

RESEARCH

Open Access



Genome wide identification, structural characterization and phylogenetic analysis of High-Affinity potassium (*HAK*) ion transporters in common bean (*Phaseolus vulgaris* L.)

Afrasyab Khan¹, Zamarud Shah^{2*}, Sajid Ali², Nisar Ahmad³, Maaz Iqbal⁴, Arif Ullah¹ and Firdous Ayub⁵

Abstract

Background High-Affinity Potassium ions represent one of the most important and large group of potassium transporters. Although *HAK* genes have been studied in a variety of plant species, yet, remain unexplored in common bean.

Results In the current study, 20 *HAK* genes were identified in common bean genome. Super-family “K_trans” domain was found in all *PvHAK* genes. Signals for localization of *PvHAK* proteins were detected in cell membrane. Fifty three *HAKs* genes, across diverse plant species, were divided into 5 groups based on sequential homology. Twelve pairs of orthologs genes were found in various plant species. *PvHAKs* genes were distributed unequally on 7 chromosomes with maximum number (7) mapped on chromosome 2 while only 1 *PvHAK* found on each chromosome 1, 4, and 6. Tandem gene duplication was witnessed in 2 paralog pairs while 1 pair exhibited segmental gene duplication. Five groups were made in *PvHAK* gene family based on Phylogeny. Maximum *PvHAKs* (10) were detected in Group-V while group-II composed of only 1 *PvHAK* gene. Variation was witnessed in number and size of motifs, and structure of *PvHAKs* associated with different groups. Light and hormone responsive elements contributed 57 and 24% share, respectively, to *cis* regulatory elements. qRT-PCR based results revealed significant increase in expression of all 4 *PvHAK* genes under low-potassium stress.

Conclusion The current study provides valuable information for further functional characterization and uncovering the molecular mechanism associated with Potassium transportation in plants.

Keywords Common bean, *HAKs*, Drought stress, Genome wide analysis, Expression analysis, Evolutionary analysis

*Correspondence:

Zamarud Shah

zamarud_gd@yahoo.com; zamarud@awkum.edu.pk

¹Department of Biotechnology, University of Science and Technology, Bannu 28100, Pakistan

²Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan

³Department of Biotechnology and Genetic Engineering, Hazara University, Mansehra 21300, Pakistan

⁴Institute of Biotechnology and Genetic Engineering, University of Agriculture, Peshawar 25130, Pakistan

⁵Department of Computer Science, Women University Swabi, Swabi 23430, Pakistan



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Introduction

Potassium is one of the most important macronutrients which accounts for up to 10% of plants dry weight [1]. Decrease in amount of potassium below 10 g/kg of dry weight is associated with severe defects in plant growth and development [2]. Metabolism of protein, carbohydrate and activities of enzymes are some of the biochemical processes affected by potassium. Similarly physiological processes including regulation of stomata and photosynthetic operation are halted by inadequate supply of potassium [3]. Moreover, potassium acts as main player in enhancing plant resistance to a number of biotic and abiotic stresses [4]. More than 50% decline in soybean yield has been witnessed, due to potassium deficiency, across a wide range of soil type. (**Louisiana State University soil fertility specialist Rasel Parvej**). Stunted growth that eventually leads to reduced productivity is one the major symptom associated under supply of potassium.

A large number of K^+ channels and transporters have been designated to carry out the absorption and translocation of potassium inside the plants [5]. High potassium concentration has been recorded as the driving force for K^+ channels to undertake its transportation with comparatively low efficiency. On the other hand, K^+ transporters are good enough to draw and transport the nutrients at low external concentrations and were truly called as high affinity system [6]. In plants, potassium transporters have been grouped into 4 families: KT (K^+ transporter)/HAK (high-affinity K^+), Trk (Transport of K^+), KEA (K^+ efflux anti-porter), and CHX (cation/hydrogen exchanger) [7]. The size of HAK proteins ranged from 300 to 900 amino acid have pronounced role in K^+ transport. Among the legume crops, common bean (*Phaseolus vulgaris*) is regarded as the most important crop grown for human consumption at global level [8]. Historically a large number of developing countries across Africa, Latin America, and Asia have remained as the main consumer of common bean [9]. Even today, common bean is rated within the top ten most consumed vegetables/pulses worldwide [10]. Common bean is nutritionally rich with high concentration of protein, fiber, iron, magnesium and folate [11].

Potassium (K) is one of the major yield limiting nutrients of common bean in South America [12]. The growth of common bean varieties with efficient K transportation system supplemented with K fertilizer would prove helpful in improving the yield and reduce the production cost. Since, common bean have shown better response to the uptake and transportation of potassium, thus, this crop is assumed to have repository gene for K^+ transportation. Though, some genomic data of common bean is available in literature, yet, little is known about of HAK transporter family in common bean. The objective of current

work was to identify HAK transporter genes across the genome of common bean and characterize for important physiochemical, structural, phylogenetic and expression analysis.

Materials and methods

Detection of HAK gene family in *Phaseolus vulgaris* genome
HAK sequence of *Arabidopsis thaliana* (NP_187864.1) was taken from “National Centre Biotechnology Information”. The presence of HAK domain was found in the sequence of *Arabidopsis thaliana* using Pfam finder (<http://pfam.sanger.ac.uk>) [13]. Domain of *AtHAK* was utilized for obtaining HAK transcripts from genome of common bean using online phytozome v.13 database (<https://phytozome-next.jgi.doe.gov>) [14]. Short and redundant protein sequences were removed. Motif finder (online tool) was used for confirmation of HAK domain in the transcripts.

Physiochemical characterization of *PvHAK* genes

Online database phytozome (<https://phytozome-next.jgi.doe.gov>) [14] was used for finding start-end point, strand nature (forward or reverse), CDS and protein length. Likewise, various other physical characters including molecular weight, PI, GRAVY, Instability index and aliphatic index of protein sequences were uncovered by subjecting the data to expasy protparam (online tool: <https://web.expasy.org/protparam>) [15].

Conserved domains within the protein sequence of common bean (domain architecture)

Rename file and Hitdata files were generated by using TBtool.v1.09854 software [16] and conserved domain database [17] respectively. Domain architecture [16] was generated by subjecting these files to TBtool.v1.09854.

Predicted subcellular localization of *PvHAKs* protein

Protein sequences of all *PvHAKs* were subjected to online tools CELLO Life and Wolf Psort for predicting their subcellular localization. Heatmap was generated using TBtool [16] for visualization of protein in the cell using Wolf Psort method.

Phylogenetic, motif and Gene Structure analysis of *PvHAK* family

Molecular Evolutionary Genetics Analysis (MEGA 7) software [18] was used for multiple alignments of protein sequences. Maximum Likelihood (ML) approach with 1,000 bootstrap replicates was adopted for exploring phylogenetic relationship among members of *PvHAK* gene family. Conserved motifs in protein sequences of *PvHAK* family were visualized by using online tool MEME (<http://memesuite.org>) [19]. Maximum number of 10 motifs per sequence was adjusted by default and

subjected to Pfam database (<http://pfam.sanger.ac.uk>) [13] for annotation analysis. Online database phytozome was used for extracting genomic and CDS sequence of *PvHAK* genes. Structural features of *PvHAK* genes were shown using Gene Structure Display Server 2.0 (GSDS).

Comparison of HAK genes among different species

Comparative analysis of *HAK* genes across diverse plant species including *P. vulgaris*, *A. thaliana*, *Z. Mays*, *O. sativa*, *H. vulgare*, *S. lycopersicum*, *C. annuum*, *P. australis*, *P. patens*, *C. nodosa* and *T. halophila*, was carried out using MEGA7.0.26 software [18]. Two genes from the same species placed in the same clade were considered as paralog while those belong to different plant species were designated as orthologs. Both paralog and ortholog gene pairs have high level of homology.

Chromosome mapping, duplication, Ka/Ks ratio and selection analysis of *PvHAKs*

The information necessary for chromosome mapping such as chromosome number, position and length of *PvHAK* genes on chromosome was obtained from phytozome [14] (online database) The data was incorporated in PhenoGram Plot (<http://visualization.ritchielab.psu.edu/phenograms/plot>) for mapping genes on the respective chromosomes [20]. Online tool SIAS (<http://imed.med.ucm.es> > Tools > sias) was used to determine the level of identity (homology) between 2 genes. The coding genes with $\geq 50\%$ identity and covering $\geq 90\%$ protein length were regarded as duplicated genes [21]. Further analysis of genes with same species and clade of phylogenetic tree was carried out for determining the pattern of duplication (tandem or segmental). All the homologous genes with physical distance of < 50 Kilobase (kb) were denoted as tandemly duplicated genes while 2 genes separated by more than 50 Kilobase (kb) were considered as segmentally duplicated genes [22]. The values of non-synonymous (Ka) and synonymous (Ks) substitutions, of all segmentally duplicated gene pairs, were obtained from online available Plant genome duplication database [23]. The Ka/Ks ratio was analyzed for determining selection pressure on duplicated genes. Less than 1 Ka/Ks ratio was regarded as less negative selection while greater than 1 value were considered as positive selection. The values equal to 1 added to neutral evolution. TBTool [16] was used for getting Ka and Ks values of the *PvHAKs* paralogous pairs. Selecton version 2.2 was utilized to find amino acids within the *PvHAKs* proteins under positive and purifying selection pressure [24].

Promoter region analysis

The 1500 bp upstream sequence from the translation initiation site (ATG) of each *PvHAK* gene was obtained from Phytozome and analyzed for *cis*-regulatory

elements using online tool Plant Cis-Acting Regulatory Element (PlantCARE); [25].

Growing plant materials, low-potassium stress treatments and qRT-PCR

Transcriptomic/expression data of common bean was extracted from Gregorio Jorge et al., 2020, GSE123381 [26] using NCBI GEO database. The data was filtered for transcripts belong to *HAK* family using TBtool software. Common bean (cv: Gorilla) seeds were sterilized with sodium hypochlorite (10%) for 15 min and rinsed with tap water for 30 min [27]. The seeds were germinated on basic salt medium (BSM, 0.5mM KCl+0.1 mM CaCl₂) for 2 d, and then BSM was changed to Hoagland solution (20%) for another 8 d in growth room with a photoperiod of 14/10 h, light intensity of $200 \pm 25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature of 23/18°C (day/night) and relative humidity of 60%. Ten days old seedlings were subjected potassium stress (0.01 mM K⁺) [28] in background of Hoagland solution (20%) while seedlings growing in Hoagland solution (20%) were marked as control. The solutions were renewed every 2 days. After treatments for 1 h, 3 h, 6 and 9 h samples from both treated and control plants were collected for RNA extraction and qRT-PCR. All samples were taken in three replicates.

Total RNA was extracted from the samples using Mini-BEST Plant RNA Extraction Kit (9769, TaKaRa, Japan) following the manufacturer's instructions. The cDNA was synthesized from total RNA (1 μg) using PrimeScript RT Master Mix (RR036A, TaKaRa, Japan) and was used as templates for qRT-PCR amplification. qRT-PCR amplification was performed with LightCycler 480 II (Roche, Basel, Switzerland) using iTaq Universal SYBR Green Supermix (1,725,124, Bio-Rad, USA). The relative gene expression was calculated based on the $2^{-\Delta\Delta\text{CT}}$ method using actin as the internal standard [29]. Primers used for qRT-PCR are listed in Supplementary Table S7.)

Statistical analysis

The experiments were performed in triplicate and the data was subjected to student's t-test for analysis. The data with the error bars represent 95% confidence interval.

Results

Genome wide identification of *HAK* gene family members in common bean and their physico-chemical characterization

A total of 20 *HAK* unique full length genes were identified in common bean genome and were designated as *PvHAK-1* to *PvHAK-20* on the basis of location on chromosome in ascending order (Table S1). The CDS sequence, protein length and molecular weight of *PvHAK* genes ranged from 1047 to 2541 (bp), 348–846 (aa)

and 39.6–94.1 (Kda), respectively (Table S1). Start-end point and strand (forward or reverse) of each transcript have been presented in the Table S1. Maximum isoelectric point (PI, 9.44) and GRAVY (0.44) were witnessed in *PvHAK-6* and *PvHAK-14*, respectively. Similarly, 6 *PvHAK* proteins exhibited higher than 40 instability index while *PvHAK-12* was recorded to have the highest aliphatic index (111.32). For subcellular localization, heat map generated through wolf psort (online tool) revealed a wide range (7–13) of *PvHAK* proteins in the plasma membrane. Maximum signals (13) were detected for *PvHAK-18* while *PvHAK-3* emitted minimum signals (7) in plasma membrane (Table S2, Fig. 1). Cello life (online tool) based analysis predicted the localization of all *PvHAK* proteins in the plasma membrane (Table S1). Trans-membrane (TM) helices in each *PvHAK* protein were found out. Maximum TMs (14) were found in 2 genes (*PvHAK-4* and *PvHAK-19*) while only 3 TMs were detected in *PvHAK-16*. The number of TM helices in other *PvHAK* genes fall in the range of 6–13 (Table S1).

Phylogenetic analysis of HAK genes across the genomes of different plant species

Phylogenetic analysis of HAK gene family was carried out across 11 plant species including *P. vulgaris*, *A. thaliana*, *Z. Mays*, *O. sativa*, *H. vulgare*, *S. lycopersicum*, *C. annum*, *P. australis*, *P. patens*, *C. nodosa*, and *T. halophila*. A total of 53 HAKs were shown in phylogenetic tree with 5 subgroups (I, II, III, IV and V). Subgroup-V was found to be largest one with 16 genes, followed by subgroup-I and subgroup-IV with 13 and 10 genes respectively. Each of subgroup-II and subgroup-III contains the least number of 7 genes. Three paralog gene pairs (*PvHAK-6–PvHAK-7*, *PvHAK-16–PvHAK-18*, and *PvHAK-5–PvHAK-12*) were witnessed in *PvHAK* family. Similarly, 12 ortholog gene pairs were identified in different plant species. Maximum numbers of 7 orthologs pairs were found in *P. vulgaris* and *A. thaliana*. One pair each was found in *C. nodosa–O. sativa*, *P. australis–Z. mays*, *S. lycopersicum–C. annum*, *O. sativa–H. vulgare* and *A. thaliana–T. halophila*.

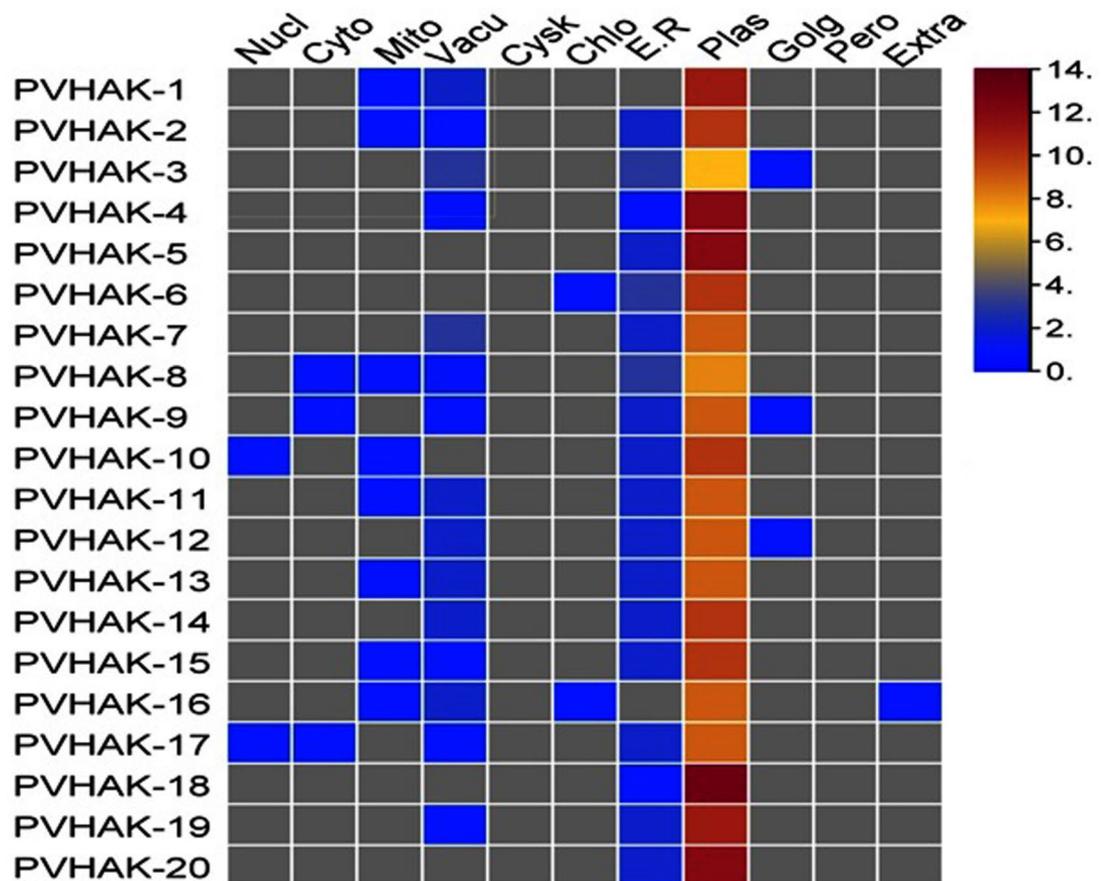


Fig. 1 Wolf PSORT based sub-cellular localization of *PvHAK* proteins. Quantitative heat map of *PvHAK* gene represents sub-cellular localization. Red color bar represents high number of proteins, blue represents low numbers and yellow shows intermediate number of proteins

Z. mays, *O. sativa*, *H. vulgare*, *S. lycopersicum*, *C. annuum*, *P. australis*, *P. patens*, *C. nodosa* and *T. halophila* were recorded to have no common ancestral gene (ortholog pair) with host plant (*P. vulgaris*) and thus were regarded as out groups in the present study (Fig. 2).

Gene location on chromosome, duplication analysis, Ka/Ks ratio and positive selection analysis

Twenty genes identified across the common bean genome were mapped unequally on 7 chromosomes. Maximum number of genes (7) were found on chromosome 2 while chromosomes 1, 4, and 6 revealed minimum number of genes. Similarly, results showed the presence of 6 genes on chromosome 9 and 2 genes on each of the chromosomes 3 and 8 (Fig. 3). Two paralog pairs (*PvHAK-6–PvHAK-7* and *PvHAK-16–PvHAK-18*) exhibited a physical distance of 30.459 and 13.974 Kb, respectively, and, hence were marked as tandemly duplicated genes.

On the other hand, one paralog gene pair (*PvHAK-5–PvHAK-12*) revealed a physical distance of 7403.474 Kb and was counted as segmentally duplicated gene pair (Fig. 3). The Ka/Ks ratio of duplicated genes ranged from 0.10 to 0.30 (less than 1 (Table S3) suggesting the passage of paralogous *PvHAK* pairs under purifying process. Consistently evolving location of amino acid is very important for conservation of protein structure and function. In the current study, 471 out of 785 amino acids of *HAK* protein were under purifying selection while the rest (314) were influenced with neutral selection (Fig. 4).

Phylogenetic analysis, Exon/intron, motifs and domain structure

Maximum likelihood based Phylogenetic tree of 20 *PvHAK* genes was constructed for showing evolutionary divergence. *PvHAK* gene family was grouped into 5 clusters using phylogenetic tree. Subgroup-V was found

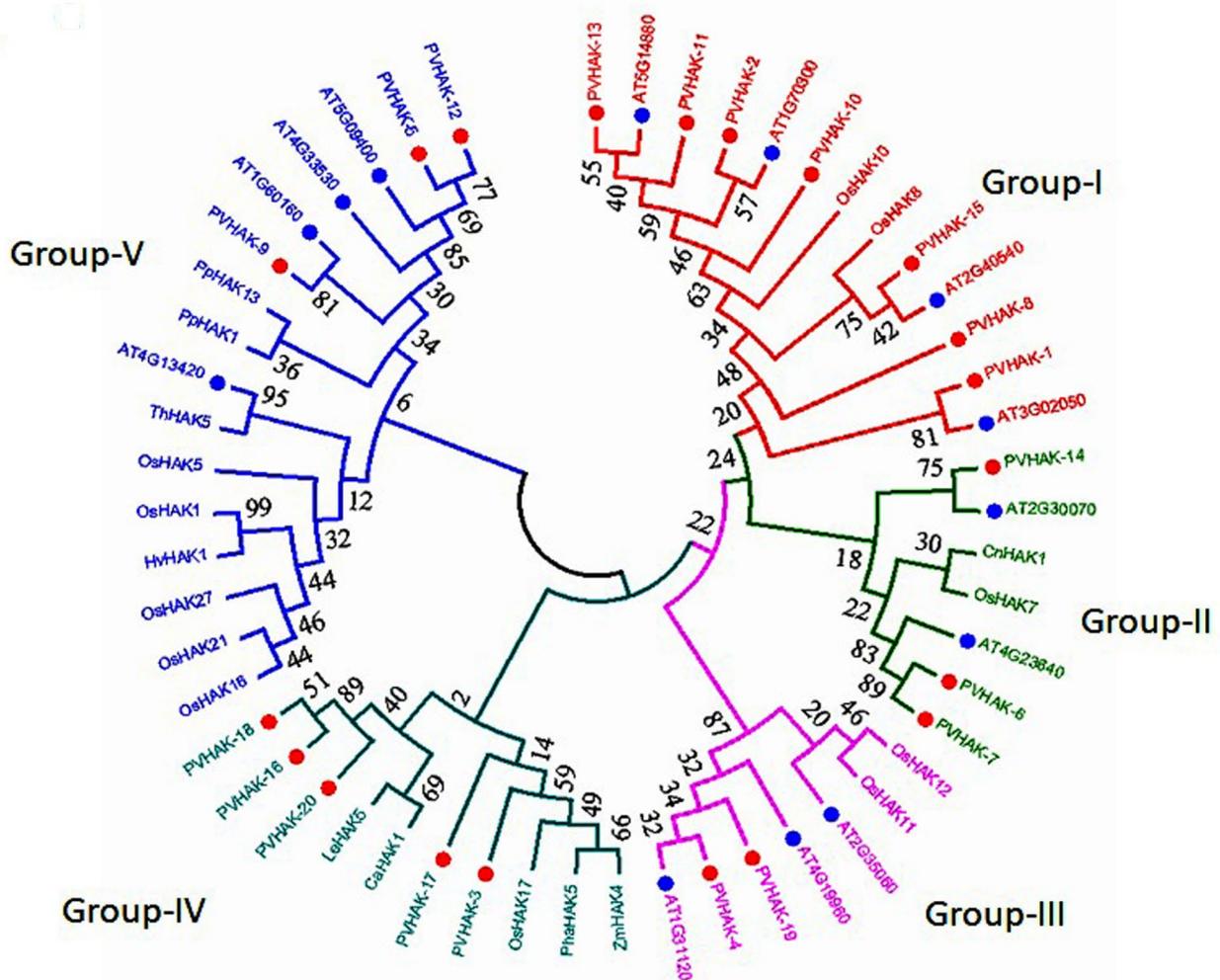


Fig. 2 Comparative phylogenetic analysis of *HAK* proteins across different plant species. Bootstrap values in percentage are indicated on the nodes. Different groups are highlighted with different colors

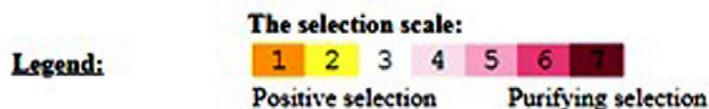


Fig. 4 Analysis of evolutionary selection pressure on amino acids of *PvHAK* protein. Yellow and brown highlight positive selection, gray and white represent neutral selection while purple indicates purifying or negative selection pressure

regulatory elements, respectively. Environmental stress, development and site-binding related elements made 10% (40), 4% (17) and 3% (12), respectively, of cis elements in upstream region of *PvHAK* (Table S5, Fig. 7A).

Among environmental stress related elements, 63% were composed of anaerobic induction regulatory elements (AIRE) followed by drought related (MYB) with 17% and defense-stress related element with 12% elements. Low

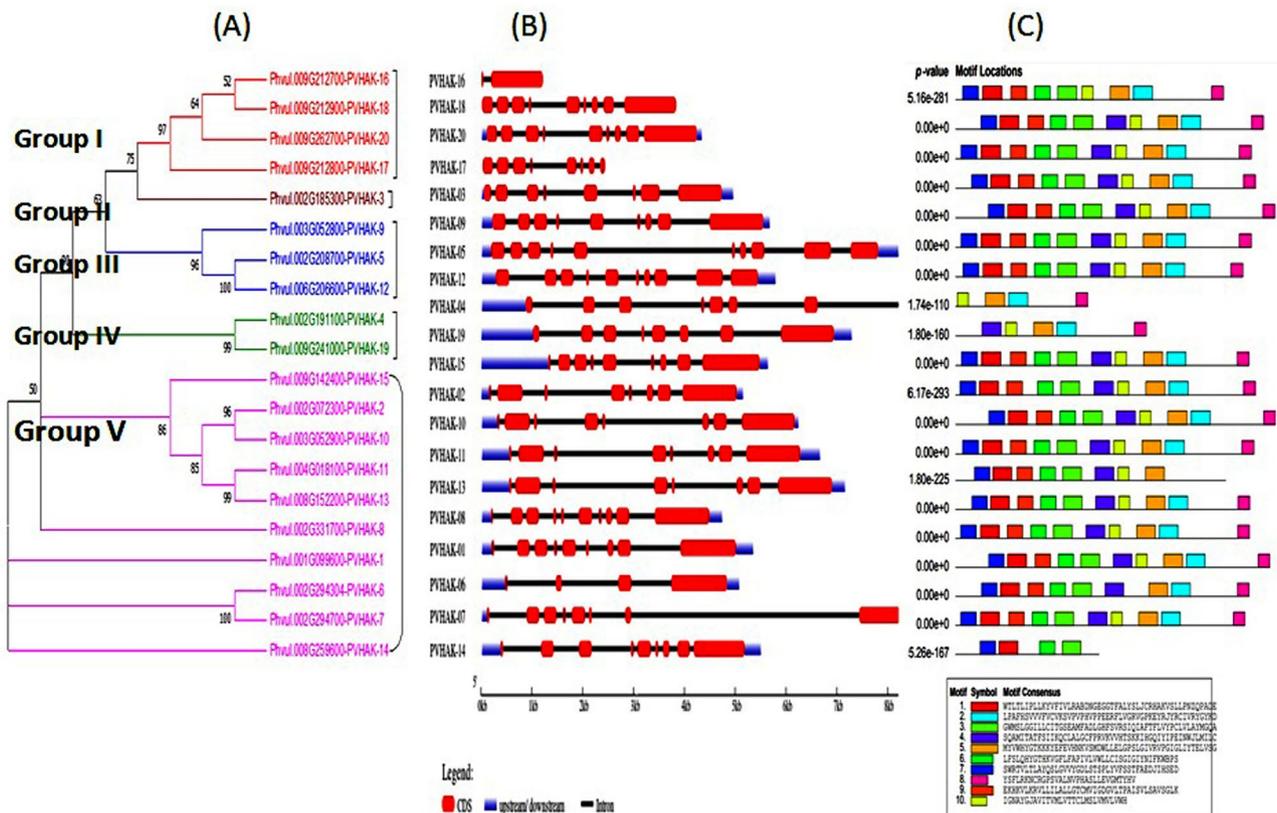


Fig. 5 Phylogenetic, structural and conserved motif analysis of *PvHAK* genes **A**) Group of Phylogenetic tree highlighted with different colors (5 groups) **B**) Red boxes represent exons/CDS (coding DNA sequence), black lines show introns while un-translated region (UTR) highlighted with blue boxes **C**) Conserved motifs of *PvHAK* proteins are shown as colored boxes. The scale used is given at the bottom [kilobase (kb)]

temperature and wound related elements constituted only 5 and 3% elements, respectively (Fig. 7B). Among development related cis acting element, highest percentage of endosperm related element (23%) was detected followed by meristem related (18%) and zein metabolism related elements (17%). Each of the 3 kind of elements i.e. MYBHv1 binding site, palisade mesophyll cells differentiation and circadian control, were found to constitute 12% elements. Only 6% elements involved in cell cycle regulation were detected (Fig. 7C). Among the hormone related elements, 43% were detected as MeJA-RE, followed by ABRE with 25% and salicylic related elements with 13% elements. Axuin and gibberelins-RE were detected to constitute 13 and 11% elements, respectively (Fig. 7D).

Expression analysis of *PvHAK* genes under low-potassium stress

According to transcriptomic data of common bean extracted from Gregorio Jorge et al. [26], *PvHAK*-15 showed maximum expression in 5 major tissues including flower buds, leaves, stem, young pods and young trifoliolate. Similarly, an elevated level *PvHAK*-11 expression was recorded in young trifoliolate, young pods, leaves and flowers buds with maximum expression detected

in flowers. Likewise, *PvHAK*-19 and *PvHAK*-1 exhibited higher expression in all the tissues investigated under low-potassium stress. Some other genes including *PvHAK*-9, *PvHAK*-12, *PvHAK*-13 and *PvHAK*-14 presented high expression upon exposure to low potassium stress (Table S6). The expression profile of 4 genes including *PvHAK*-1, *PvHAK*-11, *PvHAK*-15, *PvHAK*-19 under potassium stress was investigated to validate the results experimentally. Significant elevation in expression of *PvHAK*-15 and *PvHAK*-11 after 1, 3 and 6 h K treatment, compared to control, was witnessed. However, no such increase was observed after 9 h K treatment (Fig. 8A, C). Similarly, relative expression of *PvHAK*-1 and *PvHAK*-19 was significantly enhanced, compared to control, after 3 and 6 h treatment however, non significant increase in their expression was recorded after 1 and 9 h treatment (Fig. 8B, D).

Discussion

Potassium is one of the most indispensable micro-nutrients required for plant growth and development. An efficient transportation system within the plant is as important as availability of potassium in rhizosphere.

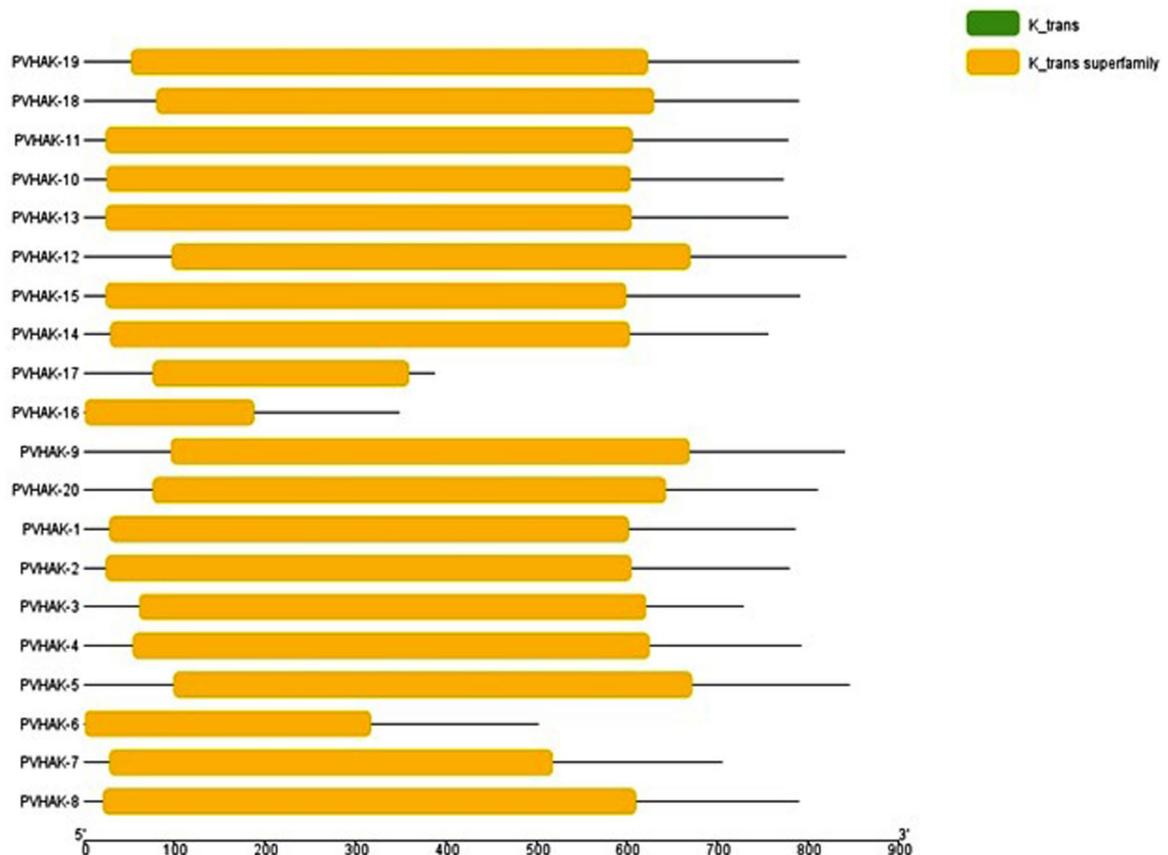


Fig. 6 Domain analysis of *PvHAK* proteins. The rectangular yellow boxes represent *K_trans* superfamily domains

HAK has been reported as the largest gene family responsible for K⁺ transportation [30].

The *HAK* family was first detected in bacteria [31] followed by kingdom Plantae and Fungi [32] but were not reported in Protista and Animalia [33]. In plantae, both monocots and dicots were found to have homologues of *HAK* in the form of multigene families [34]. In the current study, a comprehensive set of 20 unique full length *HAK* genes was identified in common bean's genome which is more than those reported in barley (5) and Arabidopsis (13). The strength of *HAK* genes family in common/french bean was found less than poplar (21) [35], rice (27) [9] and maize (27) [36]. The uneven mapping of 20 *PvHAK* genes on 7 out of chromosomes indicate that gene duplication has occurred in common bean genome. These results are in agreement with [37]. Gene duplication as evident in the present study has been reported to be the main cause for expansion gene families of plants [38]. The presence of “*K_trans*” super-family domain in all *PvHAKs* further strengthens the role of this family in K⁺ transportation. The localization of *PvHAK*

proteins in the plasma membrane, as found in the current study, provides strong channels for passage of these K ions across the membrane.

Twenty members of *PvHAK* gene family were clustered into 7 groups based phylogenetic analysis. A narrow range of exon/introns was recorded in *HAK* gene family. The number of exons in common bean *PvHAK* genes ranged from 2 to 10 which is very similar to that of rice (2–10) [9] and close to maize (3–10) [36]. The *PvHAK* genes on account of its length were detected to decode proteins with 348 to 846 amino acids. Of these, 569 amino acids constitute the characteristic “*K_trans*” domain (PF02705, in Arabidopsis T). Varied number and size of motifs in *PvHAKs* were detected with 4–10 and 29–50 amino acids, respectively. Nine out of 10 motifs were detected to have role in potassium transportation except motif 8 with unknown function. The motifs with different features may contribute to divergence of *PvHAKs*. Results revealed that members belong to the same groups shares the same gene features and motif configuration. Contrary to this, variation was observed

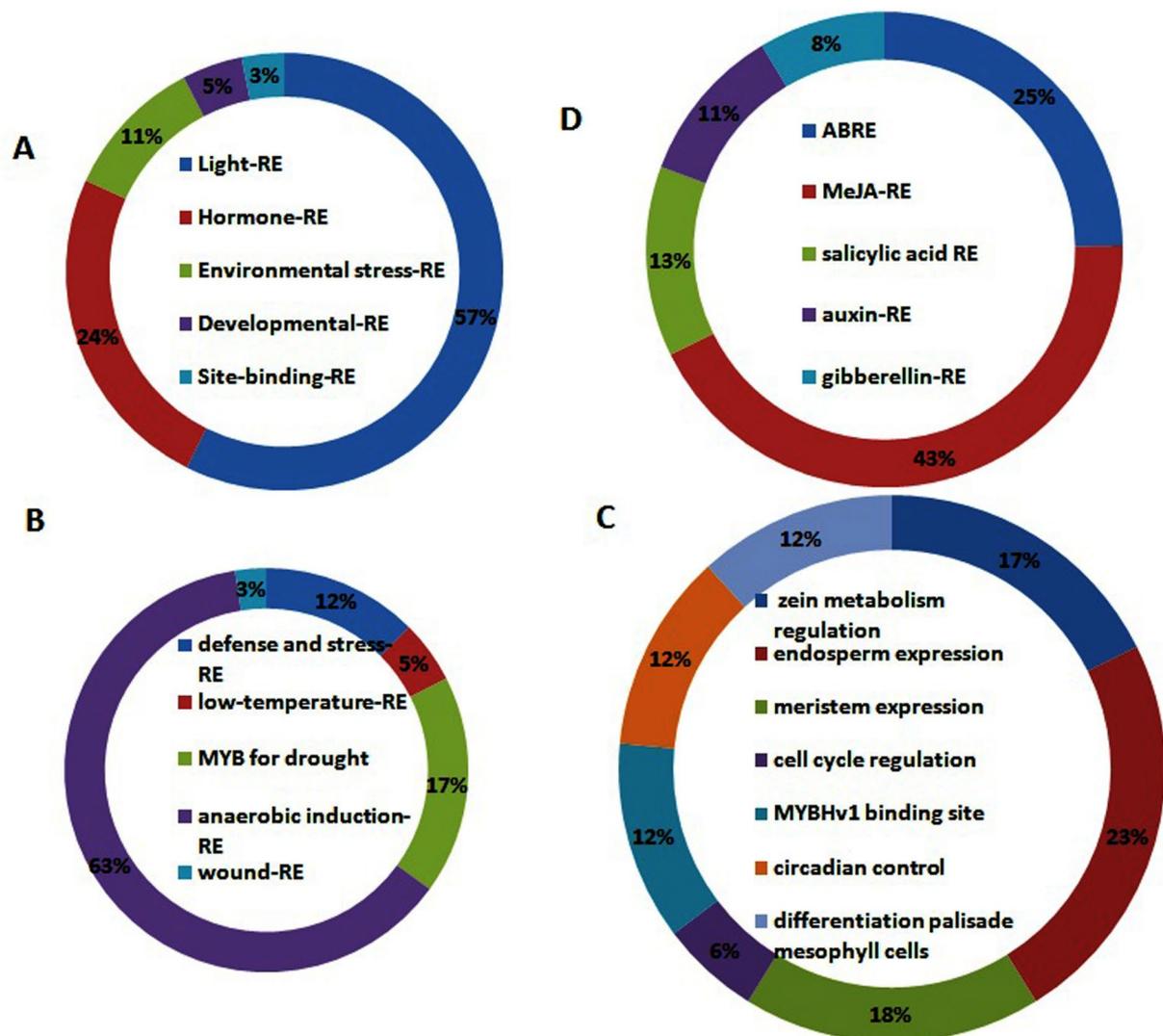


Fig. 7 Promoter region (1500 bp upstream) analysis of *PvHAK* genes. **(A)** Percentage of different cis-elements **(B)** Percentage of different stress responsive cis-element **(C)** Percentage of different hormone responsive cis-element **(D)** Percentage of different development related cis-element

in number of coding and non coding regions of *PvHAK* genes associated with different groups. This difference in expression pattern of *PvHAK* genes strengthened the view that gain or loss of introns during evolution might be the causal agent of such variability.

During comparative analysis in diverse plant species, 53 *HAKs* were divided into 5 groups based on maximum likelihood. Twelve pairs of orthologous genes detected in different plants exhibited that these genes have been derived from a common ancestral gene. *Arabidopsis thaliana* was found as the closest relative to *P. vulgare* on account of sharing maximum numbers of orthologous pairs (7). The other crops (*Z. Mays*, *O. sativa*, *H. vulgare*, *S. lycopersicum*, *C. annuum*, *P. australis*, *P. patens*, *C. nodosa* and *T. halophila*) exhibited no ortholog pair with the host plant and thus were regarded out groups. The presence of one pair of ortholog as witnessed

in each of the 5 combinations (*C. nodosa* and *O. sativa*, *P. australis* and *Z. mays*, *S. lycopersicum* and *Capsicum annuum*, *Oryza sativa* and *Hordeum vulgare*, and, *Arabidopsis thaliana* and *Thellungiella halophila*) suggest some degree of resemblance. Similarly, the detection of 3 paralogous exhibited gene duplication within *PvHAKs* gene family.

In the current study, 380 cis regulatory elements in upstream regions of 20 *PvHAK* genes were detected. Light and hormone responsive elements were found as the major contributor to cis regulatory elements with 57% (218 elements) and 24% (93 elements) share, respectively. Ten (40), 4% (17) and 3% (12) of cis regulatory elements occupied by environmental stress related, development related and Site-binding related elements, respectively. These results are in line with various stress and hormones responsive elements reported in promoter region of

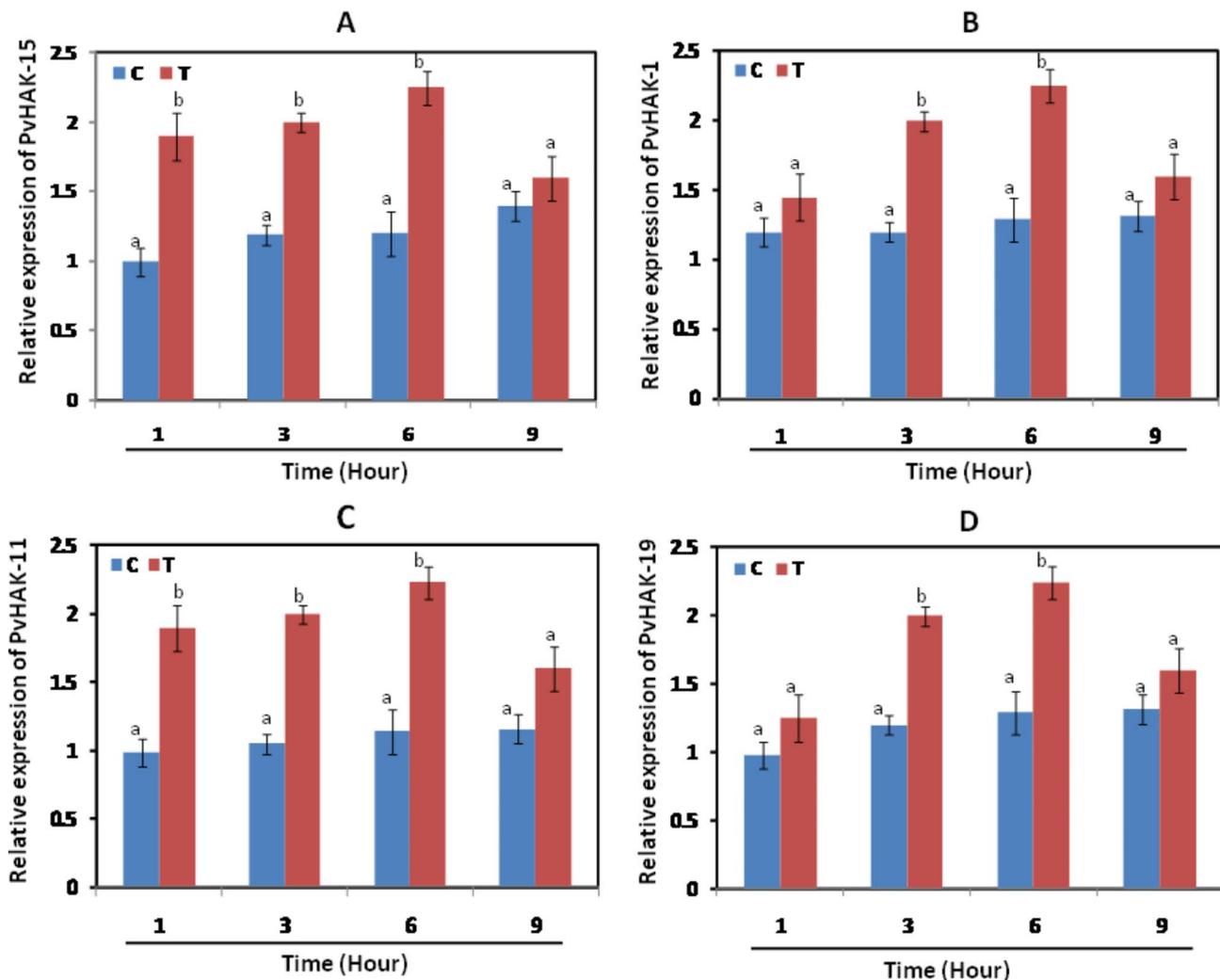


Fig. 8 Expression profiling of four PvHAK genes in response to potassium deficiency (0.01mM K^+). Lowercase letters indicated the significant difference at $p < 0.05$

many HAKs [39]. A huge number of promoter related cis regulatory elements (1410) were found in the promoter region of *PvHAKs*. Among the cis acting light responsive elements, conserved DNA module (G-box), cis-acting regulatory elements (ACE), light responsive element (GT1-motif) and part of a light responsive element shared 97, 28, 22 and 58 elements, respectively. Abscisic acid responsive elements (ABRE), methyl jasmonate-responsive element (CGTCA motif & TGACG motif) and salicylic responsive elements (TCA-element) were found in 40, 23, and 12 number, respectively. Very few auxin, gibberline responsive elements and no ethylene responsive elements (ERE) were detected in the promoter region of *PvHAK* genes. Maximum number of hormone responsive elements was found in promoter region of *PvHAK-15*. Anaerobic inducing responsive element (ARE) is the major stress responsive cis acting elements detected in the current study. Time-dependent increase in expression of all 4 genes (*PvHAK-1*, *PvHAK-11*,

PvHAK-15, *PvHAK-19*) after low-potassium treatment for 1, 3 and 6 h treatment was found in line with earlier reports in rice [40, 41]. These results strengthened earlier findings of Gregorio Jorge et al. (2020) [26]. A common pattern of decline in expression of all 4 genes after exposure to potassium stress for 9 h could possibly be attributed to negative feedback mechanism and cross-talk with other pathways. These results were found in agreement with previous report in barley [37]. Up-regulation of HAK genes, under potassium stress, enhances the uptake of potassium by the roots and translocation to other parts of the plants [42, 43]. The absorption of more potassium in response to elevated expression of HAK genes not only assist optimum plant growth but also help plants to encounter the detrimental effect of different stresses by invoking the ROS scavenging system. These results strengthened earlier reports in two different crops, tea [44] and willow [45].

Conclusion

Twenty *HAK* genes in the common bean genome were mapped unequally on 7 chromosomes. Fifty three *HAK* detected across diverse plant species were divided into 5 groups. Twelve pair of orthologs and 3 pair of paralog were detected during comparative analysis. Tandem duplication was witnessed in 2 paralog pair while 1 paralog pair was segmentally duplicated. Five groups of *PvHAK* gene family were made based on phylogenetic analysis. Variation was observed in number and size of motifs and structure of *PvHAKs* associated with different groups. Light and hormone responsive elements were found as the major contributor to *cis* regulatory elements. Expression profile of *PvHAK* genes under low-potassium stresses revealed that many genes of this family are involved in uptake and translocation of potassium across the plant body.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12863-023-01163-0>.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7

Acknowledgements

The authors are thankful to Afrasyab Khan and Dr. Zamarud Shah for assistance in experiments and data analyses.

Author contributions

Zamarud Shah designed the research. Afrasyab Khan extracted the data under the supervision of Zamarud Shah. Nisar Ahmad and Maaz Iqbal helped in expression analysis during final revision. Arif Ullah contributed in data analysis while Sajid Ali and Firdous Ayub reviewed and approved the manuscript.

Funding

The authors declare that no funds, grants or other support were received during the preparation of this manuscript.

Data Availability

The data is available in the respective web links and accession numbers. *HAK* sequence of *Arabidopsis thaliana* (Accession No: NP_187864.1). National Centre Biotechnology Information (<https://www.ncbi.nlm.nih.gov>). Pfam finder (<http://pfam.sanger.ac.uk>). Phytozome v.13 database (<https://phytozome-next.jgi.doe.gov>). ExPasy protparam (online tool: <https://web.expasy.org/protparam>). (TBtools.v1.09854 software) <https://github.com/CJ-Chen/TBtools/releases>. MEME online tool (<http://memesuite.org>). Pfam database (<http://pfam.sanger.ac.uk>). PhenoGram Plot (<http://visualization.ritchielab.psu.edu/phenograms/plot>). Online tool SIAS (<http://imed.med.ucm.es> > Tools > sias).

Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

All authors did not have any conflict of interest.

Ethical approval and consent to participate

Not applicable.

Received: 3 March 2023 / Accepted: 5 October 2023

Published online: 14 November 2023

References

1. Ragel P, Raddatz N, Leidi EO, Quintero FJ, Pardo JM. Regulation of K + Nutrition in plants. *Front. Plant Sci.* 2019. <https://doi.org/10.3389/fpls.2019.00281>.
2. Xu X, Du X, Wang F, Sha J, Chen Q, Tian G, Zhu Z, Ge S, Jiang Y. Effects of Potassium levels on Plant Growth, Accumulation and distribution of Carbon, and Nitrate metabolism in Apple dwarf rootstock seedlings. *Front Plant Sci.* 2020. <https://doi.org/10.3389/fpls.2020.00904>.
3. Houmani H, Debez A, Freitas-Silva Ld, Abdely C, Palma JM, Corpas FJ. Potassium (K+) Starvation- Induced oxidative stress triggers a General Boost of antioxidant and NADPH-Generating Systems in the Halophyte *Cakile maritima*. *Antioxidants.* 2022;11(2):401. <https://doi.org/10.3390/antiox11020401>.
4. Hasanuzzaman M, Bhuyan MHMB, Nahar K, Hossain MS, Mahmud JA, Hossen MS, Masud AAC, Mountima, Fujita M. Potassium: a vital Regulator of plant responses and tolerance to Abiotic stresses. *Agronomy.* 2018;8(3):31. <https://doi.org/10.3390/agronomy8030031>.
5. Han M, Wu W, Wu WH, Wang Y. Potassium transporter KUP7 is involved in K+ Acquisition and Translocation in Arabidopsis Root under K+-Limited conditions. *Mol Plant.* 2016;9(3):437–46. <https://doi.org/10.1016/j.molp.2016.01.012>.
6. Rodríguez-Navarro A, Rubio F. High-affinity potassium and sodium transport systems in plants. *J Exp Bot.* 2006;57(5):1149–60. <https://doi.org/10.1093/jxb/erj068>.
7. Cheng X, Liu X, Mao W, Zhang X, Chen S, Zhan K, Bi H, Xu H. Genome-wide identification and analysis of HAK/KUP/KT Potassium Transporters Gene Family in Wheat (*Triticum aestivum* L). *Int J Mol Sci.* 2018;19(12):3969. <https://doi.org/10.3390/ijms19123969>.
8. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (*Phaseolus* spp.)—model food legumes. *Plant Soil.* 2003;252(1):55–128.
9. Gupta M, Qiu X, Wang L, Xie W, Zhang C, Xiong L, Lian X, Zhang Q. KT/HAK/KUP potassium transporters gene family and their whole-life cycle expression profile in rice (*Oryza sativa*). *Mol Genet Genomics.* 2008;280(5):437–52.
10. Drewnowski A, Rehm CD. Vegetable cost metrics show that potatoes and beans provide most nutrients per penny. *Plos One*15. 2013;8(5):e63277. <https://doi.org/10.1371/journal.pone.0063277>.
11. Winham D, Webb D, Barr A. Beans and Good Health. *Nutr Today.* 2008;43:201–9. <https://doi.org/10.1097/01.NT.0000303354.21347.45>.
12. Fageria N, Filho B, M., Da Costa JJ. J. o. P. N. (2001). Potassium-use efficiency in common bean genotypes. *J Plant Nutr* 24(12): 1937–45.
13. Punta M, Coggill PC, Eberhardt RY, Mistry J, Tate J, Boursnell C, et al. The pfam protein families database. *Nucleic Acids re.* 2012;40(Database issue):D290–301. <https://doi.org/10.1093/nar/gkr1065>.
14. Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, et al. Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Res.* 2012;40(Database issue):D1178–1186. <https://doi.org/10.1093/nar/gkr944>.
15. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* 2003;31(13):3784–8. <https://doi.org/10.1093/nar/gkg563>.
16. Chen C, Chen H, He Y, Xia R. (2018) TBtools, a toolkit for biologists integrating various biological data handling tools with a user-friendly interface. *BioRxiv*, 289660.
17. Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, et al. CDD: NCBI's conserved domain database. *Nucleic Acids Res.* 2015;43(Database issue):D222–6. <https://doi.org/10.1093/nar/gku1221>.

18. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol Biol Evol.* 2016;33(7):1870–4. <https://doi.org/10.1093/molbev/msw054>.
19. Bailey TL, Williams N, Misleh C, Li WL. MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res.* 2006;34(1):1–21. <https://doi.org/10.1093/nar/gkl198>. 34(Web Server issue).
20. Wolfe D, Dudek S, Ritchie MD, Pendergrass SAJB. Visualizing genomic information across chromosomes with PhenoGram. *BioData Min.* 2013;6(1):18. <https://doi.org/10.1186/1756-0381-6-18>. <https://biodatamining.biomedcentral.com/articles/>.
21. Wang Y, Wu WH. (2017) Regulation of potassium transport and signaling in plants. 39: 123–8.
22. Cannon SB, Mitra A, Baumgarten A, Young ND, May G. The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol.* 2004;4(1):1–21.
23. Lee TH, Kim J, Robertson JS, Paterson AH. Plant Genome Duplication Database. *Methods Mol Biol.* 2017;1533:267–77. https://doi.org/10.1007/978-1-4939-6658-5_16.
24. Yang Y. An evaluation of statistical approaches to text categorization. *Inf Retr.* 1999;1(1):69–90.
25. Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Peer YV, Rouze P, Rombauts S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 2002;30(1):325–7. <https://doi.org/10.1093/nar/30.1.325>.
26. Gregorio Jorge J, Villalobos-López MA, Chavarria-Alvarado KL, Ríos-Meléndez S et al. (2020). Genome-wide transcriptional changes triggered by water deficit on a drought-tolerant common bean cultivar. *BMC Plant Biol.* 20(1), 525. PMID: 33203368.
27. Cai K, Gao H, Wu X, Zhang S, Han Z, Chen X, Zhang G, Zeng F. The ability to regulate transmembrane Potassium Transport in Root is critical for Drought Tolerance in Barley. *Int J Mol Sci.* 2019;20(17):4111. <https://doi.org/10.3390/ijms20174111>. PMID: 31443572; PMCID: PMC6747136.
28. Ye Z, Zeng J, Li X, Zeng F, Zhang G. Physiological characterizations of three barley genotypes in response to low potassium stress. *Acta Physiol Plant.* 2017;39(1):1–10. <https://doi.org/10.1007/s11738-016-2300-x>.
29. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods.* 2001;25(4):402–8. <https://doi.org/10.1006/meth.2001.1262>.
30. Li W, Xu G, Alli A, Yu L. Plant HAK/KUP/KT K⁺ transporters: function and regulation. *Semin Cell Dev Biol.* 2018;74:133–41. <https://doi.org/10.1016/j.semcdb.2017.07.009>.
31. Epstein W, Kim BS. Potassium transport loci in *Escherichia coli* K-12. *J Bacteriol.* 1971;108:639–44.
32. Anshütz U, Becker D, Shabala S. Going beyond nutrition: regulation of potassium homeostasis as a common denominator of plant adaptive responses to environment. *J Plant Physiol.* 2014;171(9):670–87.
33. Nieves-Cordones M, Martínez V, Benito B, Rubio F. Comparison between *Arabidopsis* and rice for main pathways of K⁺ and Na⁺ uptake by roots. *Front Plant Sci.* 2016. <https://doi.org/10.3389/fpls.2016.00992>.
34. Yang T, Lu X, Wang Y, Xie Y, Ma J, Cheng X, Xia E, Wan X, Zhang Z. HAK/KUP/KT family potassium transporter genes are involved in potassium deficiency and stress responses in tea plants (*Camellia sinensis* L.). *Expression and Functional Analysis.* 2020;21(1):1–18.
35. He C, Cui K, Duan A, Zeng Y, Zhang JJE. & evolution. (2012). Genome-wide and molecular evolution analysis of the P oplar KT/HAK/KUP potassium transporter gene family. *Ecol Evol* 2(8): 1996–2004.
36. Zhang Z, Zhang J, Chen Y, Li R, Wang H, Wei J. Genome-wide analysis and identification of HAK potassium transporter gene family in maize (*Zea mays* L.). *Mol Biol Rep.* 2012;39(8):8465–73.
37. Cai K, Zeng F, Wang J, Zhang G. Identification and characterization of HAK/KUP/KT potassium transporter gene family in barley and their expression under abiotic stress. *BMC Genomics.* 2021;22(1):1–14.
38. Yuan G, Vasavda C, Peng YJ, Makarenko VV, Raghuraman G, Nanduri J, et al. Protein kinase G-regulated production of H₂S governs oxygen sensing. *Sci Signal.* 2015;8(373):ra37–7.
39. Zhao Y, Wang L, Zhao P, Liu Z, Guo S, Li Y, Liu H. Genome-wide identification, characterization and expression analysis of HAK genes and decoding their role in responding to potassium deficiency and abiotic stress in *Medicago truncatula*. *PeerJ.* 2022;10:e14034. <https://doi.org/10.7717/peerj.14034>.
40. Okada T, Nakayama H, Shinmyo A, Yoshida K. Expression of OsHAK genes encoding potassium ion transporters in rice. *Plant Biotechnol.* 2008;25(3):241–5. <https://doi.org/10.5511/plantbiotechnology.25.241>.
41. Wang Y, Wu WH. Regulation of potassium transport and signaling in plants. *Curr Opin Plant Biol.* 2017;39:123–8. <https://doi.org/10.1016/j.pbi.2017.06.006>.
42. Cheng X, Liu X, Mao W, Zhang X, Chen S, Zhan K, Bi H, Xu H. Genome-wide identification and analysis of HAK/KUP/KT potassium transporters gene family in wheat (*Triticum aestivum* L.). *Int J Mol Sci.* 2018;19(12):3969. <https://doi.org/10.3390/ijms19123969>.
43. Chen G, Hu Q, Luo L, Yang T, Zhang S, Hu Y, Yu L, Xu G. Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant Cell Environ.* 2015;38(12):2747–65. <https://doi.org/10.1111/pce.12585>.
44. Yang T, Lu X, Wang Y, Xie Y, Ma J, Cheng X, Xia E, Wan X, Zhang Z. HAK/KUP/KT family potassium transporter genes are involved in potassium deficiency and stress responses in tea plants (*Camellia sinensis* L.). *BMC Genomics.* 2020;21:556. <https://doi.org/10.1186/s12864-020-06948-6>.
45. Liang M, Gao Y, Mao T, Zhang X, Zhang S, Zhang H, Song Z. (2020) Characterization and Expression of KT/HAK/KUP Transporter. Family Genes in Willow under Potassium Deficiency, Drought, and Salt Stresses. *Biomed Res Int.* ID 2690760. <https://doi.org/10.1155/2020/2690760>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.