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**THE PHYLOGENETIC DIVERSITY ANALYSIS OF  
PLANT NHX Na<sup>+</sup>/H<sup>+</sup> EXCHANGERS**

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*The bioinformatic such and selection of existing putative sequences of plant NHX Na<sup>+</sup>/H<sup>+</sup> exchangers were conducted. By application of wide range of bioinformatic tools and online services the comparative analysis and sequence alignment of selected amino acid sequences of NHX transporters with additional confirmation of domain architecture were performed. As result of this study the phylogenetic diversity of NHX family was summarized. After secondary analysis of full sequences of putative NHX Na<sup>+</sup>/H<sup>+</sup> exchangers the 403 amino acid sequences of those transporters from different plant species were selected. According to cladistical analysis of selected NHX transporter sequences these proteins are distributed between 4 different clades. 3 clades from 4 belong to the type II NHX transporters and 1 to the type II of Na<sup>+</sup>/H<sup>+</sup> exchangers. The cladistical analysis and further classification of NHX horologes indicate the mismatching of database NHX transporters given names to the clade and group which they have belonged.*

**Key words:** *Na<sup>+</sup>/H<sup>+</sup> exchangers of NHX family, functional diversity, cladistical analysis, phylogenetic tree*

The influence of various abiotic stress may affect and significantly inhibit the plant growth, development and productivity. The main types of this kind of stress are salinity, drought and high or low temperatures. It worth to note, that salt stress is one of the most important environmental factors that can significantly reduce yields of crops worldwide [1]. In order to grow crops on degraded or marginal lands the urgent need for world agriculture and other economical areas is to improve the salt tolerance of plants by molecular breeding or transgenic technology [1]. Salt stress leads to inhibition or complete disruption of plant water uptake by roots and to significant changes in key metabolic reactions in cell due to osmotic stress and toxic concentrations of sodium ions in the cytosol [2, 3]. In order to minimize the negative impact of high salt concentrations the plants employ several mechanisms

[3]. In particular, the plants are able to remove toxic sodium ions by sequestration into vacuole or outside the cell through the plasma membrane [3, 4]. The removal of cytotoxic sodium ions is mediated by work of proton-sodium ( $\text{Na}^+/\text{H}^+$ ) antiporters. The genome of *Arabidopsis thaliana* encodes more than 40 different types of  $\text{Na}^+/\text{H}^+$  antiporters [5, 6]. The  $\text{Na}^+/\text{H}^+$  antiporters are divided into three families SPA1 or NHX, NhaD and CPA2 (CHX and KEA) [7].

The first plant transport proteins of NHX family were described as tonoplast transporters involved in vacuolar deposition of  $\text{Na}^+$  from cytosol. It has been shown for many plant species that NHX transporters are main determinants of plant salt tolerance [4, 8, 9, 10]. The NHX family of  $\text{Na}^+/\text{H}^+$  exchangers NHX is divided into two main types (see. Table. 1) [11]. First type of transporters are similar to SOS1 (Salt overlay sensitive 1) proteins with plasma membrane localization. They had evolved into functional transport proteins independently from other NHX transporters [12]. The NHX transporters of the type I could play an important role in the  $\text{Na}^+$  transport over long distances, particularly in retranslocation of this toxic ion from the stem to the roots [13]. The AtNHX7/SOS1 transporter from *Arabidopsis thaliana* is homologue of yeast NHA1  $\text{Na}^+/\text{H}^+$  antiporter, which localized in the plasma membrane. AtNHX8 is phylogenetically close to AtNHX7/SOS1, but functions of this transport proteins are still not completely understood. However, there are some experimental data suggest that this transport protein is involved in toxic  $\text{Li}^+$  removal from cell (see. Table. 1) [14].

The  $\text{Na}^+/\text{H}^+$  antiporters of type II or “true” NHX transporters, also divided into two classes (see. Table. 1). The class 1 includes NHX isoforms of *A. thaliana* (AtNHX1-4), with strong vacuolar localization [15, 16]. However NHX proteins belonging to the class 2, namely AtNHX5-6, exhibit endosomal localization in cells [16, 17]. The first plant NHX transporter - AtNHX1, was identified by its sequence similarity to the yeast SsNHX1 [18] (see. Table. 1). This protein has vacuolar localization. It is responsible for the transport of  $\text{K}^+$  and  $\text{Na}^+$  sodium into vacuoles [19]. Elevation of gene expression level of this transporter leads to improvement of

salt tolerance of plants [20]. The functional studies of the class II antiporters were conducted only for *A. thaliana* AtNHX5-6 and tomato LeNHX2 [11, 21].

NHX transporters play an important role in many important cellular and physiological processes. They are responsible for cellular pH regulation, vesicular traffic, cellular elongation and plant development in general [17, 22]. The main role of most NHX, which have been so far, identified are Na<sup>+</sup> transport and accumulation in vacuoles or in other cellular compartments under conditions of salt stress, for the minimizing of toxic effects of this ion in cell metabolism. [4]. Under normal conditions, the low concentration of Na<sup>+</sup> in the cytosol, NHX antiporters are responsible for transport of K<sup>+</sup> into vacuoles or other membrane cellular compartments. Thus they help to maintain the ion homeostasis and cell elongation [23, 24, 25] (see. Table. 1). Despite that, exist some exceptions from the rule, when NHX antiporter mediate transport of Na<sup>+</sup> from the vacuole into the cytoplasm [25].

Over the last decade the large number of NHX homologs has been identified in different plant species [4]. Unfortunately, for the many of them the functions and their phylogenetic position remain unknown.

### 1. The main physiological and functional features of Na<sup>+</sup>/H<sup>+</sup> exchangers of NHX family *Arabidopsis thaliana*

Type	Class	Protein	Reference number TAIR	Cellular localisation	Function	Reference
I		AtNHX7/SOS1	At2g01980	Plasma membrane	Na <sup>+</sup> /Li <sup>+</sup> removal from cell	14
		AtNHX8	At1g14660	Plasma membrane	Li <sup>+</sup> removal from cell	14
II	1	AtNHX1	At5g27150	Vacuolar tonoplast	Na <sup>+</sup> /K <sup>+</sup> sequestration into vacuole	11, 15, 20
		AtNHX2	At3g05030	Vacuolar tonoplast	Na <sup>+</sup> /K <sup>+</sup> sequestration into vacuole	15
		AtNHX3	At5g55470	Vacuolar tonoplast	K <sup>+</sup> sequestration into vacuole	25
		AtNHX4	At3g06370	Vacuolar tonoplast	Na <sup>+</sup> transport from vacuole to cytosol	25
		AtNHX5	At1g54370	Мемрани ендосом	Na <sup>+</sup> /K <sup>+</sup> transport to endosomes	16,17
		AtNHX6	At1g79610	Мемрани ендосом	Na <sup>+</sup> /K <sup>+</sup> transport to endosomes	21

**The aim of study** - selection and classification of known NHX transporters sequences. Verification of these sequences by secondary check of domain architecture of these transport proteins. Conducting multiple sequence alignment and phylogenetic tree construction.

**Material and methods.** The search of NHX Na<sup>+</sup>/H<sup>+</sup> exchangers plant homologs were performed by keywords and on the basis of BLASTp-scanning database UniProtKB (<http://www.uniprot.org/>, SIB BLAST Network Service) with restriction only for plants and GenBank ([www.blast.ncbi.nlm.nih.gov](http://www.blast.ncbi.nlm.nih.gov)) [26, 27]. The search of plant homologs was carried out by algorithm BLASTp with «Viridiplantae» group restriction and application of the following parameters: weight matrix - BLOSUM62, the number of matches in the expected random sample of threshold E = 10, with the active filtering of low-constructed sites and aligned

fragments containing haps [26, 27, 28]. The primary sequence selection of

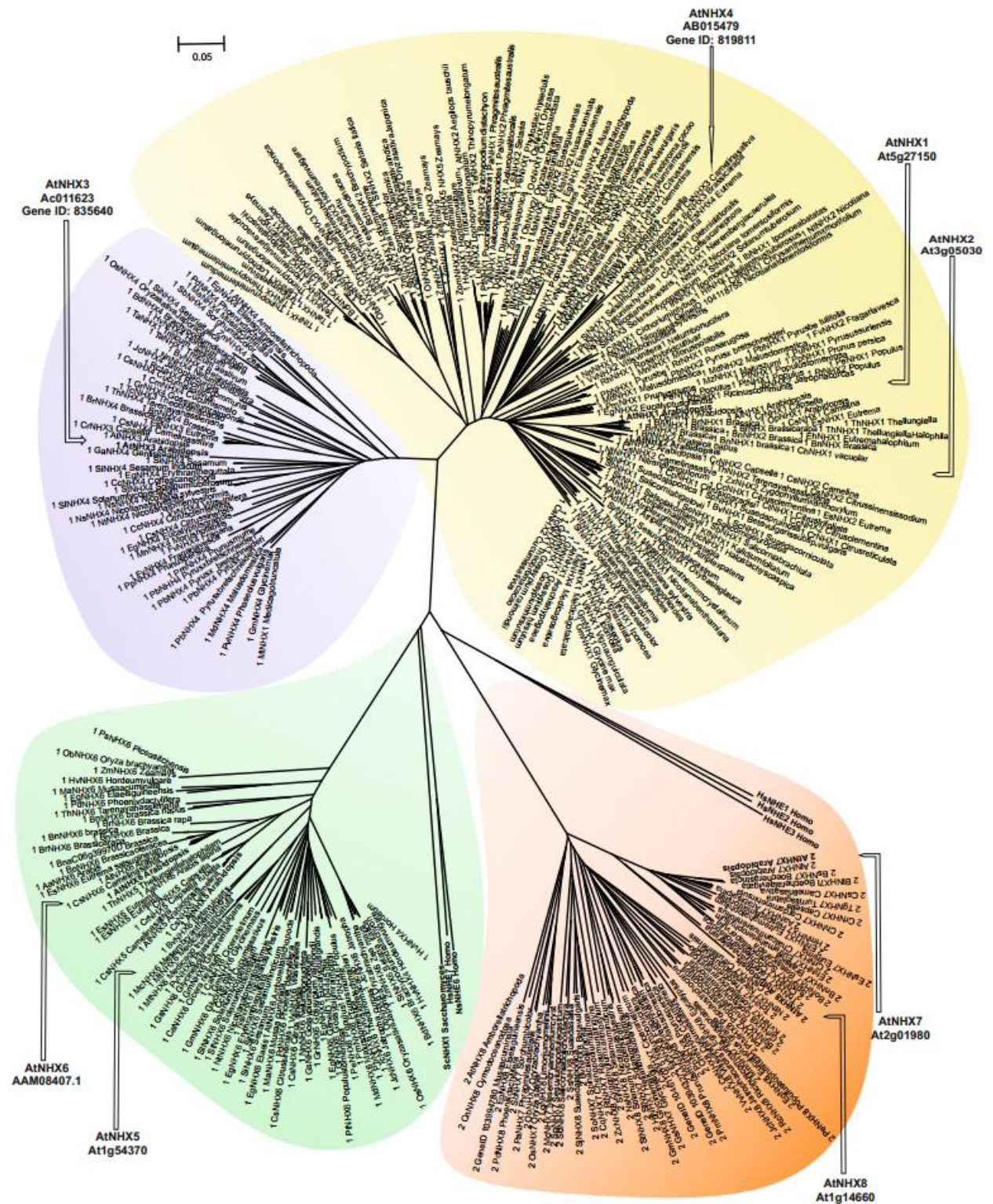


Fig.1. The cladistical analysis results of consolidated group of putative plant NHX sodium- proton exchangers. Notes: selected representatives of the NHX transporters from *A. thaliana* are marked, cladistical algorithm - neighbor-joining method (NJ).

**2. Classification of NHX Na<sup>+</sup>/H<sup>+</sup> antiporters homologues of *Arabidopsis thaliana***

Organism / Clade / Type	Type II						Type I	
	Clade 1			Clade 2	Clade 3		Clade 4	
	group 4	group 1	group 2	group 3	group 5	group 6	group 7	group 8
1	2	3	4	5	6	7	8	9
<i>Homo sapiens</i>					HsNHE6 HsNHE7		HsNHE1 HsNHE2 HsNHE3	
<i>Saccharomices cerevisiae</i>					ScNHX1			
<i>Arabidopsis thaliana</i>	AtNHX 4	AtNH X1	AtNHX 2	AtNHX 3	AtNHX 5	AtNHX6	AtNHX 7	AtNHX 8
<i>Aegilops tauschii</i>	AtNHX2 AtNHX2							
<i>Aeluropus lagopoides</i>	AINHX1							
<i>Aeluropus littoralis</i>	AINHX1							
<i>Amborella trichopoda</i>	AtNHX1 AtNHX1			AtNHX 4	AtNHX6			AtNHX8
<i>Arabidopsis lurata</i>	AINHX4	AINHX 1	AINHX 2 AINHX 2	AINHX 3	AINHX5	AINHX6	AINHX 7	
<i>Arabis alpina</i>					AaNHX 6	AaNHX6	AaNHX 7	
<i>Arachis hypogaea</i>	AhNHX 1							
<i>Atriplex dimorphostegia</i>			AdNH X1					
<i>Atriplex gmelini</i>			AgNH X1					
<i>Atriplex halimus</i>			AhNH X1					
<i>Atriplex patens</i>			ApNH X1					
<i>Beta vulgaris</i>			BvNH X1	BvNHX 4	BvNHX 6			BvNHX 8
<i>Boechera laevigata</i>							BINHX 7	
<i>Boechera stricta</i>							BsNHX 7	
<i>Brachypodium distachyon</i>	BdNHX 2 BdNHX 2 BdNHX 2			BdNHX 4	BdNHX 6			
<i>Brassica napus</i>	BnNHX	BnNH		BnNHX		Bna-	BnNHX	

	3	X BnNH X2 BnNH X1 BnNH X1 BnNH X2		4		C06g3997 0D BnNHX6 BnNHX6 BnNHX6	7	
<i>Brassica rapa</i>	BrNHX3	BrNHX 2 BrNHX BrNHX 1		BrNHX 4		BrNHX6 BrNHX6	BoNHX 7	
<i>Brassica oleracea</i>						BoNHX6		
<i>Bruguiera gymnorrhiza</i>								BgNHX 7
<i>Camelina sativa</i>	CsNHX3	CsNHX 1	CsNHX 2 CsNHX 2	CsNHX 4	CsNHX5 CsNHX5	CsNHX6	CsNHX 7	
<i>Capsella rubella</i>	CrNHX4	CrNHX 1	CrNHX 2	CrNHX 3	CrNHX5 CrNHX5		CrNHX 7	
<i>Cardamine hirsuta</i>							ChNHX 7	
<i>Chalcanthus renifolius</i>							CrNHX 7	
<i>Chenopodium quinoa</i>								CqNHX 7
<i>Chrysanthemum x morifolium</i>	CmNHX 1							
<i>Cichorium intybus</i>	CiNHX1							
<i>Cicer arietinum</i>					CaNHX 6 CaNHX 6			CaNHX 7
<i>Citrus clementina</i>	CcNHX 1		CcNHX 1 CcNHX 2	CcNHX 4				
<i>Citrus sinensis</i>	CsNHX1 CsNHX2 CsNHX2			CsNHX 4	CsNHX6 CsNHX6		CsNHX 7	
<i>Citrus reticulata</i>			CrNHX 1					
<i>Citrus trifoliata</i>			CtNHX 1					
<i>Citrus x paradisi</i>			CpNH X1					
<i>Cochlearia</i>		ChNH					ChNHX	

<i>hollandica</i>		X1					7	
<i>Coffea canephora</i>	CcNHX 1			CcNHX 4				
<i>Cucumis melo</i>				CmNH X4				CmNHX 8
<i>Cucumis sativus</i>	CsNHX2			CsNHX 4	CcNHX 6			CsNHX8
<i>Cymodocea nodosa</i>								CnNHX 8
<i>Diplachne fusca</i>	DdNHX 1							
<i>Elaeis guineensis</i>	EgNHX 2 EgNHX 2			EgNHX 4	EgNHX 6	EgNHX6		EgNHX 8
<i>Eucalyptus grandis</i>	EgNHX 3 EgNHX 2			EgNHX 4	EgNHX 6 1044457 88			EgNHX 8 EgNHX 8
<i>Eutrema salsugineum</i>	EsNHX4	EsNHX 1	EsNHX 2	EsNHX 3	EsNHX5 EsNHX6	EsNHX6	EsNHX 7	
<i>Eutrema halophilum</i>		EhNH X1					EhNHX 7	
<i>Eutrema heterophyllum</i>							EhNHX 7	
<i>Erythranthe guttata</i>				EgNHX 4	EgNHX 6 EgNHX 6			
<i>Festuca arundinacea</i>	FaNHX1							
<i>Fragaria vesca</i>	FvNNH X2			FvNHX 4 FvNHX 4				FvNHX8
	FvNHX2							
<i>Genlisea aurea</i>				GaNHX 4				
<i>Glehnia littoralis</i>	GINHX1							
<i>Glycine max</i>	GmNHX 1 GmNHX 1			GmNH X4	GmNHX 6 GmNHX 6 GmNHX 6			GmNHX 8
<i>Glycine soja</i>					GsNHX 6			GsNHX 7
<i>Gossypium raimondii</i>	GrNHX3 GrNHX1 GrNHX1			GrNHX 4	GrNHX6 GrNHX6			GrNHX8



<i>Gossypium hirsutum</i>	GhNHX 1							
<i>Gossypium boreum</i>					GsNHX 6			
<i>Halostachys caspica</i>			HcNH X1					
<i>Helianthus tuberosus</i>	HtNHX1							HtNHX7
<i>Hordeum brevisubulatum</i>	HbNHX 1							
<i>Hordeum vulgare</i>	HvNHX 3 HvNHX 1 HvNHX 2			HvNHX 4	HvNHX 4 HvNHX 4	HvNHX6		
<i>Hesperis matronalis</i>							HmNH X7	
<i>Ipomoea batatas</i>	IbNHX1							
<i>Ipomoea nil</i>	InNHX1 InNHX1							
<i>Ipomoea tricolor</i>	ItNHX1 ItNHX1							
<i>Iris lactea</i>	IINHX2 IINHX2							
<i>Jatropha curcas</i>	JcNHX3 JcNHX2			JcNHX 4	JcNHX6 JcNHX6			JcNHX8
<i>Kalidium ohiatum</i>			KfNHX 1					
<i>Kosteletzkya virginica</i>								KvNHX 7
<i>Lilium tenuifolium</i>	LtNHX1							
<i>Limonium gmelinii</i>	LgNHX 1							LgNHX 8
<i>Lolium perenne</i>	LpNHX 1							
<i>Lophopyrum elongatum</i>	LeNHX1 LeNHX2							
<i>Malus domestica</i>	MdNHX 2 MdNHX 1			MdNH X4	MdNHX 6			MdNHX 8
<i>Malus zumi</i>	MzNHX 1							
<i>Mesembryanthemum crystallinum</i>			McNH X1		McNHX 6			McNHX 7
<i>Medicago sativa</i>				MsNHX 4				
<i>Medicago truncatula</i>				MtNHX 1	MtNHX 6			

					MtNHX 6			
<i>Medicago falcata</i>				MfNHX 1				
<i>Morus notabilis</i>	MnNHX 2			MnNH X4				MnNHX 7
	MnNHX 1							
<i>Musa acuminata</i>	MaNHX 2 MaNHX 2 MaNHX 2			MaNH X4	MaNHX 6	MaNHX6		1039947 80
<i>Nelumbo nucifera</i>	NnNHX 1 1045889 31			NnNHX 4	NnNHX 6			1046110 75
<i>Nicotiana benthamiana</i>	NbNHX 1							
<i>Nicotiana tomentosiformis</i>	NtNHX1 NtNHX2 NtNHX2 1041187 55			NtNHX 4	NtNHX6			
<i>Nicotiana sylvestris</i>	NsNHX 2 NsNHX 1 NsNHX 1			NsNHX 4 NsNHX 4	NsNHX 6			NsNHX 8
<i>Nierembergia caerulea</i>	NcNHX 1							
<i>Nitraria tangutorum</i>			NtNHX 1					NtNHX
<i>Olimarabidopsis pumila</i>		OpNH X1 OpNH X2						
<i>Oryza brachyantha</i>	ObNHX 2 ObNHX 2 ObNHX 2				ObNHX 6	ObNHX6		ObNHX 7
<i>Oryza coarctata</i>	OcNHX 1							
<i>Oryza sativa Indica</i>	OsNHX 1							



<i>trigyna</i>								
<i>Ricinus communis</i>	RcNHX 3 RcNHX 1			RcNHX 4				RcNHX 8
<i>Rosa hybrid</i>	RhNHX 1							
<i>Rosa rugosa</i>	RrNHX1							
<i>Sesamum indicum</i>	SiNHX1			SiNHX 4 SiNHX 4	SiNHX6 SiNHX6			SiNHX8
<i>Salicornia bigelovii</i>			SbNHX 1					
<i>Salicornia brachiata</i>			SbNHX 1					
<i>Salicornia europaea</i>			SeNHX 1					
<i>Salicornia dolichostachya</i>								SdNHX8
<i>Salsola komarovii</i>			SkNHX 1					
<i>Setaria italica</i>	SiNHX2 SiNHX2 SiNHX2			SiNHX 4	SiNHX6			
<i>Sesuvium portulacastrum</i>			SvNHX 1					SpNHX7
<i>Schrenkiella parvula</i>							SpNHX 7	
<i>Solanum lycopersicum</i>	SINH3			SINH 4 SINH 4	SINH6			StNHX8
<i>Solanum tuberosum</i>	StNHX2			StNHX 4	StNHX6			StNHX7
<i>Sorghum bicolor</i>	SbNHX2 SbNHX3			SbNHX 4				SbNHX7
<i>Spinacia oleracea</i>								SoNHX7
<i>Suaeda corniculata</i>			ScNHX 1					
<i>Suaeda japonica</i>			SjNHX 1					SjNHX8
<i>Suaeda pruinosa</i>			SpNHX 1					
<i>Suaeda salsa</i>								SsNHX8
<i>Tarenaya hassleriana</i>	ThNHX 3		ThNHX 2	ThNHX 4		ThNHX6	ThNHX 7	
<i>Theobroma cacao</i>	TcNHX1 TcNHX1			TcNHX 3	TcNHX6			TcNHX7
<i>Thellungiella halophila</i>		ThNH X1			ThNHX 5			

		ThNH X1						
<i>Thinopyrum elongatum</i>	TeNHX1 TeNHX2							
<i>Thinopyrum intermedium</i>	TiNHX1 TiNHX							
<i>Triticum aestivum</i>	TaNHX1 TaNHX1 TaNHX2			TaNHX 1 TaNHX 1				
<i>Triticum urartu</i>	TuNHX 2							
<i>Tetragonia tetragonioides</i>			TtNHX 1 TtNHX 1					
<i>Turritis glabra</i>							TgNHX 7	
<i>Vitis vinifera</i>	VvNHX 1 VvNHX 2			VvNHX 4	VvNHX 6			VvNHX 8
<i>Vigna radiata</i>	VrNHX1							
<i>Vigna unguiculata</i>	ViNHX1							
<i>Zea mays</i>	ZmNHX 2 ZmNHX 1 ZmNHX 4 ZmNHX 1 ZmNHX 2 ZmNHX 3 ZmNHX 5			ZmNH X4	ZmNHX 6 ZmNHX 5	ZmNHX6		

plant homologs was performed by the weight alignment and identity values in percentage of similarity and availability haps [26].

The potential protein specificity was estimated by analyzing domain architecture using SMART7 network tool ([www.smart.embl-heidelberg.de](http://www.smart.embl-heidelberg.de)) [28].

Multiple alignment of amino acid sequences was performed using ClustalX (2.0.5) ([www.clustal.org](http://www.clustal.org)) using a series of BLOSSUM matrixes, cladistical analysis of NHX Na<sup>+</sup>/H<sup>+</sup> exchangers conducted by neighbor-joining method [29, 30].

Visualization and analysis of phylogenetic trees was performed using Dendroscope 3.2.8 and MEGA5 programs [31, 32].

**Results and discussion.** Initially, the bioinformatic selection of full amino acid sequences of NHX transporters from *A. thaliana* using UniProtKB databases and GenBank were performed. The further BLASTp search of other plant homologs in UniProtKB and GenBank databases was performed against the complete amino acid sequences with additional check of the catalytic domain Na<sup>+</sup>/H<sup>+</sup> antiporters (SMART- Na\_H\_Exchanger: PF00999). The cases of multiple depositions were determined by comparing the gene loci coordinates of corresponding proteins.

In order to limit the size range of selected sequences the additional analysis of domain architecture were performed. The sequences with Na<sup>+</sup>/H<sup>+</sup> antiporter domain were selected. The analysis was conducted by SMART tool with employment of additional scripts from other databases. As a result of sequences selection sequences the 403 proteins containing functional domain defined by HMM-profile were chosen.

The results of our multiple alignment of 403 NHX sequences exhibit high heterogeneity degree of selected Na<sup>+</sup>/H<sup>+</sup> antiporter homologues but clear conserved motifs allocation of the this group catalytic domain.

Despite of high level of heterogeneity within the selected group, the results of neighbor-joining method of clustering NHX transporters complete amino acid from *A. thaliana* and other plant homologs indicate the existence of common clades, which may indicate their evolutionary closeness. It was shown that all selected putative sequences of Na<sup>+</sup>/H<sup>+</sup> antiporters form four clades (Fig. 1). The first clade comprises reference NHX transporter - AtNHX1 and two other members of this family - AtNHX2 and AtNHX4. Additionally, this clade includes 219 homologs from different plant species of this type (Fig. 1) (see. Table. 2). It worth to note that cladistical analysis of NHX transporter revealed the existence of a small new clade comprising reference protein - AtNHX3 of *A. thaliana*. According to the results of our analysis, the appearance of novel NHX clade included AtNHX3 was shown for the first time. As was noted earlier, unlike other NHX transporters of Type II the

AtNHX3 provides import  $\text{Na}^+$  from the vacuole to the cytosol. Perhaps this functional difference is the key element of appearance separate clade comprising AtNHX3 like  $\text{Na}^+/\text{H}^+$  antiporters (Fig. 1) (see. Table. 1, 2) [25]. According to our study to this clade belong 58 known putative NHX3 plant homologues. The third clade includes the NHX homologues of AtNHX5 and AtNHX6 belonging to Type II of  $\text{Na}^+/\text{H}^+$  antiporters. Unlike other NHX transporters the  $\text{Na}^+/\text{H}^+$  antiporters of this clade from *A. thaliana* have endosomal localization and play important role in intracellular trafficking (Fig. 1) (see. Table. 1, 2) [21]. It should be noted, that in addition to 81 plant homolog belonging to this clade, the yeast ScNHX1 and human HsNHE6 and HsNHE7 connected to this group by separate branches. This phylogenetic closeness of these transport proteins from yeast and human homologues with plant homologues is one of possible evidence of early evolutionary functionalization of this protein group (Fig. 1) (see. Table. 2).

To the last fourth clade of NHX transporters belong the group of  $\text{Na}^+/\text{H}^+$  antiporters of type I with high homology to AtNHX7/SOS1 and AtNHX8. This clade comprises the 28 known plant proteins of NHX family. Moreover, the NHX transporters of this clade have high level of homology to human HsNHE1, HsNHE2 and HsNHE3.

It should be noted that by contrast with NHX transporters of other clades the transport proteins of fourth clade, particularly AtNHX7 / SOS1 and AtNHX8, exhibit the differences in cellular and physiological functions. These  $\text{Na}^+/\text{H}^+$  antiporters are responsible for removal of excess of  $\text{Na}^+$  and  $\text{Li}^+$  from the cell and retranslocation these toxic ions from the stem and photosynthetic tissues to the root (Fig. 1) ( See. Table. 1, 2).

### **Conclusions**

1. The selection and classification of 403 putative NHX  $\text{Na}^+/\text{H}^+$  antiporters unique sequences the mismatches of database annotated names of these transport proteins with the group to which they belong were revealed. The high level of annotated name mismatches were observed for the proteins belonging to clade I. For other clades the mismatches of annotated names with protein group were detected also.

2. The result of multiple alignment indicates a high degree of sequence divergence among potential Na<sup>+</sup>/H<sup>+</sup> antiporters of NHX family.

3. The cladistical analysis exhibits existence of 4 different clades comprising NHX - like sequences with clear features and united with more studied referent proteins from *A. thaliana*.

4. The one of result of cladistical analysis is emergence of a new clade (clade 3) comprising AtNHX3 from *A. thaliana* and its homologues.

5. The results revealed the need to make our detailed systematization and assigning appropriate and correct names of potential NHX conveyors.

6. Despite of the distribution of Na<sup>+</sup>/H<sup>+</sup> antiporters into 4 different homology groups, the physiological and cellular functions of these transport proteins might be different.

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## **АНАЛІЗ ФІЛОГЕНЕТИЧНОГО РІЗНОМАНІТТЯ ПРОТОННО-НАТРІЄВИХ ОБМІННИКІВ РОДИНИ NHX РОСЛИННОГО ПОХОДЖЕННЯ**

**С. В. Ісаєнков, Д. О. Самофалова**

*Було проведено біоінформатичний пошук та відбір наявних потенційних послідовностей протонно-натрієвих обмінників родини NHX, що належать рослинним організмам. За допомогою низки спеціалізованих біоінформаційних програм та он-лайн сервісів проведено порівняльний аналіз та множинне вирівнювання відібраних повних амінокислотних послідовностей NHX-транспортерів з уточненням доменної архітектури. В наслідок проведеної роботи було узагальнено філогенетичне різноманіття родини NHX. Після вторинного аналізу повних послідовностей потенційних протонно-натрієвих обмінників родини NHX, було відібрано 403 амінокислотні послідовності цих транспортних протеїнів з різних видів рослин. Кладистичний аналіз відібраних послідовностей NHX транспортерів вказує на існування 4 клад. 3 клади належать до NHX транспортерів типу II, а 1 клада містить протонно-натрієвих обмінники типу I. В ході проведення кладистичного аналізу та подальшої класифікації було виявлено неспівпадіння назв NHX транспортерів у базах даних із типом кладу та групи з маркерним типом, до якого ці протонні обмінники належать насправді.*

**Ключові слова:** протонно-натрієві обмінники родини NHX, функціональне різноманіття, кладистичний аналіз, філогенетичне дерево

## **АНАЛИЗ ФИЛОГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ ПРОТОННО-НАТРИЕВЫХ ОБМЕННИКОВ СЕМЕЙСТВА NHX РАСТИТЕЛЬНОГО ПРОИСХОЖДЕНИЯ**

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*Был проведен биоинформатический поиск и отбор существующих потенциальных последовательностей протонно-натриевых обменников семейства NHX, которые принадлежат растительным организмам. С*

*помощью ряда специализированных биоинформатических программ и он-лайн сервисов был проведен сравнительный анализ и множественное выравнивание полных аминокислотных NHX-транспортеров с уточнением доменной архитектуры. Вследствие проделанной работы обобщено филогенетическое разнообразие транспортеров семейства NHX. После вторичного анализа полных последовательностей было отобрано 403 аминокислотных последовательности этих транспортных протеинов из разных видов растений. Кладоистический анализ отобранных последовательностей NHX транспортеров показывает существование 4-клад. 3 клады принадлежат к NHX транспортерам типа II, а 1 клада включает в себя протонно-натриевые обменники типа I. В ходе проведения кладоистического анализа и дальнейшей классификации было обнаружено несоответствие названий NHX транспортеров в базах данных с типом клады и группы с маркерным типом, к которому эти протонно-натриевые обменники принадлежат на самом деле.*

**Ключевые слова:** *протонно-натриевые обменники семейства NHX, функциональное разнообразие, кладоистический анализ, филогенетическое дерево*