

Full Length Research Paper

In-silico identification and phylogenetic analysis of auxin efflux carrier gene family in *Setaria italica* L.

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The phytohormone auxin is crucial for plant growth and development. Transportation and distribution of auxin throughout the plant is very important to maintain the auxin homeostasis. Auxin efflux carrier genes play significant roles in auxin transport. In this study, we identified 12 auxin efflux carrier genes from the *Setaria italica* genome, which are similar in number with that of monocotyledonous plant *Oryza sativa*. Twelve (12) *SiPIN* genes are distributed in eight scaffolds. All the identified genes possess the transmembrane auxin efflux carrier domain. Phylogenetic analysis shows that *SiPIN* genes are much closer to *Sorghum bicolor* and *O. sativa* *PIN* genes of the grass family.

Key words: Auxin efflux carrier, auxin efflux carrier domain, phosphorylation, transmembrane domain.

INTRODUCTION

In the model plant *Arabidopsis thaliana*, auxin plays a crucial role in regulating and coordinating plant growth and is involved in many developmental processes, including embryogenesis, meristem maintenance, organogenesis, lateral root initiation, vascular tissue differentiation and tropisms. Specific auxin influx carriers (*AUX/LAX* proteins) and efflux carriers (*PIN* and *PGP/MDR* proteins) mediate a directional, active, cell-to-cell auxin transport, creating auxin concentration maxima in specific tissues or cells. *PIN* auxin efflux carriers play a major role in mediating and regulating polar auxin transport (PAT), creating the auxin gradients that provide positional information for cells and tissues development (Benkova et al., 2003; Michniewicz et al., 2007; Reinhardt et al., 2000).

In *A. thaliana*, there are eight *PIN* genes (*AtPIN1–AtPIN8*) coding for proteins that differ in the length of the hydrophilic loop in the middle of their polypeptide chain (Krecek et al., 2009a; Zazimalova et al., 2007). The long *PIN* proteins of *Arabidopsis* viz., *PIN1*, *PIN4* and *PIN7* show plasma membrane localization and their polar localization determines direction of auxin flux (Friml 2010). The three *PIN* proteins *PIN5*, *PIN6*, and *PIN8*, have a shorter central hydrophilic domain and both *PIN5* and *PIN8*, have been shown to localize in the endoplasmic reticulum, suggesting a possible role in regulating intracellular auxin homeostasis (Wabnik et al., 2010; Wabnik et al., 2011). The classification of *AtPIN6* is more controversial since it has a partially reduced hydrophilic loop with high sequence similarities at trans-membrane

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Abbreviations: *AtPIN*, *Arabidopsis thaliana* auxin efflux carrier; *OsPIN*, *Oryza sativa* auxin efflux carrier; *PpPIN*, *Physcomitrella patens* auxin efflux carrier; *PtPIN*, *Populus trichocarpa* auxin efflux carrier; *SbPIN*, *Sorghum bicolor* auxin efflux carrier; *SiPIN*, *Setaria italica* auxin efflux carrier.

regions (Krecek et al., 2009a; Mravec et al., 2009). In addition to the eight *AtPIN* proteins, *Arabidopsis* encodes seven *PIN* like genes and they form a different clusters and the role of these is yet to find out (Paponov et al., 2005).

Many homologous *PIN* genes were well characterized in monocot species like rice (*Oryza sativa*) and maize (*Zea mays*). Both specific features and homologies between monocots and *Arabidopsis* (eudicot) *PIN* families have been shown. Monocot-specific features comprise both sequence clustering in phylogenetic analyses and expression pattern at transcript and protein level. In rice, the sequence analysis of the 12 *PIN* genes present in the genome showed that rice has four *PIN1* genes and one *OsPIN2*, while no *OsPIN* protein was grouped into the *AtPIN3*, *AtPIN4* and *AtPIN7* cluster. Four *OsPIN* genes encode for rice *PIN* proteins with a short central hydrophilic domain: three *OsPIN5* and one *OsPIN8*. Furthermore, three *OsPIN* proteins appear monocot-specific: *OsPIN9*, *OsPIN10a*, and *OsPIN10b*. *OsPIN9* has a central hydrophilic domain intermediate in length between long and short *PINs* of *Arabidopsis* and its expression analysis at transcription level suggests a possible function in adventitious root differentiation. *OsPIN10a* and *OsPIN10b* have a long central hydrophilic domain (Carraro et al., 2006; Forestan et al., 2012; Forestan and Varotto 2010; 2012; Xu and Scheres 2005). So far, three *PIN1* genes were described in maize using an antibody raised against *AtPIN1* protein (Forestan and Varotto, 2010). Recent studies of *PIN* genes on *Sorghum bicolor* revealed the presence of 11 *PIN* genes; at least three members were grouped in the *AtPIN1* cluster and another three in the *AtPIN5* cluster (Shen et al., 2010; Wang et al., 2010).

S. italica [(L.) P. Beauv.] commonly known as foxtail millet is one of the most cultivated millet species grown worldwide including India, China, Japan, Australia North and South America (Devos et al., 1998). Foxtail millet is a diploid grass with small genome (~515 Mb) and its draft sequences has been published recently (Bennetzen et al., 2012). The major phytohormone auxin is central to plant growth and development. Availability of publicly accessible genome sequences of *S. italica* lead us to find the auxin efflux carrier genes (*PIN*) using an in-silico approach. Here, we used bioinformatics and comparative genomics approaches to find auxin efflux carrier genes in *S. italica*.

MATERIALS AND METHODS

Auxin efflux carrier (*PIN*) genes of *S. italica* were identified from plant genome database (<http://www.plantgdb.org>) and phytozome (www.phytozome.net) database (Dong et al., 2004; Duvick et al., 2008; Goodstein et al., 2012). To identify *PIN* genes, orthologous auxin efflux carrier genes from *A. thaliana* were used as search query. *Arabidopsis* *PIN* genes were downloaded from "The Arabidopsis Information Resources" (<http://www.arabidopsis.org/>). Hidden markov model approach was carried out to find the auxin

efflux carrier genes of *S. italica* (Altschul et al., 1997). Identified *StPIN* genes were again confirmed by running BLASTP searches in "The Arabidopsis Information Resources" and presence of auxin efflux carrier domains were confirmed by SWISS MODEL Workspace (www.swissmodel.expasy.org/workspace/). Nomenclature of identified *StPIN* genes were carried out according to BLASTP similarity found with *A. thaliana* *AtPIN* genes. TMMOD (The Hidden Markov Model for Transmembrane Protein Topology Prediction) (<http://www.cbs.dtu.dk/services/TMHMM/>) analyses were carried out to confirm the presence of transmembrane domains in *SiPIN* proteins (Kahsay et al., 2005; Kahsay et al., 2004). Orthologous *PIN* genes from *A. thaliana* (*AtPIN*), *O. sativa* (*OsPIN*), *Physcomitrella patens* (*PpPIN*), *Populus trichocarpa* (*PtPIN*), and *S. bicolor* (*SbPIN*) were used to analyze protein sequence similarity and construction of phylogenetic trees. *OsPIN* genes were downloaded from The TIGR Rice Genome Annotation Resources (Ouyang et al., 2007) whereas, *PpPIN*, *PtPIN*, *SbPIN* genes were downloaded from plant genome database and phytozome database. Multiple alignments of *PIN* genes from the above mentioned species were carried out by using the online available software Multalin (<http://multalin.toulouse.inra.fr/multalin/>). Phylogenetic tree was constructed by using MEGA5.2 software.

RESULTS AND DISCUSSION

Genome wide analysis of the *S. italica* genome led to the identification of 12 auxin efflux carrier (*SiPIN*) genes (Table 1). This result shows, *Setaria* has the same number of *SiPIN* genes as of rice and has four more *SiPIN* genes than *A. thaliana*. The major genome assembly of *S. italica* is arranged in 336 scaffolds. The first nine scaffolds are pseudomolecules and 98.9% of sequence data is presented in the nine pseudomolecule. Besides, the *Setaria* genome has 35,471 loci containing 40,599 protein coding transcripts (Bennetzen et al., 2012). The 12 identified *S. italica* auxin efflux carrier genes are distributed in eight scaffolds. Scaffold five contains four auxin efflux carrier genes (*SiPIN4a*, *SiPIN5a*, *SiPIN5b* and *SiPIN8*). The biggest *SiPIN* gene was *SiPIN2* with an ORF (open reading frame) length of 1890 nucleotides present in scaffold 4, whereas the smallest one was *SiPIN5c* which was present in scaffold 6. Among the 12 *SiPIN* genes, seven *SiPIN* genes (*SiPIN1a*, *SiPIN1b*, *SiPIN4a*, *SiPIN4b*, *SiPIN4c*, *SiPIN4d* and *SiPIN8*) contained five introns each and *SiPIN2* and *SiPIN5d* contained six introns each (Figure 1). *SiPIN1* transcript organization matched with that of *OsPIN1* and *AtPIN1* indicating their close homology (Wang et al., 2009).

Auxin efflux carrier genes are characterized by the presence of a transmembrane auxin efflux carrier domain (Carraro et al., 2006; Forestan et al., 2012). All *SiPIN* genes contain transmembrane auxin efflux carrier domains (Figure 2). Among the 12 *SiPINs*, seven *SiPIN* genes (*SiPIN1a*, *SiPIN1b*, *SiPIN2*, *SiPIN4a*, *SiPIN4b*, *SiPIN4c* and *SiPIN4d*) contain a long transmembrane domain. The other *SiPIN* genes (*SiPIN5a*, *SiPIN5b*, *SiPIN5c*, *SiPIN5d* and *SiPIN8*) contained a short transmembrane domain. In *Arabidopsis*, *AtPIN4* has a short transmembrane domain which is different from that

Table 1. Phytozome locus ID and transcript information of SiPIN. Naming of SiPIN were done as found by BLASTP against the Arabidopsis Information Resources database.

Locus ID	Gene name	ORF Length	Number Of a.a	Number of Introns	5'-3' Coordinates
Si016714m	<i>SiPIN1a</i>	1785	594	5	scaffold_1: 37807344 - 37810727
Si006110m	<i>SiPIN1b</i>	1794	597	5	scaffold_4: 8906772 - 8909697
Si006060m	<i>SiPIN2</i>	1890	629	6	scaffold_4: 30687696 -30691064
Si000693m	<i>SiPIN4a</i>	1863	620	5	scaffold_5: 30444777 - 30448448
Si009737m	<i>SiPIN4b</i>	1677	558	5	scaffold_7: 34823548 - 34826535
Si025109m	<i>SiPIN4c</i>	1773	590	5	scaffold_3: 9460738 - 9463047
Si026177m	<i>SiPIN4d</i>	1683	560	5	scaffold_8: 1266812 - 1269757
Si001955m	<i>SiPIN5a</i>	1095	364	4	scaffold_5: 44864303 - 44869298
Si003879m	<i>SiPIN5b</i>	1272	423	4	scaffold_5: 39347962 - 39350295
Si015697m	<i>SiPIN5c</i>	1029	342	2	scaffold_6: 33597186 - 33599056
Si033365m	<i>SiPIN5d</i>	1134	377	6	scaffold_2: 36679971 - 36681750
Si003769m	<i>SiPIN8</i>	1081	361	5	scaffold_5: 35148382 - 35150803

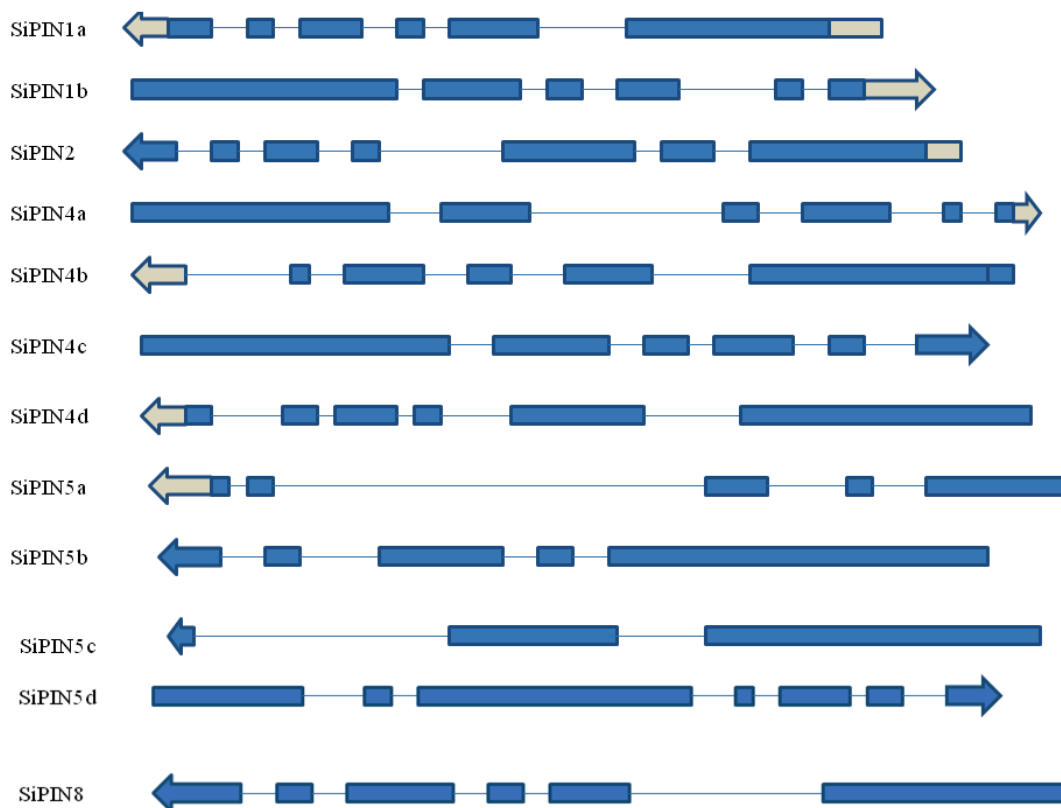


Figure 1. Transcript organization of *SiPIN* genes. Blue color boxes indicate the exons and lines indicate the introns of respective *SiPIN* genes. The arrow mark indicate the direction of expression of transcript.

of *SiPIN* genes showing diversification of PIN genes among species. *Arabidopsis AtPIN1*, rice *OsPIN1* and *OsPIN2* were grouped under long transmembrane auxin efflux carrier domain (Wang et al., 2009). *SiPIN1* and *SiPIN2* genes contain a long transmembrane domain, showing close relationship with that of *AtPIN1*, *OsPIN1*

and *OsPIN2*.

Phylogenetic analysis with orthologous PIN genes from *A. thaliana (AtPIN)*, *O. sativa (OsPIN)*, *Physcomitrella patens (PpPIN)*, *Populus trichocarpa (PtPIN)*, *S. bicolor (SbPIN)* resulted in clustering into three major groups (group I, II and III) (Figure 3). In group I, *SiPIN1a* and

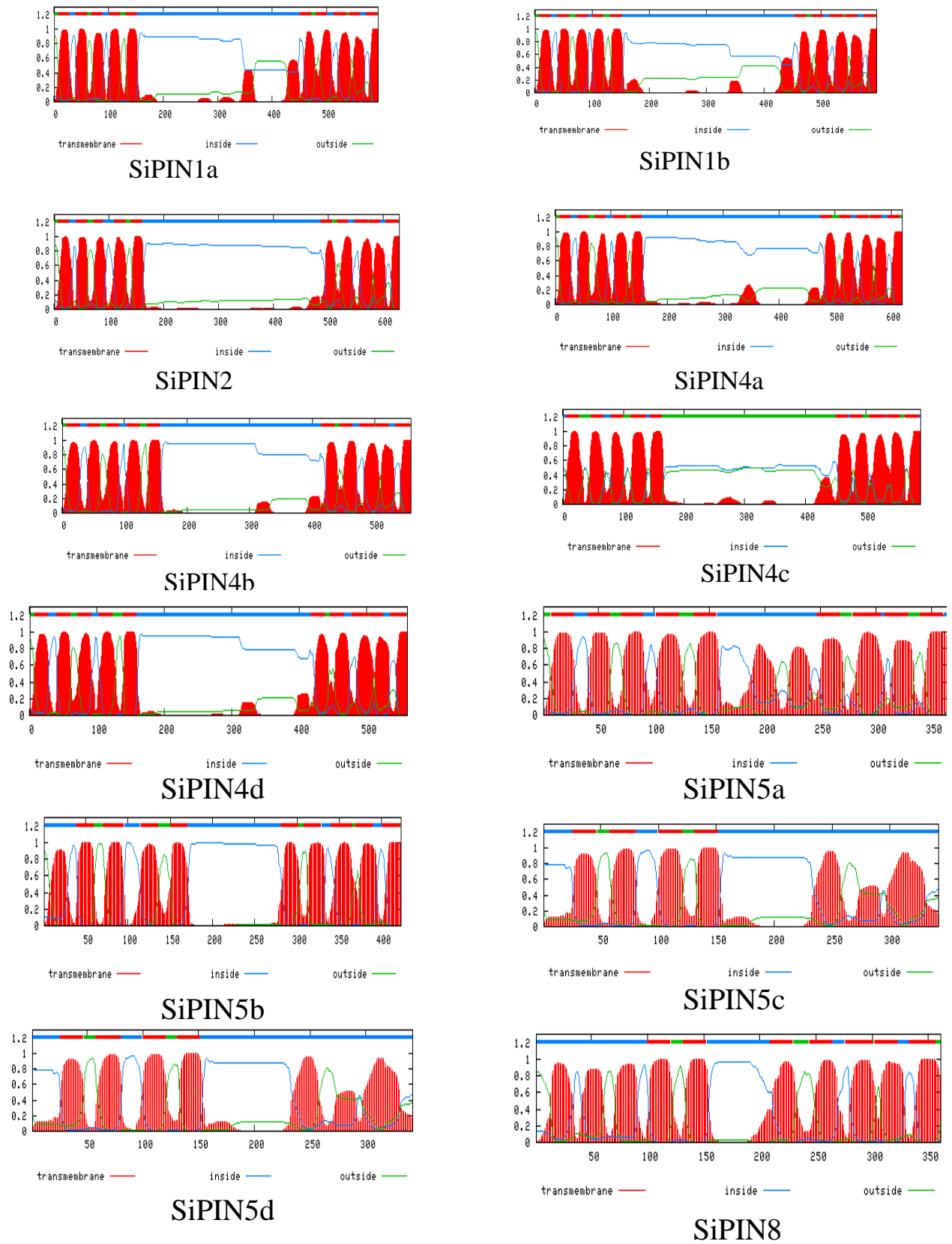


Figure 2. Transmembrane domain prediction of *SiPIN* genes by TMMOD (Hidden Markov Model for transmembrane protein topology prediction). *SiPIN1a*, *SiPIN1b*, *SiPIN2*, *SiPIN4a*, *SiPIN4b*, *SiPIN4c* and *SiPIN4d* contains long transmembrane domain where as *SiPIN5a*, *SiPIN5b*, *SiPIN5c*, *SiPIN5d* and *SiPIN8* contains short transmembrane domain.

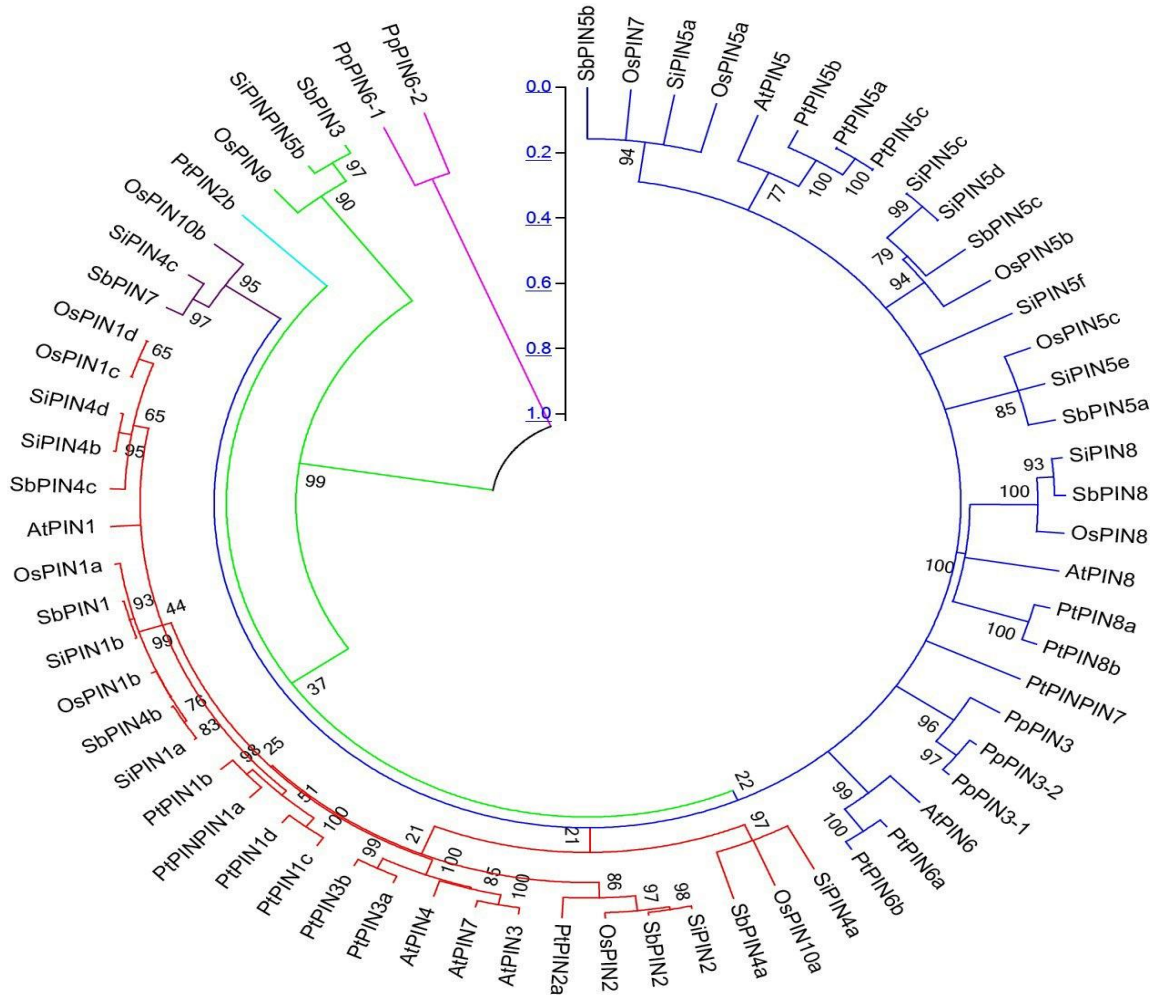


Figure 3. Phylogenetic tree of *SiPIN* genes with orthologous PIN genes of *Arabidopsis thaliana* (*AtPIN*), *Oryza sativa* (*OsPIN*), *Sorghum bicolor* (*SbPIN*), *Populus trichocarpa* (*PtPIN*) and *Physcomitrella patens* (*PpPIN*). Statistical method used to construct the phylogenetic tree was neighbor joining method; test phylogeny-boot strap method; no. of boot strap replication -500; substitution type-amino acids and model used was Jones-Taylor-Thornton (JTT).

SiPIN1b clustered with *OsPIN1*; *SiPIN4b* and *SiPIN4d* clustered with *SbPIN4c*; *SiPIN4a* clustered with *SbPIN4a*; *SiPIN4c* clustered with *SbPIN7* and *OsPIN10b*; *SiPIN2* clustered with *ObPIN2* and *OsPIN2*; *SiPIN8* clustered with *SbPIN8* and *OsPIN8*. In group II, *SiPIN5a* clustered with *SbPIN5b* and *OsPIN5b*; *SiPIN5c* clustered with *SbPIN5c* and *OsPIN5c*; *SiPIN5d* clustered with *SbPIN5a* and *OsPIN5c*; *SiPIN5b* clustered with *SbPIN3* and *OsPIN9*. In group III, *AtPIN6* clustered with *PtPIN6*. There is no gene of *Setaria* or any other grass in this cluster, showing diversification of *PIN* genes. Cluster analysis reflects, *S. italica* *PIN* genes are much closer to *PIN* genes of grasses *Sorghum bicolor* and *Oryza sativa*. Multiple alignment of amino acid sequences shows conserved N and C-terminal domains (Supplementary Figure 1). The N-terminal region shows a conserved S-P/T-P motif, a potential target phosphorylation site for

mitogen activated protein kinases (MAPK) (Sinha et al., 2011). The central hydrophilic loop is dynamic in nature and differs from each other in terms of sequence homology, but some *PIN* genes are conserved in this dynamic region with a T-P-R motif (Supplementary Figure 1). The T-P-R motif is a target phosphorylation site of mitogen activated protein kinase 3 and mitogen activated protein kinase 6 (Sorensson et al., 2012). The T-P-R motif is conserved only in the case of long transmembrane auxin efflux carrier domains. This shows that, although the central hydrophilic loop is diverse in nature, its phosphorylation events are conserved to carry out specific function suggesting that evolution of protein phosphorylation is conserved.

The *PIN*-Formed (*PIN*) proteins are a plant-specific family of transmembrane proteins that transport the phytohormone auxin as substrate molecule. There is very

limited data available which suggests auxin is a signaling molecule of ancient origin. The *PIN* gene family is found only in genomes of land plants. They act as regulator and play key roles in developmental process including embryogenesis, morphogenesis and organogenesis (Krecek et al., 2009b). The number of *PIN* genes present in *S. italica* (12) is equal to that of rice (12) and more than that of *Arabidopsis* (8) suggesting that the presence of more *PIN* genes may have some extra role in development and morphogenesis. The predicted structure of a *PIN* protein is similar to the structure of membrane transport proteins that use the electrochemical gradient across the membrane to transport molecules. All the identified *PIN* proteins have two hydrophobic domains with cytoplasmic orientation. The transmembrane helices of hydrophobic domains are highly conserved in their amino acid sequence. But substantial differences are present between the long and short *PIN*s. The hydrophobic domains of all long *PIN* proteins contain the amino acids at invariant position, but these positions are not invariants in short *PIN*s. The presence of invariant amino acid sequences in long *PIN*s may play major roles, which has not been retained in short *PIN*. The loop between the transmembrane helices being present within the hydrophobic domain exhibits dynamic variability in size and sequence.

Conclusion

S. italica popularly known as foxtail millet is one of the best studied millet species in the world. The genome sequencing project of this plant is going to be completed in the near future. This will open the door for progressing research of this plant at the molecular level. Auxin efflux carrier genes identified in this report will help to understand the role of auxin signaling and its role in growth, development as well as response to different biotic and abiotic stresses. Phylogenetic analysis shows that auxin efflux carrier genes in species of grass family are conserved.

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REFERENCES

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.

Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, Jurgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115:591-602

Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, Estep M, Feng L, Vaughn JN, Grimwood J, Jenkins J, Barry K, Lindquist E, Hellsten U, Deshpande S, Wang X, Wu X, Mitros T, Triplett J, Yang X, Ye C-Y, Mauro-Herrera M, Wang L, Li P, Sharma M, Sharma R, Ronald PC, Panaud O, Kellogg EA, Brutnell TP, Doust AN, Tuskan GA, Rokhsar D, Devos KM (2012) Reference genome sequence of the model plant *Setaria*. *Nat. Biotechnol.* 30:555-+

Carraro N, Forestan C, Canova S, Traas J, Varotto S (2006) ZmPIN1a and ZmPIN1b encode two novel putative candidates for polar auxin transport and plant architecture determination of maize. *Plant Physiol.* 142:254-264

Devos KM, Wang ZM, Beales J, Sasaki Y, Gale MD (1998) Comparative genetic maps of foxtail millet (*Setaria italica*) and rice (*Oryza sativa*). *Theor. Appl. Genet.* 96:63-68

Dong QF, Schlueter SD, Brendel V (2004) PlantGDB, plant genome database and analysis tools. *Nucleic Acids Res.* 32:D354-D359

Duvick J, Fu A, Muppirala U, Sabharwal M, Wilkerson MD, Lawrence CJ, Lushbough C, Brendel V (2008) PlantGDB: a resource for comparative plant genomics. *Nucleic Acids Res.* 36:D959-D965

Forestan C, Farinati S, Varotto S (2012) The Maize PIN Gene Family of Auxin Transporters. *Front. Plant Sci.* 3: 16

Forestan C, Varotto S (2010) PIN1 auxin efflux carriers localization studies in *Zea mays*. *Plant Signal. Behav.* 5: 436-439

Forestan C, Varotto S (2012) The role of PIN auxin efflux carriers in polar auxin transport and accumulation and their effect on shaping maize development. *Mol. Plant* 5:787-798

Friml J (2010) Subcellular trafficking of PIN auxin efflux carriers in auxin transport. *Europ. J. Cell Biol.* 89:231-235

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Res.* 40:D1178-D1186

Kahsay RY, Gao G, Liao L (2005) An improved hidden Markov model for transmembrane protein detection and topology prediction and its applications to complete genomes. *Bioinformatics* 21:1853-1858

Kahsay RY, Liao L, Gao G (2004) An improved hidden Markov model for transmembrane topology prediction. In: Khoshgoftaar TM (ed) *Ictai 2004: 16th IEEE International Conference on Tools with Artificial Intelligence*, Proceedings. pp. 634-639

Krecek P, Skupa P, Libus J, Naramoto S, Tejos R, Friml J, Zazimalova E (2009a) The PIN-FORMED (PIN) protein family of auxin transporters. *Genome Biol.* 10:249

Krecek P, Skupa P, Libus J, Naramoto S, Tejos R, Friml J, Zazimalova E (2009b) The PIN-FORMED (PIN) protein family of auxin transporters. *Genome Biol.* 10:249

Michniewicz M, Zago MK, Abas L, Weijers D, Schweighofer A, Meskiene I, Heisler MG, Ohno C, Zhang J, Huang F, Schwab R, Weigel D, Meyerowitz EM, Luschnig C, Offringa R, Friml J (2007) Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell* 130:1044-1056

Mravec J, Skupa P, Bailly A, Hoyerova K, Krecek P, Bielach A, Petrasek J, Zhang J, Gaykova V, Stierhof YD, Dobrev PI, Schwarzerova K, Rolcik J, Seifertova D, Luschnig C, Benkova E, Zazimalova E, Geisler M, Friml J (2009) Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* 459:1136-1140.

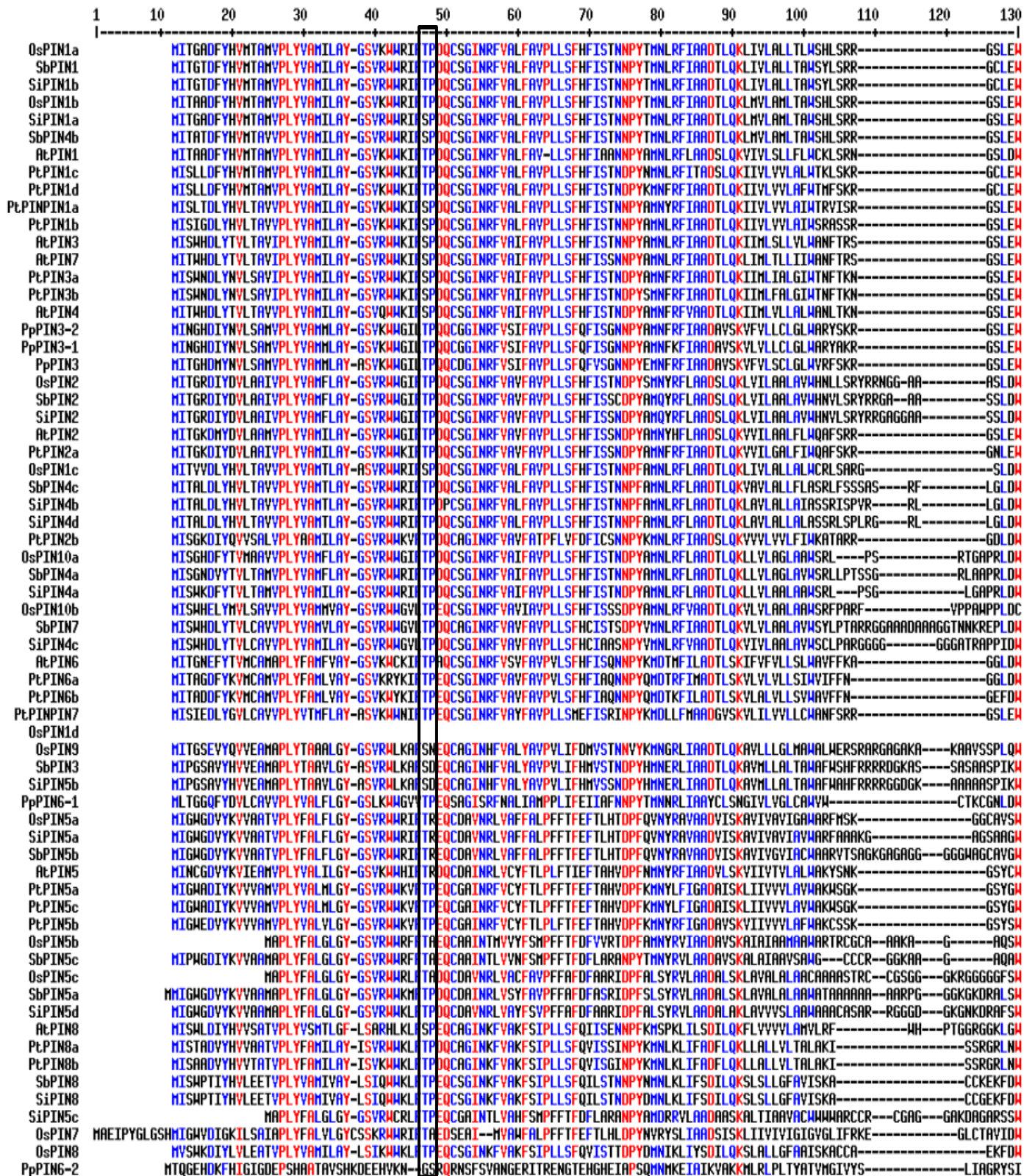
Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K, Thibaud-Nissen F, Malek RL, Lee Y, Zheng L, Orvis J, Haas B, Wortman J, Buell CR (2007) The TIGR Rice Genome Annotation Resource: Improvements and new features. *Nucleic Acids Res.* 35:D883-D887

Paponov IA, Teale WD, Trebar M, Bliou I, Palme K (2005) The PIN auxin efflux facilitators: evolutionary and functional perspectives. *Trends Plant Sci.* 10:170-177

Reinhardt D, Mandel T, Kuhlemeier C (2000) Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* 12:507-518

Shen C, Bai Y, Wang S, Zhang S, Wu Y, Chen M, Jiang D, Qi Y (2010) Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in *Sorghum bicolor* under phytohormone and abiotic stress.

- FEBS J. 277:2954-2969
- Sinha AK, Jaggi M, Raghuram B, Tuteja N (2011) Mitogen-activated protein kinase signaling in plants under abiotic stress. *Plant Signal. Behav.* 6:196-203
- Sorensson C, Lenman M, Veide-Vilg J, Schopper S, Ljungdahl T, Grotli M, Tamas MJ, Peck SC, Andreasson E (2012) Determination of primary sequence specificity of Arabidopsis MAPKs MPK3 and MPK6 leads to identification of new substrates. *Biochem. J.* 446:271-278
- Wabnik K, Kleine-Vehn J, Balla J, Sauer M, Naramoto S, Reinohl V, Merks RM, Govaerts W, Friml J (2010) Emergence of tissue polarization from synergy of intracellular and extracellular auxin signaling. *Mol. Syst. Biol.* 6:447
- Wabnik K, Kleine-Vehn J, Govaerts W, Friml J (2011) Prototype cell-to-cell auxin transport mechanism by intracellular auxin compartmentalization. *Trends Plant Sci.* 16:468-475
- Wang J-R, Hu H, Wang G-H, Li J, Chen J-Y, Wu P (2009). Expression of PIN Genes in Rice (*Oryza sativa* L.): Tissue specificity and regulation by hormones. *Mol. Plant* 2:823-831
- Wang S, Bai Y, Shen C, Wu Y, Zhang S, Jiang D, Guilfoyle TJ, Chen M, Qi Y (2010) Auxin-related gene families in abiotic stress response in *Sorghum bicolor*. *Funct. Integr. Genomics* 10:533-546
- Xu J, Scheres B (2005) Cell polarity: ROPing the ends together. *Curr. Opin. Plant Biol.* 8:613-618
- Zazimalova E, Krecek P, Skupa P, Hoyerova K, Petrasek J (2007) Polar transport of the plant hormone auxin - the role of PIN-FORMED (PIN) proteins. *Cell. Mol. Life Sci.* 64:1621-1637

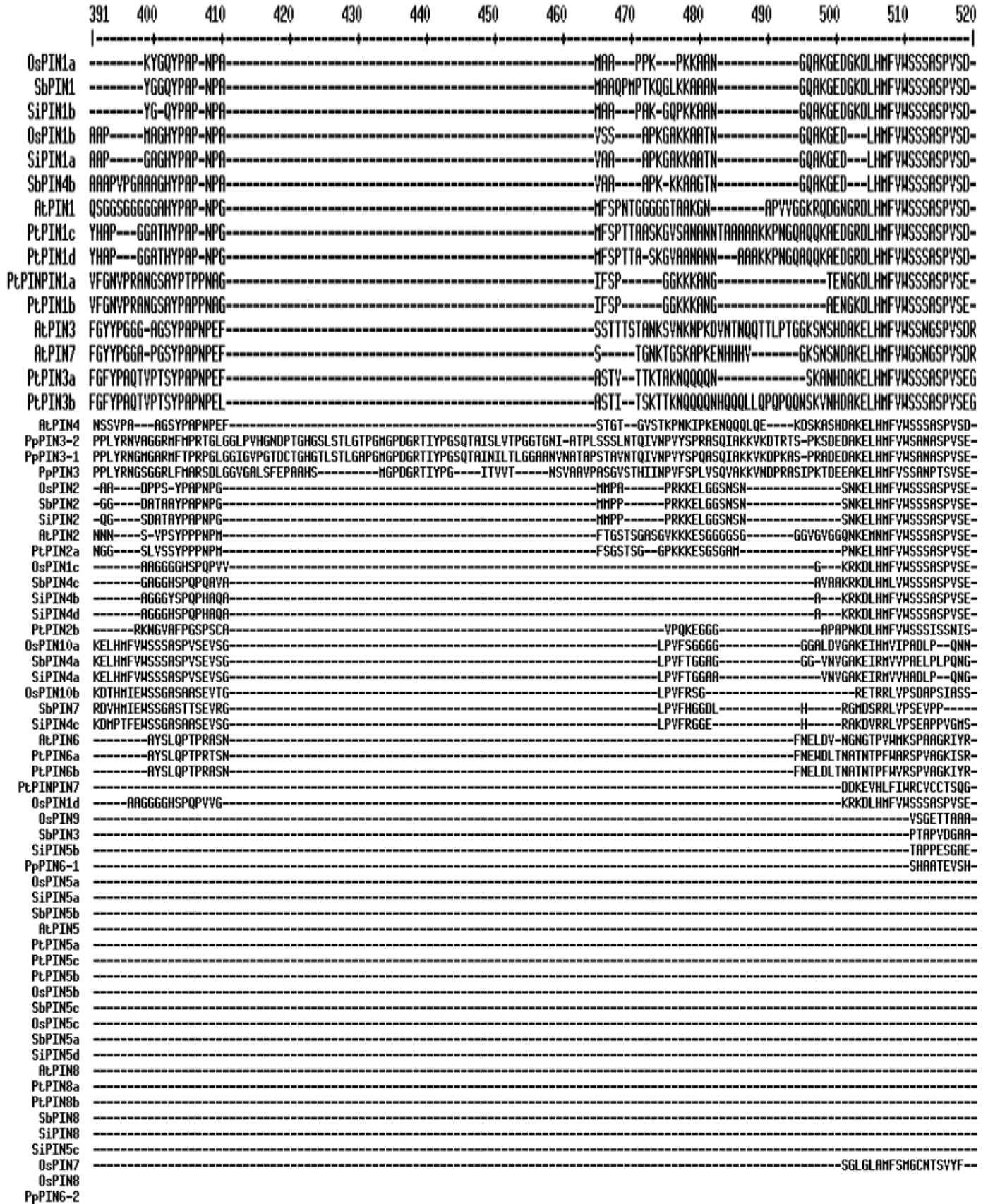


Supplementary Figure 1. Multiple sequence alignment of amino acid shows presence of N and C-terminal conserved region. N-terminal region shows presence of conserved S-P/T-P motif, target phosphorylation site of mitogen activated protein kinase. The central hydrophilic region is very dynamic in nature and differ from each other, but still contains conserved S-P-R/T-P-R motif, target phosphorylation site of mitogen activated protein kinase 3 and mitogen activated protein kinase 6. S-P-R/T-P-R motifs are conserved only in long transmembrane domain containing PIN gene.

	131	140	150	160	170	180	190	200	210	220	230	240	250	260																																																																						
OspIN1a	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	R	I	L	T	E	Q	F	P	T	A	G	A	---	I	A	S	I	V	V	D	A	D	V	S	L	-	D	G	R	R	D	I	E	T	E	A	E	V	K	---	E	D	G	K	I	H	V	T	V	R	R	S	N	A	S				
SbPIN1	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	R	I	L	T	E	Q	F	P	T	A	G	A	---	I	A	S	I	V	V	D	P	V	S	L	-	D	G	R	D	A	I	E	T	E	A	E	V	K	---	E	D	G	K	I	H	V	T	V	R	R	S	N	A	S					
SiPIN1b	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	R	I	L	T	E	Q	F	P	T	A	G	A	---	I	A	S	I	V	V	D	P	V	S	L	-	D	G	R	D	A	I	E	T	E	A	E	V	K	---	E	D	G	K	I	H	V	T	V	R	R	S	N	A	S					
OspIN1b	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	R	I	L	T	E	Q	F	P	T	A	G	A	---	I	A	S	I	V	V	D	P	V	S	L	-	D	G	R	D	A	I	E	T	E	A	E	V	K	---	E	D	G	R	I	H	V	T	V	R	R	S	N	A	S					
SiPIN1a	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	R	I	L	T	E	Q	F	P	T	A	G	A	---	I	A	S	I	V	V	D	P	V	S	L	-	D	G	R	D	A	I	E	T	E	A	E	V	K	---	E	D	G	K	I	H	V	T	V	R	R	S	N	A	S					
SbPIN4b	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	R	I	L	T	E	Q	F	P	T	A	G	A	---	I	A	S	I	V	V	D	P	V	S	L	-	D	G	R	D	A	I	E	T	E	A	E	V	K	---	E	D	G	K	I	H	V	T	V	R	R	S	N	A	S					
AtPIN1	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GDL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	I	H	V	D	S	D	I	S	L	-	D	G	R	Q	P	-	L	E	T	E	A	E	I	K	---	E	D	G	K	L	H	V	T	V	R	R	S	N	A	S			
PtPIN1c	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	I	H	V	D	S	D	I	S	L	-	D	G	R	Q	P	-	L	E	T	E	A	E	I	K	---	E	D	G	K	L	H	V	T	V	R	R	S	N	A	S			
PtPIN1d	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FM	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	I	H	V	D	S	D	I	S	L	-	D	G	R	Q	P	-	L	E	T	E	A	E	I	K	---	E	D	G	K	L	H	V	T	V	R	R	S	N	A	S			
PtPIN1e	MITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	F	R	V	D	S	D	I	S	L	-	D	G	R	E	P	-	L	Q	T	E	A	E	V	G	---	E	D	G	R	I	H	V	T	V	R	R	S	T	S				
PtPIN1b	SITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	F	R	V	D	S	D	I	S	L	-	D	G	R	E	P	-	L	Q	T	E	A	E	V	G	---	E	D	G	K	L	H	V	T	V	R	R	S	T	S				
AtPIN3	SITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	H	-	D	F	L	E	T	D	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	N	A	S			
AtPIN7	SITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	H	-	D	F	L	E	T	D	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	N	A	S			
PtPIN3a	MITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	R	-	D	F	L	E	T	D	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	N	A	S			
PtPIN3b	MITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	T	A	G	A	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	R	-	D	F	L	E	T	D	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	N	A	S			
AtPIN4	MITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GDL	T	Q	A	V	V	L	Q	II	WY	T	L	L	L	M	Y	E	R	A	K	I	L	I	M	Q	F	P	E	N	A	G	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	R	E	P	V	L	-	T	E	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	V	S
PpPIN3-2	MITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GDL	T	Q	A	V	V	L	Q	II	WY	T	L	L	L	M	Y	E	R	A	K	I	L	I	M	Q	F	P	E	N	A	G	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	R	E	P	V	L	-	T	E	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	V	S
PpPIN3-1	MITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GDL	T	Q	A	V	V	L	Q	II	WY	T	L	L	L	M	Y	E	R	A	K	I	L	I	M	Q	F	P	E	N	A	G	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	R	E	P	V	L	-	T	E	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	V	S
PpPIN3	VITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GDL	T	Q	A	V	V	L	Q	II	WY	T	L	L	L	M	Y	E	R	A	R	I	L	I	M	Q	F	P	E	N	A	G	---	I	V	S	F	K	V	E	S	D	V	S	L	-	D	G	R	E	P	V	L	-	T	E	A	E	I	G	---	D	D	G	K	L	H	V	T	V	R	R	S	V	S
OspIN2	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	P	D	V	G	A	---	I	A	S	F	R	V	D	S	D	V	S	L	-	N	G	R	E	A	-	L	Q	A	D	A	E	V	G	---	R	D	G	R	V	H	V	I	R	R	S	A	S				
SbPIN2	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	P	D	V	G	A	---	I	A	S	F	R	V	D	S	D	V	S	L	-	N	G	R	E	A	-	L	Q	A	D	A	E	V	G	---	R	D	G	R	V	H	V	I	R	R	S	A	S				
SiPIN2	TITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	P	D	V	G	A	---	I	A	S	F	R	V	D	S	D	V	S	L	-	N	G	R	E	A	-	L	Q	A	D	A	E	V	G	---	R	D	G	R	V	H	V	I	R	R	S	A	S				
AtPIN2	MITL	FSL	STL	PNTL	VNGI	PL	PKG	MY	GDFS	-----	GSL	MV	QIV	VVL	QC	II	WY	TL	ML	FL	F	Y	R	G	A	K	L	I	S	E	Q	F	P	E	T	A	G	A	---	I	T	S	F	R	V	D	S	D	V	S	L	-	N	G	R	E	P	-	L	Q	T	D	A	E	I	G	---	D	D	G	K	L	H	V	V	R								

	261	270	280	290	300	310	320	330	340	350	360	370	380	390
OspIN1a	RSDVYSRSMGFS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MVG	RSSNF	FAG	DAFGV	RTGATPRPS	NYEEDA	APNKAGS
SbPIN1	RSDIYSRRSMGFS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MVG	RSSNF	FAG	DAFGL	RTGATPRPS	NYEEDA	GGKANK
SIPIN1b	RSDIYSRRSMGFS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MVG	RSSNF	FAG	DAFGV	RTGATPRPS	NYEEDA	GKANK
OspIN1b	RSDIYSRRSMGFS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MVG	RSSNF	GAA	DAFGV	RTGATPRPS	NYEEDAS	KPKYPLPASN
SIPIN1a	RSDIYSRRSMGFS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MVG	RSSNF	GAA	DAFGI	RTGATPRPS	NYEEDAS	KPKYPLPVYN
SbPIN4b	RSDIYSRRSMGFS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MVG	RSSNF	GAA	DAFGI	RTGATPRPS	NYEEDAA	KPAKYPLPVYN
AtPIN1	RSDIYSRRSQGLS	A	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MMA	SGGGR	NSNFGPG	EA	VFGSKGP	TPRPS	NYEEDGGPAKPTAAGTAAGAGRFHY
PtPIN1c	RSDIFSRRSQGLS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MMA	AG	RNSNFGAS	DVYGL	SASRGP	TPRPS	NFEEEHG
PtPIN1d	RSDIFSRRSQGLS	S	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MMA	AG	RNSNFGAS	DVYGL	SASRGP	TPRPS	NFEENEHG
PtPIMPIN1a	RSDVFSRSMHGLN	SGLS	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	K	N		ASN	SRHS	NFSNLQF	DEESGGLG
PtPIN1b	RSEVFSHSHGLN	SGLS	TPRSNL	TNAE	IYSLQSSRN	TPRSS	FNHTDFYS	MV	N		GKN	SRHS	NFTNLQF	DEESGGLG
AtPIN3	RRSFCG	PMM	TPRSNL	TGAE	IYSLST	TPRSN	FNHSDFYS	MGFP	GRL	SNFGPA	DMSY	QSSRGP	TPRPS	NFEENCA
AtPIN7	RRSFYGGGGTMM		TPRSNL	TGAE	IYSLNT	TPRSN	FNHSDFYS	MGFP	GRL	SNFGPA	DMSY	QSSRGP	TPRPS	NFEESCA
PtPIN3-1	RRSLGPGFSFGM		TPRSNL	TGAE	IYSLSSRN	TPRSN	FNPSDFYS	MGVQGF	GRHS	NLGP	DL	YSVQSSRGP	TPRPS	NFEENCA
PtPIN3b	RRSLGPGFSFGL		TPRSNL	TGAE	IYSLSSRN	TPRSN	FNPSDFYS	MGVQGF	GRHS	NLGP	DL	YSVQSSRGP	TPRPS	NFEENCA
AtPIN4	RRSLM	M	TPRSNL	TGAE	IYSLSS	TPRSN	FNHSDFYS	MGFP	GRL	SNFGPA	DL	YSVQSSRGP	TPRPS	NFEENCA
PpPIN3-2	RSQGMHSAHSHMP	SSKAL	TPRSNL	TGAE	IYSHSSVNL	TPRSS	FNQGEFYS	MMAQRS	PHRQ	SNFDT	DVYSL	QSSRGP	TPRPS	NFEENKDMHTHRGLNLTSPRFV
PpPIN3-1	RSQGMHSAHSHMP	SSKAL	TPRSNL	TGAE	IYSHSSVNL	TPRSS	FNQGEFYS	MMAQRS	PHRQ	SNFDT	DVYSL	QSSRGP	TPRPS	NFEENKDMHTHRGLNLTSPRFV
PpPIN3	RSQGMHSAHSHIP	SSKAL	TPRSNL	TGAE	IYSHSSVNL	TPRSS	FNQGEFYS	MMAQRS	PHRQ	SNFDT	DVYSL	QSSRGP	TPRPS	NFEENKDMHTHRGLNLTSPRFV
OspIN2	STTGGGGGAARSGVY	RAYGASNAH	TPRSNL	TGVE	IYSLQTSRE	TPRSS	FNQDFYAMF	NGSKMASQ			ASP	MAHQGGAGGR	RAQLDEQVTN	KFASGK
SbPIN2	STTGGGGGARSGVGGYRPGYSSAN		TPRSNL	TGVE	IYSLQTSRE	TPRSS	FNQDFYAMF	NGSKMASPL			AQP	G	ARAPGLDEQVAN	KFASGK
SIPIN2	STTG	HGAARSGVGGYRPGYSSAN	TPRSNL	TGVE	IYSLQTSRE	TPRSS	FNQDFYAMF	NGSKMASPL			AQP	G	ARAPGLDEQVAN	KFASGK
AtPIN2	SSHTSSFNKSHGGGL	NSSM	TPRSNL	TGVE	IYVQSSRE	TPRSS	FNQDFYAMF	NASKAPSRHGYTNSYGGAGAGPGGDVYSLQSSKGVTPRTSNFDEEYMKAKKAGRGRSMGELY						
PtPIN2a	SSIVSSFNKSHGLNS	ITSM	TPRSNL	TGVE	IYVQSSRE	TPRSS	FNQDFYAMF	ASKARSPKHGYTNSFQGGI			GDVYSL	QSSKATPRTSNFDEEYMKAKKAGRGRSMGELY		
OspIN1c	RSEARCSHGTSQSH	SQSMQ	TPRSNL	SGVE	IYSLQSSRN	TPRSS	FNHAEFFNIVG				NGKQ			DEEK
SbPIN4c	RSEARCSH-SHSH	SQSMQ	TPRSNL	SGVE	IYSLQSSRN	TPRSS	FNHAEFFNIVG				AGAK	GAAARAGDEEK		
SIPIN4b	RSEARCSH-SHSH	SQSMQ	TPRSNL	SGVE	IYSLQSSRN	TPRSS	FNHAEFFNIVG				AKG			AGDEEK
SIPIN4d	RSEARCSH-SHSH	SQSMQ	TPRSNL	SGVE	IYSLQSSRN	TPRSS	FNHAEFFNIVG				GAK			AGDEEK
PtPIN2b	SSNFSSRDRFDGAWP	VLSVHL	TPRSNL	SGVE	IYVQSSRE	TPRSS	YRQDLP	NLNSFG			DIYSL	QSSRNSVPRISN	LEEMR	
OspIN10a	RRSLLVTT		TPRSNL	TGAE	IYSLSSRN	TPRSN	FNHADFFA	MYGGGPPPTPARV	RGSS	FAGSEL	YSLQSS	RGPT	PRQSNFDEHSARP	PKPPATTGALNHA
SbPIN4a	RRSLLMVTT		TPRSNL	TGAE	IYSHSSRN	TPRSN	FNHADFFA	MYD	GAPPP	TPAGG	RGSS	FAGAEVFS	SHSS	RGPT
SIPIN4a	RRSLMGVT		TPRSNL	TGAE	IYSHSSRN	TPRSN	FNHADFFA	MYD	GAPPP	TPAGG	RGSS	FAGAEVFS	SHSS	RGPT
OspIN10b	SRSSRAAA		TPRSNL	TGVE	IYSLSSRN	TPRSN	FTLADI	PGHQPPN	SAL	RASSF	GADL	SLHSSRQHT	TPRPS	SFDEHAAA
SbPIN7	PSRRSLAAT	AA	TPRSNL	TGVE	IYSHSSRN	TPRSS	GIAHADIGW	PARAP	LHGASS	LMS	SFGADL	SLHP	TPR	SSFDEQAV
SIPIN4c	SRRLA		TPRSNL	TGVE	IYVSSRN	TPRSS	FAYGDVSA	TGAR	PLHGAS	HRMSS	FADL	SLHSS	RQHT	TPR
AtPIN6	VPDSVSSSLCL		TPRSNL	SNAE	IFSVN	TPN	RFFHGGGG	SLQFYNGS	NEIM	FCNGDL	GG	FTRPLG	ASPR	LSGYSSD
PtPIN6a	APDSALSSSICL		TPRSNL	SNAE	IFSVS	TPN	PLQYHGYNGRFS	HGN	NEIM	FCNGDL	G	FYHR	SG	TSPR
PtPIN6b	APDSALSSSICL		TPRSNL	SNAE	IFSVN	TPN	PLQYHGYNGRFS	HGN	NEIM	FCNGDL	G	LAY	RS	TSPR
PtPININ7	EITENVTKV	AD	QFRSM	YAAAVDG										
OspIN1d	RSEARCSHGTSQSH	SQSMQ	TPRSNL	SGVE	IYSLQSSRN	TPRSS	FNHAEFFNIVG				NGK	HGDEEK		
OspIN9	NGNAVADR		PQEV	YVNI	EIT	EAAR	AKD							
SbPIN3	SIHEHDRS		HHQ	VYVNI	EIT	EAAR	AVST							
SIPIN5b	I	HAERS	Q	VYVNI	EIT	EAAR	ADSP							
PpPING-1	HGFEHESG		IP	ALL	TQ	EHDFRIG	IGDEP							
OspIN5a	DGAERAAA		AG	KDVE	AR	AAAGT	VYVAAA							
SIPIN5a	AREAPPVK		D	EAR	AD	AAAVV	VYVVS							
SbPIN5b	DVPDSPVK		D	EAR	AD	AAAVV	VYVVS							
AtPIN5	NISDVQVD		N	I	ES	G	KRET	VVY						
PtPIN5a	SMPDKOLE		G	N	A	D	S	T	V	S				
PtPIN5c	SMPDKOLE		G	N	A	D	S	T	V	S				
PtPIN5b	NNSDKOLE		G	S	V	D	N	T	E	S				
OspIN5b	PVYSSSS	PPEK	Q	S	D	V	E	M	G	A	V	A	P	G
SbPIN5c	PVSSSSSSSSPPSPPPK	DAAR	E	K	D	V	E	N	A	E	T	A	A	A
OspIN5c	PPPPPTGT		D	D	D	D	D	D	D	D	D	D	D	D
SbPIN5a	ARVEPADG		D	V	E	S	G	G	G	G	G	G	G	G
SIPIN5d	PAARE		E	E	G	G	D	V	E	S	G	G	E	T
AtPIN8	NDQEEANI		E	D	E	P	K	E	E	D	E	E	V	A
PtPIN8a	TGDQEARLQ		E	S	Q	Y	K	E	G	G	E	V	H	S
PtPIN8b	TGDLEARLQ		E	A	Q	H	K	D	E	G	V	Q	R	T
SbPIN8	TNEAESGT		T	G	P	H	Q	R	H	E	E	G	K	R
SIPIN8	NEGESGT		P	G	P	E	E	R	H	E	E	G	R	K
SIPIN5c	SSPPPAE		K	G	D	V	E	N	A	A	E	P	D	A
OspIN7	GARLPLFKSVAR		K	L	A	C	N	P	L	H	A	S	V	I
OspIN8	AKRMLLALAIRFFLGPAL		M	G	S	S	A	I	G	R	G	V	L	L
PpPING-2														

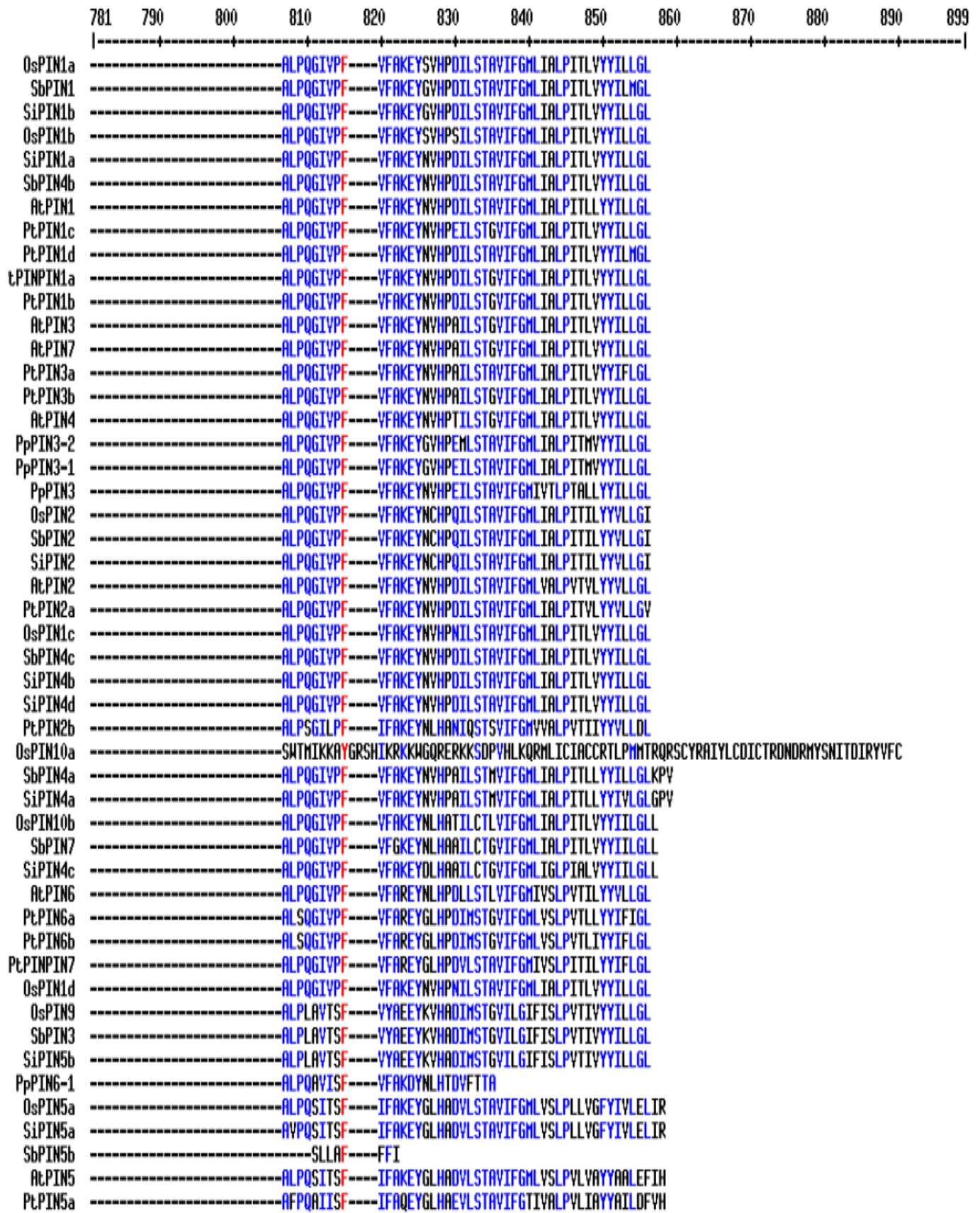
Supplementary Figure 1. Contd.



Supplementary Figure 1. Contd.

	651	660	670	680	690	700	710	720	730	740	750	760	770	780
OspIN1a	SSLIGLW	---SLVCFRWNFEMPAILKLSI	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN1	SSLIGLY	---SLVCFRWNFEMPAILKLSI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN1b	SSLIGLW	---SLVCFRWNFEMPAILKLSI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN1b	SSLIGLW	---SLVCFRWNFEMPAILVLSI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN1a	SSLIGLW	---SLVCFRWNFEMPAILVLSI	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN4b	SSLIGVW	---SLVCFRWNFQMPAIVLQSI	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN1	SSLFGITW	---SLISFKWNIEMPALIAKSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN1c	SSLIGLW	---SLVSFRWVVKMPLTIAKSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN1d	SSLIGLW	---SLVSFRWVVKMPLTIAKSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN1a	SSLIGLW	---SLVSFKWLDLNPQIIAHSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN1b	SSLIGLW	---SLVSFKWIMMPAIIAHSI	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN3	SSLIGLW	---ALVAFRWVHVMPKIIQSSI	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN7	SSLIGLW	---ALVAFRWVHVMPKIIQSSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN3a	SSLIGLW	---SLVAFRWVHVMPKIIKQSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN3b	SSLIGLW	---SLVAFRWVHVMPKIIKQSI	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN4	SSLIGLW	---ALVAYRWVHVMPKILQSSI	---	---	---	---	---	---	---	---	---	---	---	---
PpPIN3-2	SSLIGVW	---SLVANRWHTMPLILYKSYHI	---	---	---	---	---	---	---	---	---	---	---	---
PpPIN3-1	SSLIGVW	---SLVANRWHTMPLILYKSYHI	---	---	---	---	---	---	---	---	---	---	---	---
PpPIN3	SSLIGVW	---SLISFKCHLDMPILYKSYHI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN2	SSLIGLY	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN2	SSLIGLY	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN2	SSLVGLW	---ALVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN2	SSLFGLW	---SLVSFKWIKMPTIHSGSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN2a	SSLIGVW	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN1c	SSLIGVW	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN4c	SSLIGVW	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN4b	SSLIGVW	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN4d	SSLIGVW	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN2b	ASLLGLW	---SLISFRWIKMPLIIVDGSYRI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN10a	SSLIGLW	---SLVAFRIYHGAAQHHRYVQSRRL	HGRPL	PRGRCRHRVNRHRTPRDAPARRRCSGGSTTRDCAFCKRQCPGHPHESGNFHHANSSNHLAVLHP	---	---	---	---	---	---	---	---	---	---
SbPIN4a	SSLIGLW	---SLIAFRWHISMPVAVKSI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN4a	SSLIGLW	---SLVAFRWVHVMPVAVKSI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN10b	ASLIGLW	---SLIAFRFHITMPIIVAKSI	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN7	ASVVGITW	---SLISFRWVHVMPVVKNSI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN4c	ASVVGITW	---SLISFRFHIAPIIVKNSI	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN6	SSLIGLY	---SLISFKWNIQMPVIVDFSIKI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN6a	SSLIGLY	---SLVAFRWVHVMPVIVDFSIKI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN6b	SSVGLLW	---SLASFKWVWVMPVIVDFSIKI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN17	ASLIGLW	---ALVSCRYGIMKPIVIVDFSIKI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN1d	SSLIGVW	---SLVSFRWNIQMPSTIKGSI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN9	ASFLGLW	---SLIAFKCGFSMPKIVEDSLFT	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN3	ASFLGLW	---SLIAFKCGFSMPKIVEDSLFT	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN5b	ASFLGLW	---SLIAFKCGFSMPKIVEDSLFT	---	---	---	---	---	---	---	---	---	---	---	---
PpPIN6-1	ATVNGIVY	---SLTAGRWGFDPLRILRNSLDI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN5a	ASVVGITW	---ACLANRLHIALPSAFEGSVLI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN5a	ASVVGITW	---ACVANRLHLELPSAFEGSVLI	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN5b	ASVVGITW	---ACVANRLHLELPSAFEGSVLI	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN5	SCILGIW	---AFISNRWHLLELPGIEGSLI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN5a	ACIIGLY	---AFIAKRWRFEMPSIIEGSLI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN5c	ACIIGLY	---AFIAKRWRFEMPSIIEGSLI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN5b	ACIIGLY	---AFISNRWHLLELPGIEGSLI	---	---	---	---	---	---	---	---	---	---	---	---
OspIN5b	ASVVGITW	---ACIAYRWHLSPGIVTGSQV	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN5c	ASVVGITW	---ACIAYRWHLSPGIVTGSQV	---	---	---	---	---	---	---	---	---	---	---	---
OspIN5c	AGVVGITW	---ACVTRWVHETPSIIEGSLV	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN5a	AGVVGITW	---ACVTRWVHETPSIIEGSLV	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN5d	AGVVGITW	---ACVTRWVHETPSIIEGSLV	---	---	---	---	---	---	---	---	---	---	---	---
AtPIN8	ATLIGIITW	---ATLHFRGLWMLPEMIDKSIHL	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN8a	ATVVALW	---ASTHFRWVGLPDIIVDKSVI	---	---	---	---	---	---	---	---	---	---	---	---
PtPIN8b	ATVVALW	---ASTHFRWVGLPDIIVDKSVI	---	---	---	---	---	---	---	---	---	---	---	---
SbPIN8	ASLIGLW	---ALISFRWRIQLPSIVNNSIRI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN8	ASLIGLW	---ALISFRWRIQLPSIVNNSIRI	---	---	---	---	---	---	---	---	---	---	---	---
SiPIN5c	ASVVGITW	---ACIAYRWHLSPGIVTGSQV	---	---	---	---	---	---	---	---	---	---	---	---
OspIN7	AINRWVYVFAFPFFTFEFTLHLDPNVYRCSL	TAADSTAKLIT	---	---	---	---	---	---	---	---	---	---	---	---
OspIN8	AINRWVYVFAFPFFTFEFTLHLDPNVYRCSL	TAADSTAKLIT	---	---	---	---	---	---	---	---	---	---	---	---
PpPIN6-2	AINRWVYVFAFPFFTFEFTLHLDPNVYRCSL	TAADSTAKLIT	---	---	---	---	---	---	---	---	---	---	---	---

Supplementary Figure 1. Contd.



Supplementary Figure 1. Contd.

SbPIN5b -----SLLAF---FFI
 AtPIN5 -----ALPQSITSF---IFAKEYGLHADVLSTAVIFGMLVSLPVLVAYYAALFEIH
 PtPIN5a -----AFPQAISF---IFAQYGLHAEVLSTAVIFGTIVALPVLIAYYAILDFYH
 PtPIN5c -----AFPQAISF---IFAQYGLHAEVLSTAVIFGTIVALPVLIAYYAILDFYH
 PtPIN5b -----ALPQSITSF---IFAKEYGLHAEVLSTAVIFGMLAALPVLITYYAILDFVP
 OsPIN5b -----ALPQSIASF---VFAKEYGLHADVLSTAVIFGTLISLPVLIYAYVLFVY
 SbPIN5c -----ALPQSIASF---VFAKEYGLHADVLSTAVIFGTLVSLPVLIAYYAVLGIL
 OsPIN5c PEICLNSNGCMHDTSPHDETARDEQAALPQSITTF---VFAKEYGLHAEILSTAVIFGTLASLPVLIYYYIVLGFIR
 SbPIN5a -----ALPQSITTF---VFAKEYGLHADVLSTAVIFGTLASLPVLIYYYIVLGLIRC
 SiPIN5d -----ALPQAITTF---VFAKEYSLHADVLSTAVIFGTLASLPVLIYYYIVLGLIRC
 AtPIN8 -----ALPQGVYVF---VFAKEYNLHPEIISTGVIFGMLIALPTTLAYYFLDL
 PtPIN8a -----ALPQGIYVF---VFAKEYNVHPDTLSTGVIFGMLIAMPIALAYYSLAL
 PtPIN8b -----ALPQGIYVF---VFAKEYNVHPDTLSTGVIFGMLISMPIALAYYSLAL
 SbPIN8 -----ALPQGIYVF---VFAKEYNVHADILSTAILGHIYAVVPVALGYFVMDHPRL
 SiPIN8 -----ALPQGIYVF---VFAKEYNVHADIVSTAIIVGMMVAVVPVALGYFVIDHPRF
 OsPIN7 -----VYVLEVRKAFVSDAHDESNSYEEGSFIDDDTVYGSSTSEDMSLEEGVSDATNQDLRGEERYSVAVVNGARLPLFKSVARRTSLCHQLWRGRC
 OsPIN8
 PpPING-2

Supplementary Figure 1. Contd.