IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF KUP/HAK/KT GENE FAMILY IN DAUCUS CAROTA (WILD CARROT)

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Abstract. Potassium is essential macronutrient of plant. It is uptaken by plant through channels and transporters. Plants specie genomes contain a number of KUP/HAK/KT transporters having the primary function to mediate K^+ fluxes. In this study, we have identified 13 gene members of KUP/HAK/KT transporter gene family. In most of the plant species, these genes have been characterized but uncharacterized in *D. carota*. This study has been done to identify KUP/HAK/KT gene family in *D. carota* plant species to study its phylogeny. This gene family is important for potassium uptake and plays an important role in translocation, osmotic potential regulation, plant development and growth. Different tools like MEGA 7.0.21, pfam, SMART and NCBI-BLASTp has been used to characterize the gene family. This study covers the phylogeny and evolution of KUP/HAK/KT transporters in *D. carota* with reference to *A. thaliana*.
Keywords: *K^+ ion, A. thaliana, D. carota, KUP/HAK/KT, phylogeny*

Introduction

Potassium ion (K+) being the essential macronutrient is found very important for various aspects of plant life. In plants, potassium concentration may reach up to 8% of dry cell weight. Potassium is the most plentiful cation that maintains the electrical charge balance and transport of sugar and nitrate in plant cells (Li et al., 2018). It may act as protein synthesis stabilizer or enzyme activator. It is involved in metabolic and physiological processes, such as photosynthesis, cellular osmoregulation and respiration (Liang et al., 2020). Potassium deficiency highly affects the plant growth and development as they pertains many abiotic stresses like salth stress, drought stress and cold stress (Xie et al., 2020). Potassium is found in bulk amount in earth crust and the uptake of potassium from soil is not as good as it to be because potassium is not in ionic form. The concentration of potassium at root surface is mostly lower than in bulk soil solution which may fall down to µM range. To enhance the potassium uptake, plant has developed various mechanisms for potassium acquisition with >1000-fold concentration gradients (Grabov, 2007).

Usually there are two mechanisms for potassium uptake in plants. These are high affinity transporter system via potassium transporters and low affinity potassium transporters via potassium channels. On the basis of function and structure, potassium transporters are divided into five families: (1) shaker channels; (2) TDK (tandem pore-k+); (3) HAK (high affinity K+) /KUP (K+ uptake permease)/KT (K+ transporter); (4)

HKT transporters; and (5) CPAs (cation proton-antiporters). KUP/HAK/KT is the largest and widely distributed family in bacteria, fungi, and plants (*Table 1*). The homology with bacterial KUP and fungal HAK transporters show that the plants KUP/HAK/KT transporter members AtKUP1 and HvHAK1 were first cloned from Arabidopsis and Barley. Comparative genomic analysis showed that 13, 27 and 27 KUP/HAK/KT genes were identified in Arabidopsis, Rice and Maize respectively (Feng et al., 2020). Mutation analysis has shown that the 8th transmembrane domain and C-terminus of KUP/HAK/KT gene family has a key role in determining the K+ transport capacity. A series of KUP/HAK/KT genes were identified in past decades and a number of physiological roles of these genes were characterized in plant species. At present, KUP/HAK/KT genes have been identified in other species like poplar (Populus trichocarpa), tomato (Solanum lycopersicum), pear (Pyrus bretschneideri) and soybean (Glycine max) (MaoNi et al., 2017).

Table 1. *Some major functions related to KUP/HAK/KT.*

Category	Citation
Regulation of cell size, auxin distribution or osmotic stress adaptation.	Very et al. (2014)
Mediate K^+ fluxes.	Ahn et al. (2004)
Subcellular localization (plasma membrane, tonoplast or other endomembranes).	Osakabe et al. (2013); Rigas et al. (2001)
Expression patterns (root meristems, vascular tissues, guard cells, fruits or specialized organs such as flytraps).	Osakabe et al. (2013); Scherzer et al. (2015)

Expression of KUP/HAK/KT is regulated by K+ starvation, abiotic stresses and phytohormones including ABA, cytokinin, ethylene and NAA. These KUP/HAK/KT proteins show a great variety of subcellular localization in tonoplast, plasma membrane and endoplasmic reticulum (*Figure 1*). This gene family have role in plant growth and development. The ubiquitous nature of these genes shows that they are very important for plants as they help them in survival in potassium poor environment and in nutrients uptake (Grabov, 2007). Daucus carota is a popular taproot vegetable, a plant rich in many bioactive pigments like carotenoids and lutein, cultivated all over the world (Fikselová et al., 2008). Carotenoid found in flowers and fruits of plant playing major role in photosystems of higher plants. It can enhance the pollination ability of plant and seed dispersal activity. It accumulates in carrot taproot being the major nutrient of carrot taproot and also contributes to carrot taproot color (Ma et al., 2017). The analysis is done in comparison with the features of this gene family in Arabidopsis. This comprehensive study may provide important information about lineage of KUP/HAK/KT gene family in Daucus carota to other species.

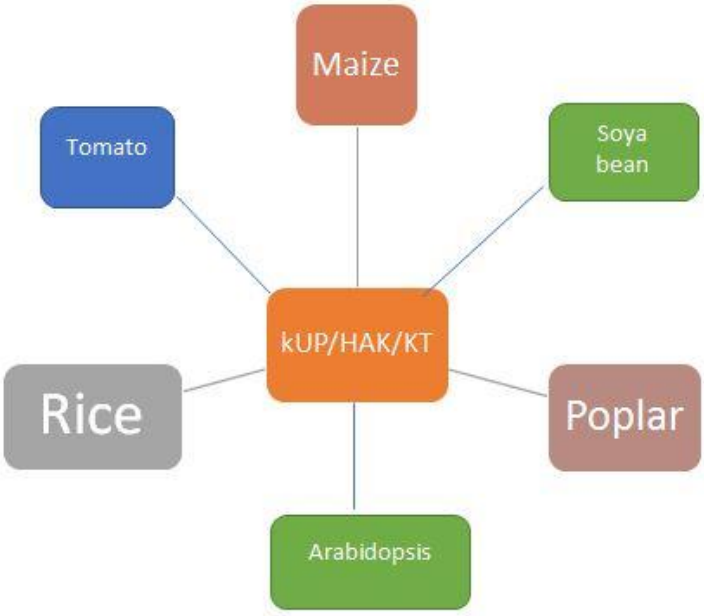


Figure 1. *Genome wide analysis conducted in plants.*

Materials and Methods

Identification and evolutionary analysis of KUP/HAK/KT genes in D. carota

The KUP/HAK/KT potassium transporting genes of *D. carota* were retrieved from NCBI database. The protein sequence of KUP/HAK/KT potassium transporting genes was retrieved from already known protein sequence of potassium transporter genes in *A. thaliana*. Protein blast (Blastp) was performed against genome database and GenBank to retrieve the protein and DNA sequences of *D. carota*. *A. thaliana* gene sequence was used as query sequence against non-redundant protein sequence of *D. carota*. The retrieved data was manually checked to eradiacte the non-identical or false positives. The sequences having similarity less than 60% were discarded. Both candidate protein sequences were examined for conserved domains using pfam domain (El-Gebali et al., 2019; Bateman et al., 2002) and SMART (Letunic et al., 2012) and sequences were filtered to get finer collection of non-redundant gene sequences present in *D. carota*. This process was done to eliminate the sequences having no conserved domains. Multiple sequence alignment was done to confirm the conserved domains in the target plant species *D. carota*. The full length KUP/HAK/KT protein from *A. thaliana* and *D. carota* were aligned in MEGA 7.0.21 software and neighbor joining evolutionary tree were constructed with bootstrapping value of 1000 for phylogenetic analysis (Hall, 2013; Kumar et al., 2008). NCBI database was used to recover all genomic information of these potassium transporting genes like exon numbers, chromosomal location of gene, proteina and RNA accession numbers, amino acid length and ORF length

Gene structure analysis

To predict gene structure and their schematic representation, an online freely available server called Gene Structure Display server (GSDS) was used (Hu et al., 2015).

Trace the different restriction maps and multiple sequence alignment

Different restriction maps were generated of each sequence by using SerialCloner2-6 software (Amara, 2017). It generated different graphic maps of each sequence of identifies gene sequence. For multiple sequence alignment, a web-based tool called ClustalW was used to align the sequence of target specie (Thompson et al., 2003).

Results and Discussion

Identification of KUP/HAK/KT in D. Carota and conserved domain profile

The KUP/HAK/KT protein sequences of *A. thaliana*, *C. arietinum* and *O. sativa* were retrieved from NCBI. The retrieved AtKUP/HAK/KT sequences were used in NCBI-BLAST search against *D. carota* sequence. After making all queries, 13 members of KUP/HAK/KT gene family were identified out of more than 100 sequences and named as:

(1) DcKUPHAKKT2,
(2) DcKUPHAKKT3,
(3) DcKUPHAKKT5.1,
(4) DcKUPHAKKT5.2,
(5) DcKUPHAKKT6.1
(6) DcKUPHAKKT6.2,
(7) DcKUPHAKKT6.3,
(8) DcKUPHAKKT7.1,
(9) DcKUPHAKKT7.1,
(10) DcKUPHAKKT8,
(11) DcKUPHAKKT10,
(12) DcKUPHAKKT11,
(13) DcKUPHAKKT12.

The nomenclature was proposed according to functions of homologous *Arabidopsis* genes. The evolution process tells that the similar structure and similar function of genes arise through replication event and gene duplication is the process which leads towards evolution (Shen and Yuan, 2021). The analysis revealed that *D. carota* contains 13 members of KUP/HAK/KT gene family as in *A. thaliana*. The AtHAK2, AtHAK6 and AtHAK11 are responsible for salinity response because their expression gets affected when salt concentration is increased (Li et al., 2018). Salinity is caused by reduced Na+ controlled by AtHKT1 and AtHAK5 which is regulated by external K+ concentration (Wang et al., 2015).

The gene location on the chromosome from top to bottom was analysed. From the analysis, it was analyzed that all of the genes are present on chromosomes having number of 1-9 except 5 and 8. No gene is present on chromosome number 5 and 8. Chromosome location is helpful to find the location of gene on chromosome. Similarly, the exon count analysis reveals that its average range is 8-11, four genes have 9 exons, 4 genes have 8 exons, two genes contain 10 exons and 1 gene contains 11 exons. The presence of K_trans domain in the identified sequence was confirmed using an online tool pfam (El-Gebali et al., 2019) and SMART (Schultz et al., 2000). Pfam and SMART

server searches revealed that the most conserved domain in all sequences is K_trans which show that it belongs to potassium transporter gene family (*Table 2*).

Table 2. *Whole genomic information about identified genes.*

PN	GL	PAN	RNAAN	E	CN	OL	AAL	SGL	CDPS
DcKUPHAKKT2	DCAR_019813	KZM92822.1	XM_017399321.1	9	6	2349	782	36563422	K trans
DcKUPHAKKT3	DCAR_014456	XP_017247390.1	XM_017391901.1	9	4	2349	782	21753814	K_trans
DcKUPHAKKT5.1	DCAR_007762	XP_017231954.1	XM_017376465.1	8	2	2277	758	36900730	K_trans
DcKUPHAKKT5.2	DCAR_023264	XP_017255168.1	XM_017399679.1	9	6	2475	824	196829	K_trans
DcKUPHAKKT6.1	DCAR_007096	XP_017232096.1	XM_017376607.1	8	2	2403	800	31460152	K_trans
DcKUPHAKKT6.2	DCAR_031093	XP_017224518.1	XM_017369029.1	9	9	2340	779	32656696	K_trans
DcKUPHAKKT6.3	DCAR_024139	XP_017218890.1	XM_017363401.1	8	7	2343	780	9796649	K_trans
DcKUPHAKKT7.1	DCAR_001786	XP_017225142.1	XM_017369653.1	10	1	2535	844	20502453	K_trans
DcKUPHAKKT7.2	DCAR_021857	XP_017258440.1	XM_017402951.1	10	6	2532	843	19110565	K_trans
DcKUPHAKKT8	DCAR_011216	XP_017241396.1	XM_017385907.1	8	3	2325	774	33669288	K_trans
DcKUPHAKKT10	DCAR_001295	XP_017229797.1	XM_017374308.1	8	1	2364	787	4149046	K_trans
DcKUPHAKKT11	DCAR_007784	XP_017236764.1	XM_017381275.1	11	2	2352	783	37055812	K_trans
DcKUPHAKKT12	DCAR_007095	XP_017235837.1	XM_017380348.1	9	2	2526	841	31449412	K_trans

**PN= Proposed Names; GL=Gene Locus; PAN=Protein Accession No.; RNAAN=RNA Accession No.; E=Exons; CN=Chromosome No.; OL=Orf Length; AAL=Amino Acid Length; SGL= Start of Genomic Location; CDPS= Conserved Domains in Protein Sequence*

Phylogenetic analysis

To characterize the evolutionary relationship between KUP/HAK/KT gene family of *A. thaliana*, *C. arietinum*, *O. sativa* and *D. carota*, MEGA 7.0.21 was used to generate the rooted tree by the neighbor joining method by the alignment of KUP/HAK/KT from both species (*Figure 2*). It is concluded that the KUP/HAK/KT gene family is common in almost all plant species. The evolutionary tree showed a close relationship between *O. sativa*, *C. arietinum*, *A. thaliana* and *D. carota*. This gene family helps in mediating the inward transport of K+ from Dilute K+ solutions as shown in OsHAK1 and OsHAK5 transporters (Santa-María et al., 2018). As the KUP/HAK/KT transporters are multi gene family. For example, Arasbidopsis has 13 KUP/HAK/KT genes and Oryza sativa has 27 KUP/HAK/KT genes in total (Gierth et al., 2005). Investigation shows that KUP/HAK/KT can be divided into four clusters which is confirmed by the phylogenetic analysis of full-length sequences of KUP/HAK/KT transporter gene family. The most studied gene members of KUP/HAK/KT transporter gene family belongs to clusterI and clusterII (Gupta et al., 2008). ClusterI gene members play essential role in potassium uptake when potassium concentration is low. So, it may be designated as high affinity potassium uptake transporters in Arabidopsis which may be have the same role in *D. carota* (Grabov, 2007). Phylogenetic analysis results indicate that evolutionary branches were constant with their assigned gene names. Introns have

been found essential traits of eukaryotic gene families during the evolutionary process of multiple gene families (Liang et al., 2020)

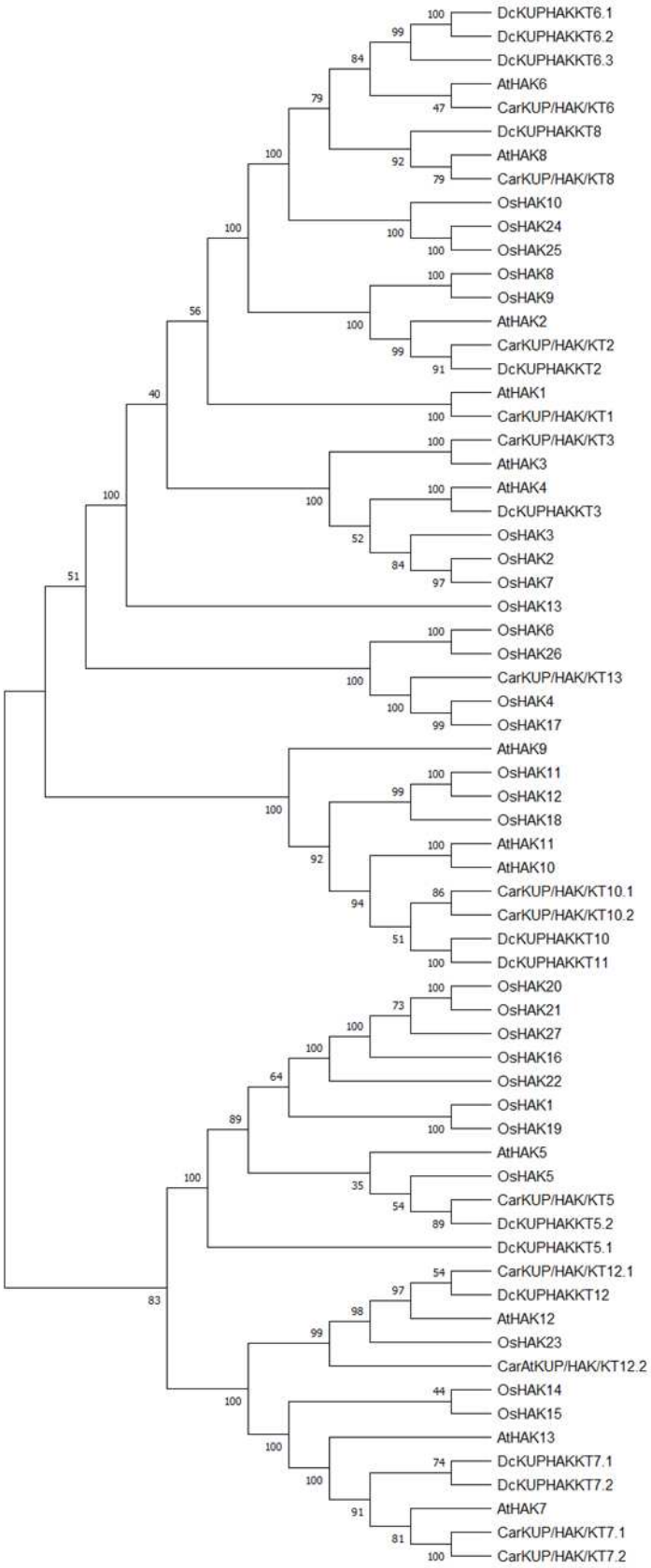


Figure 2. *Phylogenetic analysis of A. thaliana, O. sativa and D. carota. The phylogenetic tree of potassium transporter genes was constructed with MEGA 7 using Neighbor-Joining method with 1,000 bootstrap replicates.*

Gene structure analysis

Usually it is found that the position of introns/exons in orthologous genes are commonly well conserved in evolutionary times, but the structure of introns/exons is comparatively less conserved in paralogus genes. To study the structural differences of KUP/HAK/KT gene family we used Gene Structure Display tool which analyzed the arrangements of introns and exons by comparing CDS sequences and Genomic sequences (Aslam et al., 2019). It shows that DcKUPHAKKT5.2 has more exonic regions than others. In contrast the longest intronic regions are present in DcKUPHAKKT10 and DcKUPHAKKT7.2 gene. The highest exon count is 11 in DcKUPHAKKT11 (*Figure 3*). It has been reported that AtKUP2 gene plays important role in maintaining cell size. Because K+ is the major solute in cell so any diminishing effect to the potassium homeostasis leads to the weakened cell turgor which causes reduction in cell size. Similarly, the gene in *D. carota* will also affect the cell size (Grabov, 2007).

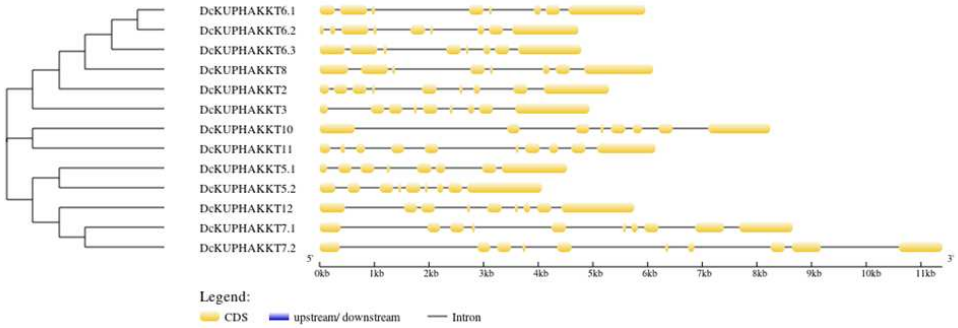
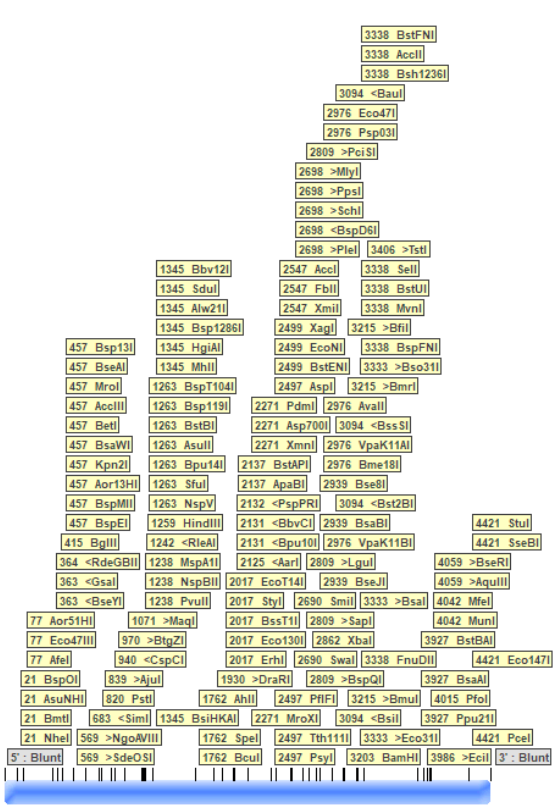
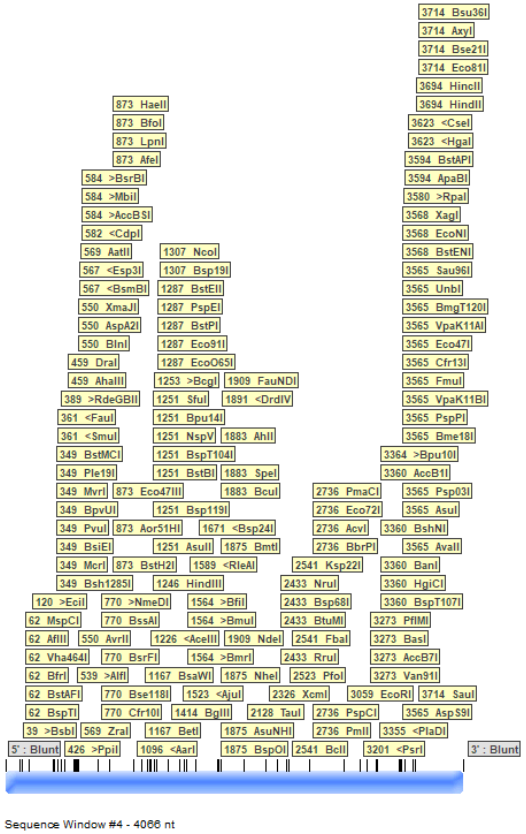


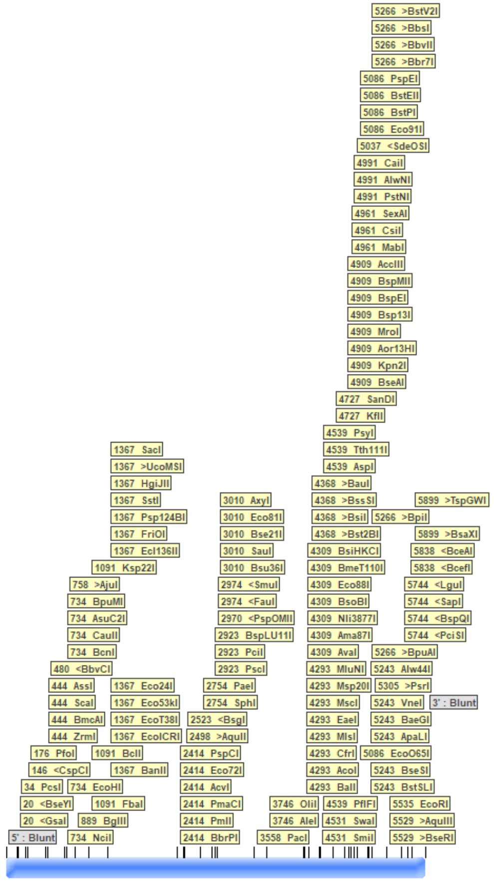
Figure 3. *The structure of introns/exons in gene structure analysis.*

Mapping restriction sites

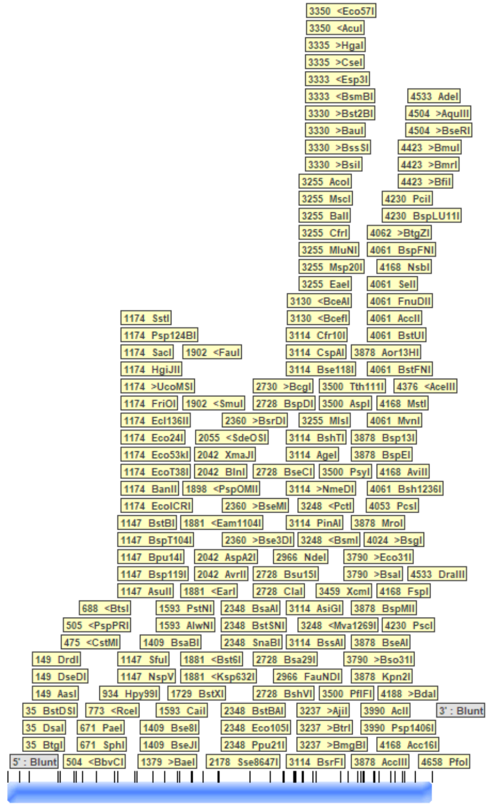
To find the restriction sites of identified sequence, SerialCloner2-6 was used. The genomic sequence of the entire 13 gene members were saved in FASTA format and then each sequence was submitted to SerilCloner2-6 software and a graphic map of restriction sites were generated (*Figure 4*).





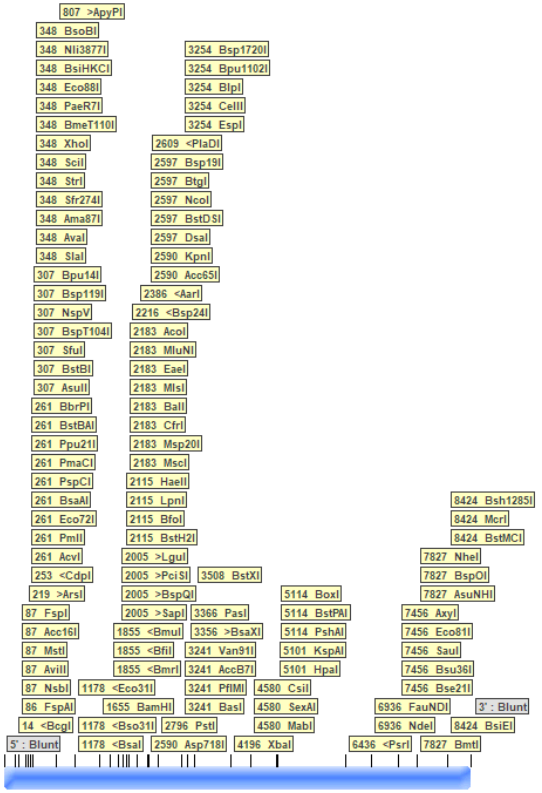


Sequence Window #6 - 5960 nt

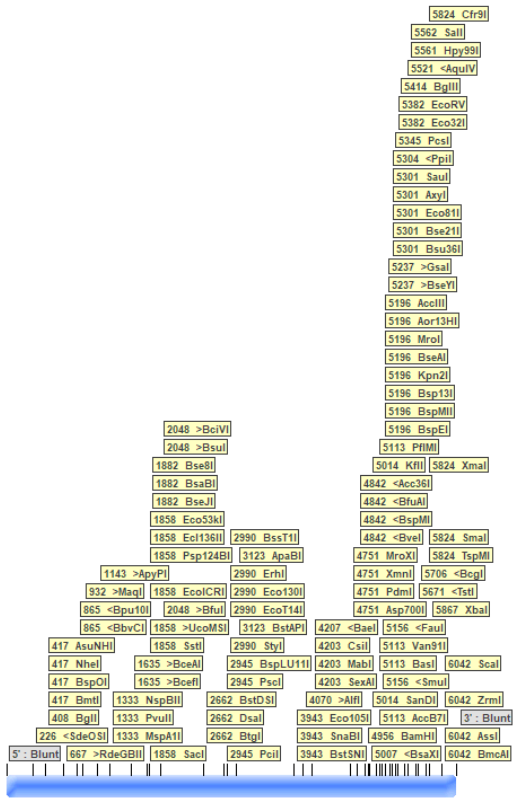


Sequence Window #7 - 4739 nt

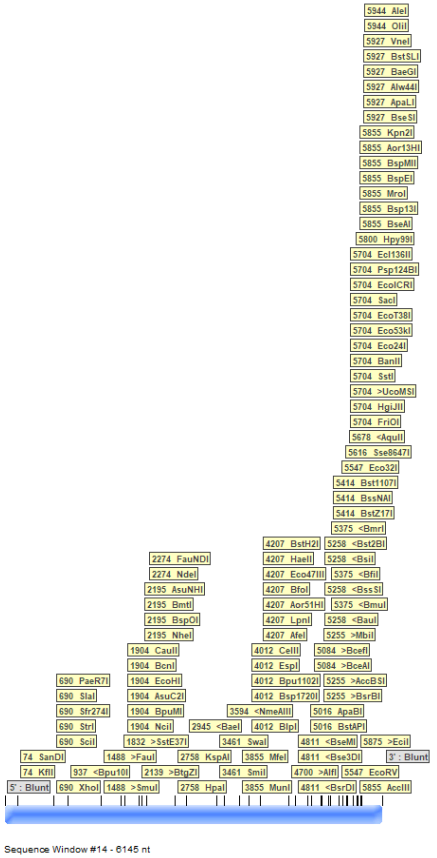
Sequence Window #8 - 4789 nt



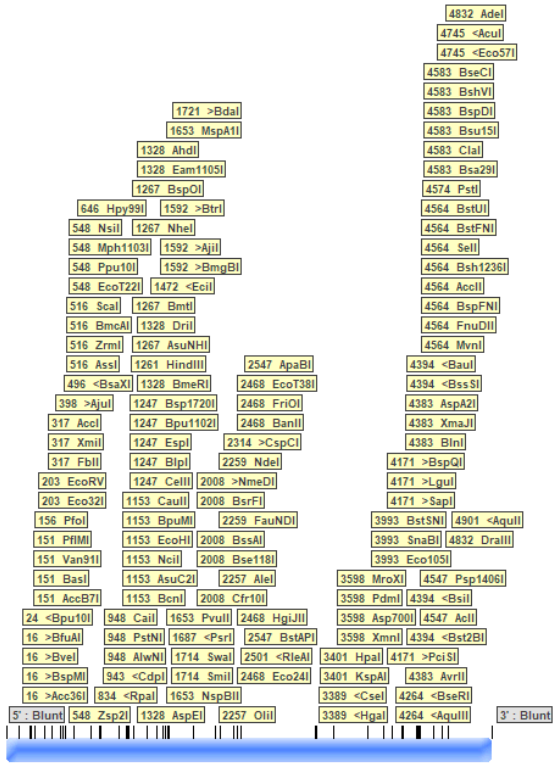
Sequence Window #9 - 8657 nt



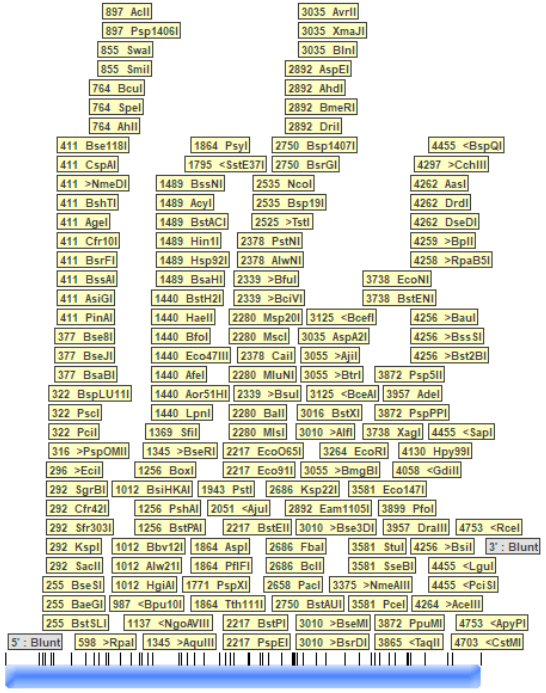
Sequence Window #13 - 6572 nt



Sequence Window #14 - 6145 nt



Sequence Window #2 - 5274 nt



Untitled Sequence #1 - 4938 nt

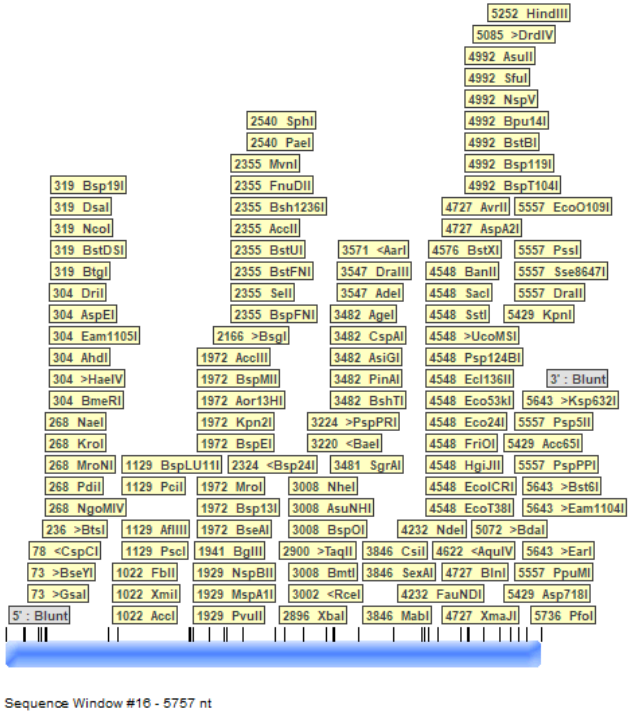


Figure 4. Graphic map of restriction sites for 13 gene members.

Conclusion

Potassium is the macronutrient of plants, it is uptaken by plants through various mechanism. High affinity potassium uptake through Potassium transporter is one of them. This study reported 13 gene members of KUP/HAK/KT potassium transporter gene family in *D. carota* (wild carrot). This study covers the genomic information, domain prediction, gene structure prediction and phylogenetic analysis. These genes showed a close phylogenetic relationship with *A. thaliana*, *C.arietinum* and *O. sativa*. This gene family is important for potassium transport. Different software like NCBI-BLASTp, pfam, SMART and SerialCloner 2-6 was used. Gene structure display server software was also used to conduct gene structure analysis. MEGA 7.0.21 software was used to make the phylogenetic tree for evolutionary study.

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Conflict of interest

There are no conflict of intrest involve any parties in this research study.

REFERENCES

[1] Amara, A.A.A.F. (2017): α Globin Hemoglobinopathy as A Case Study: Mutants and Prototype Mutants: Are They Absent α0, Reduced α+ or Recombinant? – Cellular & Molecular Medicine 3(2): 14p.

[2] Aslam, M., Fakher, B., Jakada, B.H., Zhao, L., Cao, S., Cheng, Y., Qin, Y. (2019): Genome-Wide Identification and Expression Profiling of CBL-CIPK Gene Family in Pineapple (Ananas comosus) and the Role of AcCBL1 in Abiotic and Biotic Stress Response. – Biomolecules 9(7): 19p.

[3] Bateman, A., Birney, E., Cerruti, L., Durbin, R., Etwiller, L., Eddy, S.R., Griffiths-Jones, S., Howe, K.L., Marshall, M., Sonnhammer, E.L.L. (2002): The Pfam Protein Families Database. Nucleic Acids Research 30(1): 276-280.

[4] El-Gebali, S., Mistry, J., Bateman, A., Eddy, S.R., Luciani, A., Potter, S.C., Qureshi, M., Richardson, L.J., Salazar, G.A., Smart, A., Sonnhammer, E.L.L., Hirsh, L., Paladin, L., Piovesan, D., Tosatto, S.C.E., Finn, R.D. (2019): The Pfam protein families database in 2019. – Nucleic Acids Research 47(D1): D427–D432.

[5] Feng, X., Wang, Y., Zhang, N., Wu, Z., Zeng, Q., Wu, J., Wu, X., Wang, L., Zhang, J., Qi, Y. (2020): Genome-wide systematic characterization of the HAK/KUP/KT gene family and its expression profile during plant growth and in response to low-K+ stress in Saccharum. – BMC Plant Biology 20(1): 1-17.

[6] Fikselová, M., Šilhár, S., Mareček, J., Frančáková, H. (2008): Extraction of carrot (Daucus carota L.) carotenes under different conditions. – Czech Journal of Food Sciences 26(4): 268-274.

[7] Gierth, M., Mäser, P., Schroeder, J.I. (2005): The Potassium Transporter AtHAK5 Functions in K+ Deprivation-Induced High-Affinity K+ Uptake and AKT1 K+ Channel Contribution to K+ Uptake Kinetics in Arabidopsis Roots. – Plant Physiology 137(3): 1105-1114.

[8] Grabov, A. (2007): Plant KT/KUP/HAK Potassium Transporters: Single Family – Multiple Functions. – Annals of Botany 99(6): 1035-1041.

[9] Gupta, M., Qiu, X., Wang, L., Xie, W., Zhang, C., Xiong, L., Lian, X., Zhang, Q. (2008): KT/HAK/KUP potassium transporters gene family and their whole-life cycle expression profile in rice (Oryza sativa). – Molecular Genetics and Genomics: MGG 280(5): 437-452.

[10] Hall, B.G. (2013): Building Phylogenetic Trees from Molecular Data with MEGA. – Molecular Biology and Evolution 30(5): 1229-1235.

[11] Hu, B., Jin, J., Guo, A.Y., Zhang, H., Luo, J., Gao, G. (2015): GSDS 2.0: An upgraded gene feature visualization server. – Bioinformatics 31(8): 1296-1297.

[12] Kumar, S., Nei, M., Dudley, J., Tamura, K. (2008): MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. – Briefings in Bioinformatics 9(4): 299-306.

[13] Letunic, I., Doerks, T., Bork, P. (2012): SMART 7: Recent updates to the protein domain annotation resource. – Nucleic Acids Research 40(D1): D302-D305.

[14] Li, W., Xu, G., Alli, A., Yu, L. (2018): Plant HAK/KUP/KT K+ transporters: Function and regulation. – Seminars in Cell & Developmental Biology 74: 133-141.

[15] 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 Research International 12p.

[16] Ma, J., Xu, Z., Tan, G., Wang, F., Xiong, A. (2017): Distinct transcription profile of genes involved in carotenoid biosynthesis among six different color carrot (Daucus carota L.) cultivars. – Acta Biochimica et Biophysica Sinica 49(9): 817-826.

[17] MaoNi, C., QingYu, W., JinYu, Z., ZhiYong, Z., Jie, D., YaXin, Y. (2017): Identification and expression analysis of KUP/HAK/KT potassium transporter gene family in soybean [Glycine max (L.) Merr.]. – Acta Botanica Boreali-Occidentalia Sinica 37(2): 239-249.

[18] Santa-María, G.E., Oliferuk, S., Moriconi, J.I. (2018): KT-HAK-KUP transporters in major terrestrial photosynthetic organisms: A twenty years tale. – Journal of Plant Physiology 226: 77-90.

[19] Schultz, J., Copley, R.R., Doerks, T., Ponting, C.P., Bork, P. (2000): SMART: A web-based tool for the study of genetically mobile domains. – Nucleic Acids Research 28(1): 231-234.

[20] Shen, C., Yuan, J. (2021): Genome-Wide Investigation and Expression Analysis of K+-Transport-Related Gene Families in Chinese Cabbage (Brassica rapa ssp. Pekinensis). – Biochemical Genetics 59(1): 256-282.

[21] Thompson, J.D., Gibson, T.J., Higgins, D.G. (2003): Multiple Sequence Alignment Using ClustalW and ClustalX. – Current Protocols in Bioinformatics 00(1): 22p.

[22] Wang, Q., Guan, C., Wang, P., Lv, M.L., Ma, Q., Wu, G.Q., Bao, A.K., Zhang, J.L., Wang, S.M. (2015): AtHKT1;1 and AtHAK5 mediate low-affinity Na+ uptake in Arabidopsis thaliana under mild salt stress. – Plant Growth Regulation 75(3): 615-623.

[23] Xie, Q., Ma, L., Tan, P., Deng, W., Huang, C., Liu, D., Lin, W., Su, Y. (2020): Multiple High-Affinity K+ Transporters and ABC Transporters Involved in K+ Uptake/Transport in the Potassium-Hyperaccumulator Plant Phytolacca acinosa Roxb. – Plants 9(4): 19p.