Genome Wide Identification of Auxin Efflux Carrier Gene Family in Solanum tuberosum L

Article · October 2013

Tapan Kumar Mohanta
Yeungnam University

Research Paper
Journal of Natural Sciences.JNS 1(2): 44-51 (2013)

ISSN 2308-5061

Genome Wide Identification of Auxin Efflux Carrier Gene Family in *Solanum tuberosum* L.

Tapan Kumar Mohanta[1], Nibedita Mohanta[2]

[1]National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, India, 110067.
[2]Dept. di Biologia Vegetale, Viale Mattioli, University of Turin, Turin, 10125, Italy

Keywords: Auxin efflux carrier, gravitropic response, phydrophilic domain, organogenesis

Abstract

Phytohormone auxin is one of the most important growth regulator in plant and maintains its growth, development and organogenesis by maintaining auxin distribution and homeostasis. Auxin efflux carrier (PIN) protein plays vital role in transport of auxin from aerial parts to basal parts. They are asymmetrically localized within the cells and their polarity determines the directionality of intercellular auxin flow. In this study, we reported identification of auxin efflux carrier gene family of *Solanum tuberosum*. We found that *S. tuberosum* posses 10 PIN genes in its genome and represented by multigene family in plants. All PIN genes are membrane localized and shows similar structure with amino- and carboxy terminal hydrophobic domain. Membrane spanning domain is separated by non conserved central hydrophilic domain. Phylogenetic analysis shows, StPIN genes are closer to *Arabidopsis thaliana* AtPIN gene.

Corresponding author, Tapan Kumar Mohanta, National Institute of Plant Genome Research, Aruna Asaf Ali Marg,
New Delhi, India, 110067. E- mail: nostoc.tapan@gmail.com

Introduction

Phytohormone auxin plays important roles in plant growth and development including cell division, cell elongation, vascular differentiation, apical dominance, tropic growth, embryogenesis, cell polarity, root architecture and root organogenesis Benkova et al. (2003); (Feraru et al. 2011; Friml and Palme 2002; Sabatini et al. 1999). It synthesizes in the aerial parts of the plant and transported to root tip via vascular system, thus maintaining auxin homeostasis and distribution (Forestan and Varotto 2010a; Forestan and Varotto 2010b; Forestan and Varotto 2012; 2013; Peer et al. 2004). Besides vascular transport, cell to cell transport is present in the tissue covering long and short distance auxin distribution (Zazimalova et al. 2010). Polar auxin efflux carrier (PIN) genes plays important roles in auxin distribution and homeostasis including root gravitropic response (Abas et al. 2006; Barbez et al. 2012; Friml and Palme 2002). AtPIN1gene is the first PIN gene to be cloned (Galweiler et al. 1998). When it has reported that Arabidopsis pin-formed1 (pin1) mutant defective in auxin transport develops pin-like inflorescence, it became clear that PIN protein plays significant role in auxin efflux from the cells (Galweiler et al. 1998). There are twelve PIN genes found in rice and eight PIN genes in found Arabidopsis thaliana (Blilou et al. 2005; Wang et al. 2009). PIN genes shows distinct pattern of cellular and sub-cellular localization in root and shoot and shows tissue specific expression (Blilou et al. 2005; Friml et al. 2002; Paponov et al. 2005). AtPIN1 localizes polarly in plasma membrane and upon pharmacological disruption, it immediately relocalizes, suggesting conceptual basis of auxin flux affects tropic response and patterning (Geldner et al. 2001). It has been found that rice OsPIN1 gene expressed in root cap, OsPIN1b, OsPIN1c and OsPIN9 predominantly expressed in stele whereas OsPIN1b, OsPIN1c, OsPIN5a and OsPIN5b expressed in meristem (Wang et al. 2009). Solanum lycopersicum shows presence of ten PIN gene and predominant in tissue specific expression (Pattison and Catala 2012).

The plant specific PIN gene family of efflux carriers is integral membrane proteins with transmembrane domain Krecek et al. (2009); (Petrasek et al. 2006). The N-terminal and C-terminal region of PIN gene family are conserved, where as the central hydrophilic loop is very much dynamic among different PIN gene. Based on the divergence of central hydrophilic loop, PIN genes are divided into different groups (Krecek et al. 2009). In this paper, we are reporting the finding of PIN gene family of StPIN gene from Solanum tuberosum, the most important tuber crop.

Materials and Methods

The auxin efflux carrier gene family of Solanum tuberosum was downloaded from publicly available potato and phytozome (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml, http://www.phytozome.net/) database using BLASTP protocol (Altschul et al. 1997) as well as using hidden

Received Manuscript 20-06-2013 Accepted Manuscript 04-10-2013

markov model approach. Auxin efflux carrier gene family from Arabidopsis thaliana was downloaded from The Arabidopsis Information Resources (www.arabidopsis.org) and used as orthologous gene for the query search. The PIN gene found from Solanum tuberosum genome was named according to nomenclature of Arabidopsis thaliana PIN genes. To analyze the domain structure of StPIN gene, online available software TMMOD (Hidden Markov Model for Transmembrane Domain) model was used (Kahsay et al. 2005; Kahsay et al. 2004) . Multiple sequence alignment of StPIN genes with orthologous PIN genes of Arabidopsis thaliana with Solanum tuberosum (StPIN) was carried out using Multalin software and programme used was BLOSUM62 (http://multalin.toulouse.inra.fr/multalin/). Phylogenetic tree of StPIN genes with AtPIN and OsPIN gene was constructed using MEGA5.05 software (Tamura et al., 2011). Sub-cellular localization of StPIN gene was predicted using online available software CELLO v.2.5: sub-cellular localization predictor (Yu et al., 2006).

Results and Discussion

Solanum tuberosum, the most important tuber crop posses 800 Mb genome arranged in 12 chromosome with 35,119 loci coding for 51,472 protein-coding transcript. Among them there are 10 StPIN gene codes for auxin efflux carriers. In eukaryotic organisms, auxin efflux carriers known to regulate diverse developmental and physiological process as well as biotic and abiotic stress. Auxin efflux carrier gene in some plant species already been identified and reported, but yet to be identified and reported from Solanum tuberosum (potato). Availability of potato genome sequencing project in public leads to carry out genome wide identification of PIN gene family. Major objective of this study was to utilize in-silico and phylogenetic approach to identify and asses' functional divergence and evolutionary relationship of identified StPIN gene with other publicly available PIN genes. Bioinformatics approaches and comparative genomics studies were carried out to assess homologies based on phylogenetics and sequence identities and named the newly identified genes according to nomenclature of model plant Arabidopsis thaliana. Total of 10 Solanum tuberosum PIN (StPIN) gene were identified and named them accordingly (table 1). Amino acid sequence alignment of PIN gene of Arabidopsis thaliana (AtPIN), with Solanum tuberosum (StPIN) shows presence of conserved N- and C-terminal domain. The central hydrophilic domain is diverse from each other (figure 1) and very dynamic in nature. Domain prediction shows StPIN1a, StPIN1b, StPIN1c, StPIN2, StPIN3b, StPIN6 posses longer central hydrophilic loop as compared to StPIN3a, StPIN5a, StPIN5b and StPIN7 which posses shorter central loop domain as in AtPIN5 and AtPIN8 (figure 2) (Paponov et al. 2005). Phylogenetic analysis with above mentioned species shows cluster of 6 groups (figure 3). StPIN1a clustered with AtPIN1, OsPIN1a and OsPIN1b. StPIN3a and StPIN3b clustered independently. StPIN3a and StPIN3b grouped with AtPIN3 and AtPIN4 where as StPIN2 clustered with AtPIN2. StPIN6clustered with AtPIN6, whereas StPIN5a stay independently. Overall analysis found in phylogenetic tree shows, Solanum tuberosum PIN genes are much closer to Arabidopsis thaliana PIN gene and hence may evolved for dicot specific function. Transcript organization shows StPIN gene averagely posses 5 introns; StPIN2 and StPIN6 posses maximum of 6 introns each where as StPIN5 and StPIN8 posses 4 introns each (figure 4) (Wang et al. 2009). Chromosome 10 posses two StPIN genes, i.e. StPIN1b and StPIN1c where as chromosome 4 posses StPIN3a and StPIN5b (table 1). There is not absolute correlation between length of PIN genomic sequences and length of protein.

Sub-cellular localization of StPIN gene shows all StPIN genes are plasmamembrane bound and maximum of them are extra-cellularly localized (table 2). The group of PIN genes which have small transmembrane domain like AtPIN5 usually presents either in membrane of endoplasmic reticulum or chloroplast (Mravec et al., 2009). Solanum tuberosum StPIN2, StPIN3a and StPIN7 are predicted to be present in membrane of chloroplast (table 2). Study of sub-cellular localization of proteins are important as proteins functions is directly related to its sub-cellular localization (Yu et al., 2006).

Auxin regulates growth and vascular differentiation is a quite clear phenomena, but role of specific proteins in basipetal auxin transport is limited. AtPIN and OsPIN gene plays significant role in this context (Barbez et al. 2012; Geldner et al. 2001) . Carraro et al (2012) reported, greater number of putative transporters expressed in young internodes where cambial growth plays significant role. PtaPIN1 and PtaPIN6 are highly expressed in the stellar region of internodes. In the developing xylem parenchyma of stellar region PtaPIN1 gene is highly expressed and PtPIN8a and PtPIN8b highly expressed by auxin in intermodal region, suggesting their important roles. Expression of different gene in different tissue indicates tissue specific expression and hence their tissue specific roles (Shen et al. 2010). Although the domains of PIN gene varied among them in closely evolutionary group, evolutionary relationship is not related to variation in function of PIN gene (Wang et al. 2009).

Shen et al. (2010) reported, although SbPIN1 and SbPIN5 expressed highly, most SbPIN gene are induced by application of exogenous auxin. Studies from jain and Khurana (2009) suggests that PIN genes also involved in abiotic stress response (Jain and Khurana 2009). Different environmental signal modulates polarity of PIN protein and mediates distribution. Upon treatment with ABA, salt and drought SbPIN1-6 undergoes up-regulation in all three treatment suggesting their role in stress response (Shen et al. 2010). Identification of StPIN gene will give quite broad idea

Received Manuscript 20-06-2013 Accepted Manuscript 04-10-2013

about tissue specific expression and developmental role in Solanum tuberosum. Besides this, it will help to explain, role of different StPIN gene and their differential response to different biotic and abiotic stress.

Conclusion

Solanum tuberosum, the most studied tuber crop posses ten StPIN gene in its genome. The diversity of presence of StPIN gene could explain its diverse role including growth and development. Besides this StPIN gene can also explain its response towards different abiotic and biotic stress response. Further studies necessary to elucidate more about potential role of these genes.

Acknowledgements

I would like to say thanks to Dr. Alok Krishna Sinha, Staff Scientist, National Institute of Plant Genome Research, New Delhi, India for providing necessary laboratory facility to carry out this work.

References

Abas, L., Benjamins, R., Malenica, N., Paciorek, T., Wiśniewska, J., Moulinier-Anzola, J.C., Sieberer, T., Friml, J., Luschnig, C. (2006) Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. Nat Cell Biol 8(3): 249-256.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25(17): 3389-3402.

Barbez, E., Kubes, M., Rolcik, J. et al (2012) A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. Nature 485(7396): 119-U155.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G., Friml, J. (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115(5): 591-602.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K., Scheres, B. (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 433(7021): 39-44.

Carraro, N., Tisdale-Orr, T.E., Clouse, R.M. et al (2012) Diversification and expression of PIN, AUX/LAX, and ABCB families of putative auxin transporters in Populus. Front Plant Sci. 3:17.

Feraru, E., Feraru, M.I., Kleine-Vehn, J., Martinière, A., Mouille, G., Vanneste, S., Vernhettes, S., Runions, J., Friml, J. (2011) PIN Polarity Maintenance by the Cell Wall in Arabidopsis. Current Biology 21(4): 338-343.

Forestan, C., and Varotto, S. (2010) PIN1 auxin efflux carriers localization studies in Zea mays. Plant Signal Behav 5(4): 436-439.

Forestan, C., and Varotto, S. (2010) Unraveling the role of PIN auxin efflux carriers in mediating polar auxin transport and accumulation in Zea mays: phylogenetic analysis, expression patterns and subcellular trafficking. Journal of Biotechnology 150: S110-S111.

Forestan, C., and Varotto, S. (2012). The Role of PIN Auxin Efflux Carriers in Polar Auxin Transport and Accumulation and Their Effect on Shaping Maize Development. Molecular Plant 5(4): 787-798.

Forestan, C., and Varotto S (2013) Auxin immunolocalization in plant tissues. Methods in molecular biology (Clifton, N.J.) 959: 223-233.

Friml, J., Benková, E., Blilou, I., Wisniewska, J., Hamann, T., Ljung, K., Woody, S., Sandberg, G., Scheres, B., Jürgens, G., Palme, K. (2002) AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. Cell 108(5): 661-673.

Friml, J., and Palme, K. (2002) Polar auxin transport - old questions and new concepts? Plant Molecular Biology 49(3-4): 273-284.

Friml, J., Yang, X., et al (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science 282(5397): 2226-2230.

Geldner, N., Friml, J., Stierhof, Y.D., Jürgens, G., Palme, K. (2001) Auxin transport inhibitors block PIN1 cycling and vesicle traficking. Nature 413(6854): 425-428.

Jain, M., and Khurana, J.P. (2009) Transcript profiling reveals diverse roles of auxin-responsive genes during reproductive development and abiotic stress in rice. Febs Journal 276(11): 3148-3162.

Kahsay, R.Y., 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(9): 1853-1858.

Krecek, P., Skupa, P., Libus, J. et al (2009) The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biology 10(12).

Received Manuscript 20-06-2013 Accepted Manuscript 04-10-2013

Mravec, J., Skupa, P., Bailly, A., et al. (2009). Subcellular homeostasis of phytohormone auxin is mediated by ER-localized PIN5 transporter. Nature, 459(7250): 1136-1140.

Paponov, IA., Teale, W.D., Trebar, M., Blilou, I., Palme, K. (2005) The PIN auxin efflux facilitators: evolutionary and functional perspectives.Trends in plant science 10(4): 170-177.

Pattison, R.J., and Catala. C (2012) Evaluating auxin distribution in tomato (Solanum lycopersicum) through an analysis of the PIN and AUX/LAX gene families. Plant Journal 70(4): 585-598.

Peer, W.A., Bandyopadhyay, A., Blakeslee, J.J., Makam, S.N., Chen, R.J., Masson, P.H., Murphy, A.S. (2004) Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutants with altered auxin transport in Arabidopsis thaliana. Plant Cell 16(7): 1898-1911.

Petrásek, J., Mravec, J., Bouchard, R., Blakeslee, J.J., Abas, M., Seifertová, D., Wisniewska, J., Tadele, Z., Kubes, M., Covanová, M., Dhonukshe, P., Skupa, P., Benková, E., Perry, L., Krecek, P., Lee, O.R., Fink, G.R., Geisler, M., Murphy, A.S., Luschnig, C., Zazímalová, E., Friml, J. (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. Science 312(5775): 914-918.

Sabatini, S., Beis, D., Wolkenfelt, H. et al (1999) An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. Cell 99(5): 463-472.

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 Journal 277(14): 2954-2969.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. Mol. Biology and Evolution 28: 2731-2739.

Wang, J., Hu, H., Wang, G.H. et al (2009) Expression of PIN Genes in Rice (Oryza sativa L.): Tissue Specificity and Regulation by Hormones. Molecular Plant 2(4): 823-831.

Yu, C.S., Chen, Y.C., Lu, C.H., Hwang, J.K.. (2006). Prediction of protein subcellular localization. Proteins: structure, function, and bioinformatics 64(3): 643-651.

Zazimalova, E., Murphy, A.S., Yang, H. et al (2010) Auxin Transporters - Why So Many? Cold Spring Harbor Perspectives in Biology 2(3).

Received Manuscript 20-06-2013 Accepted Manuscript 04-10-2013

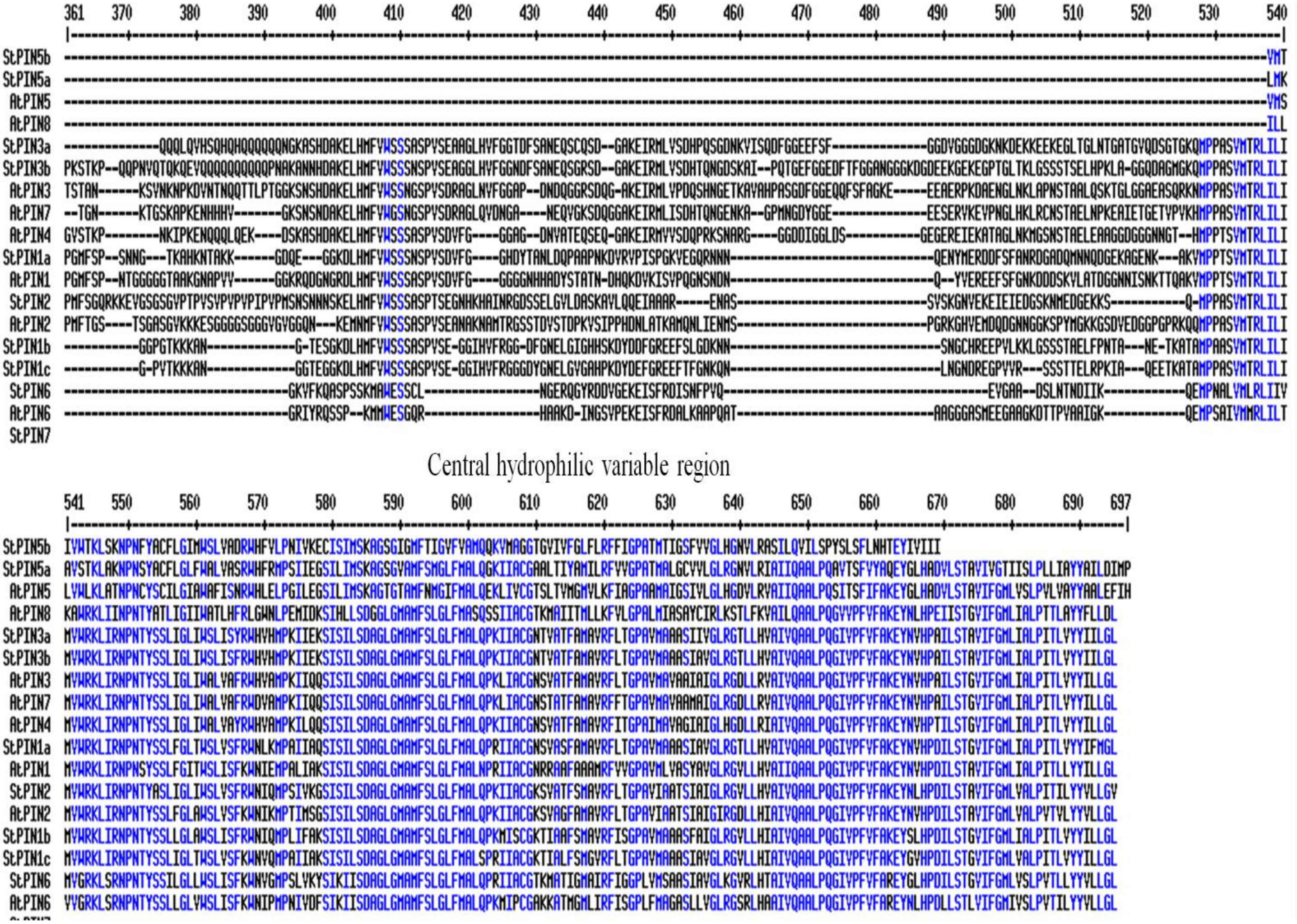
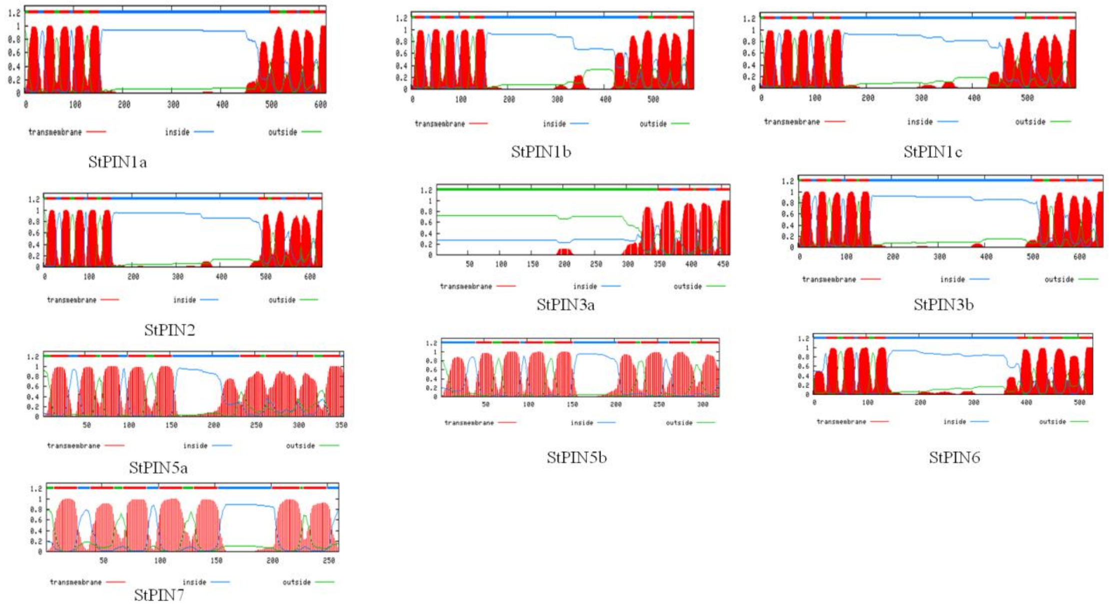


Figure 1. Amino acid sequence alignment of Solanum tubersoum auxin efflux carrier gene and Arabidopsis thaliana auxin efflux carrier gene. Alignment shows N- and C-terminal conserved region and central variable region. Multiple sequence alignment was done using Multalin software (http://multalin.toulouse.inra.fr/multalin/) using programme BLOSUM62.

Figure 2. Predicted transmembrane helices of StPIN gene. The transmembrane domain was estimated using TMMOD (Hidden Markov Model for Transmembrane Domain) http://liao.cis.udel.edu/website/servers/TMMOD/scripts/frame.php?p=submit. Red peaks shows predicted transmembrane domain.



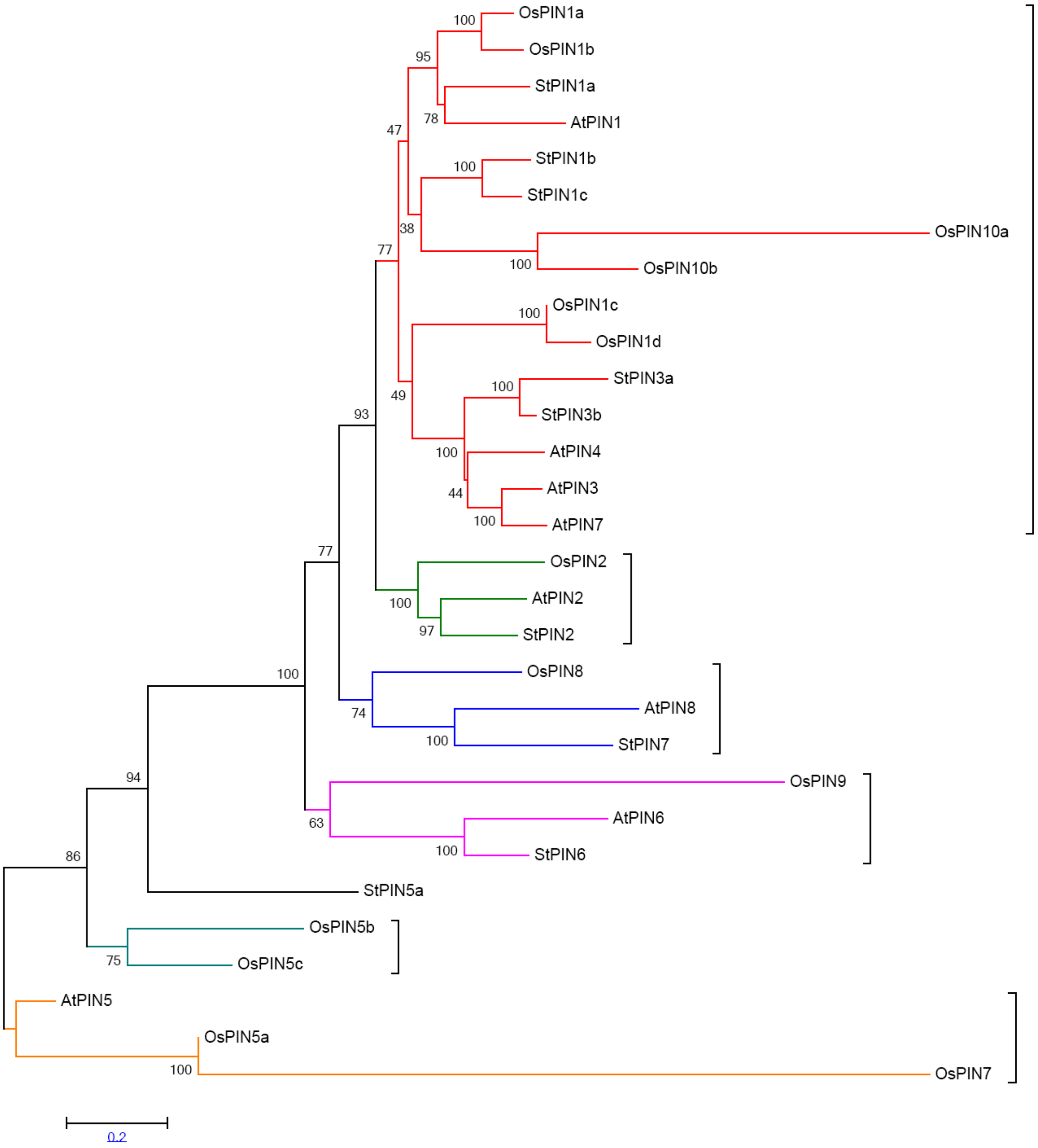
StPIN1a StPIN1b StPIN1c

StPIN2 StPIN3a StPIN3b

StPIN5a StPIN5b StPIN6

StPIN7

Figure 3. Phylogenetic tress of Solanum tuberosum PIN genes with Arabidopsis thaliana and Oryza sativa PIN genes. Phylogenetic tress shows StPIN genes are much more closer to AtPIN genes. Phylogenetic tree was constructed using MEGA5 software. Statistical programme used for construction of this phylogenetic tress was; Test phylogeny-bootstrap method, number of boot strap replication-100, model/method-poisson model, rate among site-gamma distributed (G), gap/missing data treatment-pairwise deletion.



Received Manuscript 20-06-2013 Accepted Manuscript 04-10-2013

Figure 4. Transcript organization of Solanum tuberosum StPIN gene. Box indicates the exon and line indicates the intron.

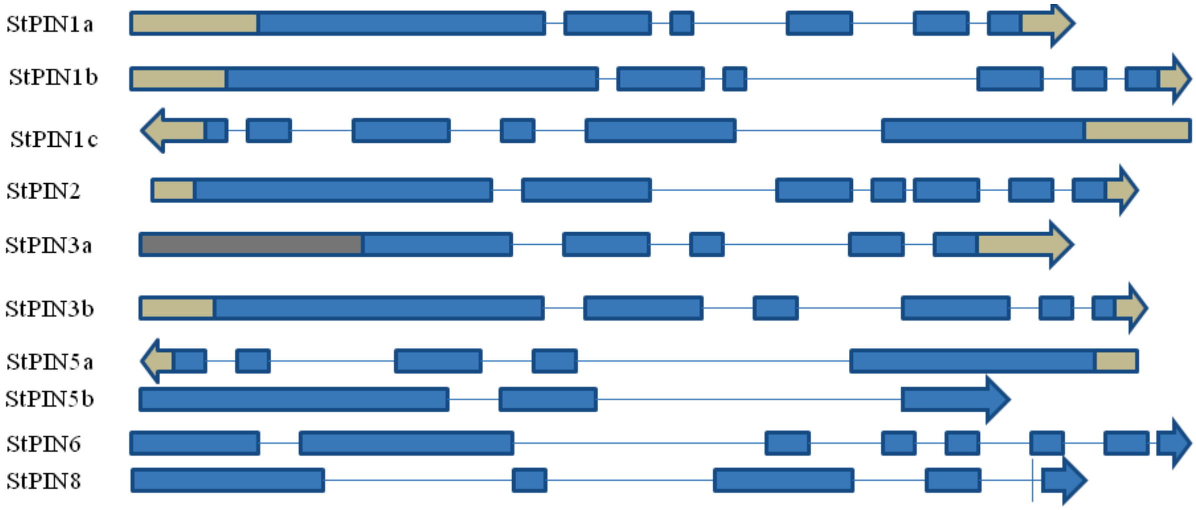


Table 1. Table showing Phytozome locus ID, nomenclature and chromosomal distribution of Solanum tuberosum PIN gene in its genome.

Phytozome Locus ID	Gene name	Chr. No.	ORF	No. a.a	No. of Introns	5'-3' Coordinates
PGSC0003DMT400005750	PIN1a	3	1845	614	5	43940359 - 43944589
PGSC0003DMT400028191	PIN1b	10	1764	587	5	49863370 - 49867160
PGSC0003DMT400008379	PIN1c	10	1785	594	5	44140537 - 44144107
PGSC0003DMT400018745	PIN2	7	1896	631	6	3512224 - 3515144
PGSC0003DMT400005956	PIN3a	4	1395	464	5	858407 - 862015
PGSC0003DMT400030495	PIN3b	5	1950	649	5	4075695 - 4079392
PGSC0003DMT400017955	PIN5a	1	1068	355	4	70198879 - 70202239
PGSC0003DMT400010532	PIN5b	4	966	321	2	42485475 - 42487082
PGSC0003DMT400030755	PIN6	6	1587	528	6	37166650 - 37173029
PGSC0003DMT400001412	StPIN8	2	783	260	4	67705982 - 67708171

Table 2: Sub-celluar localization of different PIN gene of Solanum tuberosum as predicted by CELLO v 2.5 subCELlular Localization predictor.

Gene Name	Sub-cellular localization	Sub-cellular compartment	Reliability
StPIN1a	Plasmamembran	Extracellular	4.310*
StPIN1b	Plasmamembrane	Extracellular	4.863*
StPIN1c	Plasmamembrane	Extracellular	4.842*
StPIN2	Plasmamembrane	Chloroplast	4.489*
StPIN3a	Plasmamembrane	Chloroplast	3.304*
StPIN3b	plasmamembrane	Extracellular	3.916*
StPIN5a	Plasmamembrane	Extracellular	4.920*
StPIN5b	Plasmamembrane	Extracellular	4.958*
StPIN6	Plasmamembrane	Extracellular	4.880*
StPIN7	Plasmamembrane	Chloroplast	4.785*

Received Manuscript 20-06-2013 Accepted Manuscript 04-10-2013