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PHYLOGENETIC ANALYSIS OF PUTATIVE PLANT POTASSIUM CHANNELS BELONGING TO TPK FAMILY

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The bioinformatic sequence hunting of putative TPK-cahennels and summarizing of phylogenetic diversity TPK family were conducted. By application of wide range of bioinformatic tools and online services the comparative analysis and aminoacid sequence alignment of putative TPK-channels were performed. After secondary analysis of pore domain architecture the 157 sequences of TPK-channels from different plants species were selected. The further cladististical analysis of selected TPK sequences exhibited division of TPK family on 4 different clades.

Key words: *two-pore potassium channels (TPK), TPK-channel domain architecture, cladistical analysis, phylogenetic tree*

The members of two-pore potassium channels (TPK) family have wide range of physiological functions. Except single-pore relative AtKCO3, all other members of membrane channels that involved in potassium transport have two pore regions with the characteristic amino acid signature GYGD, responsible for the selectivity to potassium (fig. 1). It is possible that single-pore KCO3 channel were formed by partial division of pore region with selective filter for potassium and two transmembrane domains (fig. 1). It was shown that single-pore Arabidopsis AtKCO3 channel could have functionality only if in dimeric form [6, 15].

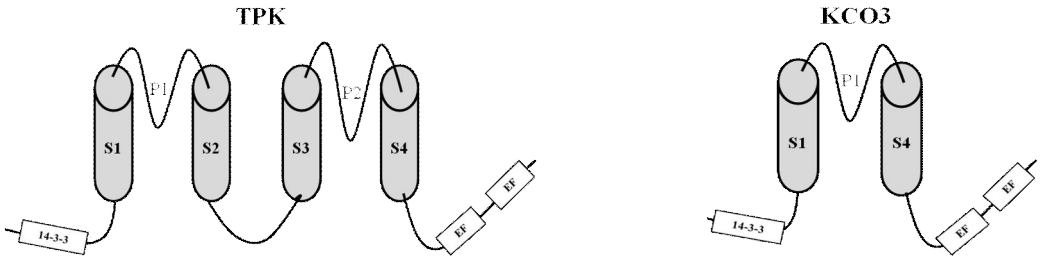

Fig. 1. Structure of the TPK and KCO3 channels in plants. The channels consist of pore domain (P) and transmembrane domains (S). Channels TPK family and KCO3 have at its N-terminus 14-3-3 domains that can bind with14-3-3 proteins and thus regulate the activity of these channels. Typically, the C-terminus of channels containing EF-hands responsible for binding of calcium ions, therefore there is regulation of these channels.

The TPK family channels have the structural similarity to the tandem pore of mammalian potassium channel (fig. 1). Channels of TPK family contain 4 transmembrane domains and two pore regions with amino acid sequence GYGD. This amino acid "signature" is responsible for the potassium selectivity of pore regions. It should be noted also, that the majority of these channels have the EF-hands in the C-terminus, therefore these proteins could be regulated by calcium ions. Many members of TPK family could also contain 14-3-3 binding site in their N-terminus.

Due to their structural differences, the TPK channels might have the different physiological functions and different cellular localization. Vacuolar channels of TPK family were found in the genomes of many species from *Physcomitrella* moss to eucalyptus. The most studied channel of TPK family is AtTPK1 from *A. thaliana*. The activity of this channel depends on cytosolic pH and intracellular calcium concentration. Like the majority of TPKs, AtTPK1 localized in the tonoplast of large lytic vacuole [3, 15]. Recent studies of functions and cellular localization of some TPK family members indicate existence of TPK channels with different to tonoplast of lytic vacuole of membrane specialization. It is known, that

AtTPK4 of Arabidopsis has no EF-domains in the C-terminus and localized in the plasma membrane [1]. It was supposed, that AtTPK3 localized in the tonoplast of central vacuole, but recent data demonstrate the chloroplast localization of this channel [2, 15]. An interesting fact is that the rice TPKs (OsTPKa and OsTPKb) localized in different types of vacuoles. OsTPKa is specific to the lytic vacuole tonoplast, whereas OsTPKb is localized in the membrane of prtotein storage vacuole [6, 7]. A similar pattern of vacuolar localization has a member of TPK family from tobacco - NtTPK1b (see table. 1) [8, 14]. It was shown that this type of channels is localized in small vesicular structures resemble to protein storage vacuoles. It should be noted that although other members of the Arabidopsis TPK family, AtTPK2 and AtTPK5 localized in membranes of lytic vacuole, there are some experimental data suggesting, that these channels can also be localized in smaller vesicular structures [15].

The physiological role of TPKs is very diverse. They are involved in maintaining of potassium homeostasis and turgor generation. Channels of this family are involved in many other processes of plant response to abiotic stress [6]. For example, it was shown that AtTPK1 involved in the movement of stomata cells [3]. Unlike AtTPK1, which gene is expressed in all types of plant tissues, AtTPK2 is a specialized channel of pollen and thus is an important element in the process of pollination [1]. Due to the rice OsTPKb unique localization, this channel is important in processes of seed formation, germination and mineral enrichment. There are a number of data indicating that the function of this channels is extremely important for the salt and water stress processes in plants [6]. It is known that the activity NtTPK1a is sensitive to spermidine and spermine, and the transcript level of gene encoding this channel increases with hyperosmotic conditions (Table. 1) [4]. It has been shown that gene expression of poplar TPK channel in tobacco cells increased their resistance to salt stress [16].

1. The main physiological and functional characteristics of some members of the TPK channels

Protein	Gene expression	Cellular localization	Functions	Reference
AtTPK1	All tissues	Tonoplast of central lytic vacuole	Homeostasis K^+, K + release during movement stomata cells, seed germination	1, 3
AtTPK2	Pollen	Tonoplast of central lytic vacuole	Unknown	1, 2
AtTPK3	Root tips, pollen	Chloroplast membrane	K^+ homeostasis in chloroplasts	2, 5
AtTPK4	Pollen	Plasma membrane	Homeostasis K^+	1,4
AtTPK5	Vacsular tissues	Tonoplast of central lytic vacuole	Unkbown	1, 2
OsTPKa	All tissues	Tonoplast of central lytic vacuole	Unknown	1, 6
OsTPKb	All tissues, seeds	Tonoplast of protein storage vacuole	Homeostasis K^+ in seeds	1, 6
NtTPK1a	Unknown	Tonoplast of central lytic vacuole	Regulation salt and osmotic shock	1, 7, 9
NtTPK1b	Unknown	Potentilay, tonoplast of protein storage vacuole	Cell division, drought	1, 7, 8

The aim - the study and summarization of the known potassium channel sequences of TP family. Conducting of sequences verification by the secondary analysis of domain structure. Performing the cladistic analysis and construction of phylogenetic tree. Evaluation of prospective of further studies of these channels.

Material and methods research. Search by plant homologues performing keywords and on the basis of BLASTp-scanning database UniProt (SIB BLAST Network Service) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/) [9, 17].

The boundaries of the catalytic domain were determined according to GenBank, UniProt protocols and SMART7 analysis of domain architecture (http://smart.embl-heidelberg.de/) [12]. Search of plant homologues were conducted by BLASTp algorithm with limitation the group by «Viridiplantae» and

using the following parameters: weight matrix - BLOSUM62, the expected number of matches in a random sample of the threshold E = 10 at the active sites and filtering adjustment fragments containing spaces (www .expasy.org; www.blast.ncbi.nlm.nih.gov) [9, 12, 17]. Initial bulk of sequence homologs was performed by weight adjustment, and also yielding of percentage identity, similarity and gaps availability [9].

Estimation of the potential and specific blast proteins architecture was performed by analyzing the use of network tools SMART7 (www.smart.embl-heidelberg.de) [13]. Multiple alignment of amino acid sequences were performed using ClustalX (2.0.5) (www.clustal.org) using a series of matrices BLOSSUM [11].

Phylogenetic analysis of potassium channel TPK family was based on clustering of proteins with the architecture of the two catalytic domains combined linker region using the method of binding two nearest neighbors (Neighbor-Joining) [11, 13].

Visualization, analysis and identification of root phylogenetic tree was performed using software Dendroscope 3.2.8 and MEGA5 [16, 17]. The general methodology of the research presented in Figure 2.

Results and discussion. Based on the literature [3, 6, 15] and UniProtKB database and GenBank appropriate the complete amino acid sequences and sequence of the catalytic domain potassium channels TRK family of *Arabidopsis thaliana* were selected. Further BLASTp-scan of the other plant homologues in UniProtKB database and GenBank, was made relatively complete amino acid sequences and catalytic domains of ion transporters (SMART-Ion_trans_2: PF07885). Cases of multiple deposit found by comparing the coordinates of the corresponding gene loci proteins.

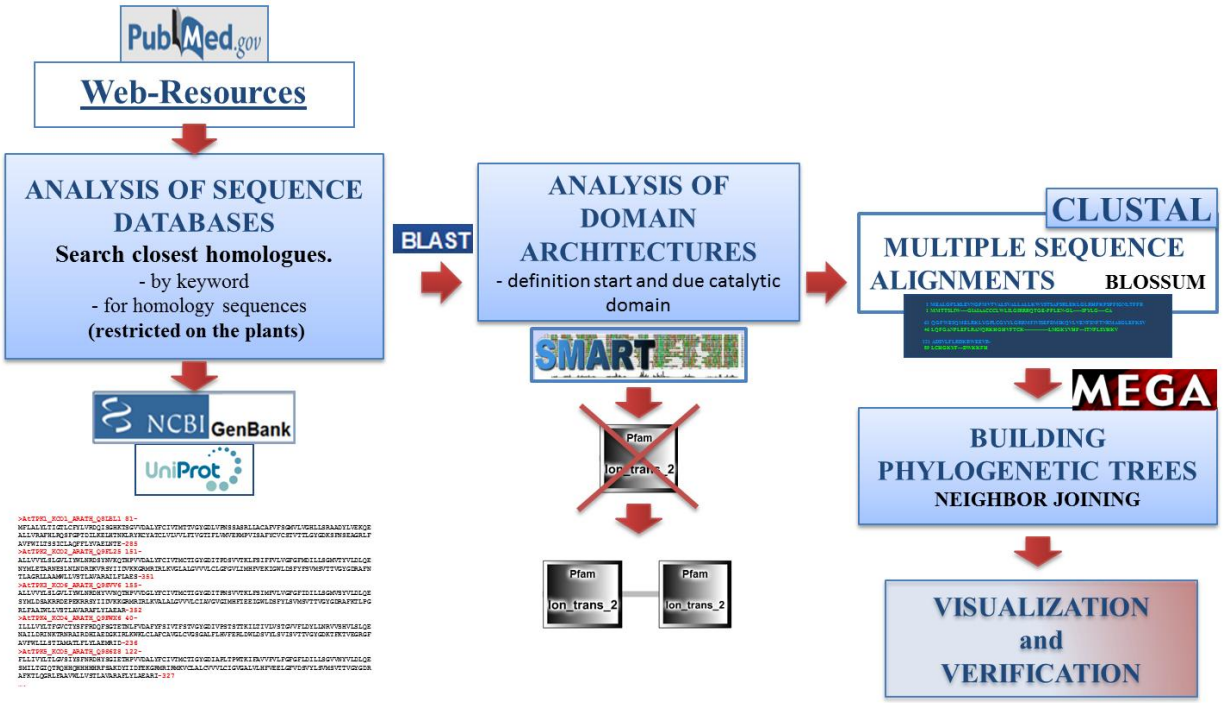

Fig.2. General research methodology of potential two-pore potassium channel vegetable of plant origin.

To limit the selected bulk of sequences, the 300 protein sequences were passed selection by domain architecture analysis. Only those sequences that have two domains of ion transporters Ion_trans_2: PF07885, United unstructured linker site were selected. The work performed by SMART tool connectivity analysis scripts for all other additional databases. As a result of this selection sequences 157 proteins containing domains identified by HMM-profiles and are appropriate structure were passed selection process (fig. 2).

According to results of multiple sequence alignment of 157 selected sequences, the high degree of heterogeneity selected group of homologues were shown with 3.5% of identity and 7.7% of similarity, but with clear allocation of conserved motifs.

Despite the high heterogeneity in the middle of the sample, the results of joint NJ - clustering catalytic domains of Arabidopsis potassium channels and other homologues plant indicate the existence of their common clade that could indicate

their evolutionary closeness. In particular, it was found that all selected sequences of potential plant TPKs comprise four large clades (fig. 3). The first clade group includes control channel TPK1 (UniprotKB: KCO1, Q8LBL1) of *A. thaliana* and 69 plant homologs of this type. It should be noted that cladytic analysis revealed the existence of separate branche represented one protein - TPK4 from Arabidopsis (UniprotKB: KCO4, Q9FWX6), which is closer to the hypothetical root phylograms (fig. 3). It should be noted, that Arabidopsis TPK4 is localized in the plasma membrane and contains EF-hands in the C-terminus (see table. 1) [1]. The second group of TPK channel family of three branches with very similar in protein conserved motifs and includes 25 putative channels (fig. 3). The third group included 23 plant proteins combined with the control protein of TPK5 from Arabidopsis (UniprotKB: KCO5, Q9S6Z8). The fourth group of TPKs contains potential root of phylogramm, a group of 35 potential plant potassium channels. This group is combined with two control Arabidopsis proteins TPK2 (UniprotKB: KCO2, Q9FL25) and TPK3 (UniprotKB: KCO6, Q9SVV6) (fig. 3).

Conclusions

1. As a result of this work the 300 proteins were analyzed. By analysis of the domain specific architecture, the 157 unique sequences of putative plant TPK potassium channels were selected.

2. Results of sequence multiple alignments indicate a high degree of sequence divergence among putative plant TPK potassium channels.

3. According to results of cladystic analysis, the 4 clades of TPK like protein sequences with distinct characteristics and associations with more control proteins from *A. thaliana* were determined.

4. Despite the distribution of potassium channels TRK family in 4 different groups of homologues, physiological function and cellular localization of these channels may be different. Therefore, further study of the functions and the cellular localization of these transport proteins and application of molecular modeling is very important.

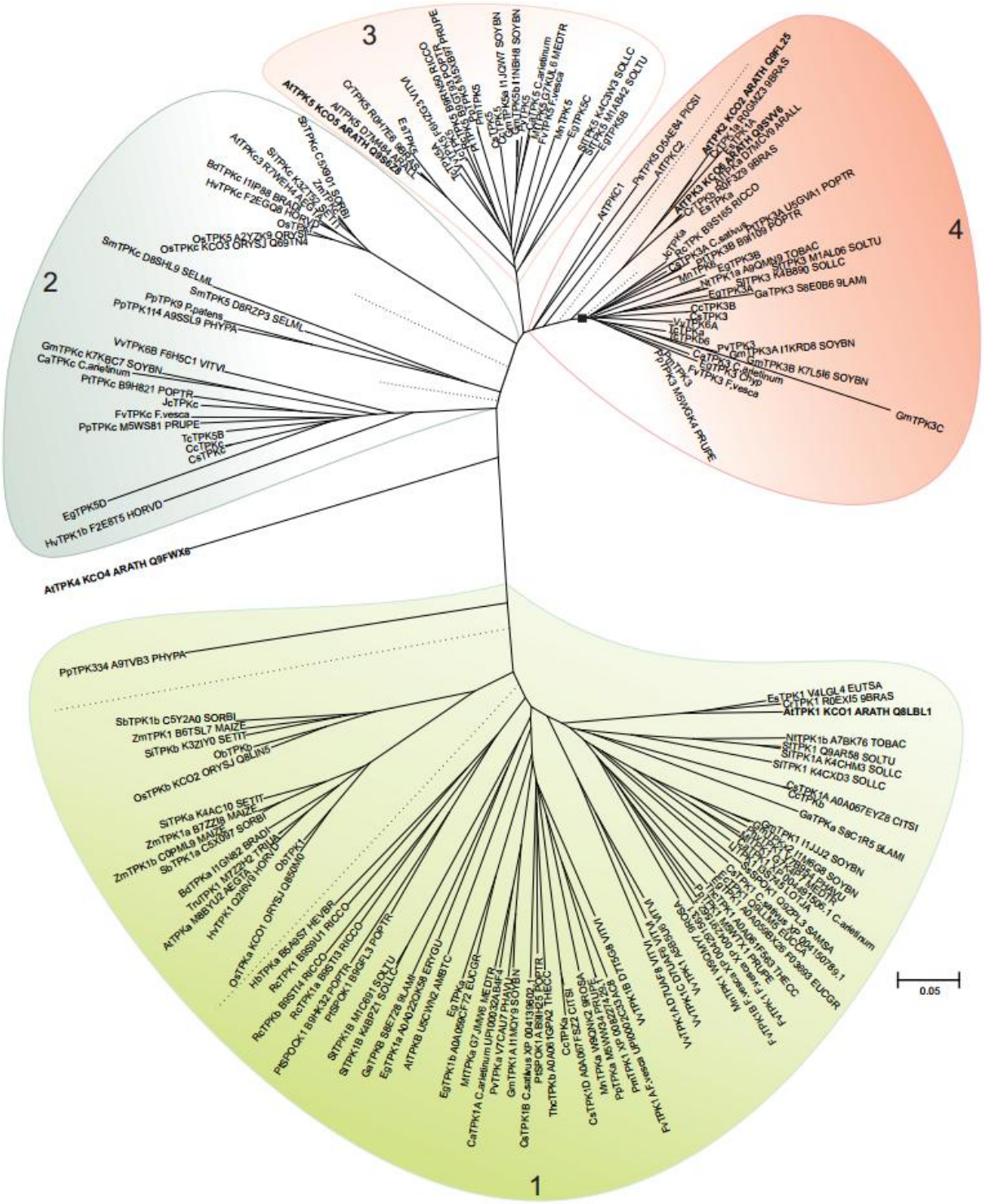

Fig. 3. The results of phylogenetic analysis of selected group of plant putative two-pore potassium channels. Notes: algorithm - binding nearest neighbors, square root of the tree marked potential.

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ФІЛОГЕНЕТИНИЙ АНАЛІЗ ПОТЕНЦІЙНИХ КАЛІЄВИХ КАНАЛІВ РОДИНИ TPK РОСЛИННОГО ПОХОДЖЕННЯ

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Проведено біоінформаційний пошук послідовностей потенційних ТРК-каналів та узагальнення філогенетичного різноманіття родини ТРК. За допомогою низки спеціалізованих програм та онлайн сервісів проводився порівняльний аналіз та вирівнювання амінокислотних послідовностей потенційних представників ТРК-каналів. Після вторинного аналізу доменної архітектури порових ділянок 300 потенційних білків, було відібрано 157 каналів родини ТРК різних видів рослин. Подальший кладистичний аналіз показав, що родина ТРК-каналів рослин поділяється на чотири різних клади, показуючи ділення за типовими ознаками.

Ключові слова: *двопорові калієві канали родини ТРК, доменна структура ТРК-каналів, кладистичний аналіз, філогенетичне дерево*

ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ ПОТЕНЦИАЛЬНЫХ РАСТИТЕЛЬНЫХ КАЛИЕВЫХ КАНАЛОВ СЕМЕЙСТВА ТРК

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Был проведен биоинформационный поиск потенциальных последовательностей ТРК-каналов и обобщение филогенетического разнообразия семейсва ТРК.

Проведено біоінформаційний пошук та узагальнення філогенетичного різноманіття рослинних калієвих каналів родини ТРК. За допомогою низки спеціалізованих програм та онлайн сервісів проводився порівняльний аналіз та вирівнювання амінокислотних послідовностей потенційних представників ТРК-каналів. Після відбору на основі вторинного аналізу доменної архітектури порових ділянок було відібрано 157 каналів родини ТРК різних видів рослин. Подальший кладистичний аналіз показав, що родина ТРК-каналів рослин поділяється на 4 різних клади.

Ключові слова: *двопорові калієві канали родини ТРК, доменна структура ТРК-каналів, кладистичний аналіз, філогенетичне дерево*