Pak. J. Agri. Sci., Vol. 58(1), 81-94; 2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.9703
http://www.pakjas.com.pk

GENOME-WIDE ANALYSIS AND EXPRESSION PROFILING OF POTASSIUM TRANSPORT RELATED GENES IN *Solanum tuberosum*

Farrukh Azeem[1,2], Mudassar Hussain[1], Sabir Hussain[3], Muhammad Zubair[1], Habibullah Nadeem[1], Muhammad Amjad Ali[4], Muhammad Afzal[1], Muhammad Hussnain Siddique[1,]*

[1]Department of Bioinformatics and Biotechnology, Govt. College University, Faisalabad, Pakistan; [2]Haikou Experimental Station, Chinese Academy of Tropical Agricultural Sciences (CATAS)-Hainan Key Laboratory of Banana Genetic Improvement, Haikou 570102, Hainan Province, China. [3]Department of Environmental Sciences and Engineering, Govt. College University, Faisalabad, Pakistan; [4]Department of Plant Pathology, University of Agriculture, Faisalabad.
***Correspondence author's e-mail: mhs1049@gmail.com**

Potassium (K^+) is one of the vital macronutrients for the plant kingdom. It constitutes almost 10% of total plant biomass. Therefore, it is evident that K^+ takes part in diverse biological processes throughout plant life. A number of molecular and functional studies have demonstrated the existence of a very organized system for K^+ uptake and transport in model plants. However, very scanty information is available related to the potassium transport system in *Solanum tuberosum*. Current study involves genome wide identification, *in silico* characterization and expression analysis of K^+ transport related genes in *S. tuberosum*. In this study, we identified 43 genes encoding transporters and channels involved in potassium transport. These genes were categorized on the basis of their conserved motifs and domain structure. There are twenty three potassium transorters (1 HKT, 4 KEAs and 18 KUP/HAK/KTs) and twenty channels (5 KCOs/TPKs and 15 Shakers). Chromosomal positioning of these genes showed that these genes are randomly spread over all the 12 chromosomes of *S. tuberosum*. The phylogenetic analysis showed that K^+ transporters and channels in *S. tuberosum* are orthologous to genes that are in charge of K^+ transport in *Arabidopsis thaliana* and *Solanum lycopersicum*. To validate the *in silico* data, we performed expression analysis of these genes under salinity stress. Some genes were differentially expressed in response to salt stress, which indicates the significance of these genes for stress response of *S. tuberosum*. Current study is the first insight into the potassium transport system in *S. tuberosum* and it will work as a baseline for functional analysis of these genes.

Keywords: Potassium, *S. tuberosum*, Channel, Transporter, Ion homeostasis.

INTRODUCTION

Potassium (K^+) is a significant macronutrient and almost 10% of plant dry weight is composed of this ion (Clarkson and Hanson, 1980). This cation is abundantly (60-150 mM) present in the cytosol of a plant cell (Schroeder *et al.*, 1994; Walker *et al.*, 1996; Leigh, 2001), where it is used in fundamental processes including cell elongation, stomatal movements, osmoregulation, control of membrane polarization and electrical balance of anionic groups. K^+ is also required to maintain pH of the cytosol, which is essential for standard functioning of majority of the enzymes (Véry and Sentenac, 2003; Lebaudy *et al.*, 2007). Apart from cytosol, K^+ is additionally gathered in mitochondria and in different other organelles like chloroplasts, nucleus and vacuoles as well. Capacity of K^+ in vacuole is useful in keeping up the concentration of K^+ in cytosol through its interchange between these two parts. In spite of its abundance and significance in the cell, an ideal cell concentration of K^+ must be kept up for proper cell working. For instance, maintenance of potassium concentration in the cytoplasm is related with plant resistance to drought stress (Gupta *et al.*, 1989) and salinity (Shi *et al.*, 2000). In addition, K^+ application decreases the accumulation of reducing sugars (Chapman *et al.*,1992; Mohr and Tomasiewicz, 2012) and enzymes used for starch synthesis needs a particular concentration of potassium and tubers rely on a dynamic supply of potassium for increased starch content (Lindhauer and De Fekete, 1990). Biomass and increased number of potato tubers are positively influenced by synthesis and gathering of starch. Potassium assumes a key job in stimulating the starch synthase enzyme activity and bring the inclusion of simple glucose molecules into complex molecules of starch (Moinuddin *et al.*, 2004). Starch gathering is combined with cell and tissue development of the tubers as potassium improves the general development of the plants and encourages the translocation of assimilates to the tubers/ sinks (Moinuddin *et al.*, 2005), which could ultimately increment the tuber bulking capability and, accordingly, its biomass and yield. The application of K^+ activates various enzymes engaged in starch digestion, photosynthesis, and proteins, and aids the translocation of sugars from leaves to tubers, which expands the size of tubers however not their number (Trehan *et al.,* 2001). It is likewise significant for anions neutralization, involved in the balance and modification of membrane potential (Leigh, 2001). An intricate transport system comprising of different channels and transporters is associated with homeostasis of K^+ in the cytosol. This system intervenes the assimilation and translocation of potassium from soil to various parts of the plant (Maathuis, 2009). In model plant *A. thaliana,* nearly thirty five genes have been reported to code for potassium transport proteins (fifteen channels and twenty transporters) (Mäser *et al.,* 2001; Véry and Sentenac, 2003).

K^+ channels are multimeric proteins consisting of specific trans-membrane (TM) segments (α-segments) and Pore domains. There are four Pore domains in a functional multimeric channel protein that play a role in the channel activity. A highly conserved motif (GYGD/E) is the hallmark of Pore domains in K^+ selective channel proteins. There are fifteen K^+ selective channels in *A. thaliana*. On the basis of sequence features, these channel proteins are classified into three families as Voltage Gated Shaker channels (nine members), Tandem Pore Potassium (TPK) channels (five members) and one K^+ inward rectifier or Kir like channel (Mäser *et al.,* 2001; Véry and Sentenac, 2003; Lebaudy *et al.*, 2007). Likewise, there are three families of potassium transporters as High affinity K^+ Transporters or HKTs (1 member), K^+ Uptake Permeases or KUP/HAK/KTs (13 members) and K^+ Efflux Anti-porters or KEAs (6 members) (Mäser *et al.*, 2001).

During the last century, potato (*S. tuberosum*) has become a worldwide elementary staple food due to higher nutritional value as a carbohydrate source, storability and divergent uses. *S. tuberosum* is a species, which needs high potassium levels for development and growth (Kang *et al.,* 2014; Tein *et al.,* 2014). At functional level, K^+ affects the quality characteristics of *S. tuberosum*. It becomes even more important for industrial processing of potatoes (Gerendás *et al.,* 2007; Zörb *et al.,* 2014). An inverse connection between K^+ and sugar concentration in tubers is essential for better human nutrition and industrial processing (Westermann *et al.,* 1994). Therefore, a comprehension of K^+ uptake and transport procedure is important for potato crop improvement. A number of K^+ transporters and channels have been well characterized in *O. sativa* and *A. thaliana*. Genome wide analysis has been used to identify and characterize several gene families in different plant species (Yasmeen *et al.,* 2016; Nawaz *et al.,* 2017; Rehman *et al.,* 2017; Lee *et al.,* 2019). However, very limited information is available for potassium transport related proteins in *S. tuberosum*. Current study was performed to recognize potassium transport related genes, to find phylogenetic relationship of these genes and to study their abiotic stress response in *S. tuberosum.*

MATERIALS AND METHODS

***Protein sequence retrieval of potassium transporters and channels*:** NCBI-Genbank and Solanaceae genome database (http://solanaceae.plantbiology.msu.edu/index.shtml) were used to retrieve potassium transport related genes in *S. tuberosum.* For this purpose, already identified protein sequences of potassium transporting genes in *O. sativa and A. thaliana* were used as query (Véry and Sentenac, 2003; Amrutha *et al.,* 2007; Véry *et al.,* 2014). Moreover, to further improve these findings, we also employed PSI-BLAST and DELTA-BLAST. In addition, by using TBLASTX program, *S. tuberosum* genome was also screened in six frame translations. The resulting raw data were manually curated for removing false positives or redundant sequences. Later on, these sequences were reviewed for the presence of K^+ selectivity filter motif (G-Y-G). The selected sequences were additionally examined through SMART (http://smart.embl-heidelberg.de/), NCBI conserved domain database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and Pfam database (http://pfam.janelia.org/) for the presence of relevant domians and motifs. All the genomic data, chromosomal location, number of exons in every gene and the total number of amino acids in each protein were taken from NCBI and Solanaceae genome database.

***Motif identification, prediction of gene structure, multiple sequence alignment, chromosomal mapping and phylogenetic analysis*:** Conserved motifs inside the protein sequences of potassium channels and transporters were found through an online server MEME (http://meme.sdsc.edu/meme/meme.html). For motif analysis, the default parameters were utilized. The coding and genomic sequences of all the recognized genes were retrieved from NCBI. Further, these sequences were then utilized for gene structure examination. The schematic description of all gene structures were taken out utilizing Gene Structure Display Server (GSDS) (http://gsds.cbi.pku.edu.cn/). Multiple sequence alignment was carried out by utilizing ClustalW. Phylogenetic analysis were performed by using MEGA 7.0. Phylogenetic trees were constructed via Neighbor Joining and Maximum Likelihood methods with 1000 bootstrap duplicates. A chromosomal map of putative potassium transporters and channels loci was generated by using the MapChart program (http://www.biometris.wur.nl/ UK/ Software/ MapChart/download).

***Plant material, growth conditions and application of stress*:** Commercially available *cv* Desiree of *S. tuberosum* were grown under controlled circumstances (08h dark period/16h light, 25±1 °C under light/ 20±1°C in dark) within 65% humidity in Hoagland’s solution. After the development of first true leaf, salinity stress was applied by adding 100 mM NaCl Hoagland’s solution. After 24 hours, plant leaves and stem were collected in liquid nitrogen for further analysis. Each sample was the blend of leaves from three different plants and three biological repeats were used for control/treatment samples.

***RNA isolation, reverse transcriptase PCR and quantitative real-time PCR (qRT-PCR)*:** The total RNA was extracted from leaf samples utilizing TRIzol reagent (Invitrogen & Co.) according to the manufacturer's instructions and quantified utilizing Nano-Drop spectrophotometer (Colibri spectrometer, Titertek Berthold, Germany). RNA was treated with dsDNase (cat#K1681) to remove contamination of genomic DNA. Later on, Maxima H Minus First Strand cDNA synthesis kit was used for the reverse transcription of one microgram of total RNA. For the quantification of gene specific transcripts, qRT-PCR was performed using iTaq Universal SYBR Green Supermix in CFX96 Touch™ Real-Time PCR Detection System. The gene specific primers were designed using an online server named "Oligo Calculator" (http://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html) and specificity of primers was checked by NCBI Primer-BLAST program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primer sequences for the evaluation of expression of various genes are provided in Table 1.

Table 1. List of primers designed for Shaker and HKT family genes in *S. tuberosum*

Gene Name	Primer Sequence	Primer length
StHKT **F**	TCATCCCTAGCGCGAAACAA	20
StHKT **R**	TGCAAATGTTGAAACTGTGGTG	22
StAKT1.1 **F**	CGAGGAACGAGGGGTTAGAT	20
StAKT1.1 **R**	AGACTCCATGGGATGCAGGG	20
StAKT1.2 **F**	CACTGATAGGATTTGTGGCTGC	22
StAKT1.2 **R**	TCCTCCTGCCAAGTTTCCAC	20
StAKT1.3 **F**	CTCTTGGCGGCCATAGTGAT	20
StAKT1.3 **R**	AAGCACCAAAAATGCGTCCC	20
StAKT1.4 **F**	TCCCCCAGAAGAATCTCCGT	20
StAKT1.4 **R**	TGAGGGGAGTGTTAGATTCGC	21
StAKT2.1 **F**	AACCAGTCGCACCATGGAAT	20
StAKT2.1 **R**	GCTCCTTCAGTCTTGGAGGC	20
StAKT2.2 **F**	GCTCACTGGCAAACACCAAG	20
StAKT2.2 **R**	GGCGAAGACGCCAAAATCTG	20
StGORK **F**	TTCTAGGTTGCATGCCCTGG	20
StGORK **R**	CCCTGCTCAACCGAATCCAT	20
StKAT1.1 **F**	CACGGGTCACAAAGAAAGCG	20
StKAT1.1 **R**	CTTGCAAACAGGGCACTGAC	20
StKAT1.2 **F**	TTGTAGTTCACTGGACCGGC	20
StKAT1.2 **R**	TTCGCGAATTCTTGTGCTGC	20
StKAT1.3 **F**	GTTGGCCGAGATCATAGGGG	20
StKAT1.3 **R**	TCCACAACCAACCTCGTTCT	20
StKAT2 **F**	AGTGAAAGTGGTGGACGTGA	20
StKAT2 **R**	CGGAGCCATTTCAATATGCCC	21
StKAT3.1 **F**	CCTGAGTCAGTTGAAGGTCTCC	22
StKAT3.1 **R**	GGAGCCATCTGCCATGAGAA	20
StKAT3.2 **F**	GAGGAGCTAGCAGAGATGCC	20
StKAT3.2 **R**	GAGCTCCCGCCTCATTCATT	20
StSKOR.1 **F**	GGAGATTTTAGCTCATTGTGGC	22
StSKOR.1 **R**	TGTACCACGTCGTTGGACTG	20
StSKOR.2 **F**	GGACAGCGAGTTAGATGCAGA	21
StSKOR.2 **R**	AGGTACCCATAGAACGACTCCT	22

For normalization of the expression data, the *S. tuberosum tubulin alpha chain* gene (Liu *et al.,* 2018) (NM_001318688.1, forward primer, 5'-CCACCATCAAGACTAAGCG-3'; reverse primer, 5'-AGAAGACCTCAGCAACACTC-3') was utilized as internal control. For each sample, an equal amount of cDNA template was used including the internal control. The qPCR was performed in triplate independently.

RESULTS

Identification of K^+ ***channels and transporters in S. tuberosum*:** Genes related to K^+ transport have been reported in different plants (Mäser *et al.,* 2001; Azeem *et al.,* 2018). Amino acid sequences of these genes were utilized as a query to find potassium transporters/channels in non-redundant protein sequence database of *S. tuberosum*. As a result, almost 56 hits were observed. Later on, we removed the redundant sequences. Finally, the result of BLASTP search demostrated a total of 43 putative proteins. Based on sequence identity and domain resemblance to *A. thaliana* genes, 23 proteins were classified as K^+ transporters and the other 20 proteins were placed in the K^+ channels group. Individual members of each family were named according to their homologs in *A. thaliana*. The descriptive information of the potassium transport related genes and respective proteins is presented in Table 2.

***Potassium transporters*:** In *A. thaliana*, 20 K^+ transporters have been reported. These genes are classified as KEA family (6 genes), HKT/Trk family (1 gene) and KT/HAK/KUP family (13 genes). In current genome-wide analysis on *S. tuberosum*, it was found that there are 23 genes, which are potentially K^+ transporters. One of these transporters is similar to Trk/HKT family, 18 to KT/HAK/KUP family and 4 members belong to KEA family.

The HAK/KUP/KT is a large gene family and there are different names for this family in literature. For example, K^+ transporters (KT), higher affinity potassium transporters (HAKs) and potassium up-take permeases (KUP) (Azeem *et al.,* 2018). This group is labeled as KT/HAK/KUP in the current research to show all of them as an one family. In *S. tuberosum* genome, 18 members of this family were distinguished. The number of genes found in this family is moderately higher than *A. thaliana* (13 members) and smaller as compared to *Solanum lycopersicum* (24 members).

HKT family is placed in the Trk superfamily. It is topologically related to potassium channels. When this gene family was compared with *A. thaliana*, only 1 member

Table 2. Nomenclature and protein properties of *S. tuberosum* K$^+$ potassium channels and transporters

Sr. No.	Gene	Chr	Start	End	Exons	Genomic DNA Accession*	Transcript Accession*	Protein Size (aa)	I.C point	Mol. weight
St-KUP/HAK/KT Family										
1	StKUP1.1	9	52,332,154	52,333,867	3	PGSC0003DMG401011387	PGSC0003DMT400029610	433	8.5754	49,477.34 g/mol
2	StKUP1.2	9	52,350,669	52,354,458	8	PGSC0003DMG400011388	PGSC0003DMT400029616	745	8.6091	83,237.21 g/mol
3	StKUP2	6	32,099,078	32,106,917	9	PGSC0003DMG400024311	PGSC0003DMT400062466	790	7.5864	87,899.87 g/mol
4	StKUP3.1	12	11,740,112	11,748,755	10	PGSC0003DMG400020386	PGSC0003DMT400052541	792	9.0347	87,607.80 g/mol
5	StKUP3.2	8	8,181,069	8,187,150	9	PGSC0003DMG400025230	PGSC0003DMT400064965	817	9.1174	91,021.82 g/mol
6	StKUP3.3	12	58,199,834	58,206,143	10	PGSC0003DMG400029362	PGSC0003DMT400075514	818	8.3811	90,788.86 g/mol
7	StKUP5.1	12	6,853,045	6,858,344	8	PGSC0003DMG400013739	PGSC0003DMT400035722	784	8.9374	88,240.53 g/mol
8	StKUP5.2	6	38,062,351	38,064,920	1	PGSC0003DMG400028891	PGSC0003DMT400074329	227	9.5667	25,981.22 g/mol
9	StKUP5.3	12	6,844,567	6,846,235	12	PGSC0003DMG400013740	PGSC0003DMT400035725	422	7.1962	48,181.69 g/mol
10	StKUP6.1	2	46,898,734	46,904,934	8	PGSC0003DMG400000501	PGSC0003DMT400001329	772	7.9211	87,023.98 g/mol
11	StKUP6.2	4	22,582,407	22,586,026	4	PGSC0003DMG400013819	PGSC0003DMT400035889	494	8.627	55,579.14 g/mol
12	StKUP6.3	5	7,716,491	7,722,639	8	PGSC0003DMG400004113	PGSC0003DMT400010525	779	8.3664	87,104.18 g/mol
13	StKUP7.1	1	81,875,391	81,886,308	10	PGSC0003DMG400012585	PGSC0003DMT400032781	849	6.2182	94,549.18 g/mol
14	StKUP7.2	4	22,319,412	22,327,197	9	PGSC0003DMG400013821	PGSC0003DMT400035898	848	7.0358	93,979.80 g/mol
15	StKUP7.3	10	29,642,596	29,643,783	2	PGSC0003DMG400016667	PGSC0003DMT400042977	265	8.3073	30,167.80 g/mol
16	StKUP10	2	27,417,702	27,427,277	8	PGSC0003DMG400010431	PGSC0003DMT400027038	792	8.1942	89,079.77 g/mol
17	StKUP11	2	15,282,512	15,293,961	8	PGSC0003DMG400017862	PGSC0003DMT400046034	784	7.5763	88,145.59 g/mol
18	StKUP12	6	38,066,821	38,068,432	4	PGSC0003DMG400002354	PGSC0003DMT400006056	356	8.4063	39,469.73 g/mol
St-HKT Family										
19	StHKT	7	8,188,850	8,194,714	3	PGSC0003DMG400029966	PGSC0003DMT400077052	501	9.4789	57,034.22 g/mol
St-TPK/KCO Family										
20	StKCO1.1	7	55,454,825	55,460,725	4	PGSC0003DMG400022284	PGSC0003DMT400057395	349	8.3711	39,036.75 g/mol
21	StKCO1.2	10	1,001,834	1,006,086	3	PGSC0003DMG400014411	PGSC0003DMT400037347	349	5.5162	39,128.56 g/mol
22	StKCO1.3	4	8,871,713	8,874,510	3	PGSC0003DMG400023600	PGSC0003DMT400060672	353	5.7948	39,612.87 g/mol
23	StKCO5	6	327,518	330,128	2	PGSC0003DMG400007275	PGSC0003DMT400018768	379	6.9887	42,400.91 g/mol
24	StKCO6	2	30,336,970	30,345,525	2	PGSC0003DMG400009702	PGSC0003DMT400025112	424	7.1681	46,978.71 g/mol
St-Shaker Family										
25	StAKT1.1	12	4,997,465	5,005,723	11	PGSC0003DMG400001066	PGSC0003DMT400002749	872	7.2916	98,443.30 g/mol
26	StAKT1.2	8	39,973,288	39,979,045	12	PGSC0003DMG400029083	PGSC0003DMT400074787	861	7.4715	96,733.51 g/mol
27	StAKT1.3	9	8,918,340	8,920,175	1	PGSC0003DMG400040591	PGSC0003DMT400091020	611	6.919	69,471.36 g/mol
28	StAKT1.4	9	9,168,237	9,171,797	3	PGSC0003DMG400025670	PGSC0003DMT400065948	314	4.4804	34,512.98 g/mol
29	StAKT2.1	10	27,377,725	27,383,564	11	PGSC0003DMG400024168	PGSC0003DMT400062100	842	6.6595	96,735.27 g/mol
30	StAKT2.2	1	80,681,088	80,687,276	11	PGSC0003DMG400023769	PGSC0003DMT400061090	824	7.0058	94,261.80 g/mol
31	StGORK/TORK	11	1,149,991	1,156,528	13	PGSC0003DMG400013243	PGSC0003DMT400034446	828	6.8913	94,655.97 g/mol
32	StKAT1.1	2	29,708,438	29,712,521	12	PGSC0003DMG400021091	PGSC0003DMT400054339	688	7.3743	78,864.94 g/mol
33	StKAT1.2	8	10,201,719	10,205,237	11	PGSC0003DMG400009648	PGSC0003DMT400024968	689	6.3377	79,528.20 g/mol
34	StKAT1.3	0	35,389,325	35,390,755	5	PGSC0003DMG400021079	PGSC0003DMT400054318	315	4.9474	113.811 g/mol
35	StKAT2	1	12,665,467	12,666,784	5	PGSC0003DMG400009614	PGSC0003DMT400024866	289	6.0469	32,801.15 g/mol
36	StKAT3.1	8	42,731,150	42,736,229	13	PGSC0003DMG400014505	PGSC0003DMT400037608	623	9.2368	70,937.73 g/mol
37	StKAT3.2	0	19,798,323	19,808,676	13	PGSC0003DMG400025678	PGSC0003DMT400065964	631	9.3184	72,411.83 g/mol
38	StSKOR1	3	45,339,090	45,341,406	2	PGSC0003DMG400033682	PGSC0003DMT400083961	106	4.3996	12,051.42 g/mol
39	StSKOR2	3	45,308,625	45,309,119	1	PGSC0003DMG400033692	PGSC0003DMT400083985	82	4.4692	9,288.50 g/mol
St-KEA Family										
40	StKEA1	1	71,866,659	71,868,287	5	PGSC0003DMG400000153	PGSC0003DMT400000448	179	6.1091	19,226.86 g/mol
41	StKEA5	3	41,054,206	41,062,755	20	PGSC0003DMG400031029	PGSC0003DMT400079671	577	7.5516	62,964.48 g/mol
42	StKEA6.1	8	36,226,462	36,242,516	20	PGSC0003DMG400029945	PGSC0003DMT400076994	599	7.7644	64,771.97 g/mol
43	StKEA6.2	5	21,898,023	21,906,651	7	PGSC0003DMG400009808	PGSC0003DMT400025403	209	4.2249	22,512.51 g/mol

* http://plants.ensembl.org/Solanum_tuberosum/Info/Index

(*AtHKT1*) was found analogous to *StHKT* gene of *S. tuberosum*.

There genome of *A. thaliana* has six K$^+$/H$^+$ antiporter members of KEA family and these genes are termed as *KEA1* to *KEA6*. Genome-wide study in *S. tuberosum* suggests that there are four KEA members in this family. These are named according to respective homologues in *A. thaliana*. Genes corresponding to *KEA2, KEA3* and *KEA4* are absent in *S. tuberosum* but two genes were found to be homologs of *KEA6*.

***Potassium channels*:** There are two families of potassium channels as TPK/KCO and Shaker. Genome-wide analysis revealed that *A. thaliana* and *S. tuberosum* contain 15 and 20 potassium channel genes respectively.

The members of Shaker family were the primary potassium channels, which were well studied and characterized compared to other groups. In *A. thaliana*, nine members of Shaker family have been reported (Maathuis, 2009). After genomic analyses, it was found that *S. tuberosum* has 15 sequences to encode Shaker type genes. In comparison with *A. thaliana*, Shaker family genes of *S. tuberosum* are *GORK/SKOR-like* (3 genes), *AKT1-like* (4 genes), *AKT2-like* (2 genes), *KAT1/KAT2-like* (4 genes) and *KAT3-like* (2 genes). Similarly, five members of TPK/KCO were identified in *S. tuberosum* (Table 1).

***Phylogenetic analyses of K$^+$ transporters in S. tuberosum and other plant species*:** Protein sequences of K$^+$ channels and transporters relating to various species like *A. thaliana*, *S. lycopersicum* and *S. tuberosum* were utilized for analyzing the phylogenetic and evolutionary relationship via cladogram (Fig. 1) and phylogram (Fig. 2). On the basis of sequence similarity, the cladograms are used to group

Table S1. Complete list of Arabidopsis K^+ transport related genes used as reference

Sr. No.	Gene Name	Gene ID	Chm	Exon	Protein Accession	Length	Domains
At-KUP/HAK/KT Family							
1	KT1	AT2G30070	2	9	NP_001324058	592	1
2	KT2	AT2G40540	2	11	NP_001324081	794	2
3	KUP3	AT3G02050	3	9	NP_186854	789	1
4	KUP4	AT4G23640	4	9	NP_194095	775	1
5	KUP5	AT4G13420	4	9	NP_567404	785	2
6	KUP6	AT1G70300	1	6	NP_177187	782	1
7	KUP7	AT5G09400	5	10	NP_568213	858	2
8	KUP8	AT5G14880	5	8	NP_196992	781	2
9	KUP9	AT4G19960	4	9	NP_001190775	807	1
10	KUP10	AT1G31120	1	7	NP_174397	796	1
11	KUP11	AT2G35060	2	8	NP_181051	792	1
12	KUP12	AT1G60160	1	8	NP_176222	827	1
13	KUP13	AT4G33530	4	10	NP_195079	855	1
At-HKT Family							
14	HKT1	AT4G10310	4	3	NP_567354	506	2
At-KCO Family							
15	KCO1	AT5G55630	5	3	NP_200374	363	2
16	KCO2	AT5G46370	5	2	NP_199449	443	2
17	KCO3	AT5G46360	5	3	NP_001190480	260	1
18	KCO4	AT1G02510	1	2	NP_171752	284	1
19	KCO5	AT4G01840	4	2	NP_192093	408	1
20	KCO6	AT4G18160	4	2	NP_193550	436	2
At-Shaker Family							
21	AKT1	AT2G26650	2	11	NP_180233	857	6
22	AKT2	AT4G22200	4	10	NP_567651	802	6
23	AKT3	AT3G56290	3	3	NP_191188	173	3
24	AKT4	AT5G14890	5	7	NP_196993	482	4
25	AKT5	AT4G32500	2	12	NP_194976	880	6
26	AKT6	AT2G25600	2	12	NP_180131	888	6
27	GORK	AT5G37500	5	11	NP_001332118	842	6
28	KAT1	AT5G46240	5	9	NP_199436	677	4
29	KAT2	AT4G18290	4	11	NP_001329801	708	4
30	KAT3	AT4G32650	4	13	NP_194991	662	5
31	SKOR	AT3G02850	3	11	NP_186934	828	6
At-KEA Family							
32	KEA1	AT1G01790	1	21	NP_171684	1193	4
33	KEA2	AT4G00630	4	20	NP_191972	1174	7
34	KEA3	AT4G04850	4	18	NP_001190675	776	2
35	KEA4	AT2G19600	2	20	NP_849990	592	1
36	KEA5	AT5G51710	5	20	NP_568763	568	1
37	KEA6	AT5G11800	5	20	NP_196741	597	1

protein sequences. While phylograms are used to group protein sequences on the basis of evolutionary distances. According to both tree types, all channels and transporters were divided into five families. Moreover, predictions were consistent in both tree types. Potassium transport related proteins of *S. tuberosum* shared clades with *S. lycopersicum* as compared to *A. thaliana*. It is also evident from both tree types that members of *A. thaliana* diverged earlier than both of *S. lycopersicum* and *S. tuberosum* (Fig. 1 and Fig. 2).

According to Fig. 1, KUP/HAK/KT family is further sub-divided into three major categories as group I, II and III. Among these, the group I is the largest group, which contains 12 KUP/HAK/KT members. While group II and III contain 4 and 2 members respectively. Similarly, Shaker channels were grouped into AKT1-type, KAT1-type, KAT3-type, GORK/SKOR (outward-type) and AKT2-type.

According to Fig. 2, KUP/HAK/KT family is composed of three major categories as gI, gII and gIII. Moreover, number of members in each group was also persistent likewise Fig. 1. Least evolutionary distances were observed between members of *S. lycopersicum* and *S. tuberosum* in each clade as compared to *A. thaliana.*

***Analysis of exon-intron structure*:** Over long evolutionary time intervals, the exon and intron position is commonly highly conserved in orthologous genes, while intron/exon structure is somewhat less, however adequately conserved in paralogous genes (Li *et al*., 2014). To examine the gene structural diversity of potassium channels and transporters in *S. tuberosum*, we investigated the intron/exon organization. In general, there was extensive diversity in the number of exons (1–20) and the length of exons in the potassium channels and transporters (Fig. 3). But, it was observed that

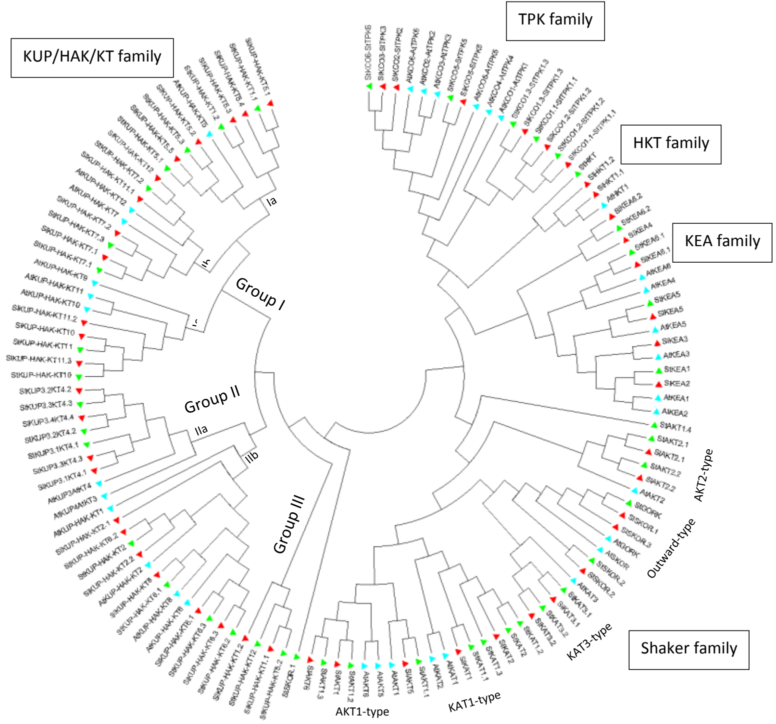

Figure 1. **Cladogram of K⁺ transporter Gene families in *Arabidopsis thaliana* (At), *Solanum lycopersicum* (Sl) and *Solanum tuberosum* (St)**

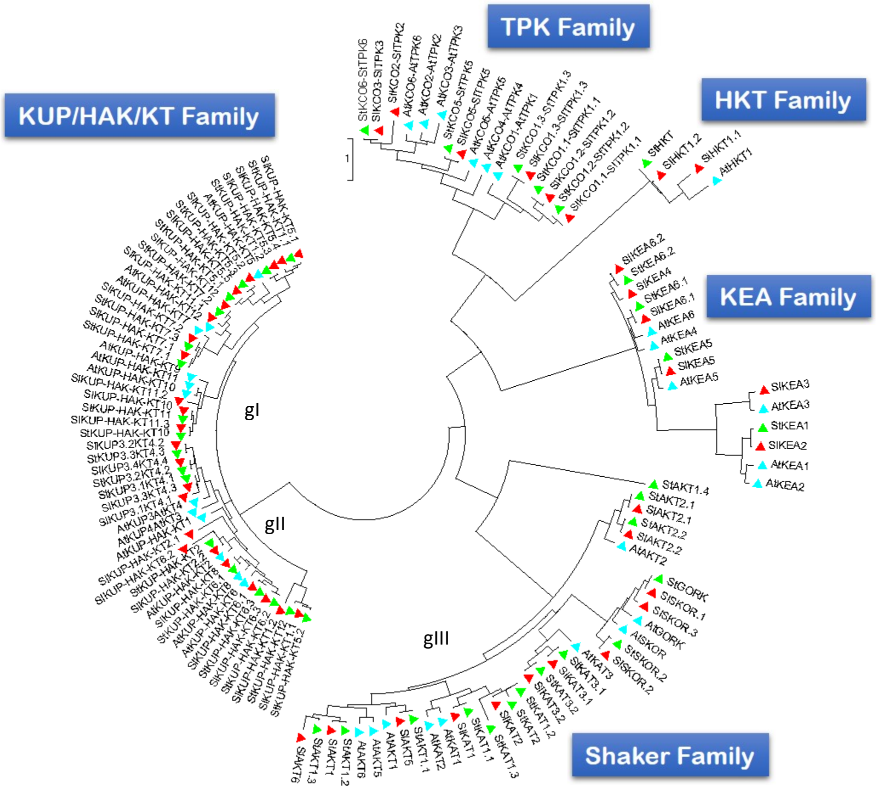

Figure 2. **Phylogram of K^+ transporter Gene families in *Arabidopsis thaliana* (At), *Solanum lycopersicum* (Sl) and *Solanum tuberosum* (St)**

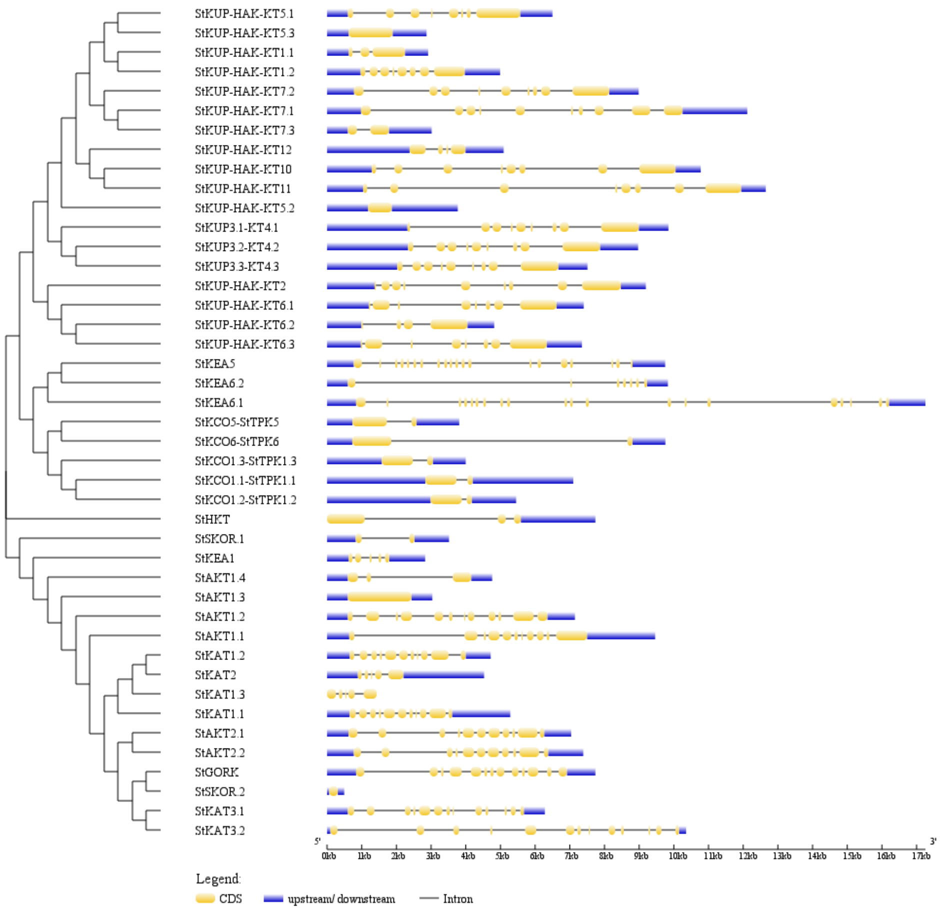

Figure 3. Exon-intron structure in *S.tuberosum* potassium transport related genes.

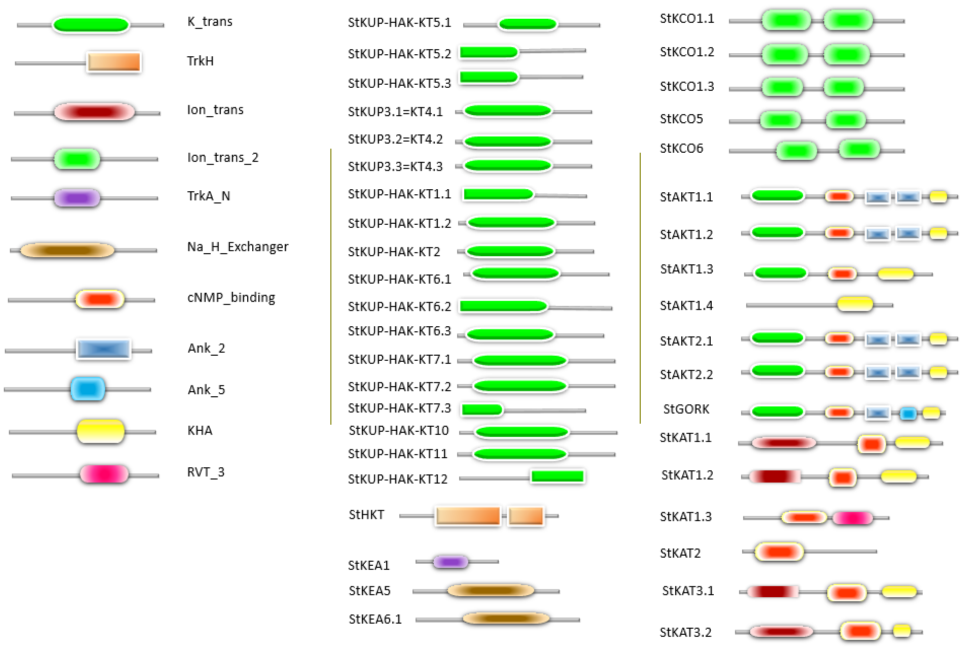

Figure 4. Conserved domains in potassium transport related genes in *S. tuberosum*

members in a subfamily shared variable gene structure with regard to intron number. The members *StKUP/HAK/KT1.1*, *StKUP/HAK/KT5.2*, *StKUP/HAK/KT5.3*, *StKUP/HAK/KT7.3*, *StAKT1.3*, *stAKT1.4* and *StSKOR2* are either intron-less or have 1 to 2 introns. However, a close inspection suggests that these sequences are incomplete or a rudimentary part of relevant sequence present on another branch. All members of TPK/KCO family have a single intron, which seems specific for this family. It is also important to notice that there exists least variation within family for exon length and/or intron patterns (Fig. 3).

Conserved domains analysis**:** For an insight of potential functional diversity in K^+ transporter/channel proteins, the conserved domains of these proteins were investigated in *S. tuberosum* using Pfam online tool. Pfam predicted 11 types of different conserved domains (Fig. 4). Proteins in the same group or subgroup comprise similar domains, while the domains also showed some structure deviation between several groups or subgroups. For example, domain "Ion trans 2" solitarily appears in the members of the KCO/TPK family, it suggests that this domain is exclusively associated with the KCO/TPK family. Inversely, domain "K trans" is distributed in the members of HAK/ KUP /KT family and also present in the members of AKT family. Shaker family members exhibited the highest number of domains for every member as compared to other families. On the other hand, HKT family contains only two domains in protein structure. Domains "Ion trans" found to be very specific to the only KAT group members while "Ank2" domain to only AKT channels group. The domain "cNMP binding" is possessed by every member of the Shaker family. The domain "Na H Exchanger" is present in only KEA family. There are some domains that are present in some genes exclusively and not found in any other transporter like KEA1 has "TrkA N", GORK has "Ank 5" and KAT1.3 has "RVT 3".

It is interesting to notice that few proteins have incomplete domains like StKUP/HAK/KT1.1, 5.2, 5.3, 6.2, 7.3 and 12. While others lack key functional domains like StKEA1 lack Na_H exchanger domain, StAKT1.3, 1.4 and StKAT2 lack ion/K_trans domain (Fig. 4). These findings show that the arrangement of the structural domains varies among different K^+ transporter/channel families but is similar within the same families. Mostly, proteins with parallel domain patterns were assembled in the same group that demonstrates the similar purposes of domains among the members of the same group.

Distribution of K^+ transport genes on chromosomes**:** Positions of 41 (out of 43) K^+ transporter/channel genes were detected on different chromosomes of *S. tuberosum* genome. Location of genes was detected with the help of *S. tuberosum* genome browser on Spud Database (Potato Genomics Resources) (Fig. 5). These genes are irregularly present across the chromosomes of the *S. tuberosum* genome: Chromosome 11 holds only one gene "*StGORK*", while a comparatively high number of potassium transport genes were detected on Chromosome 02, Chromosome 08 and Chromosome 12 (five transporter/channel genes on each). Chromosome 02 have *StKUP6.1*, *StKUP 10*, *StKUP11*, *StKAT1.1* and *StKCO6*. Chromosome 08 have *StKUP3.2*, *StAKT1.2*, *StKAT1.2*, *StKAT3* and *StKEA6.1*. Chromosome 12 have *StKUP 3.1*, *StKUP 3.3*, *StKUP5.1*, *StKUP5.3* and *StAKT1.1*.

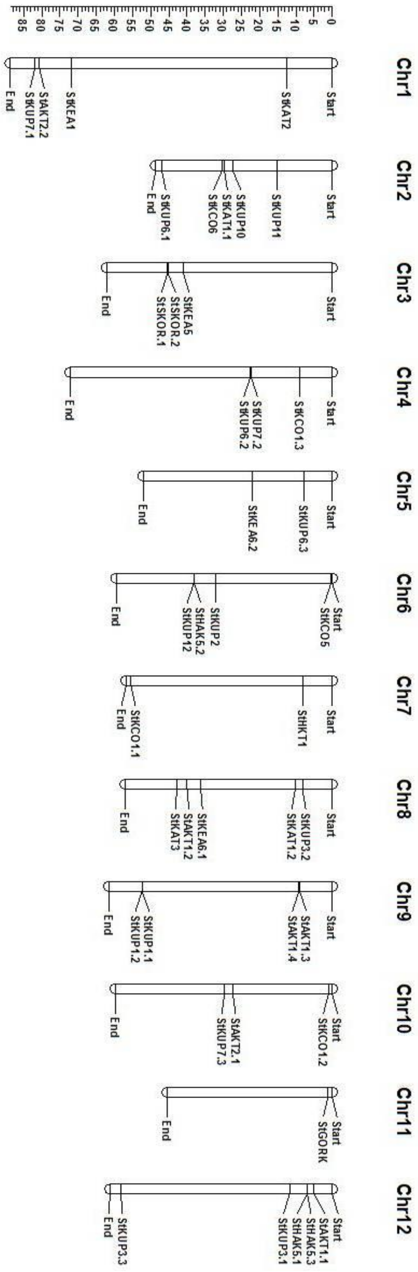

Figure 5. Chromosomal map showing positions of potassium transport related genes on chromosomes of *S. tuberosum*

Some chromosomes contain three members (Chromosome 03, Chromosome 04 and Chromosome 10). Chromosome 03 contains *StSKOR1*, *StSKOR2* and *StKEA5*. Chromosome 04 has *StKCO1.3*, *StKUP6.2* and *StKUP7.2*. Chromosome 10 has *StKUP 7.3, StKCO1.2* and *StAKT2.1*. Three chromosomes possess four K^+ transporters/channels genes (Chromosome 01, Chromosome 06 and Chromosome 09). Chromosome 01 has *StKUP7.1*, *StKEA1*, *StAKT2.2* and *StKAT2*. Chromosome 06 has *StKUP2, StKUP5.2, StKUP12* and *StKCO5*. Chromosome 09 has *StKUP1.1*, *StKUP1.2, StAKT1.3* and *StAKT1.4*. On the other hand, chromosome number 05 and 07 contain only two genes. Chromosome 05 has *StKUP6.3* and *StKEA6.2*. Chromosome 07 has *StHKT* and *StKCO1.1*.

a)

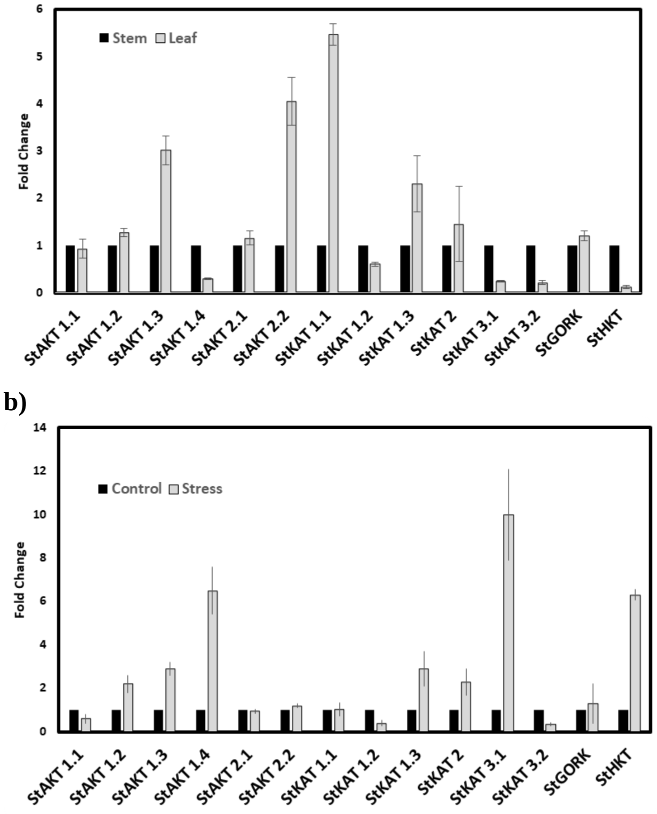

Figure 6. Expression analysis of Shaker and HKT genes in response to salinity stress. a) Comparative expression level of genes in stem and leaf tissues. b) Comparative expression level of genes in control and salinity treated leaf tissues. These figures represent an average of three biological repeats while each biological repeat was quantified three times in real time qRT-PCR analysis.

***Tissue-specific and stress-responsive expression analysis of HKT transporter and Shaker channels genes*:** Expression of potassium transporters and channels is influenced by abiotic and biotic stresses (Gaymard *et al.*, 1998; Lacombe *et al.*, 2000; Reintanz *et al.*, 2002; Becker *et al.*, 2003; Pilot *et al.*, 2003). With an aim to predict the potential role of potassium transport related genes in *S. tuberosum*, relative real time qRT-PCR was carried out for the approximation of tissue-specific expression of Shaker (13 genes) and HKT (1 gene) family members in leaf and stem tissues (Fig. 6a). Moreover, to understand potential role of these genes in stress response, expression of these genes was also estimated in response to salt stress (100 mM NaCl, 24H) (Fig. 6b).

Interestingly, genes under study exhibited higher expression in leaves (*StAKT1.3, StAKT2.2, StKAT1.1* and *StKAT1.3*) or stem (*StAKT1.4, StKAT1.2, StKAT3.1, StKAT3.2* and *StHKT*). Moreover, for some genes (*StAKT1.1, StAKT1.2, StAKT2.1, StKAT2 and StGORK*), there was almost no difference of expression in both tissues (Fig. 6a) or gene expression was not detected in both tissues (*StSKOR1* and *StSKOR2*). Similarly, based on expression analysis under salt stress conditions, the results of this analysis can be grouped into three categories: gene expression remained unchanged (*StAKT1.1, StAKT1.2, StAKT2.1, StAKT2.2, StKAT1.1* and *StGORK*), gene expression decreased almost 50% (*StKAT1.2* and *StKAT3.2*), and gene expression increased more than two folds (*StAKT1.3, StAKT1.4, StKAT1.3, StKAT2, StKAT3.1* and *HKT)*. The gene expression of *StKAT3.1* was very high (almost 10 folds) in leaves of salt-treated plants as compared to normal plants (Fig. 6b). It is interesting to notice that the expression of this gene is very low under controlled conditions. It predicts the potential significance of this gene in plant stress response.

DISCUSSION

K^+ is one of the three principal nutrients (NPK) for plants, yet it is given minimum significance both at researcher and farmer ends. Potassium plays different roles in plants like regulation of protein synthesis, balancing of charges and activation of enzymes. Potassium channels in mitochondria are responsible for cyto-protection and cell necrosis (Laskowski *et al.*, 2016). Studies have revealed that potassium helps plants for pH maintenance and regulation of water potential. Potassium also assists in modulation of turgor pressure and polarization of plasma membrane (Ward *et al.*, 2009). A well-organized system is present in plants that helps in translocation of potassium from soil to various parts of the plant (Lebaudy *et al.*, 2007, 2008). Among all of potassium-selective channels/transporters in plants, Shaker and HKTs have been well characterized (Chérel and Gaillard, 2019; Véry *et al.*, 2014). Therefore, we selected only these two families for expression analysis.

Here, we report 18 members of HAK/KUP/ KT/ family in genome of *S. tuberosum*. Previous studies have reported that *A. thaliana* has 13 members and rice has 17 members of

KUP family (Gupta *et al.*, 2008). So, a higher number of (18 KUP members) genes are found in *S. tuberosum*. This gene family shows almost the similar domain pattern to its homologues in *A. thaliana*. There were eleven trans-membrane (TM) domains and a "K trans" domain that have a very important role in ion transport. In *A. thaliana*, nine genes were reported as members of Shaker family that are voltage dependent for potassium transport (Lebaudy *et al.*, 2007). These transporters have different responses for voltage potential. One group is hyperpolarized group known as K_{in} (inward rectifying potassium channels). This group comprises AtAKT1, AtAKT5, AtAKT6, AtKAT1 and AtKAT2. Similarly, AtAKT2 is a week inward rectifier (involved in two-way movement of potassium), AtKAT3 is a regulatory sub-unit of inward rectifiers, AtSKOR and AtGORK are outward rectifiers (Véry and Sentenac, 2003). Genome-wide study established that 15 Shaker channels are present in *S. tuberosum* plant. Number of genes (Shaker channels) found in *S. tuberosum* (15 genes) is much higher as compared to other plants like *Vitis vinifera* (9 genes), *A. thaliana* (9 genes) and rice (11 genes) (Pilot *et al.*, 2003; Davies *et al.*, 2006; Amrutha *et al.*, 2007). Contrary to two out-ward rectifying channel genes (*AtSKOR* and *AtGORK*) in *A. thaliana*, three outward rectifying channel genes (*StGORK*, *StSKOR1* and *StSKOR2*) were identified in *S. tuberosum*. It predicts an important role of these outward rectifiers in long distance K^+ transport in stomatal movements and from roots to shoots (Hosy *et al.*, 2003). These outcomes are strengthened by phylogenetic analysis. K^+ channels in *S. tuberosum* share close homology with *A. thaliana* and *S. lycopersicum*. KAT3 is a regulatory protein, which modulates inward rectifying voltage-gated potassium channels (Jeanguenin *et al.*, 2011). A significant increase in its expression (Fig. 6) predicts potentially very important role for this gene.

TPK and Kir-like (KCO) channels are similar to each other at some extent. Initially they were taken as separate groups although they were reported only in *A. thaliana* (Dunkel *et al.*, 2008). TPK1 activity is affected by Ca^{2+} concentration and pH of cytoplasm (Gobert *et al.*, 2007). It is believed that both groups emerged from the same group. All KCO member channels of *A. thaliana* localize on membrane of vacuoles except for AtKCO4. TPK4 is also reported to be present on cell membrane of pollen (Voelker *et al.*, 2010). Some regulatory proteins are reported to be obligatory for the regulation of TPKs in animals. When these regulatory proteins from animal were introduced in plants, TPKs showed down-regulation. HKT/Trk proteins bring adaptations in monocotyledonous and dicotyledonous plants that induce tolerance against salt stress (Almeida *et al.*, 2013). Trk/HKT family, KEA family and HAK/ KUP /KT family of potassium transporters were found similar in the most of fruit plants (Garciadeblas *et al.*, 2002). In fungi, prokaryotes and higher plants, transporters of KUP/HAK/KT family are present which help them in transportation of potassium through K^+/H^+ symport mechanism (Véry and Sentenac, 2003).

Potassium transport system was studied first time in *A. thaliana* and transporters of this system were analyzed for their functional domains. In current study, different topological patterns of domains were analyzed in *S. tuberosum* and *A. thaliana*. Total 77 and 11 different types of conserved domains are present in potassium transporter proteins of *S. tuberosum*. In almost all Shaker family genes of *S. tuberosum* "cNMP binding" domain was found. It predicts that Shaker type K^+-selective channels as the target sites for cyclic nucleotide monophosphates (cNMPs). (Kaplan *et al.*, 2007). The Na^+/H^+ exchanger domain of potassium transporters is a constituent part of transmembrane that perform by swapping one proton (H^+) with one (Na^+) sodium (Li and Fliegel, 2015). Na^+/H^+ exchanger is made up of a hydrophobic membrane that exchanges the protons in cells (Liu *et al.*, 2015). Ankyrin (ANK) repeats possess vital functions of plant cells such as growth/development and breaking biotic/abiotic stresses (Yuan *et al.*, 2013).

A broad spectrum phylogenetic study was carried out for *S. tuberosum* with other plants in which K^+ transport system was well known. So, this phylogenetic study encompasses potassium transport system of multiple plants including *S. tuberosum*, *A. thaliana* and *S. lycopersicum* for tree construction. In this study, expression profiling of *S. tuberosum* potassium transporters was made in two categories. First, expression analysis was made only to find out comparative expression level of K^+ transporters in leaf and stem under normal conditions. Second, expression analysis was made to find stress response under saline conditions. Phylogenetic study of HAK genes in rice suggests that division of monocots/dicots resulted in expansion of HAK family in lineage-specific fashion. Tandem duplication and segmental duplication are two main events that empowered the expansion of this gene family (Yang *et al.*, 2009). In soybean, some random genes were studied with quantitative RT-PCR. In situation of low potassium stress, potassium transport and absorption level were changed (Wang and Wu, 2013).

It has been reported that genes involved in potassium transport play role in plant response to environmental stimuli (Hosy *et al.*, 2003; Sharma *et al.*, 2013; Nieves-Cordones *et al.*, 2016). We studied the expression pattern of Shaker and HKT type genes from *S. tuberosum*. Most of the genes under study were expressed (in leaf or stem) except SKOR type genes. It has previously been reported that these genes possess highly tissue-specific expression (Lebaudy *et al.*, 2007). *AtSKOR* is expressed in roots where it is involved in potassium release into the xylem sap (Gaymard *et al.*, 1998) Gene expression profiles in response to salt stress revealed upregulation of AKT, KAT and HKT type genes (Fig. 6b).

Homologs of these genes were reported to take part in plants stress reaction in various plants (Becker *et al.,* 2003; Liu *et al.,* 2006; Voelker *et al.,* 2010; Osakabe *et al.,* 2013; Hamamoto *et al.*, 2015; Ahmad *et al.*, 2016). Thus, differentially expressing potassium transport related genes are likely to function in plant adaptation to environmental stresses.

***Conclusion*:** Among the three vital nutrients (NPK) for plants, the molecular mechanisms for K^+ acquisition/transport have been least understood. *S. tuberosum* is an important crop, which requires high amounts of K^+ for better growth and development. Therefore, more details are required to have comprehensive insight about molecular mechanisms of potassium homeostasis in this plant. In current study, *S. tuberosum* genome was studied for identification of K^+ transport related genes. Based on sequence and structural homology with potassium transport related genes in *A. thaliana,* total of 43 members were identified and classified as potassium channels (20 members) and transporters (23 members). At protein level, *in-silico* analysis predicted the existence of crucial residues. Furthermore, gene structure and phylogenetic analysis indicated evolutionary conservation of these genes between *S. tuberosum and A. thaliana.* Expression analysis indicated that K^+ transport related genes are potentially involved in abiotic stress. Current study provides the first understanding of molecular entities of potassium transport system in *S. tuberosum,* which will be useful in functional characterization of these genes.

REFERENCES

Ahmad, I., J. Devonshire, R. Mohamed, M. Schultze and F.J.M. Maathuis. 2016. Overexpression of the potassium channel TPKb in small vacuoles confers osmotic and drought tolerance to rice. New Phytol. 209:1040-1048.

Almeida, P., D. Katschnig and A. de Boer. 2013. HKT transporters-state of the art. Int. J. Mol. Sci. 14:20359-20385.

Amrutha, R.N., P.N. Sekhar, R.K. Varshney and P.B.K. Kishor. 2007. Genome-wide analysis and identification of genes related to potassium transporter families in rice (*Oryza sativa* L.). Plant Sci. 172:708-721.

Azeem, F., B. Ahmad, R.M. Atif, M.A. Ali, H. Nadeem, S. Hussain, H. Manzoor, M. Azeem and M. Afzal. 2018. Genome-wide analysis of potassium transport-related genes in chickpea (*Cicer arietinum* L.) and their role in abiotic stress responses. Plant Mol. Biol. Rep. 36: 451-468.

Becker, D., S. Hoth, P. Ache, S. Wenkel, M.R.G.R.G. Roelfsema, O. Meyerhoff, W. Hartung and R. Hedrich. 2003. Regulation of the ABA-sensitive *Arabidopsis* potassium channel gene GORK in response to water stress. FEBS Lett. 554:119-126.

Chapman, K., L. Sparrow, P. Hardman, D. Wright and J. Thorp. 1992. Potassium nutrition of kennebec and russet burbank potatoes in Tasmania: effect of soil and fertiliser potassium on yield, petiole and tuber potassium concentrations, and tuber quality. Aust. J. Exp. Agric. 32: 521-527.

Chérel, I. and I. Gaillard. 2019. The complex fine-tuning of k^+ fluxes in plants in relation to osmotic and ionic abiotic stresses. Int. J. Mol. Sci. 20: 715.

Clarkson, D.T. and J.B. Hanson. 1980. The mineral nutrition of higher plants. Annu. Rev. Plant Physiol. 31:239-298.

Davies, C., R. Shin, W. Liu, M.R. Thomas and D.P. Schachtman. 2006. Transporters expressed during grape berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry potassium accumulation. J. Exp. Bot. 57:3209-16.

Dunkel, M., A. Latz, K. Schumacher, T. Müller, D. Becker and R. Hedrich. 2008. Targeting of vacuolar membrane localized members of the TPK channel family. Front. Plant Sci. 1: 938-949.

Garciadeblas, B., B. Benito and A. Rodríguez-Navarro. 2002. Molecular cloning and functional expression in bacteria of the potassium transporters CnHAK1 and CnHAK2 of the seagrass *Cymodocea nodosa*. Plant Mol. Biol. 50:623-33.

Gaymard, F., G. Pilot, B. Lacombe, D. Bouchez, D. Bruneau, J. Boucherez, N. Michaux-Ferrière, J.B. Thibaud and H. Sentenac. 1998. Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. Cell. 94:647-55.

Gerendás, J., F. Heuser and B. Sattelmacher. 2007. Influence of nitrogen and potassium supply on contents of acrylamide precursors in potato tubers and on acrylamide accumulation in French fries. J. Plant Nutr. 30: 1499-1516.

Gobert, A., S. Isayenkov, C. Voelker, K. Czempinski and F.J.M. Maathuis. 2007. The two-pore channel TPK1 gene encodes the vacuolar K^+ conductance and plays a role in K^+ homeostasis. P. Natl. Acad. Sci. USA. 104:10726-31.

Gupta, A.S., G.A. Berkowitz and P.A. Pier. 1989. Maintenance of photosynthesis at low leaf water potential in wheat : role of potassium status and irrigation history. Plant Physiol. 89: 1358-65.

Gupta, M., X. Qiu, L. Wang, W. Xie, C. Zhang, L. Xiong, X. Lian and Q. Zhang. 2008. KT/HAK/KUP potassium transporters gene family and their whole-life cycle expression profile in rice (*Oryza sativa*). Mol. Genet. Genomics. 280:437-452.

Hamamoto, S., T. Horie, F. Hauser, U. Deinlein, J.I. Schroeder and N. Uozumi. 2015. HKT transporters mediate salt stress resistance in plants: from structure and function to the field. Curr. Opin. Biotech. 32:113-120.

Hosy, E., A. Vavasseur, K. Mouline, I. Dreyer, F. Gaymard, F. Porée, J. Boucherez, A. Lebaudy, D. Bouchez, A.A. Very, T. Simonneau, J.B. Thibaud and H. Sentenac. 2003. The *Arabidopsis* outward K^+ channel GORK is involved in regulation of stomatal movements and plant transpiration. P. Natl. Acad. Sci. USA. 100:5549-54.

Jeanguenin, L., C. Alcon, G. Duby, M. Boeglin, I. Chérel, I. Gaillard, S. Zimmermann, H. Sentenac and A.A. Véry. 2011. AtKC1 is a general modulator of *Arabidopsis* inward Shaker channel activity. Plant J. 67: 570–82.

Kang, W., M. Fan, Z. Ma, X. Shi and H. Zheng. 2014. Luxury absorption of potassium by potato plants. Am. J. Potato Res. 91:573-578.

Kaplan, B., T. Sherman and B. Fromm. 2007. Cyclic nucleotide-gated channels in plants. FEBS Lett. 581:2237-2246

Lacombe, B., G. Pilot, E. Michard, F. Gaymard, H. Sentenac and J.B. Thibaud. 2000. A shaker-like $K^{(+)}$ channel with weak rectification is expressed in both source and sink phloem tissues of *Arabidopsis*. Plant Cell. 12: 837-851.

Laskowski, M., B. Augustynek, B. Kulawiak, P. Koprowski, P. Bednarczyk, W. Jarmuszkiewicz and A. Szewczyk. 2016. What do we not know about mitochondrial potassium channels? Biochim. Biophys. Acta. 1857:1247-1257.

Lebaudy, A., A. Vavasseur, E. Hosy, I. Dreyer, N. Leonhardt, J.-B.Thibaud, A.A. Véry, T. Simonneau and H. Sentenac. 2008. Plant adaptation to fluctuating environment and biomass production are strongly dependent on guard cell potassium channels. P. Natl. Acad. Sci. USA. 105:5271-5276.

Lebaudy, A., A.A. Véry and H. Sentenac. 2007. K^+ channel activity in plants: genes, regulations and functions. FEBS Lett. 581:2357-66.

Lee, S.I., M. Muthusamy, M. A. Nawaz, J. K. Hong, M. Lim, J. A. Kim and M. Jeong. 2019. Genome-wide analysis of spatiotemporal gene expression patterns during floral organ development in *Brassica rapa*. Mol. Genet. Genomics. 294:1403-1420.

Leigh, R.A. 2001. Potassium homeostasis and membrane transport. J. Plant Nutr. Soil Sci. 164: 193-198.

Li, X. and L. Fliegel. 2015. A novel human mutation in the SLC9A1 gene results in abolition of Na^+/H^+ exchanger activity. PLOS ONE. 10: e0119453.

Li, W.Y., X. Wang, R. Li, W.Q. Li and K.M. Chen. 2014. Genome-wide analysis of the NADK gene family in plants. PLOS ONE. 9:e101051.

Lindhauer, M.G. and M.A.R. De Fekete. 1990. Starch synthesis in potato (*Solanum tuberosum*) tubers: Activity of selected enzymes in dependence of potassium content in storage tissue. Plant Soil. 124:291-295.

Liu, Y., A. Basu, X. Li and L. Fliegel. 2015. Topological analysis of the Na^+/H^+ exchanger. Biochim. Biophys. Acta Biomembr. 1848:2385-2393.

Liu, K., L. Li and S. Luan, 2006. Intracellular K^+ sensing of SKOR, a Shaker-type K^+ channel from *Arabidopsis*. Plant J. 46:260-268.

Liu, J., X. Pang, Y. Cheng, Y. Yin, Q. Zhang, W. Su, B. Hu, Q. Guo, S. Ha, J. Zhang and H. Wan. 2018. The Hsp70 gene family in *Solanum tuberosum*: Genome-wide identification, phylogeny and expression patterns. Sci. Rep. 8:16628.

Maathuis, F.J.M. 2009. Physiological functions of mineral macronutrients. Curr. Opin. Plant Biol. 12:250-8.

Mäser, P., S. Thomine, J.I. Schroeder, J.M. Ward, K. Hirschi, H. Sze, I.N. Talke, A. Amtmann, F.J. Maathuis, D. Sanders, J.F. Harper, J. Tchieu, M. Gribskov, M.W. Persans, D.E. Salt, S.A. Kim and M.L. Guerinot, 2001. Phylogenetic relationships within cation transporter families of Arabidopsis. Plant Physiol. 126:1646-67.

Mohr, R.M. and D.J. Tomasiewicz. 2012. Effect of rate and timing of potassium chloride application on the yield and quality of potato (*Solanum tuberosum* L. 'Russet Burbank'). Can. J. Plant Sci. 92:783-794.

Moinuddin, K. Singh and S.K. Bansal. 2005. Growth, yield and economics of potato in relation to progressive application of potassium fertilizer. J. Plant Nutr. 28:183-200.

Moinuddin, K. Singh, S.K. Bansal and N.S. Pasricha. 2004. Influence of graded levels of potassium fertilizer on growth, yield and economic parameters of potato. J. Plant Nutr. 27: 239-259.

Nawaz, M.A., H.M. Rehman, M. Imtiaz, F.S. Baloch, J. D. Lee, S. H. Yang, S. I. Lee and G. Chung. 2017. Systems identification and characterization of cell wall reassembly and degradation related genes in *Glycine max* (L.) merill, a bioenergy legume. Sci.Rep. 7:10862.

Nieves-Cordones, M., V. Martínez, B. Benito and F. Rubio. 2016. Comparison between arabidopsis and rice for main pathways of K^+ and Na^+ uptake by roots. Front. Plant Sci. 7: 992.

Osakabe, Y., K. Yamaguchi-Shinozaki, K. Shinozaki and L.S.P. Tran. 2013. Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. J. Exp. Bot. 64:445-458.

Pilot, G., F. Gaymard, K. Mouline, I. Chérel and H. Sentenac. 2003. Regulated expression of Arabidopsis shaker K^+ channel genes involved in K^+ uptake and distribution in the plant. Plant Mol. Biol. 51:773-87.

Pilot, G., R. Pratelli, F. Gaymard, Y. Meyer and H. Sentenac. 2003. Five-group distribution of the Shaker-like K^+ channel family in higher plants. J. Mol. Evo. 56:418–434.

Rehman H.M., M. A. Nawaz, Z. H. Shah, I. Daur, S.

Khatoon, S. H. Yang and G. Chung. 2017. In-depth genomic and transcriptomic analysis of five K^+ transporter gene families in soybean confirm their differential expression for nodulation. Front. Plant Sci. 8:804.

Reintanz, B., A. Szyroki, N. Ivashikina, P. Ache, M. Godde, D. Becker, K. Palme and R. Hedrich. 2002. AtKC1, a silent *Arabidopsis* potassium channel alpha-subunit modulates root hair K^+ influx. P. Natl. Acad. Sci. USA. 99:4079-84.

Schroeder, J.I., J.M. Ward and W. Gassmann. 1994. Perspectives on the physiology and structure of inward-rectifying K^+ channels in higher plants: biophysical implications for K^+ uptake. Annu. Rev. Biophys. Biomol. Struct. 23:441-71.

Sharma, T., I. Dreyer and J. Riedelsberger. 2013. The role of K^+ channels in uptake and redistribution of potassium in the model plant *Arabidopsis thaliana*. Front. Plant Sci. 4:224.

Shi, H., M. Ishitani, C. Kim and J.K. Zhu. 2000. The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. P. Natl. Acad. Sci. USA. 97:6896-6901.

Tein, B., K. Kauer, V. Eremeev, A. Luik, A. Selge and E. Loit. 2014. Farming systems affect potato (*Solanum tuberosum* L.) tuber and soil quality. Field Crops Res. 156:1-11.

Trehan, S.P., S.K. Roy and R.C. Sharma. 2001. Potato variety differences in nutrient deficiency symptoms and responses to NPK. Better Crops Int. 15:18-21.

Véry, A.A., M. Nieves-Cordones, M. Daly, I. Khan, C. Fizames and H. Sentenac. 2014. Molecular biology of K^+ transport across the plant cell membrane: What do we learn from comparison between plant species? J. Plant Physiol. 171:748-769.

{Received 10 Oct 2019; Accepted 9 Aug 2020. Published (online) 11 Jan 2021}

Véry, A.A. and H. Sentenac. 2003. Molecular mechanisms and regulation of K^+ transport in higher plants. Annu. Rev. Plant Biol. 54:575-603.

Voelker, C., J.L. Gomez-Porras, D. Becker, S. Hamamoto, N. Uozumi, F. Gambale, B. Mueller-Roeber, K. Czempinski and I. Dreyer. 2010. Roles of tandem-pore K^+ channels in plants-a puzzle still to be solved. Plant Biol. 12:56-63.

Walker, D.J., R.A. Leigh and A.J. Miller. 1996. Potassium homeostasis in vacuolate plant cells. P. Natl. Acad. Sci. USA. 93:10510-14.

Wang, Y. and W.H. Wu. 2013. Potassium transport and signaling in higher plants. Annu. Rev. Plant Biol.. 64:451-476.

Ward, J.M., P. Mäser and J.I. Schroeder. 2009. Plant ion channels: gene families, physiology, and functional genomics analyses. Annu. Rev. Physiol. 71:59-82.

Westermann, D.T., D.W. James, T.A. Tindall and R.L. Hurst. 1994. Nitrogen and potassium fertilization of potatoes: sugars and starch. Am. Potato J. 71: 433-453.

Yang, Z., Q. Gao, C. Sun, W. Li, S. Gu and C. Xu. 2009. Molecular evolution and functional divergence of HAK potassium transporter gene family in rice (*Oryza sativa* L.). J. Genet. Genomics. 36:161-172.

Yasmeen, E., M. Riaz1, S. Sultan, F. Azeem, A. Abbas, K. Riaz and M. A. Ali. 2016. Genome-wide analysis of trihelix transcription factor gene family in *Arabidopsis thaliana.* Pak. J. Agric. Sci. 53:439-448.

Yuan, X., S. Zhang, X. Qing, M. Sun, S. Liu, H. Su, H. Shu and X. Li. 2013. Superfamily of ankyrin repeat proteins in tomato. Gene. 523:126–136.

Zörb, C., M. Senbayram and E. Peiter. 2014. Potassium in agriculture–status and perspectives. J. Plant Physiol. 171:656-669.