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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 (At*PIN1*–*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 there 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 St*PIN* 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	*SiPIN1a*	1785	594	5	scaffold_1: 37807344 - 37810727
Si006110m	*SiPIN1b*	1794	597	5	scaffold_4: 8906772 - 8909697
Si006060m	*SiPIN2*	1890	629	6	scaffold_4: 30687696 -30691064
Si000693m	*SiPIN4a*	1863	620	5	scaffold_5: 30444777 - 30448448
Si009737m	*SiPIN4b*	1677	558	5	scaffold_7: 34823548 - 34826535
Si025109m	*SiPIN4c*	1773	590	5	scaffold_3: 9460738 - 9463047
Si026177m	*SiPIN4d*	1683	560	5	scaffold_8: 1266812 - 1269757
Si001955m	*SiPIN5a*	1095	364	4	scaffold_5: 44864303 - 44869298
Si003879m	*SiPIN5b*	1272	423	4	scaffold_5: 39347962 - 39350295
Si015697m	*SiPIN5c*	1029	342	2	scaffold_6: 33597186 - 33599056
Si033365m	*SiPIN5d*	1134	377	6	scaffold_2: 36679971 - 36681750
Si003769m	*SiPIN8*	1081	361	5	scaffold_5: 35148382 - 35150803

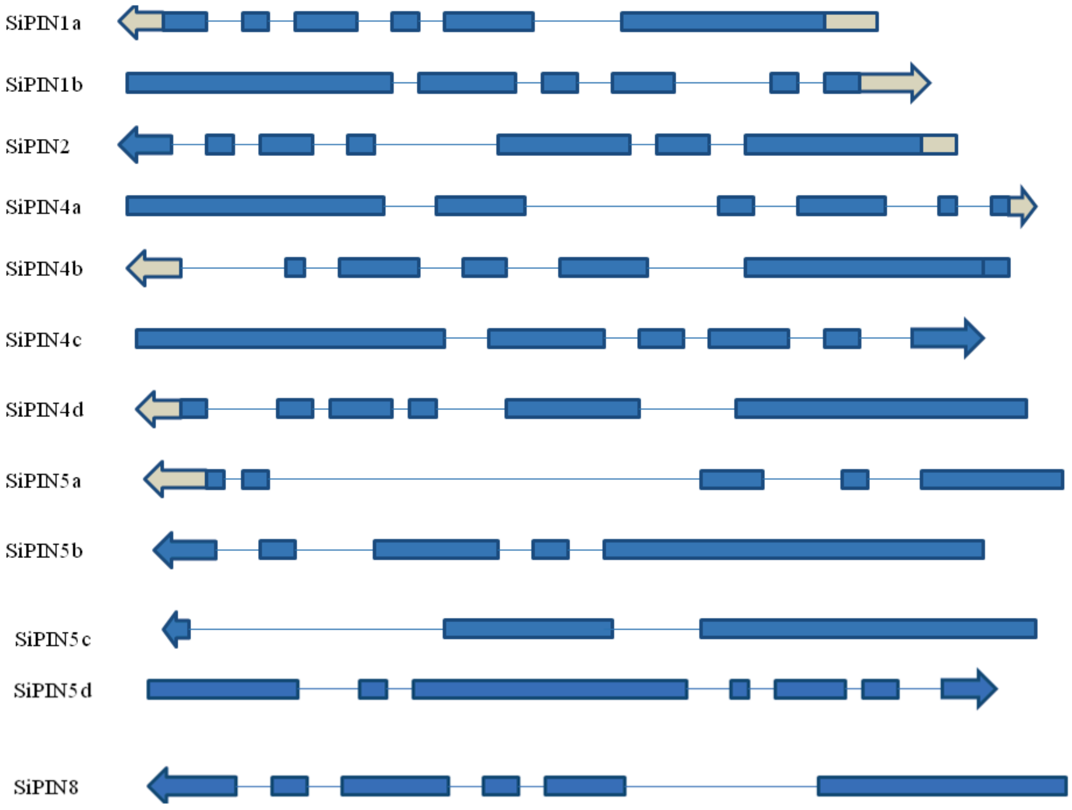
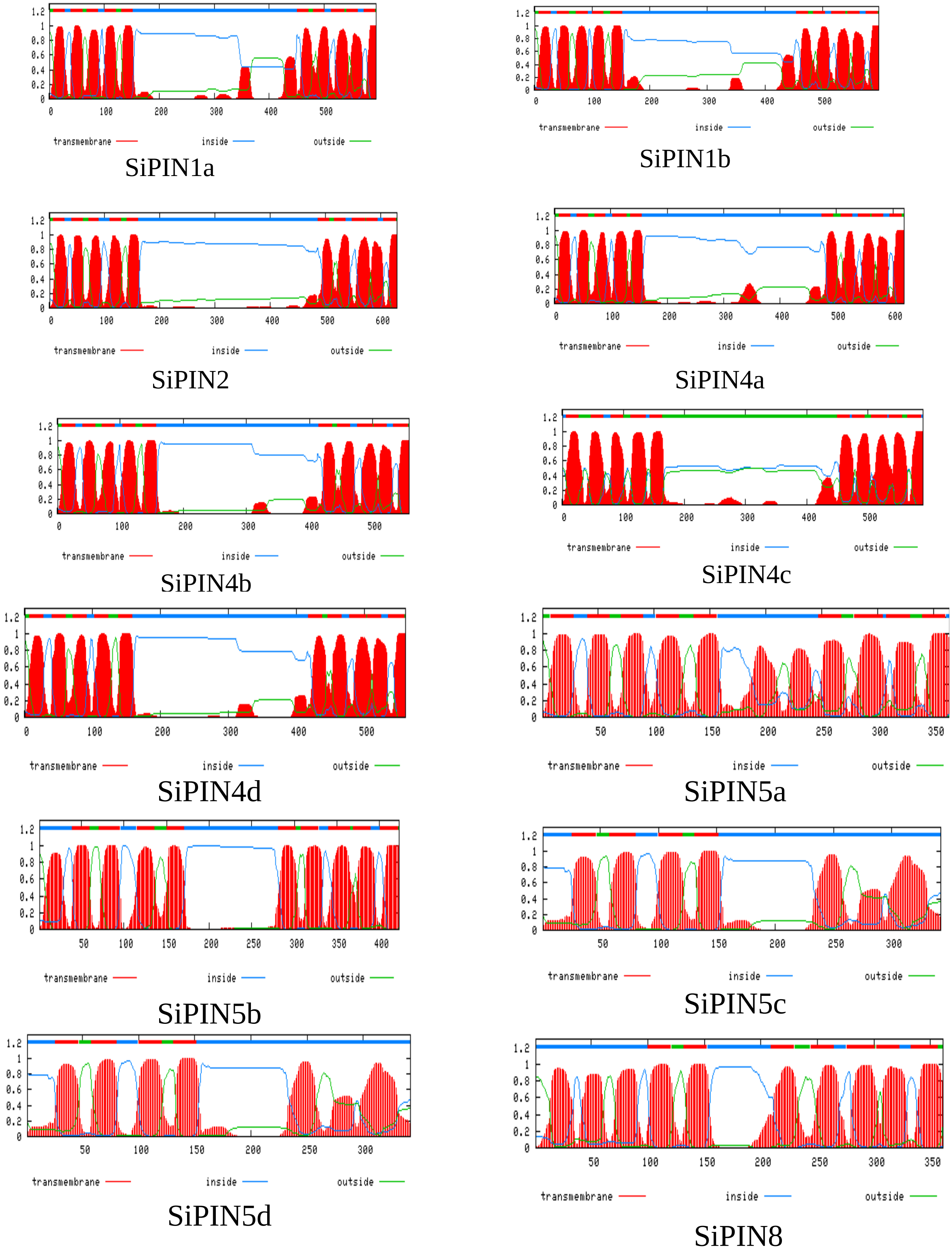


Figure 1. Transcript organization of *SiPIN* genes. Blue color boxes indicate the exons and lines indicate the introns of respective *SiPIN* genes. The arrow mark indicate the direction of expression of transcript.

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



SiPIN1a

SiPIN1b

SiPIN2

SiPIN4a

SiPIN4b

SiPIN4c

SiPIN4d

SiPIN5a

SiPIN5b

SiPIN5c

SiPIN5d

SiPIN8

Figure 2. Transmenrane 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.

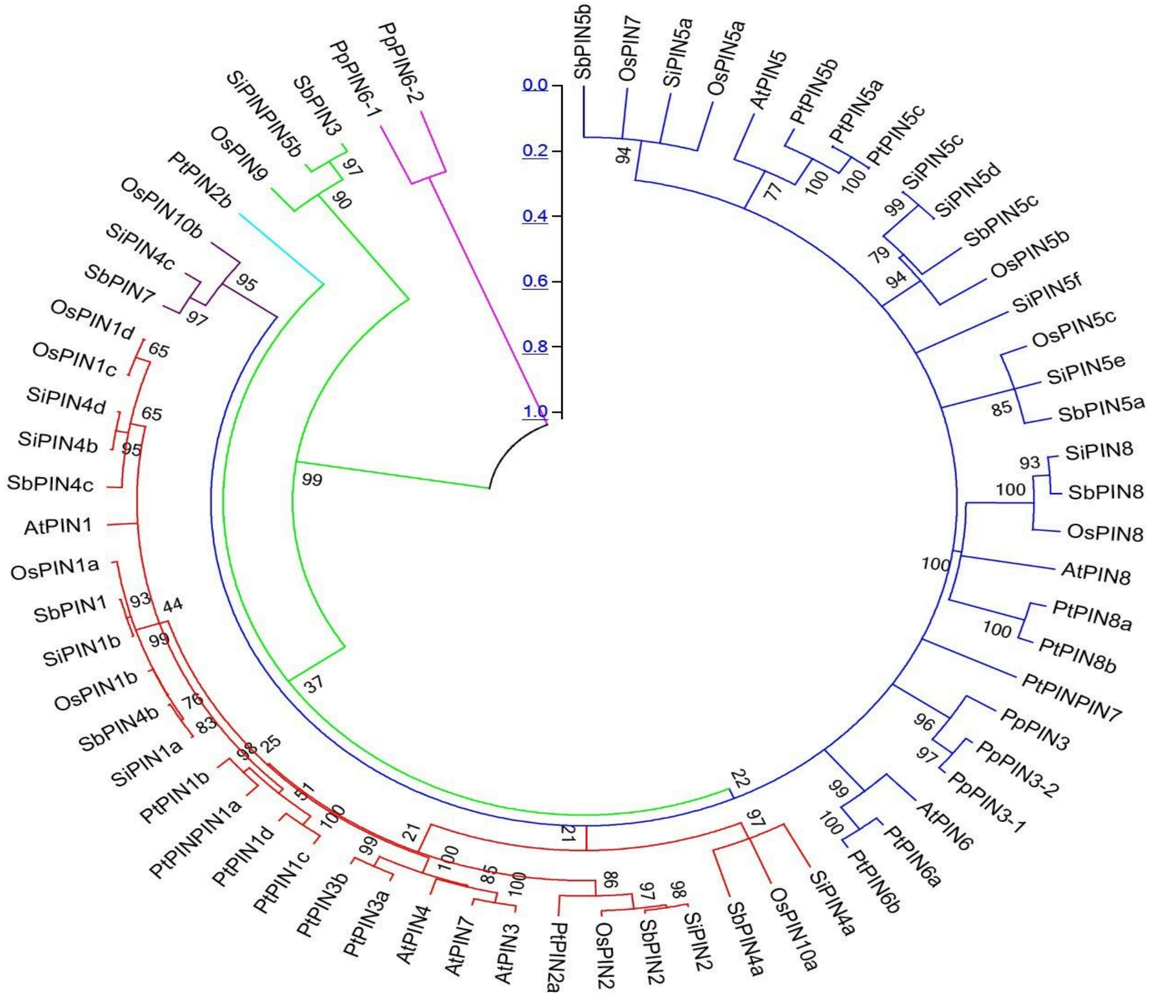


Figure 3. Phylogenetic tree of *SiPIN* genes with orthologous PIN genes of *Arabidopsis thaliana* (*AtPIN*), *Oryza sativa* (*OsPIN*), *Sorghum bicolor* (*SbPIN*), *Populus trichocarpa* (*PtPIN*) and *Physcomitrella patens* (*PpPIN*). Statistical method used to construct the phylogenetic tree was neighbor 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 electro-chemical 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 *PINs*. The hydrophobic domains of all long *PIN* proteins contain the amino acids at invariant position, but these positions are not invariants in short *PINs*. The presence of invariant amino acid sequences in long *PINs* 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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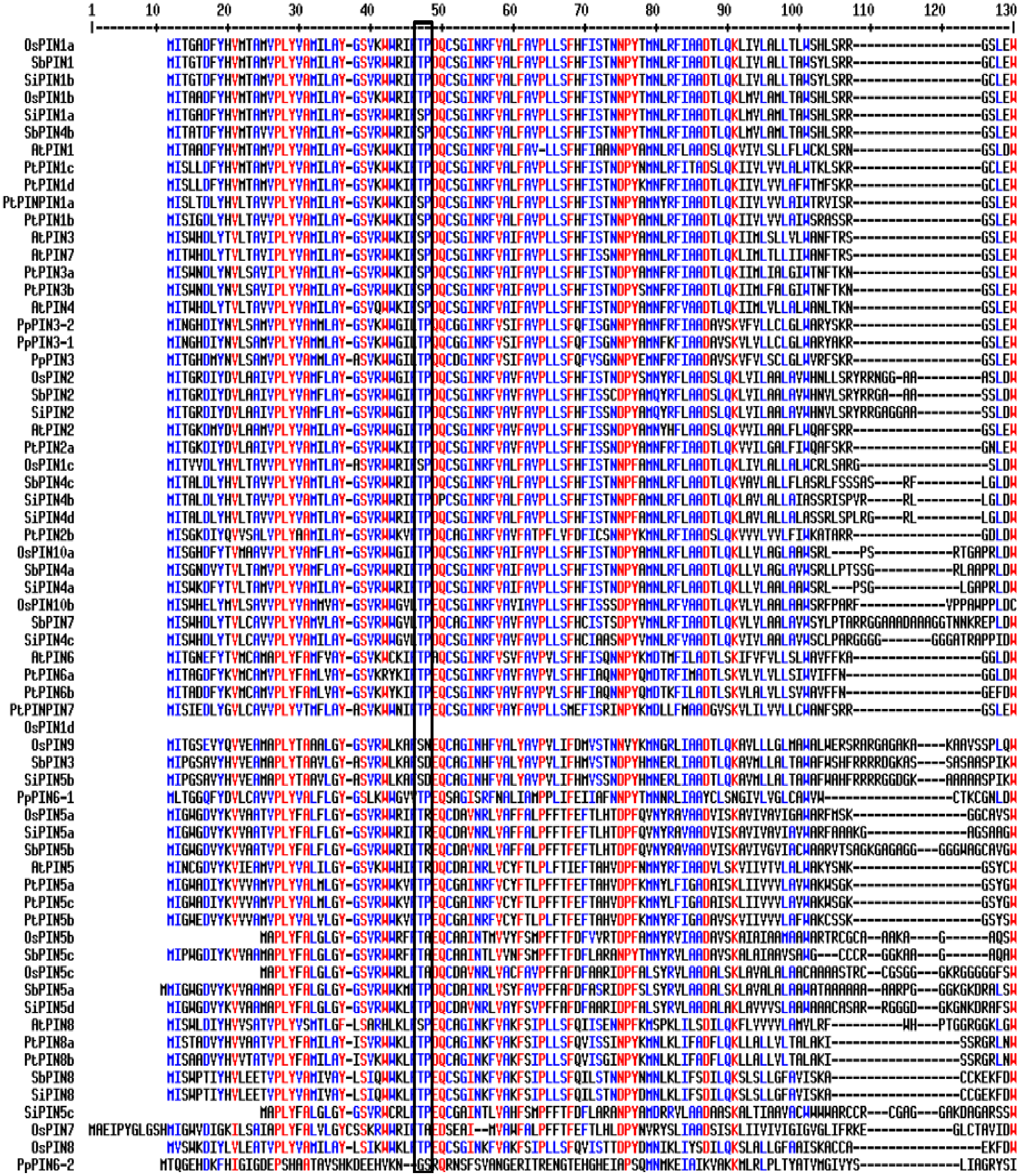

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            1       10        20        30        40        50        60        70        80        90       100       110       120       130
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
   OsPIN1a             MITGADFYHVMTAMVPLYVAMILAY-GSVKWWRIFTPDQCSGINRFVALFAVPLLSFHFISTNNPYTMNLRFIAADTLQKLIVLALLTLWSHLSRR------------------GSLEW
    SbPIN1             MITGTDFYHVMTAMVPLYVAMILAY-GSVRWWRIFTPDQCSGINRFVALFAVPLLSFHFISTNNPYTMNLRFIAADTLQKLIVLALLTAWSYLSRR------------------GCLEW
   SiPIN1b             MITGTDFYHVMTAMVPLYVAMILAY-GSVRWWRIFTPDQCSGINRFVALFAVPLLSFHFISTNNPYTMNLRFIAADTLQKLIVLALLTAWSYLSRR------------------GCLEW
   OsPIN1b             MITAADFYHVMTAMVPLYVAMILAY-GSVKWWRIFTPDQCSGINRFVALFAVPLLSFHFISTNNPYTMNLRFIAADTLQKLMVLAMLTAWSHLSRR------------------GSLEW
   SiPIN1a             MITGADFYHVMTAMVPLYVAMILAY-GSVRWWRIFSPDQCSGINRFVALFAVPLLSFHFISTNNPYTMNLRFIAADTLQKLMVLAMLTAWSHLSRR------------------GSLEW
   SbPIN4b             MITATDFYHVMTAVVPLYVAMILAY-GSVRWWRIFSPDQCSGINRFVALFAVPLLSFHFISTNNPYTMNLRFIAADTLQKLMVLAMLTAWSHLSRR------------------GSLEW
    AtPIN1             MITAADFYHVMTAMVPLYVAMILAY-GSVKWWKIFTPDQCSGINRFVALFAV-LLSFHFIAANNPYAMNLRFLAADSLQKVIVLSLLFLWCKLSRN------------------GSLDW
   PtPIN1c             MISLLDFYHVMTAMVPLYVAMILAY-GSVKWWKIFTPDQCSGINRFVALFAVPLLSFHFISTNDPYNMNLRFITADSLQKIIVLVVLALWTKLSKR------------------GCLEW
   PtPIN1d             MISLLDFYHVMTAMVPLYVAMILAY-GSVKWWKIFTPDQCSGINRFVALFAVPLLSFHFISTNDPYKMNFRFIAADTLQKIIVLVVLAFWTMFSKR------------------GCLEW
PtPINPIN1a             MISLTDLYHVLTAVVPLYVAMILAY-GSVKWWKIFSPDQCSGINRFVALFAVPLLSFHFISTNNPYAMNYRFIAADTLQKIIVLVVLAIWTRVISR------------------GSLEW
   PtPIN1b             MISIGDLYHVLTAVVPLYVAMILAY-GSVKWWKIFSPDQCSGINRFVALFAVPLLSFHFISTNNPYAMNLRFIAADTLQKIIVLVVLAIWSRASSR------------------GSLEW
    AtPIN3             MISWHDLYTVLTAVIPLYVAMILAY-GSVRWWKIFSPDQCSGINRFVAIFAVPLLSFHFISTNNPYAMNLRFIAADTLQKIIMLSLLVLWANFTRS------------------GSLEW
    AtPIN7             MITWHDLYTVLTAVIPLYVAMILAY-GSVRWWKIFSPDQCSGINRFVAIFAVPLLSFHFISSNNPYAMNLRFIAADTLQKLIMLTLLIIWANFTRS------------------GSLEW
   PtPIN3a             MISWNDLYNVLSAVIPLYVAMILAY-GSVRWWKIFSPDQCSGINRFVAIFAVPLLSFHFISTNDPYAMNFRFIAADTLQKIIMLIALGIWTNFTKN------------------GSLEW
   PtPIN3b             MISWNDLYNVLSAVIPLYVAMILAY-GSVRWWKIFSPDQCSGINRFVAIFAVPLLSFHFISTNDPYSMNFRFIAADTLQKIIMLFALGIWTNFTKN------------------GSLEW
    AtPIN4             MITWHDLYTVLTAVVPLYVAMILAY-GSVQWWKIFSPDQCSGINRFVAIFAVPLLSFHFISTNDPYAMNFRFVAADTLQKIIMLVLLALWANLTKN------------------GSLEW
  PpPIN3-2             MINGHDIYNVLSAMVPLYVAMMLAY-GSVKWWGILTPQQCGGINRFVSIFAVPLLSFQFISGNNPYAMNFRFIAADAVSKVFVLLCLGLWARYSKR------------------GSLEW
  PpPIN3-1             MINGHDIYNVLSAMVPLYVAMMLAY-GSVKWWGILTPQQCGGINRFVSIFAVPLLSFQFISGNNPYAMNFKFIAADAVSKVLVLLCLGLWARYAKR------------------GSLEW
    PpPIN3             MITGHDMYNVLSAMVPLYVAMMLAY-ASVKWWGILTPQQCDGINRFVSIFAVPLLSFQFVSGNNPYEMNFRFIAADAVSKVFVLSCLGLWVRFSKR------------------GSLEW
    OsPIN2             MITGRDIYDVLAAIVPLYVAMFLAY-GSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFISTNDPYSMNYRFLAADSLQKLVILAALAVWHNLLSRYRRNGG-AA---------ASLDW
    SbPIN2             MITGRDIYDVLAAIVPLYVAMFLAY-GSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFISSCDPYAMQYRFLAADSLQKLVILAALAVWHNVLSRYRRGA--AA---------SSLDW
    SiPIN2             MITGRDIYDVLAAIVPLYVAMFLAY-GSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMQYRFLAADSLQKLVILAALAVWHNVLSRYRRGAGGAA---------SSLDW
    AtPIN2             MITGKDMYDVLAAMVPLYVAMILAY-GSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNYHFLAADSLQKVVILAALFLWQAFSRR------------------GSLEW
   PtPIN2a             MITGKDIYDVLAAIVPLYVAMILAY-GSVRWWKIFTPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNFRFIAADTLQKVVILGALFIWQAFSKR------------------GNLEW
   OsPIN1c             MITVVDLYHVLTAVVPLYVAMTLAY-ASVRWWRIFSPDQCSGINRFVALFAVPLLSFHFISTNNPFAMNLRFLAADTLQKLIVLALLALWCRLSARG------------------SLDW
   SbPIN4c             MITALDLYHVLTAVVPLYVAMTLAY-GSVRWWRIFTPDQCSGINRFVALFAVPLLSFHFISTNNPFAMNLRFLAADTLQKVAVLALLFLASRLFSSSAS----RF---------LGLDW
   SiPIN4b             MITALDLYHVLTAVVPLYVAMTLAY-GSVRWWRIFTPDPCSGINRFVALFAVPLLSFHFISTNNPFAMNLRFLAADTLQKLAVLALLAIASSRISPVR-----RL---------LGLDW
   SiPIN4d             MITALDLYHVLTAVVPLYVAMTLAY-GSVRWWRIFTPDQCSGINRFVALFAVPLLSFHFISTNNPFAMNLRFLAADTLQKLAVLALLALASSRLSPLRG----RL---------LGLDW
   PtPIN2b             MISGKDIYQVVSALVPLYAAMILAY-GSVRWWKVFTPDQCAGINRFVAVFATPFLVFDFICSNNPYKMNLRFIAADSLQKVVVLVVLFIWKATARR------------------GDLDW
  OsPIN10a             MISGHDFYTVMAAVVPLYVAMFLAY-GSVRWWGIFTPDQCSGINRFVAIFAVPLLSFHFISTNDPYAMNLRFLAADTLQKLLVLAGLAAWSRL----PS-----------RTGAPRLDW
   SbPIN4a             MISGNDVYTVLTAMVPLYVAMFLAY-GSVRWWRIFTPDQCSGINRFVAIFAVPLLSFHFISTNNPYAMNLRFLAADTLQKLLVLAGLAVWSRLLPTSSG-----------RLAAPRLDW
   SiPIN4a             MISWKDFYTVLTAMVPLYVAMILAY-GSVRWWRIFTPDQCSGINRFVAIFAVPLLSFHFISTNDPYAMNLRFLAADTLQKLLVLAALAAWSRL---PSG------------LGAPRLDW
  OsPIN10b             MISWHELYMVLSAVVPLYVAMMVAY-GSVRWWGVLTPEQCSGINRFVAVIAVPLLSFHFISSSDPYAMNLRFVAADTLQKVLVLAALAAWSRFPARF------------VPPAWPPLDC
    SbPIN7             MISWHDLYTVLCAVVPLYVAMVLAY-GSVRWWGVLTPDQCAGINRFVAVFAVPLLSFHCISTSDPYVMNLRFIAADTLQKVLVLAALAVWSYLPTARRGGAAADAAAGGTNNKREPLDW
   SiPIN4c             MISWHDLYTVLCAVVPLYVAMILAY-GSVRWWGVLTPDQCSGINRFVAVFAVPLLSFHCIAASNPYVMNLRFVAADTLQKVIVLAALAVWSCLPARGGGG-------GGGATRAPPIDW
    AtPIN6             MITGNEFYTVMCAMAPLYFAMFVAY-GSVKWCKIFTPAQCSGINRFVSVFAVPVLSFHFISQNNPYKMDTMFILADTLSKIFVFVLLSLWAVFFKA------------------GGLDW
   PtPIN6a             MITAGDFYKVMCAMVPLYFAMLVAY-GSVKRYKIFTPEQCSGINRFVAVFAVPVLSFHFIAQNNPYQMDTRFIMADTLSKVLVLVLLSIWVIFFN-------------------GGLDW
   PtPIN6b             MITADDFYKVMCAMVPLYFAMLVAY-GSVKWYKIFTPEQCSGINRFVAVFAVPVLSFHFIAQNNPYQMDTKFILADTLSKVLALVLLSVWAVFFN-------------------GEFDW
 PtPINPIN7             MISIEDLYGVLCAVVPLYVTMFLAY-ASVKWWNIFTPEQCSGINRFVAYFAVPLLSMEFISRINPYKMDLLFMAADGVSKVLILVVLLCWANFSRR------------------GSLEW
   OsPIN1d
    OsPIN9             MITGSEVYQVVEAMAPLYTAAALGY-GSVRWLKAFSNEQCAGINHFVALYAVPVLIFDMVSTNNVYKMNGRLIAADTLQKAVLLLGLMAWALWERSRARGAGAKA----KAAVSSPLQW
    SbPIN3             MIPGSAVYHVVEAMAPLYTAAVLGY-ASVRWLKAFSDEQCAGINHFVALYAVPVLIFHMVSTNDPYHMNERLIAADTLQKAVMLLALTAWAFWSHFRRRRDGKAS----SASAASPIKW
   SiPIN5b             MIPGSAVYHVVEAMAPLYTAAVLGY-ASVRWLKAFSDEQCAGINHFVALYAVPVLIFHMVSSNDPYHMNERLIAADTLQKAVMLLALTAWAFWAHFRRRRGGDGK----AAAAASPIKW
  PpPIN6-1             MLTGGQFYDVLCAVVPLYVALFLGY-GSLKWWGVYTPEQSAGISRFNALIAMPPLIFEIIAFNNPYTMNNRLIAAYCLSNGIVLVGLCAWVW------------------CTKCGNLDW
   OsPIN5a             MIGWGDVYKVVAATVPLYFALFLGY-GSVRWWRIFTREQCDAVNRLVAFFALPFFTFEFTLHTDPFQVNYRAVAADVISKAVIVAVIGAWARFMSK----------------GGCAVSW
   SiPIN5a             MIGWGDVYKVVAATVPLYFALFLGY-GSVRWWRIFTREQCDAVNRLVAFFALPFFTFEFTLHTDPFQVNYRAVAADVISKAVIVAVIAVWARFAAAKG--------------AGSAAGW
   SbPIN5b             MIGWGDVYKVVAATVPLYFALFLGY-GSVRWWRIFTREQCDAVNRLVAFFALPFFTFEFTLHTDPFQVNYRAVAADVISKAVIVGVIACWAARVTSAGKGAGAGG---GGGWAGCAVGW
    AtPIN5             MINCGDVYKVIEAMVPLYVALILGY-GSVKWWHIFTRDQCDAINRLVCYFTLPLFTIEFTAHVDPFNMNYRFIAADVLSKVIIVTVLALWAKYSNK------------------GSYCW
   PtPIN5a             MIGWADIYKVVVAMVPLYVALMLGY-GSVRWWKVFTPEQCGAINRFVCYFTLPFFTFEFTAHVDPFKMNYLFIGADAISKLIIVVVLAVWAKWSGK------------------GSYGW
   PtPIN5c             MIGWADIYKVVVAMVPLYVALMLGY-GSVRWWKVFTPEQCGAINRFVCYFTLPFFTFEFTAHVDPFKMNYLFIGADAISKLIIVVVLAVWAKWSGK------------------GSYGW
   PtPIN5b             MIGWEDVYKVVVAMVPLYVALVLGY-GSVRWWKVFTPEQCGAINRFVCYFTLPLFTFEFTAHVDPFKMNYRFIGADAVSKVIIVVVLAFWAKCSSK------------------GSYSW
   OsPIN5b                           MAPLYFALGLGY-GSVRWWRFFTAEQCAAINTMVVYFSMPFFTFDFVVRTDPFAMNYRVIAADAVSKAIAIAAMAAWARTRCGCA--AAKA----G------AQSW
   SbPIN5c             MIPWGDIYKVVAAMAPLYFALGLGY-GSVRWWRFFTAEQCAAINTLVVNFSMPFFTFDFLARANPYTMNYRVLAADAVSKALAIAAVSAWG---CCCR--GGKAA---G------AQAW
   OsPIN5c                           MAPLYFALGLGY-GSVRWWRLFTADQCDAVNRLVACFAVPFFAFDFAARIDPFALSYRVLAADALSKLAVALALAACAAAASTRC--CGSGG---GKRGGGGGFSW
   SbPIN5a            MMIGWGDVYKVVAAMAPLYFALGLGY-GSVRWWKMFTPDQCDAINRLVSYFAVPFFAFDFASRIDPFSLSYRVLAADALSKLAVALALAAWATAAAAAA--AARPG---GGKGKDRALSW
   SiPIN5d             MIGWGDVYKVVAAMAPLYFALGLGY-GSVRWWKLFTPDQCDAVNRLVAYFSVPFFAFDFAARIDPFALSYRVLAADALAKLAVVVSLAAWAAACASAR--RGGGD---GKGNKDRAFSW
    AtPIN8             MISWLDIYHVVSATVPLYVSMTLGF-LSARHLKLFSPEQCAGINKFVAKFSIPLLSFQIISENNPFKMSPKLILSDILQKFLVVVVLAMVLRF----------WH---PTGGRGGKLGW
   PtPIN8a             MISTADVYHVVAATVPLYFAMILAY-ISVRWWKLFTPDQCAGINKFVAKFSIPLLSFQVISSINPYKMNLKLIFADFLQKLLALLVLTALAKI------------------SSRGRLNW
   PtPIN8b             MISAADVYHVVTATVPLYFAMILAY-ISVKWWKLFTPDQCAGINKFVAKFSIPLLSFQVISGINPYKMNLKLIFADFLQKLLALLVLTALAKI------------------SSRGRLNW
    SbPIN8             MISWPTIYHVLEETVPLYVAMIVAY-LSIQWWKLFTPEQCSGINKFVAKFSIPLLSFQILSTNNPYNMNLKLIFSDILQKSLSLLGFAVISKA------------------CCKEKFDW
    SiPIN8             MISWPTIYHVLEETVPLYVAMIVAY-LSIQWWKLFTPEQCSGINKFVAKFSIPLLSFQILSTNDPYDMNLKLIFSDILQKSLSLLGFAVISKA------------------CCGEKFDW
   SiPIN5c                           MAPLYFALGLGY-GSVRWCRLFTPEQCGAINTLVAHFSMPFFTFDFLARANPYAMDRRVLAADAASKALTIAAVACWWWWARCCR---CGAG---GAKDAGARSSW
    OsPIN7  MAEIPYGLGSHMIGWVDIGKILSAIAPLYFALVLGYCSSKRWWRIFTAEDSEAI--MVAWFALPFFTFEFTLHLDPYNVRYSLIAADSISKLIIVIVIGIGVGLIFRKE------------GLCTAVIDW
    OsPIN8             MVSWKDIYLVLEATVPLYVAMILAY-LSIKWWKLFTPEQCSGINKFVAKFSIPLLSFQVISTTDPYDMNIKLIYSDILQKSLALLGFAAISKACCA------------------EKFDW
  PpPIN6-2            MTQGEHDKFHIGIGDEPSHAATAVSHKDEEHVKN--GSRQRNSFSVANGERITRENGTEHGHEIAPSQMNMKEIAIKVAKKMLRLPLTYATVMGIVYS--------------LIAGRYSI
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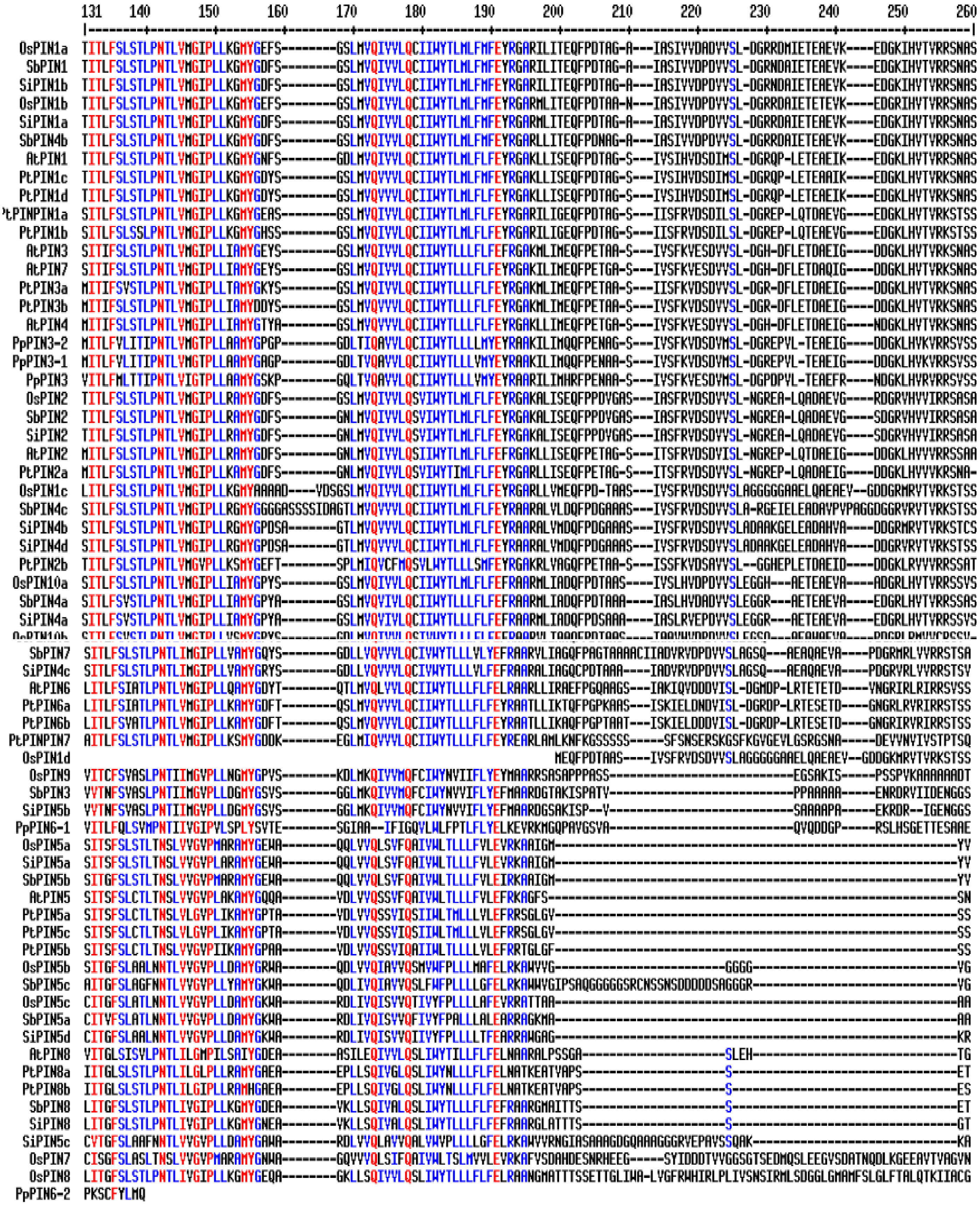
Supplementary Figure 1. Multiple sequence alignment of amino acid shows presence of N and C-terminal conserved region. N-terminal region shows presence of conserved S-P/T-P motif, target phosphorylation site of mitogen activated protein kinase. The central hydrophilic region is very dynamic in nature 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.



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            131     140       150       160       170       180       190       200       210       220       230       240       250       260
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
    OsPIN1a TITLFSLSTLPNTLVMGIPLLKGMYGEFS--------GSLMVQIVVLQCIIWYTLMLFMFEYRGARILITEQFPDTAG-A---IASIVVDADVVSL-DGRRDMIETEAEVK----EDGKIHVTVRRSNAS
     SbPIN1 TITLFSLSTLPNTLVMGIPLLKGMYGDFS--------GSLMVQIVVLQCIIWYTLMLFMFEYRGARILITEQFPDTAG-A---IASIVVDPDVVSL-DGRNDAIETEAEVK----EDGKIHVTVRRSNAS
    SiPIN1b TITLFSLSTLPNTLVMGIPLLKGMYGDFS--------GSLMVQIVVLQCIIWYTLMLFMFEYRGARILITEQFPDTAG-A---IASIVVDPDVVSL-DGRNDAIETEAEVK----EDGKIHVTVRRSNAS
    OsPIN1b TITLFSLSTLPNTLVMGIPLLKGMYGEFS--------GSLMVQIVVLQCIIWYTLMLFMFEYRGARMLITEQFPDTAA-N---IASIVVDPDVVSL-DGRRDAIETETEVK----EDGRIHVTVRRSNAS
    SiPIN1a TITLFSLSTLPNTLVMGIPLLKGMYGDFS--------GSLMVQIVVLQCIIWYTLMLFMFEYRGARMLITEQFPDTAG-A---IASIVVDPDVVSL-DGRRDAIETEAEVK----EDGKIHVTVRRSNAS
    SbPIN4b TITLFSLSTLPNTLVMGIPLLKGMYGDFS--------GSLMVQIVVLQCIIWYTLMLFMFEYRGARLLITEQFPDNAG-A---IASIVVDPDVVSL-DGRRDAIETEAEVK----EDGKIHVTVRRSNAS
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    PtPIN1c TITLFSLSTLPNTLVMGIPLLKGMYGDYS--------GSLMVQVVVLQCIIWYTLMLFMFEYRGAKLLISEQFPDTAG-S---IVSIHVDSDIMSL-DGRQP-LETEAAIK----EDGKLHVTVRKSNAS
    PtPIN1d TITLFSLSTLPNTLVMGIPLLKGMYGDYS--------GSLMVQVVVLQCIIWYTLMLFLFEYRGAKLLISEQFPDTAG-S---IVSIHVDSDIMSL-DGRQP-LETEAEIK----EDGKLHVTVRKSNAS
  'tPINPIN1a SITLFSLSTLPNTLVMGIPLLKGMYGEAS--------GSLMVQIVVLQCIIWYTLMLFLFEYRGARILIGEQFPDTAG-S---IISFRVDSDILSL-DGREP-LQTDAEVG----EDGKLHVTVRKSTSS
    PtPIN1b SITLFSLSSLPNTLVMGIPLLKGMYGHSS--------GSLMVQIVVLQCIIWYTLMLFLFEYRGARILIGEQFPDTAG-S---IISFRVDSDILSL-DGREP-LQTEAEVG----EDGKLHVTVRKSTSS
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     AtPIN7 SITIFSLSTLPNTLVMGIPLLIAMYGEYS--------GSLMVQIVVLQCIIWYTLLLFLFEYRGAKILIMEQFPETGA-S---IVSFKVESDVVSL-DGH-DFLETDAQIG----DDGKLHVTVRKSNAS
    PtPIN3a MITIFSVSTLPNTLVMGIPLLTAMYGKYS--------GSLMVQIVVLQCIIWYTLLLFLFEYRGAKMLIMEQFPETAA-S---IISFKVDSDVVSL-DGR-DFLETDAEIG----DDGKLHVTVRKSNAS
    PtPIN3b MITIFSLSTLPNTLVMGIPLLIAMYDDYS--------GSLMVQVVVLQCIIWYTLLLFLFEYRGAKMLIMEQFPETAA-S---IVSFKVDSDVVSL-DGR-DFLETDAEIG----DDGKLHVTVRKSNAS
     AtPIN4 MITIFSLSTLPNTLVMGIPLLIAMYGTYA--------GSLMVQVVVLQCIIWYTLLLFLFEYRGAKLLIMEQFPETGA-S---IVSFKVESDVVSL-DGH-DFLETDAEIG----NDGKLHVTVRKSNAS
    PpPIN3-2 MITLFVLITIPNTLVMGTPLLAAMYGPGP--------GDLTIQAVVLQCIIWYTLLLLMYEYRAAKILIMQQFPENAG-S---IVSFKVDSDVMSL-DGREPVL-TEAEIG----DDGKLHVKVRRSVSS
    PpPIN3-1 MITLFVLITIPNTLVMGTPLLAAMYGAGP--------GDLTVQAVVLQCIIWYTLLLVMYEYRAAKILIMQQFPENAA-S---IVSFKVDSDVMSL-DGREPVL-TEAEIG----DDGKLHVKVRRSVSS
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    PtPIN2a MITLFSLSTLPNTLVMGIPLLKAMYGDFS--------GNLMVQIVVLQSVIWYTIMLFLFEYRGARLLISEQFPETAG-S---ITSFRVDSDVVSL-NGREP-LQADAEIG----DDGKLHVVVKRSNA-
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     SbPIN7 SITLFSLSTLPNTLIMGIPLLVAMYGQYS--------GDLLVQVVVLQCIVWYTLLLVLYEFRAARVLIAGQFPAGTAAAACIIADVRVDPDVVSLAGSQ---AEAQAEVA----PDGRMRLVVRRSTSA
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   PtPINPIN7 AITLFSLSTLPNTLVMGIPLLKSMYGDDK--------EGLMIQVVVLQCIIWYTLLLFLFEYREARLAMLKNFKGSSSSS-----SFSNSERSKGSFKGVGEVLGSRGSNA-----DEVVNVIVSTPTSQ
    OsPIN1d                                                                      MEQFPDTAAS----IVSFRVDSDVVSLAGGGGGAAELQAEAEV--GDDGKMRVTVRKSTSS
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     SbPIN3 VVTNFSVASLPNTIIMGVPLLDGMYGSVS--------GGLMKQIVVMQFCIWYNVVIFLYEFMAARDGTAKISPATV---------------------------PPAAAAA-----ENRDRVIIDENGGS
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    SiPIN5a SITSFSLSTLTNSLVVGVPLARAMYGEWA--------QQLVVQLSVFQAIVWLTLLLFVLEVRKAAIGM-----------------------------------------------------------YV
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    PtPIN5c SITSFSLCTLTNSLVLGVPLIKAMYGPTA--------VDLVVQSSVIQSIIWLTMLLLVLEFRRSGLGV-----------------------------------------------------------SS
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    SbPIN5c AITGFSLAGFNNTLVVGVPLLYAMYGKWA--------QDLIVQIAVVQSLFWFPLLLLGFELRKAWWVGIPSAQGGGGGSRCNSSNSDDDDDSAGGGR------------------------------VG
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    SiPIN5d CITGFSLAALNNTLVVGVPLLDAMYGKWA--------RDLIVQISVVQIIVYFPLLLLTFEARRAWGAG-----------------------------------------------------------KR
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    PtPIN8b IITGLSLSTLPNTLILGIPLLRAMHGAEA--------EPLLSQIVGLQSLIWYNLLLFLFELNATKEATVAPS---------------------S---------------------------------ES
     SbPIN8 LITGFSLSTLPNTLIVGIPLLKGMYGDEA--------VKLLSQIVALQSLIWYTLLLFLFEFRAARGMAITTS---------------------S---------------------------------ET
     SiPIN8 LITGFSLSTLPNTLIVGIPLLKGMYGNEA--------VKLLSQIVALQSLIWYTLLLFLFEFRAARGLATTTS---------------------S---------------------------------GT
    SiPIN5c CVTGFSLAAFNNTLVVGVPLLDAMYGAWA--------RDLVVQLAVVQALVWVPLLLLGFELRKAWVVRNGIASAAAGDGQAAAGGGRVEPAVSSQAK------------------------------KA
     OsPIN7 CISGFSLASLTNSLVVGVPMARAMYGNWA--------GQVVVQLSIFQAIVWLTSLMVVLEVRKAFVSDAHDESNRHEEG-----SYIDDDTVVGGSGTSEDMQSLEEGVSDATNQDLKGEEAVTVAGVN
     OsPIN8 LITGFSLSTLPNTLIVGIPLLKGMYGEQA--------GKLLSQIVVLQSLIWYTLLLFLFELRAANGMATTTSSETTGLIWA-LVGFRWHIRLPLIVSNSIRMLSDGGLGMAMFSLGLFTALQTKIIACG
    PpPIN6-2 PKSCFYLMQ
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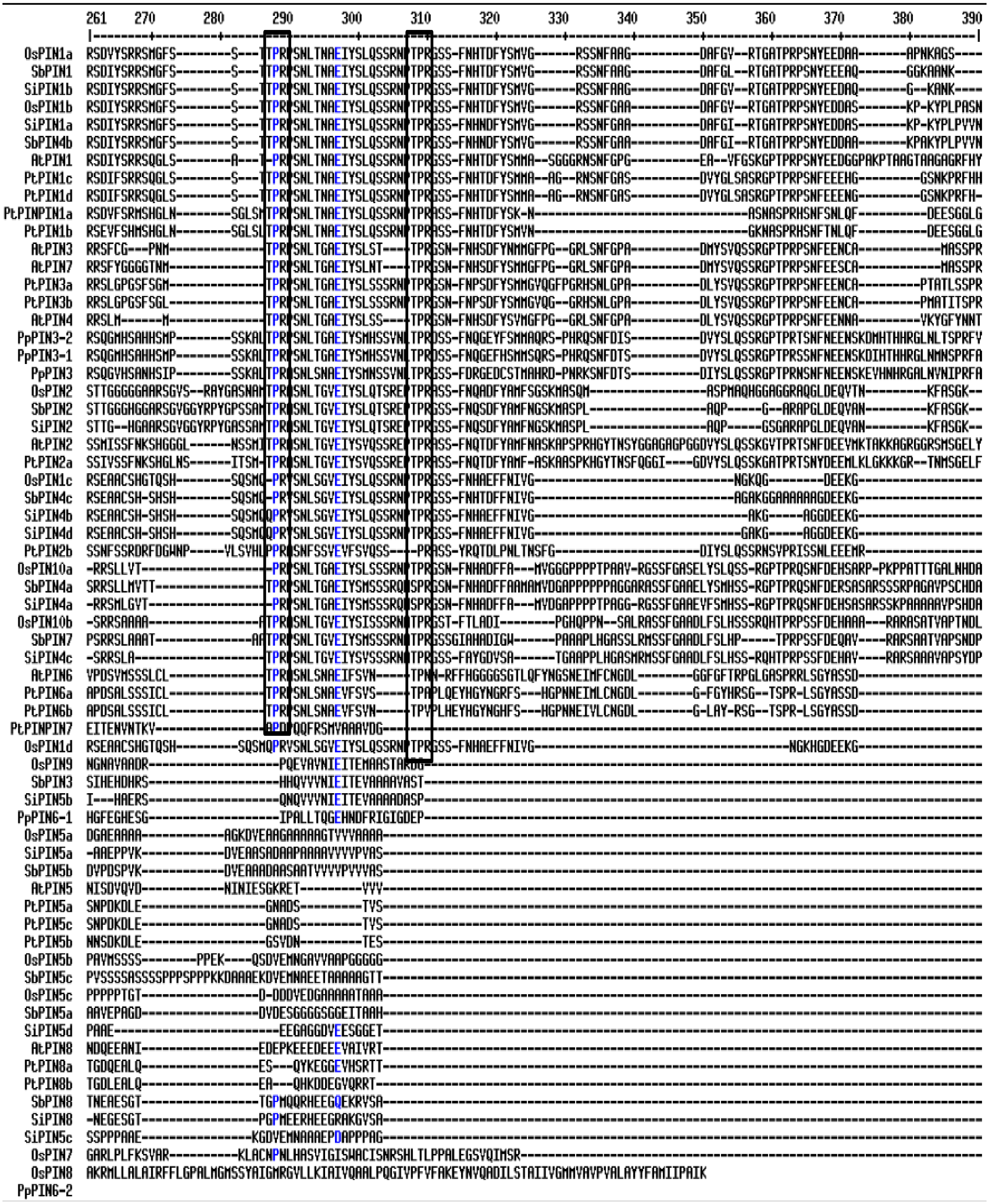
Supplementary Figure 1. Contd.



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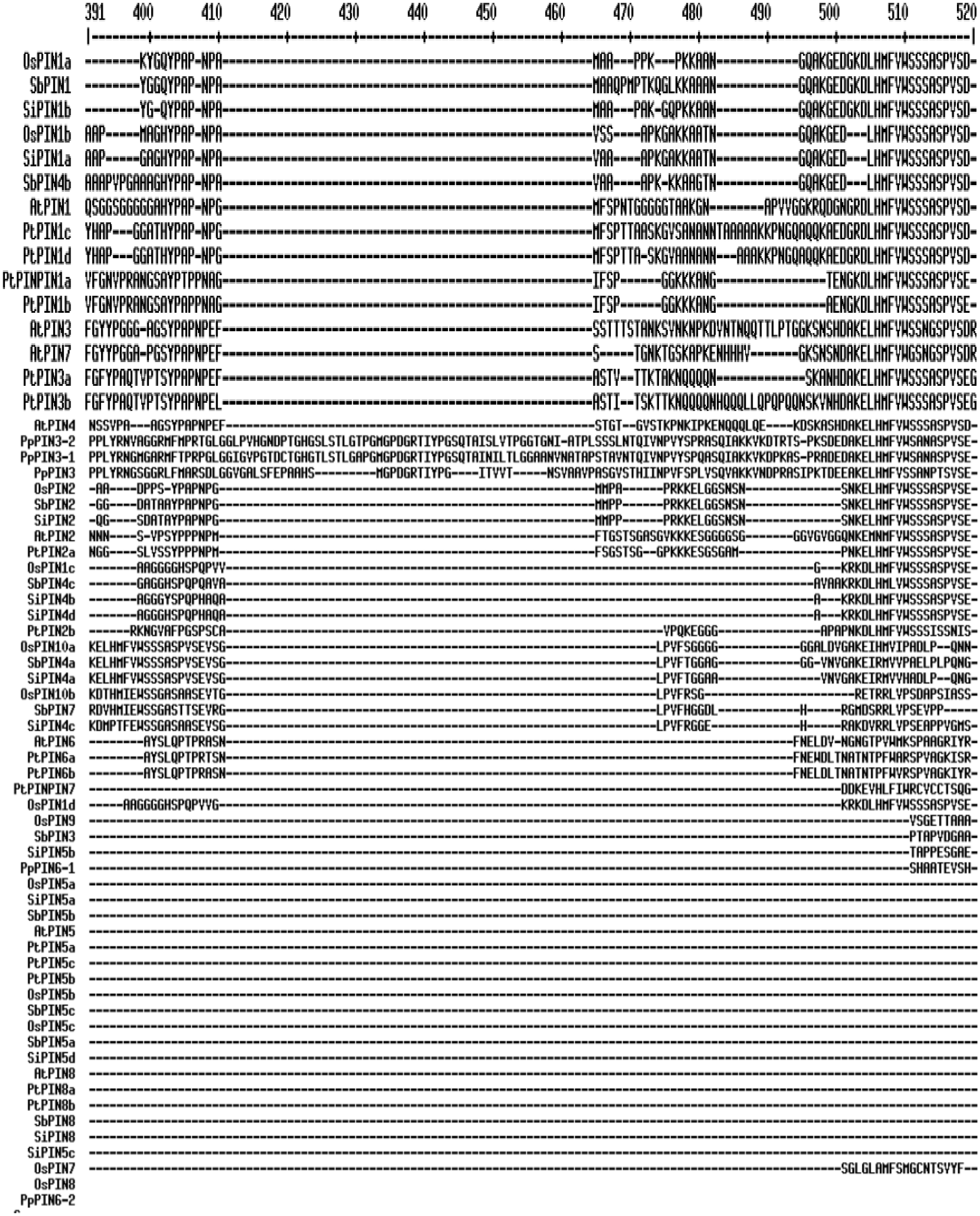
Supplementary Figure 1. Contd.



Supplementary Figure 1. Contd.

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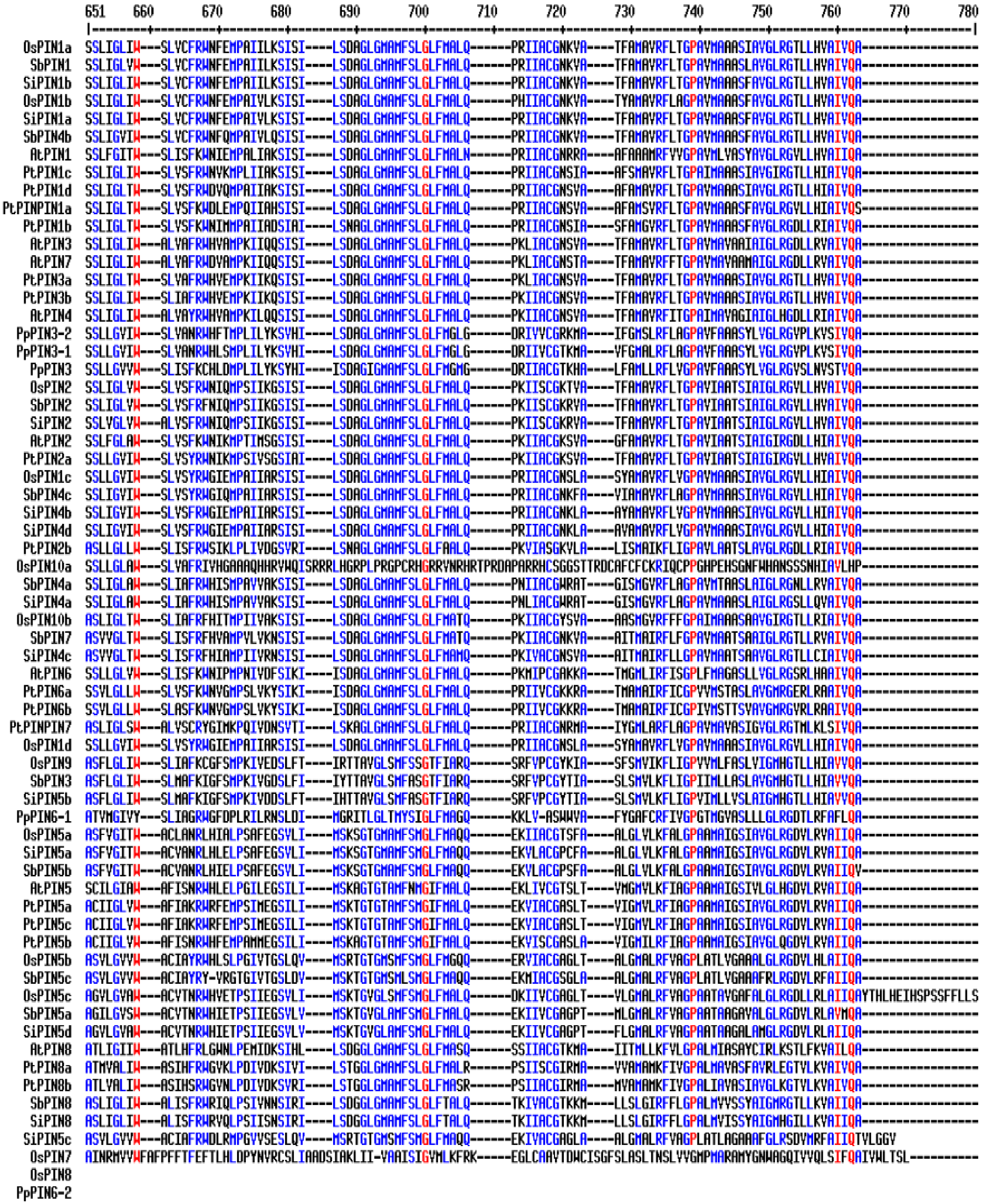

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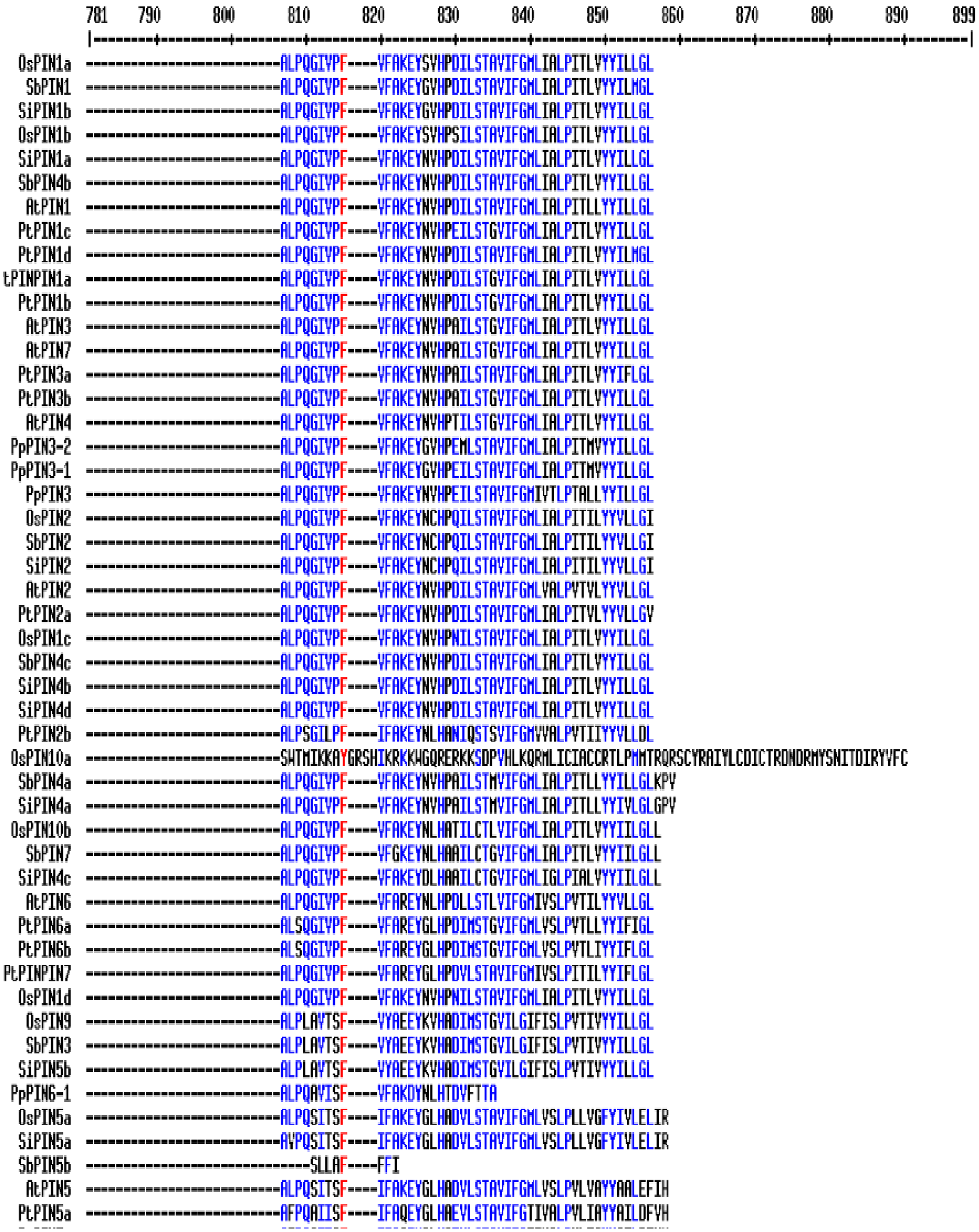
Supplementary Figure 1. Contd.



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  SiPIN1b --------------------------ALPQGIVPF----VFAKEYGVHPDILSTAVIFGMLIALPITLVYYILLGL
  OsPIN1b --------------------------ALPQGIVPF----VFAKEYSVHPSILSTAVIFGMLIALPITLVYYILLGL
  SiPIN1a --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITLVYYILLGL
  SbPIN4b --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITLVYYILLGL
   AtPIN1 --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITLLYYILLGL
  PtPIN1c --------------------------ALPQGIVPF----VFAKEYNVHPEILSTGVIFGMLIALPITLVYYILLGL
  PtPIN1d --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITLVYYILMGL
tPINPIN1a --------------------------ALPQGIVPF----VFAKEYNVHPDILSTGVIFGMLIALPITLVYYILLGL
  PtPIN1b --------------------------ALPQGIVPF----VFAKEYNVHPDILSTGVIFGMLIALPITLVYYILLGL
   AtPIN3 --------------------------ALPQGIVPF----VFAKEYNVHPAILSTGVIFGMLIALPITLVYYILLGL
   AtPIN7 --------------------------ALPQGIVPF----VFAKEYNVHPAILSTGVIFGMLIALPITLVYYILLGL
  PtPIN3a --------------------------ALPQGIVPF----VFAKEYNVHPAILSTAVIFGMLIALPITLVYYIFLGL
  PtPIN3b --------------------------ALPQGIVPF----VFAKEYNVHPAILSTGVIFGMLIALPITLVYYILLGL
   AtPIN4 --------------------------ALPQGIVPF----VFAKEYNVHPTILSTGVIFGMLIALPITLVYYILLGL
 PpPIN3-2 --------------------------ALPQGIVPF----VFAKEYGVHPEMLSTAVIFGMLIALPITMVYYILLGL
 PpPIN3-1 --------------------------ALPQGIVPF----VFAKEYGVHPEILSTAVIFGMLIALPITMVYYILLGL
   PpPIN3 --------------------------ALPQGIVPF----VFAKEYNVHPEILSTAVIFGMIVTLPTALLYYILLGL
   OsPIN2 --------------------------ALPQGIVPF----VFAKEYNCHPQILSTAVIFGMLIALPITILYYVLLGI
   SbPIN2 --------------------------ALPQGIVPF----VFAKEYNCHPQILSTAVIFGMLIALPITILYYVLLGI
   SiPIN2 --------------------------ALPQGIVPF----VFAKEYNCHPQILSTAVIFGMLIALPITILYYVLLGI
   AtPIN2 --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLVALPVTVLYYVLLGL
  PtPIN2a --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITVLYYVLLGV
  OsPIN1c --------------------------ALPQGIVPF----VFAKEYNVHPNILSTAVIFGMLIALPITLVYYILLGL
  SbPIN4c --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITLVYYILLGL
  SiPIN4b --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITLVYYILLGL
  SiPIN4d --------------------------ALPQGIVPF----VFAKEYNVHPDILSTAVIFGMLIALPITLVYYILLGL
  PtPIN2b --------------------------ALPSGILPF----IFAKEYNLHANIQSTSVIFGMVVALPVTIIYYVLLDL
 OsPIN10a --------------------------SWTMIKKAYGRSHIKRKKWGQRERKKSDPVHLKQRMLICIACCRTLPMMTRQRSCYRAIYLCDICTRDNDRMYSNITDIRYVFC
  SbPIN4a --------------------------ALPQGIVPF----VFAKEYNVHPAILSTMVIFGMLIALPITLLYYILLGLKPV
  SiPIN4a --------------------------ALPQGIVPF----VFAKEYNVHPAILSTMVIFGMLIALPITLLYYIVLGLGPV
 OsPIN10b --------------------------ALPQGIVPF----VFAKEYNLHATILCTLVIFGMLIALPITLVYYIILGLL
   SbPIN7 --------------------------ALPQGIVPF----VFGKEYNLHAAILCTGVIFGMLIALPITLVYYIILGLL
  SiPIN4c --------------------------ALPQGIVPF----VFAKEYDLHAAILCTGVIFGMLIGLPIALVYYIILGLL
   AtPIN6 --------------------------ALPQGIVPF----VFAREYNLHPDLLSTLVIFGMIVSLPVTILYYVLLGL
  PtPIN6a --------------------------ALSQGIVPF----VFAREYGLHPDIMSTGVIFGMLVSLPVTLLYYIFIGL
  PtPIN6b --------------------------ALSQGIVPF----VFAREYGLHPDIMSTGVIFGMLVSLPVTLIYYIFLGL
PtPINPIN7 --------------------------ALPQGIVPF----VFAREYGLHPDVLSTAVIFGMIVSLPITILYYIFLGL
  OsPIN1d --------------------------ALPQGIVPF----VFAKEYNVHPNILSTAVIFGMLIALPITLVYYILLGL
   OsPIN9 --------------------------ALPLAVTSF----VYAEEYKVHADIMSTGVILGIFISLPVTIVYYILLGL
   SbPIN3 --------------------------ALPLAVTSF----VYAEEYKVHADIMSTGVILGIFISLPVTIVYYILLGL
  SiPIN5b --------------------------ALPLAVTSF----VYAEEYKVHADIMSTGVILGIFISLPVTIVYYILLGL
 PpPIN6-1 --------------------------ALPQAVISF----VFAKDYNLHTDVFTTA
  OsPIN5a --------------------------ALPQSITSF----IFAKEYGLHADVLSTAVIFGMLVSLPLLVGFYIVLELIR
  SiPIN5a --------------------------AVPQSITSF----IFAKEYGLHADVLSTAVIFGMLVSLPLLVGFYIVLELIR
  SbPIN5b ------------------------------SLLAF----FFI
   AtPIN5 --------------------------ALPQSITSF----IFAKEYGLHADVLSTAVIFGMLVSLPVLVAYYAALEFIH
  PtPIN5a --------------------------AFPQAIISF----IFAQEYGLHAEVLSTAVIFGTIVALPVLIAYYAILDFVH
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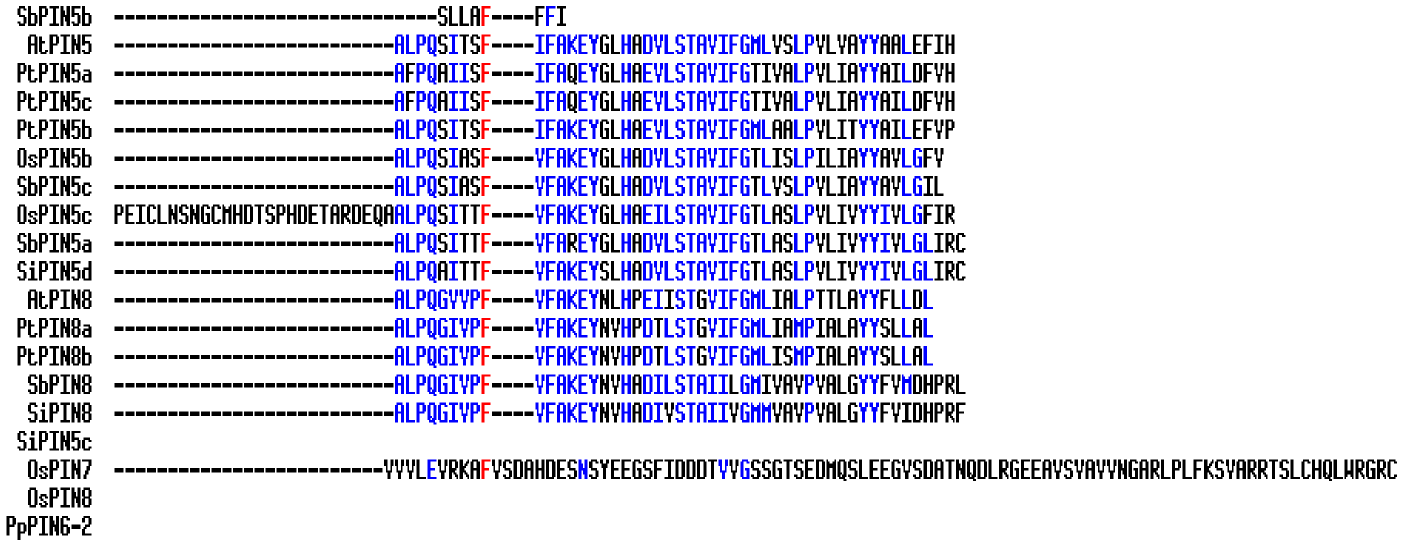
Supplementary Figure 1. Contd.



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 SbPIN5b  ------------------------------SLLAF----FFI
  AtPIN5  --------------------------ALPQSITSF----IFAKEYGLHADVLSTAVIFGMLVSLPVLVAYYAALEFIH
 PtPIN5a  --------------------------AFPQAIISF----IFAQEYGLHAEVLSTAVIFGTIVALPVLIAYYAILDFVH
 PtPIN5c  --------------------------AFPQAIISF----IFAQEYGLHAEVLSTAVIFGTIVALPVLIAYYAILDFVH
 PtPIN5b  --------------------------ALPQSITSF----IFAKEYGLHAEVLSTAVIFGMLAALPVLITYYAILEFVP
 OsPIN5b  --------------------------ALPQSIASF----VFAKEYGLHADVLSTAVIFGTLISLPILIAYYAVLGFV
 SbPIN5c  --------------------------ALPQSIASF----VFAKEYGLHADVLSTAVIFGTLVSLPVLIAYYAVLGIL
 OsPIN5c  PEICLNSNGCMHDTSPHDETARDEQAALPQSITTF----VFAKEYGLHAEILSTAVIFGTLASLPVLIVYYIVLGFIR
 SbPIN5a  --------------------------ALPQSITTF----VFAREYGLHADVLSTAVIFGTLASLPVLIVYYIVLGLIRC
 SiPIN5d  --------------------------ALPQAITTF----VFAKEYSLHADVLSTAVIFGTLASLPVLIVYYIVLGLIRC
  AtPIN8  --------------------------ALPQGVVPF----VFAKEYNLHPEIISTGVIFGMLIALPTTLAYYFLLDL
 PtPIN8a  --------------------------ALPQGIVPF----VFAKEYNVHPDTLSTGVIFGMLIAMPIALAYYSLLAL
 PtPIN8b  --------------------------ALPQGIVPF----VFAKEYNVHPDTLSTGVIFGMLISMPIALAYYSLLAL
  SbPIN8  --------------------------ALPQGIVPF----VFAKEYNVHADILSTAIILGMIVAVPVALGYYFVMDHPRL
  SiPIN8  --------------------------ALPQGIVPF----VFAKEYNVHADIVSTAIIVGMMVAVPVALGYYFVIDHPRF
 SiPIN5c
  OsPIN7  -------------------------VVVLEVRKAFVSDAHDESNSYEEGSFIDDDTVVGSSGTSEDMQSLEEGVSDATNQDLRGEEAVSVAVVNGARLPLFKSVARRTSLCHQLWRGRC
  OsPIN8
PpPIN6-2
```

Supplementary Figure 1. Contd.