |
| 3       | TaRPK1.3 (TaRPK3) | TraesCS1D02G303700 | 964 | 632–901 | 104 | 30.18 | 90.54 | -0.04 | Cell membrane |
| 4       | TaRPK1.4 (TaRPK4) | TraesCS3A02G340100 | 891 | 563–832 | 97 | 37.72 | 89.55 | -0.108 | Cell membrane |
| 5       | TaRPK1.5 (TaRPK5) | TraesCS3B02G333600 | 891 | 563–832 | 97 | 38.74 | 89.99 | -0.109 | Cell membrane |
| 6       | TaRPK1.6 (TaRPK6) | TraesCS3D02G333600 | 891 | 563–832 | 97 | 38.74 | 89.99 | -0.109 | Cell membrane |
| 7       | TaRPK1.10 (TaRPK10) | TraesCS2A02G176500 | 1,123 | 841–1,048 | 120 | 42.51 | 103.46 | 0.121 | Cell membrane |
| 8       | TaRPK1.11 (TaRPK11) | TraesCS2B02G202900 | 1,123 | 841–1,112 | 120 | 42.79 | 104.32 | 0.138 | Cell membrane |
| 9       | TaRPK1.12 (TaRPK12) | TraesCS2D02G281400 | 928 | 700–971 | 104 | 42.82 | 106.38 | 0.135 | Cell membrane |
| 10      | TaRPK1.13 (TaRPK13) | TraesCS2A02G260600 | 728 | 499–720 | 80 | 52.17 | 101.35 | 0.063 | Cell membrane |
| 11      | TaRPK1.14 (TaRPK14) | TraesCS2B02G281400 | 609 | 330–601 | 67 | 47 | 98.74 | -0.054 | Cell membrane |
| 12      | TaRPK1.15 (TaRPK15) | TraesCS2D02G263100 | 732 | 453–724 | 80 | 51.16 | 100.26 | -0.035 | Cell membrane |
| 13      | TaRPK1.17 (TaRPK17) | TraesCS3A02G340000 | 923 | 593–865 | 101 | 31.67 | 89.51 | -0.147 | Cell membrane |
| 14      | TaRPK1.18 (TaRPK18) | TraesCS3B02G333600 | 957 | 627–897 | 104 | 31.92 | 90.92 | -0.146 | Cell membrane |
| 15      | TaRPK1.19 (TaRPK19) | TraesCS3D02G333500 | 924 | 594–864 | 101 | 31.27 | 87.93 | -0.179 | Cell membrane |

Loc, location; Mol. wt., molecular weight; pl, isoelectric point; II, instability index; AI, aliphatic index; GRAVY, grand average of hydropathicity; SCL, sub-cellular localization.
Correlation coefficients on the basis of verities, treatments, and tissues were calculated in R 3.4.0. These coefficients indicate the degree of association among the terms and provide linkages among the TaRPK1 members.

Plant Material and Stress Treatment
Previously, Pakistan-13, Galaxy, and Shafaq were studied under drought stress and categorized as drought tolerant and susceptible varieties, respectively (Shabbir et al., 2015; Ulfat et al., 2017; Ahmad et al., 2021; Wasaya et al., 2021; Iqra et al., 2022). So, seeds of these varieties were obtained and sown under controlled glass-house conditions at the National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agriculture Research Center (NARC), Islamabad, Pakistan. After 2 weeks of sowing (seedling stage), the roots and leaves tissues were collected. At growth stage 8 (tillering stage), roots, stems, and leaf tissues were collected. At the grain filling stage (14 days after flowering), sampling for roots, stems, leaf, and grains was done (Hyles et al., 2020). For expression profile analysis under drought stress, seeds of selected varieties were first surface sterilized with sodium hypochlorite followed by three washings, then soaked in distilled water in a growth chamber (16 h light/8 h dark cycle at 22°C). After 2 weeks, young seedlings were treated with 20% polyethylene glycol (PEG) 6,000 (v/v). The root and leaf tissues of seedlings were harvested after 12 h of exposure to stress conditions. All the samples were collected in three replicates, and samples were frozen immediately in liquid nitrogen, and placed in −80°C storage for RNA extraction.

RNA Isolation and qRT-PCR Analysis
Approximately 100 mg of tissues were taken for total RNA extraction using an RNA mini kit (Cat # 12183018A, Invitrogen, Thermo Fischer Scientific) followed by the manufacturer’s instructions. Through agarose gel electrophoresis, the quality and concentration of RNA were determined, followed by optical density measurement through a spectrophotometer. With the help of the RT Prime-Script Reagent Kit, the cDNA was made from 1 µg of RNA. Specific primers were designed for TaRPK1 genes manually, followed by confirmation via NCBI Primer Blast software (http://www.ncbi.nlm.gov/tools/primer-blast), provided in Supplemental Table S2. The qRT-PCR was accomplished with SYBR Green I (Roche) Master Mix. Wheat β-Actin was used as a control reference gene. Three independent biological replicates were analyzed for each sample. The values were means and standard deviations (SD) were calculated from biological replicates. The relative expression levels of each gene were studied by means of 2−ΔΔCt (Schmittgen and Livak, 2008).

RESULTS
Analysis and Sequence Identification of RPK1 Genes in T. aestivum
A set of 15 candidate RPK1 genes were retrieved from Triticum aestivum based on BlastP and BlastN. A domain search with the corresponding RPK1 candidate amino acid sequences confirmed the S_TKc Domain (SM00220). Thus, a total of 15 TaRPK1 with complete structures were analyzed in T. aestivum (Table 1). Subsequent sequence identification of 15 TaRPK1 showed the protein length of 609–1,123 amino acids and a molecular mass ranged from 67–120 kDa. The iso-electric points (PI) of these proteins were 6–9. The Instability Index (II) ranged from 28.37–52.17, the Aliphatic Index (AI) was 87.93–106.38, and the grand-average of hydropathicity...
The instability index of group I was less than 40, representing stable proteins, whereas proteins of groups II and III showed instability index values of more than 40, indicating unstable proteins. The AI signified that all of the TaRPK1 proteins are thermally stable. The GRAVY indicated TaRPK1 proteins to be hydrophilic proteins except for TaRPK10, TaRPK11, and TaRPK12, which showed a value less than zero, representing them as hydrophobic proteins. The sub-cellular localizations of the TaRPK1 were anticipated, which showed that all the TaRPK1 were localized to the cell membrane (Table 2).

A detailed protein alignment of structural predictions showed that all TaRPK1 proteins are composed of the leucine-rich repeat domain, leucine-rich repeat domain, transmembrane domain I, and a serine–threonine kinase (S_TKc) domain. However, the LRR domains were

FIGURE 2 | Comparative phylogenetic tree of RPK genes between Triticum aestivum, Triticum dicoccoides, Triticum turgidum, Triticum speltoides, Aegilops tauschi, Triticum urata, Hordeum vulgare, Arabidopsis thaliana, and different species of Oryza (rupigong, indica, japonica, and glaberrima). 1,000 replicates were used for the bootstrap test, and the replication percentage is presented next to the branches.
missing in TaRPK1, TaRPK2, and TaRPK3 sequences (Supplemental Figure S1).

**Chromosomal Distribution of RPK Genes**

The physical location of RPK genes in *T. aestivum*, to the corresponding chromosomes, is shown in Figure 1. A total of 15 RPK genes were mapped on 9 out of 21 chromosomes in wheat. The genes were mainly mapped on chromosomes 1, 2, and 3 on the respective A, B, and D genomes. No RPK genes were found on the rest of the chromosomes.

**Phylogenetic Analysis of TaRPK1 Proteins**

Of the 15 identified TaRPK1 genes in this study in *Triticum aestivum*, two RPK genes from *Arabidopsis thaliana*, 16 RPK genes from rice, seven RPK genes from *Triticum dicoccoides*, three RPK genes from *Triticum urata*, seven RPK genes from *Triticum turgidum*, four RPK genes from *Aegilops tauschii*, 11 RPK genes from *Triticum speltoides*, and four RPK genes from *Hordeum vulgare* were used to construct a neighbor-joining based tree with MEGA X software in order to study the evolutionary relationships (Figure 2). The phylogenetic tree generated on the basis of similarities with protein sequences distributed RPK members into four main groups, with TaRPK1 members in three groups. Overall group I possessed nine TaRPK1 members (TaRPK1-9), that were closely associated with RPK members of rice. Group II (TaRPK10-12) and Group III (TaRPK13-15) exhibited three TaRPK1 members each, that exhibited close association with *Triticum turgidum*, *Triticum speltoides*, *Aegilops tauschii* and *Triticum dicoccoides*.
Analysis of TaRPK1 Gene Structure and Conserved Motif

The intron–exon number and arrangements of the RPK1 members were envisaged through comparing the coding sequence with the genomic DNA sequence. All of the TaRPK1 genes in group I consisted of 16–18 introns, except for the groups II and III that contained 0 and 1 intron (Figure 3A). Furthermore, the conserved motifs within TaRPK1 proteins were predicted by online MEME software. Ten conserved motifs (1-10) were analyzed (Figure 3B and Supplementary Figure S2). The motifs 1, 3, 4, 7, 8, and 10 were present in all of the RPK1 sequences. However, group II did not display motifs 3 and 10, and the motif three was also missing in group III sequences.

Gene Ontology of RPK1 Genes

GO annotation analysis was conducted for the functional analysis of RPK1 genes. In-silico functional prediction was performed, and the results displayed two types of processes involved, that is, molecular processes (MPs) and biological processes (BPs) (Figure 4 and Supplementary Table S3). Biological processes indicate that RPK members are involved actively in various metabolic processes. The molecular processes suggested the RPK1 member’s catalytic activity. Such outcomes clearly denote RPK1 genes’ significant role in growth and development via modulation of molecular and biological processes.

MicroRNA Targeting TaRPK1 Genes

We also identified putative 18 miRNAs targeting TaRPK1 genes for the generation of interaction networks by Cytoscape software in order to better understand the underlying miRNA mechanism involved in the modulation of TaRPK1 genes (Figure 5 and Supplementary Table S4). In the connection distribution and regulation network, TaRPK1, TaRPK2, and TaRPK3 were found targeted by single miRNAs, which are tae-miR9782, tae-miR9776, and tae-miR1122c-3p, respectively. TaRPK10 and TaRPK11 are the most targeted RPK1 wheat genes by tae-miR1134, tae-miR9774, tae-miR9661-5p, tae-miR9664-3p and tae-miR9777 targeting TaRPK10, and tae-miR9774, tae-miR9777, tae-miR9664-3p, tae-miR395a and tae-miR9661-5p targeting TaRPK11 genes. However, no miRNA was found targeting TaRPK13, TaRPK14, and TaRPK15 genes.

Regulatory Cis-Element Interpretation in T. aestivum

The promoter regions contain cis-modulatory elements which are critical for the binding of transcription factors for transcription initiation, which has an essential function in the expression of genes. The promoter regions of RPK1 members were used for the cis-regulatory element prediction (Figure 6A). The results indicated that the cis-regulatory elements can be distributed into several categories, such as hormone related elements, light-related elements, developmental responsive elements, abiotic stress responsive elements, promoter-related motifs, and other motifs. Amid them, the elements chiefly present were associated with photoreaction, hormone responsiveness, and abiotic stress-related motifs. The photoreaction responsive cis-regulatory elements included ACE, AE-Box, ATCT, G-Box, GATA, GT1, SP1, AT1, Box 4, Box II, I-Box, TCT, GA, L-Box, TCCC, and ATC motif. The most abundant light-responsive elements were found in TaRPK11 and TaRPK13, which had 17 and 12 members, respectively. Hormone responsive elements were also copiously present in the RPK1 promoter, mostly comprising abscisic acid response elements. The three extensively distributed cis elements were related to abiotic stress response, among which drought responsive elements were profuse. Other elements correlated to abiotic stress were also identified.

Syntenic Relationship Analysis

In order to understand the evolutionary relationship and origin of Triticum aestivum (tr) with Triticum turgidum (tg), Aegilops tauschii (at), Triticum speltoides (ts) and Triticum dicoccoides (td), a comparative synteny scrutiny of RPK protein sequences was performed. The proteins were closely related among five species and exhibited significant similarity in analysis of evolutionary correlation. It was observed that the TaRPK1 genes of T. aestivum have similar origins of evolution to other Triticum species (Figure 6B and Supplementary Table S5).

In silico 3D-Structure Prediction of TaRPK1 Proteins

Three-dimensional (3D) structures of TaRPK1 proteins were predicted by using SWISS_MODEL online computational software. 3D structures of target proteins were anticipated based on homology modeling. The SWISS MODEL predicted 15 successful models of TaRPK1 proteins with at least 30% identity to the template (4mn8.1. A, 5hyx.1. A, 5xkj.1. C, 6mOu.1. A, 4mn4.1. A, 4oh4.1. A, 6cth.1. A, 7brc.1. A, 7bos.1. A) that was a widely recognized threshold for effective modeling (Xiang, 2006). However, TaRPK2 and TaRPK3 showed sequence identity of 27.84% and 29.47%, respectively, with the template, which was less than 30%. The highest sequence identity of 45% with the template was observed by TaRPK4, TaRPK5 and TaRPK6 (Figure 7). The verification and validation of the predicted 3D structure of TaRPK1 were assessed via Ramachandran Plots (Anderson et al., 2005) that validated the backbone dihedral angles of the targeted protein. The Ramachandran plot assessment showed that 92–98% of the regions of TaRPK1 protein showed highly favorable regions, which indicates the stability and good quality of the predicted protein structure (Supplemental Table S6).

Genome Wide Expression Patterns of RPK Genes

The data of RNA-seq for all of the 15 RPK sequences were obtained from online database. A heatmap was generated showing expression levels of RPK members at different stages, namely seedling stage, vegetative stage, and reproductive stage.
(Figure 8 and Supplementary Table S7) and in various organs (root, leaf, shoot, spike, and grain) of wheat. The highest expression of TaRPK1 members was observed in root tissues compared to other tissues. TaRPK1, TaRPK2, and TaRPK3 exhibited the highest expression patterns in roots at seedling, vegetative, and reproductive stages. Higher to moderate expression was observed in grain at the developing reproductive stage by TaRPK13 and TaRPK14, respectively. Spikes, leaves, and shoots showed moderate to low expression in all of the TaRPK1 members in wheat.
Expression Analysis of RPK1 Genes in *T. aestivum*

The TaRPK1 gene expression was determined in drought-tolerant (Pakistan 13 and Galaxy) and drought-susceptible (Shafaq) varieties under normal growth conditions in order to get a baseline expression profile. The expression pattern in all of the three varieties was examined in various developmental stages, including seedling stage, tillering stage, and heading stage and in different tissues such as root, stem, leaf, and grain (Figure 9). The TaRPK1, TaRPK2, and TaRPK3 showed significant expression in the roots at the heading and seedling stages of the Pakistan-13 and Galaxy varieties. The TaRPK13 exhibited higher expression in grain tissues of all varieties compared to other TaRPK1. The TaRPK1 genes displayed higher expression in roots whereas they showed less expression in leaves and stems compared to the grain and root expression in developmental stages. Our results indicated that TaRPK1 genes had similar expression patterns in both Pakistan 13 and Galaxy varieties, unlike the Shafaq variety. The higher expression of TaRPK1 genes was observed in the heading > seedling > tillering stages in Pakistan 13, Galaxy, and Shafaq varieties. Overall, TaRPK1 exhibited significant expression in root tissues compared to leaf, shoot, and grain tissues.

Roots are a good source to study the drought mechanism. To further confirm this, qRT-PCR showed the expression of TaRPK1 members in the leaves and roots of two-week-old seedlings with drought stress through PEG simulation. PEG-6000 treatment induced an upregulated expression in roots and leaf tissues in comparison to the susceptible genotype. Higher expression was observed in root seedlings in comparison to the leaf seedlings, except for TaRPK4 and TaRPK7, where higher expression was detected in the leaf tissues compared to the root tissues under drought stress (Figure 10). The TaRPK1 genes displayed higher expression in Pakistan 13 > Galaxy > Shafaq varieties. Furthermore, we also performed co-expression (Supplementary Figure S3) and interaction network (Supplementary Figure S4 and Supplementary Table S8) analyses and the results revealed that all the RPK1 members showed highly significant associations. These results indicate TaRPK1 gene involvement in drought stress regulation.

**DISCUSSION**

RPK1 is a serine/threonine protein kinase and belongs to the subfamily LRRKs, which is the largest subfamily of RLK. The LRRKs play a crucial role in a large number of biological activities, from development and growth to stress management in plants (Dufayard et al., 2017). RPK genes play significant roles in root...
FIGURE 6 | Cis-Elements and evolutionary conservation. (A) Regulatory Cis-element prediction of 2-Kb sequence upstream of RPK1 genes in T. aestivum. (B) Syntenic relationship between Triticum aestivum (tr, black), Triticum dicoccoides (td, purple), Triticum turgidum (tg, blue), Aegilops tauschii (at, pink), and Triticum speltoides (ts, orange).
FIGURE 7 | 3D structure of TaRPK1 proteins along with Ramachandran plots in T. aestivum. In all 3D protein structures, the spirals are helices, broad strips with arrow heads are beta-pleated sheets, and thin loops are coils. In Ramachandran plots, dark black, gray, and light gray represent highly preferred conformations with Delta ≥ −2. White with a black grid denotes preferred conformations with −2 > Delta ≥ −4. White with gray grid symbolizes questionable conformations with Delta < −4. The green crosses signify highly preferred observations, brown triangles specify preferred observations, and red circles represent unfavorable observations.
system architecture (RSA), plant height, number of tillers, salt tolerance, cotyledon primordial initiation of cotyledons during embryogenesis, ABA-induced senescence, and ROS production (Shi et al., 2014; Zou et al., 2014; Dai et al., 2018). The functional characterization of RPK1 members in wheat has not been reported in previous studies. The reason for it could be a complex allohexaploid (2n = 6x = 42) genome and other factors. Since RPK1 genes are accountable for essential roles in plants, therefore a comprehensive study was performed to determine the chromosomal location, phylogenetic analysis, gene structure and expression of these genes in *T. aestivum*.

The standard process for the sequence identification of a new gene family is by a BLAST search of sequences of known proteins in model plants. A similar BLAST method was used to characterize two RPK genes from *Arabidopsis thaliana*, 16 RPK genes from different species of rice, seven RPK genes from *Triticum dicocoides*, three RPK genes from *Triticum urata*, seven RPK genes from *Triticum turgidum*, four RPK genes from
Aegilops tauschii, 11 RPK genes from Triticum speltoides, four RPK genes from Hordeum vulgare, and 15 TaRPK1 genes in Triticum aestivum. The number of TaRPKs identified in T. aestivum is similar to that of RPKs in Triticum speltoides (11). The identified RPK genes were confirmed for the conserved domains by the SMART database. A higher number of
FIGURE 10 | Expression profiling of TaRPK1 genes under 20% PEG stress in Pakistan 13, Galaxy, and Shafaq varieties. Expressions of TaRPK1 genes were determined in root (S–R) and leave (S–L) at the seedling stage (S). Error bars denote standard errors of three biological replicates. Significance was assessed by using a t-test (*p < 0.05, **p < 0.01, and ns = non-significant).
TaRPK1 genes might be because of the large allohexaploid nature of the bread wheat genome.

The allohexaploid *T. aestivum* genome was originated due to the 3A, B, and D diploid sub genomes hybridization (Marcussen et al., 2014). Three homoeologous genes at a minimum should be for each *T. aestivum* gene, that is, one from each sub genome, also named as homoeologous genes for their homologous chromosomal localization (Sharma et al., 2016). The genome-wide analysis displayed that TaRPK1 genes along with the homoeologous genes were located mainly on chromosomes 1, 2, and 3 on A, B, and D sub-genomes, which showed that there might be no deletion of TaRPK1 genes in the course of the acclimatization and evolution process of *T. aestivum*. The TaRPK1 genes were found to be with maximum number on chromosome 2 and 3 (Figure 1) which was very similar to other studied crops. Crops such as *Triticum dicoccoides*, *Aegilops tauschii*, *Hordeum vulgare*, *Triticum speltoides*, and *Triticum turgidum* also showed the distribution of RPKs on chromosome 2 and 3, in addition to chromosome 5. However, the RPKs were distributed on chromosomes 3, 4, and 7 in rice, and in *Arabidopsis thaliana* they were on chromosomes 1 and 3.

The phylogenetic relationship was studied using complete TaRPK1 protein sequences, as it indicated evolutionary inference. The known homoeologous sequences were clustered closely (Figure 2), which indicated further evolutionary relationships and homology of sequences among them. The putative paralogous sequences were grouped together by those that specified similar origins. Similarity in organization and architecture of domains and motifs in clades designates functional association between these proteins. The gene structure analysis revealed intron numbers in TaRPK1 genes that ranged from 0 to 1 and 16-18 (Figure 3A). The difference in the number of exons in TaRPK1 was analogous to the one observed in other crops. *Triticum dicoccoides*, *Aegilops tauschii*, *Hordeum vulgare*, and *Triticum speltoides* exhibited one to two coding exons, and *Triticum turgidum* exhibited one to three coding exons. *Oryza* species also exhibited one to three exons except for *Oryza rufipogon*; ORUFI04G26970 had 102 exons and Os050486100-01 RPK1 exhibited 18 exons. This points toward evolutionary conservation and hence expression of genes between these species.

Prediction of protein domain configuration revealed the similarity to the previously studied RPK proteins (Cheng et al., 2009), with conserved C-terminal Ser/Thr kinase, a transmembrane domain suggesting membrane-bound features of TaRPK1 proteins and LRR domains. The LRR domains were absent in TaRPK1, TaRPK2, and TaRPK3 proteins. However, all TaRPK1 members showed an additional LRRNT2 (leucine-rich repeat N-terminal) domain in the N-terminal region of the amino acid (Supplemental Figure S1). In addition to sequence alignment, motif analysis also displayed the conservation of the motif at the initial N-terminal region and kinase domain with the motif that remained conserved in all of the 15 TaRPK1 protein sequences (Figure 3B). For the functional analysis of TaRPK1 genes, the gene ontology enrichment analysis was performed (Figure 4). In silico prediction showed that TaRPK1 members were involved in several processes of development through regulation of molecular functions (MFs) and biological processes (BPs), and exhibited response to environmental stresses. Several prior studies also described that through monitoring expression of genes, microRNAs respond to stress stimuli (Yan et al., 2019; Rasool et al., 2021; Rehman et al., 2022). The microRNAs are 21–24 nucleotides long endogenous non-coding RNAs that regulates development, growth, and adaptive response against abiotic stresses via monitoring target genes at posttranscriptional level or translation level of protein synthesis (Bai et al., 2017). In this study, we recognized microRNAs and their target genes in order to explore specific transcripts involved in development and growth processes and in response to different stress environments. We identified that miRNAs are majorly involved in cleavage mechanisms rather than translation inhibition (Figure 5).

The cis-regulatory elements identified in TaRPK1 were mostly related to light responsiveness (Figure 6A). Other distributed cis-regulatory elements were related to stress factors, such as drought, cold stress, anaerobic response, wounding pathogens, and defensive elements. Functional relation of other cis elements was linked to plant hormones comprising auxins, abscisic, gibberellin and salicylic acid. Thus, the occurrence of various groups of cis-regulatory elements functioning in diverse physiological processes is suggestive of the dynamic RPK1 gene regulation in *T. aestivum*. Synteny analysis with other ancestral *Triticum* species revealed that the RPK1 gene family converges to a single ancestor (Figure 6B). This relationship validates that RPKs with analogous evolutionary status might have similar functions in plant growth and development. Homology models for 15 TaRPK1 proteins were made and evaluated with homologous templates. The TaRPK1 proteins exhibited 28%–45% identity to the template, which is a widely accepted threshold for successful modeling. The Ramachandran plots verification and validation displayed that a very high percentage of all 15 TaRPK1 protein regions showed highly favorable regions that denote good quality protein structure prediction (Figure 7). Previous studies have shown similar 3D structure of TATrx proteins in wheat through homology modeling along with Ramachandran plot. The proteins were compared to 2wt1.A, 2vlt1.A, 1fb01.A, 3d221.A, 2vm1.3.A, and 1fafa1.A templates, and the Ramachandran plot showed more than 95% of the thioredoxin amino acids lying in the most favored area (Bhurta et al., 2022). Another study in wheat has shown similar three-dimensional structure prediction of twenty-one TaEIL proteins via SWISS-MODEL along with Ramachandran plot analysis. The prediction model on the basis of templates heuristically enhanced percentage identification, alignment range, and confidence score of test sequences. The Ramachandran plot analysis confirmed 80% of residuals in the allowed area, signifying the quality of the model (Yi-Qin et al., 2020).

The gene expression in a specific tissue can be used as an information source for function identification in that tissue. Studies have revealed that OsRPK1 overexpression altered the total architecture of roots in transgenic seedlings along with height, tillering numbers, and apical meristem of roots (Zou et al., 2014). The larger root system might result in a substantial upsurge in water and nutrient uptake. The relative expression level in different...
CONCLUSION
We completely investigated the properties, developmental, location on chromosomes, cis-components, synteny, and expression profiles of TaRPK1 members. An aggregate of 15 TaRPK1s were distinguished in the T. aestivum genome. This work can fill in as an initial phase in the complete useful portrayal of RPK1 genes by reversible genetic methodologies. This study provides helpful assets to future investigations on the design and function of RPK1 genes and for distinguishing and describing these genes in different species. Consequently, the outcomes might offer important data to examine the role of TaRPK1 genes being developed and stress reactions through present-day practical genomics tools (next-generation sequencing) and genome editing, henceforth clearing the way toward genetic improvement of wheat.

DATA AVAILABILITY STATEMENT
The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS
MK and AR perceived the idea and planned the experiments. AR performed the research and wrote the manuscript. NZ and OR helped in data analysis. NR and MU helped in manuscript revision. MK supervised the research and acquired resources.

FUNDING
This research was conducted with funds from the Agricultural Linkages Program of Pakistan (ALP project number CS-399).

ACKNOWLEDGMENTS
We acknowledge the research facilities provided by the National Institute for Genomics and Advanced Biotechnology (NIGAB), Pakistan. We also thank the Ensembl plants, NCBI, and various other tools for the availability of sequences and data.

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.912251/full#supplementary-material

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