UDC: 581.1+ 76.314+ 57,023: 581.1+ 575.8 THE PHYLOGENETIC DIVERSITY ANALYSIS OF PLANT NHX Na⁺/H⁺ EXCHANGERS S. V. ISAYENKOV, Senior Research Fellow, D. A. SAMOFALOVA, Research assistant Institute of Food Biotechnology and Genomics National Academy of Sciences of Ukraine E-mail: stan.isayenkov@gmail.com

The bioinformatic such and selection of existing putative sequences of plant NHX Na+/H+ 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+/H+ 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+/H+ 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+/H+ 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 (Na⁺/H⁺) antiporters. The genome of *Arabidopsis thaliana* encodes more than 40 different types of Na⁺/H⁺ antiporters [5, 6]. The Na⁺/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 Na⁺ from cytosol. It has been shown for many plant species that NHX transporte3rs are mane determinants of plant salt tolerance [4, 8, 9, 10]. The NHX family of Na⁺/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 Na ⁺transport over long distances, particularly in retranslocation of this toxic ion from the stem to the roots [13]. The AtNHX7/SOS1 transporter from *Araboidopsis thaliana* is homologue of yeast NHA1 Na⁺/H⁺ antiporter, which localized in the plasma membrane. AtNHX8 is phylogeneticaly 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 Li⁺ removal from cell (see. Table. 1) [14].

The Na⁺/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 K⁺ and 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⁺ 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⁺ in the cytosol, NHX antiporters are responsible for transport of K⁺ 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⁺ 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.

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

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

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^+/H^+ 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) [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-konstructed 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^+/H^+ antiporters homologues of Araboidopsis thaliana

	Туре П							Type I	
Organism /		Clade 1		Clade 2	Cla	ade 3	Cla	de 4	
Clade / Type	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	
Homo sapiens					HsN HsN	NHE6 NHE7	HsNHE1 HsNHE2 HsNHE3		
Saccharomices cerevisiae					ScN	NHX1			
Arabidopsis	AtNHX	AtNH	AtNHX	AtNHX	AtNHX	AtNHX6	AtNHX	AtNHX	
thaliana	4	X1	2	3	5		7	8	
Aegilops tauschii	AtNHX2 AtNHX2								
Aeluropus	AlNHX1								
lagopoides									
Aeluropus	AlNHX1								
littoralis									
Amborella	AtNHX1			AtNHX	AtNHX6			AtNHX8	
trichopoda	AtNHX1			4					
Arabidopsis	AlNHX4	AlNHX	AINHX	AINHX	AlNHX5	AlNHX6	AlNHX		
lurata		1	2 AINHX 2	3			7		
Arabis alpina					AaNHX 6	AaNHX6	AaNHX 7		
Arachis	AhNHX								
hypogaea	1								
Atriplex			AdNH						
dimorphostegia			X1						
Atriplex gmelini			AgNH X1						
Atriplex halimus			AhNH X1						
Atriplex patens			ApNH X1						
Beta vulgaris			BvNH X1	BvNHX 4	BvNHX 6			BvNHX 8	
Boechera							BINHX		
laevigata							7		
Boechera stricta							BsNHX 7		
Brachypodium distachyon	BdNHX 2			BdNHX 4	BdNHX 6				
usiucnyon	BdNHX 2 BdNHX				0				
Brassica napus	2 BnNHX	BnNH		BnNHX		Bna-	BnNHX		

	3	Х		4		C06g3997	7	
	C	BnNH				0D		
		\mathbf{x}_{2}				BnNHX6		
		AL DnNU				DinvitX0 DrNHV6		
						DIINIIA0 DrNUV6		
						ΔΠΝΠΛΟ		
		BNNH						
	5.11110	X2		D 11111			D 1000	
Brassica rapa	BrNHX3	BrNHX		BrNHX		BrNHX6	BoNHX	
		2		4		BrNHX6	7	
		BrNHX						
		BrNHX						
		1						
Brassica						BoNHX6		
oleracea								
Bruguiera								BgNHX
gymnorhiza								7
Camelina sativa	CsNHX3	CsNHX	CsNHX	CsNHX	CsNHX5	CsNHX6	CsNHX	
		1	2	4	CsNHX5		7	
			CsNHX					
			2					
Capsella rubella	CrNHX4	CrNHX	CrNHX	CrNHX	CrNHX5		CrNHX	
		1	2	3	CrNHX5		7	
Cardamine							ChNHX	
hirsuta							7	
Chalcanthus							CrNHX	
renifolius							7	
Chenopodium								CqNHX
quinoa								7
Chrysanthemum	CmNHX							
x morifolium	1							
Cichorium	CiNHX1							
intvbus								
Cicer arietinum					CaNHX			CaNHX
					6			7
					CaNHX			,
					6			
Citrus	CcNHX		CcNHX	CcNHX	0			
clementina	1		1	4				
ciententina	1		CcNHX					
			2					
Citrus sinonsis	C _s NHX1		-	CsNHX	C _s NHX6		CsNHX	
Curus smensis	C _s NHX2				C _s NHX6		7	
	CsNHX2			-	CSIVIIAO		/	
Citrus reticulata			CrNHY				+	
Curus rencundu								
Citrus trifoliata			¹ CtNHY					
Citrus y naradisi			1 CpNH				+	
			X1					
Cochlearia		ChNH	211				ChNHY	
Cochieuriu								

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

Gossypium	GhNHX						
hirsutum	1						
Gossypiumar				GsNHX			
boreum				6			
Halostachys		HcNH					
caspica		X1					
Helianthus	HtNHX1						HtNHX7
tuberosus							
Hordeum	HbNHX						
brevisubulatum	1						
Hordeum	HvNHX		HvNHX	HvNHX	HvNHX6		
vulgare	3		4	4			
	HvNHX			HvNHX			
	1			4			
	HvNHX						
	2						
Hesperis						HmNH	
matronalis						X7	
Ipomoea batatas	IbNHX1						
Ipomoea nil	InNHXI						
T 1	InNHXI						
Ipomoea tricolor	ItNHX1						
	ItNHXI						
Iris lactea	IINHX2						
T	IINHX2		T	LANDA			LNUN
Jatropha curcas	JCNHX3		JCNHX	JCNHX6			JCNHX8
17 1: 1:	JCNHX2		4	JCNHX6			
Kalidium		KINHX					
Ollatum Koatolot-lava		1					V. NILIV
<i>Koslelelzkya</i>							
I ilium	I tNHX1						/
tenuifolium	LUNIIXI						
Limonium	ΙσNHX						I oNHX
omelinii	1						8
Lolium perenne	LpNHX						0
Lottan perenne	1						
Lophopyrum	LeNHX1						
elongatum	LeNHX2						
Malus domestica	MdNHX		MdNH	MdNHX			MdNHX
	2		X4	6			8
	MdNHX						
	1						
Malus zumi	MzNHX						
	1						
Mesembryanthe		McNH		McNHX			McNHX
тит		X1		6			7
crystallinum							
Medicago sativa			MsNHX				
		 	4				
Medicago			MtNHX	MtNHX			
truncatula			1	6			

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

						Co	ontinuation	n of table 2
1	2	3	4	5	6	7	8	9
Oryza sativa	OsNHX		1	OsNHX	OsNHX			OsNHX
Japonica	1			4	6			7
	OsNHX							
	2							
	OsNHX							
	2							
	OsNHX							
	3							
	OsNHX							
	2							
Oxybasis glauca			OgNH					
			X1					
Petunia x	PhNHX1							
hybrida								
Phaseolus	PvNHX1			PvNHX	PvNHX6			
vulgaris	PvNHX1			4				
Phoenix	PdNHX2			PdNHX	PdNHX6	PdNHX6		PdNHX8
dactylifera	PdNHX2			4				
Phragmites	PaNHX1							PaNHX7
australis	PaNHX2							
Phyllostachys	PeNHX1							
edulis								
Picea sitchensis	PsNHX4					PsNHX6		
Populus	PeNHX1				PeNHX6			PeNHX8
euphratica	PeNHX2							
	PeNHX2							
Populus	PtNHX1				PtNHX6			
tomentosa								
Populus	PtNHX1				PtNHX6			PtNHX7
trichocarpa	PtNHX2							PtMHX8
	PtNHX1							
Prunus persica	PpNHX1			PpNHX				PpNHX8
	PpNHX1			4				
Prunus mume	PmNHX			PmNH	PmNHX			PmNHX
	1			X4	6			8
Puccinellia	PtNHX1							
tenuiflora								
Pyrus betulifolia	PbNHX1							
	PbNHX1							
Pyrus	PuNHX1							
ussuriensis								
Pyrus x	PbNHX2			PbNHX	PbNHX6			1039432
bretschneideri				4				71
				PbNHX				
				4				
				PbNHX				
				4 DLNUNZ				
Demo				4				DANILING
кеаитигіа								KtINHX/

Ricinus communis ReNHX ReNHX ReNHX 4 ReNHX 4 ReNHX 8 Rosa hybrid RhNHX I Rosa hybrid RhNHX I Rosa nugosa RrhNX1 I Rosa rugosa RrhNX1 I Rosa rugosa RrhNX1 SiNHX Seamum SiNHX SiNHX indicum SiNHX SiNHX Salicornia SbNHX brachiaa I Salicornia SbNHX Fachiaa I Salicornia SenHX Salicornia SiNHX2 Salicornia SiNHX2 Salicornia SiNHX2 Salicornia SiNHX2 SinhX2 SiNHX SinhX2 SiNHX SinhX2 SiNHX SinhX2 SiNHX SinhX2 SiNHX SinhX3 A Scharai talica SiNHX2 SinhX3 SiNHX Scharai talica SiNHX2 SinhX3 SiNHX Scharai SiNHX3 SinhX3 SiNHX Solanum SiNHX3 Solanum SiNHX3 Solanum SiNHX3 SonHX3 SiNHX4<	trigyna								
communis 3 ReNIX 4 8 Rosa hybrid RhNIIX 1 1 Rosa rugosa RrNIIX1 5 5 Rosa rugosa RrNIIX1 5 5 Rosa rugosa RrNIX1 5 5 Sexamum SiNHX SiNHX SiNHX 5 Jailcornia SbNIX 5 5 5 Salicornia SbNIX 1 5 5 Salicornia ShNIX 1 5 5 Salicornia ShNIX 1 5 5 Salicornia SkNIX 1 5 5 Salicornia ShNIX2 SiNHX 5 5 Saliola SkNIX2 SiNHX 5 5 Saliola SkNIX2 SiNHX 5 5 Sesuvium 1 5 5 5 Solanum SiNHX3 SiNHX 5 5 Solanum SiNHX3 SiNHX SiNHX6 SiNHX7 Solanum	Ricinus	RcNHX			RcNHX				RcNHX
ReNHX I I I I I Rosa hybrid RhNHX I I I I I Rosa rugosa RrNIX1 I	communis	3			4				8
Image: scale of the second s		RcNHX							
Rosa hybrid RNNHX I I I Rosa rugosa RtNHX1 I I I Sesamum SiNHX1 SiNHX SiNHX6 SiNHX6 Sesamum SiNHX1 I SiNHX SiNHX6 Salicorriia SbNHX I I Salicorriia SbNHX I I Salicorriia SbNHX I I Salicorriia SeNHX I I Salicorriia SeNHX I I Salicorriia SeNHX I I Salicorriia SeNHX I I Salicorriia SiNHX2 SiNHX I Seavium I SiNHX2 SiNHX6 SpNHX7 Solanum SiNHX3 SiNHX SiNHX6 SiNHX8 Solanum SiNHX3 SiNHX SiNHX6 SiNHX7 Solanum SiNHX3 SiNHX SiNHX6 SoNHX7 Sorghun bicolor SbNHX2 <		1							
1IIIIRosa rugosaRrNHX1SiNHXSiNHXSiNHX6SiNHX8SesamumSiNHX1SiNHXSiNHX6SiNHX8indicumSbNHXSiNHXSiNHX6SiNHX8SalicorniaSbNHXIIISalicorniaSbNHXIISalicorniaSbNHXIISalicorniaSbNHXIISalicorniaSeNHXIISalicorniaSeNHXIISalicorniaSkNHXIISalicorniaSkNHXIISalicorniaSkNHXIISalicorniaSkNHXIISalicorniaSkNHXIISalicorniaSkNHX2SiNHX6SdNHX8SalicorniaSkNHX2SiNHX6SgNHX8SalicorniaSkNHX2SiNHX6SgNHX8SolarumSiNHX2SiNHX7SpNHX7SolarumSiNHX3SiNHX3SiNHX6SiNHX8SolarumSiNHX3SiNHX4SiNHX6SiNHX7SolarumSiNHX3SiNHX3SiNHX6SiNHX7SolarumSiNHX2SiNHXSiNHX6SiNHX7SolarumSiNHX3SiNHX4SiNHX6SiNHX7SolarumSiNHX2SiNHXSiNHX6SiNHX7SolarumSiNHX3SiNHX4SiNHX6SiNHX7Singun bicolorSiNHX2SiNHXSiNHX7SuaedaSiNHX3SiNHXSiNHX8 </td <td>Rosa hybrid</td> <td>RhNHX</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	Rosa hybrid	RhNHX							
Rosa rugosa Sesamum indicumReNHX1SiNHX SiNHX1SiNHX 4SiNHX6 4 SiNHX 4SiNHX6 4 SiNHX4 4SiNHX8 4Salicornia brachiataSbNHX 1IIIISalicornia brachiataSbNHX 1IIIISalicornia brachiataSbNHX 1IIIISalicornia brachiataSbNHX 		1							
Sexamum SiNHX1 SiNHX SiNHX6 SiNHX6 indicum SiNHX SiNHX SiNHX6 SiNHX8 Salicornia SbNHX I I I Salicornia SbNHX I I I I Salicornia SbNHX I I I I I Salicornia SeNHX SbNHX I	Rosa rugosa	RrNHX1							
indicum 4 SiNHX6 SiNHX6 SiNHX6 SiNHX6 SiNHX6 SiNHX6 SiNHX6 SiNHX6 SiNHX SiNHX6 SiNHX6 SiNHX6 SiNHX6 SiNHX6 SeNHX Servium SinHX2 SiNHX2 SiNHX6 SiNHX2 SiNHX2 SiNHX2 SiNHX2 SiNHX2 SiNHX2 SiNHX6 SiNHX6 SiNHX6 SiNHX2 SiNHX2 SiNHX6 SiNHX6 SiNHX7 Solanum SiNHX2 SiNHX3 SiNHX SiNHX6 SiNHX6 SiNHX8 SiNHX6 SiNHX7 Solanum SiNHX2 SiNHX3 SiNHX SiNHX6 SiNHX6 SiNHX7 Solanum SiNHX2 SiNHX7 SiNHX8 SiNHX8 SiNHX7 SiNHX8 SiNHX7 SiNHX8 SiNHX8 SiNHX8 SiNHX7 SiNHX8 SiN	Sesamum	SiNHX1			SiNHX	SiNHX6			SiNHX8
Salicornia bigeloviiSiNHX 4Image: sinthy 4Salicornia brachiataSbNHX 1Image: sinthy 4Image: sinthy 4Salicornia europaeaSeNHX 1Image: sinthy 4Image: sinthy 4Salicornia dolichostachyaSkNHX 1Image: sinthy 4Image: sinthy 4Setaria italica SiNHX2 SiNHX2 SiNHX2 SiNHX2SiNHX 4SiNHX6 4Image: sinthy 7Setaria italica SiNHX2 SiNHX2 SiNHX2 SiNHX2SiNHX 4SiNHX6 4Image: sinthy 7Sesuvium portulacastrum LycopersicumSiNHX3 4SiNHX6 4SpNHX7 7Solanum lycopersicumStNHX2 SbNHX3SiNHX 4SiNHX6 4StNHX7 7Solanum solanum lycopersicumStNHX2 SbNHX3SiNHX 4SiNHX6 4StNHX7 7Solanum solanum lycopersicumStNHX2 SbNHX3SiNHX 4SiNHX6 4StNHX7 7Solanum solanum lycopersicumStNHX2 SbNHX3SiNHX 4StNHX6 4StNHX7 7Solanum solanum solanum lycopersicumStNHX2 4StNHX6 4StNHX7 7Solanum solanum 	indicum				4	SiNHX6			
SalicorniaSbNHX4Image: Constraint of the system of the sy					SiNHX				
Salicornia bigeloviiSbNHX 1Image: solution in a solution i					4				
bigelovii 1 Image: style styl	Salicornia			SbNHX					
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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) [28].

Multiple alignment of amino acid sequences was performed using ClustalX (2.0.5) (www.clustal.org) using a series of BLOSSUM matrixes, cladistical analysis of NHX Na⁺/H⁺ 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⁺/H⁺ 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^+/H^+ 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^+/H^+ 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 frome *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⁺/H ⁺ antiporters form four clades (Fig. 1). The first clade comprises reference NHX transporter - AtNHX1 and two other members of this family - AtNHX2 and AtNHX4. Additionaly, this clade includes 219 homologs from different plans 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 Na⁺ from the vacuole to the cytosol. Perhaps this functional difference is the key element of appearance separate clade comprising AtNHX3 like Na⁺/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 Na⁺/H⁺ antiporters. Unlike other NHX transporters the Na⁺/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 Na⁺/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 Na^+/H^+ antiporters are responsible for removal of excess of Na^+ and Li^+ from the cell and retrainslocation 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 Na^+/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^+/H^+ 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 (clade3) 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^+/H^+ 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 NHXтранспортерів 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 І. В ході проведення кладистичного аналізу та подальшої класифікації було виявлено неспівпадіння назв NHX транспортерів у базах даних із типом клади 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 ФИЛОГЕНЕТИЧЕСКОКГО 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 филогенетичекое разнообразие транспортеров семейства NHX. После вторичного анализа полных последовательностей было отобрано 403 аминокислотных последовательности этих транспортных протеинов из разных видов растений. Кдадистический 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 кладистичекого 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 обмеменники сімейства NHX, функциональное разнообразие, кладистический анализ, филогенетическое дерево