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Genome wide identification of auxin efflux carrier gene family in physcomitrella patens

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Abstract

Phycomitrella patens is non vascular chlorophyll containing plant. The pseudo rooting system present in this plant is point of interest in terms of auxin biology. In this research, focus was given to find out auxin efflux carrier (PIN) protein from P. patens. Results shows, there is 5 different auxin efflux carrier gene in P. patens. Phylogenetic analysis shows, they belongs to different clade, but present between Arabidopsis thaliana and Oryza sativa. Amino acid sequence alignment shows presence of conserved T-P-R motif, which are potential target phosphorylation site of mitogen activated protein kinase 3 and mitogen activated protein kinase 6. This explains protein phosphorylation sites of PIN genes are conserved.

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Introduction

The emergence of land plants from an aquatic ancestor that initiated during the mid-Ordovician and lower Silurian (480–430 million years ago) was one of the major evolutionary events for the life occurred on planet earth and bryophytes were dominated the earth on that time (Ishizaki et al., 2008). Shifting from aquatic habitate to terrestrial habitate is most important event occurred in the history of life. The most important bryophyte still exists from that time is Physcomitrella patens. P. Patens is a non-vascular, chlorophyll containing, widely spread dioecious liverwort species. It has been extensively studied by developmental biologists since the 19th century (Rensing et al., 2007). The haploid gametophytic generation dominates over the diploid sporophytic generation during its life cycle. This is very much advantageous for functional genetic analyses. P. Patens can propagate not only sexually, but also by asexual bud-like structures called gemmae, allowing rapid proliferation of isogenic biomass for molecular and biochemical experiments (Cuming, 2011). Phylogenetics analysis strongly supports the sister relationship of liverworts to all extant land plants (Duff and Nickrent, 1999). These liverworts are a key group in comparative genomics to address fundamental questions in plant biology, such as the genetic basis of the key innovations that allowed land plants to evolve from aquatic ancestors and adapt to life on land, and the developmental genetic changes responsible for alterations in body plan within land plants (Reski et al., 1994). Thus M. polymorpha is being developed as a model plant occupying a critical evolutionary position to study specific molecular and cellular developmental processes in detail.

Besides this, since the time of evolution, plants are always in threats to different biotic and abiotic stress and hence they are changing themselves and making them adaptable according to changing environment (Mohanta et al., 2012, Mohanta 2012). The survival for fittest rule plays important role, and only those plant species got survived which did able to adopt themselves to the changing environment. Among different plant species that are able to adopt this change, Physcomitrella patens is one of them and still existing since the Ordovician and Silurian age.

The potential phytohormone auxin plays important role in regulation of plant growth, development and physiological process in response to diverse environmental, biotic and abiotic cues (Rahman, 2012; Pasternak et al., 2005). Most important thing among them is vascular differentiation, organogenesis, tropic growth, root and shoot architecture (Petrasek and Friml., 2009). The major naturally occurring auxin, indole-3-acetic acid (IAA), coordinates many plant processes by modulating gene expression, which leads to changes in cell division, elongation and differentiation (Ishida et al., 2010). How auxin signal is perceived, distributed, interpreted and maintains cellular homeostasis by plant has been a central question in plant biology. As Physcomitrella patens the first pseudo root bearing plant, this can answer a lot of question about auxin perception, homeostasis and regulation of gene expression. Rensing et al (2007) first reported the draft genome of Physcomitrella patens.

Auxin plays important role in development of root system in plants and its synthesis takes places in aerial parts of plants and transported to root to play its role (Zhao, 2010). It is widely reported that polar auxin efflux carrier component (PIN) are crucial for cellular coordination and auxin homoestasis. The first putative efflux carrier component to be characterized was AtPIN1 and shown to be localized polarly in plasma membranes and rapidly relocalize, thereby providing a conceptual basis for explaining how changes in auxin efflux affects tropic response and patterning (Galweiler et al., 1998;

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Paponov et al., 2005). PIN plays vital role in cell polarity, patterning, embryogenesis, gravitropism and phototropism, apical dominance, root architecture (Paponov et al., 2005; Leyser, 2005; Blakeslee et al., Palme et al., 2006; Blilou et al., 2005). Both metabolic changes and transport of auxin have been demonstrated to play major role in plant organogenesis and differentiation (Petraseka and Friml, 2009). There are eight PIN genes from the genome of Arabidopsis and 12 from the genome of Oryza sativa respectively. It has been suggested that all PIN genes in Arabidopsis and in all higher plants diverged from a single ancestral. In contrast to dicots, the wider PIN family of the monocot has more divergent phylogenetic structure (Paponov et al., 2005). Presence of PIN gene in Physcomitrella patens can explain the evolution of auxin transport career component and hence evolution of auxin signaling mechanism.

Materials and Methods

Identification of PIN gene

For identification of PIN genes in Physcomitrella patens, phytozome database (www.phytozome.net), the Institute for Genome Research (TIGR) database and The Arabidopsis Information Resource (TAIR) and rice genome database were used. The amino acid sequences of 8 PIN proteins from Arabidopsis were downloaded from The Arabidopsis Information Resource (TAIR) and 12 PIN proteins from rice were downloaded from rice genome database. These PIN proteins were used to search their homologs in phytozome database using BLASTP programme (Altschul et al., 1997).

Sequence analysis

The PIN gene identified from phytozome database were again analyzed to know their orthology with regard to higher plants. BLASTP Analysis were carried out with TIGR database and orthologous PIN gene found from Physcomitrella patens were named accordingly. Arabidopsis PIN genes were used for nomenclature of Physcomitrella patens PIN gene as Arabidopsis is considered as the model plant. Multiple alignment of proteins from Arabidopsis, rice and Physcomitrella patens were carried out to know their homology using Multalign software (http://multalin.toulouse.inra.fr/multalib/). Phylogenetic tree was constructed using CLUSTALW programme (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Larkin et al., 2007). Domain annotation was carried out using TMMOD (The Hidden Markov Model for Transmembrane Protein Topology Prediction) to confirm presence of auxin efflux carrier domain and whether they are belonged to membrane transport family protein (Kahsay et al., 2005).

Results and Discussion

Study carried out to find PIN gene from Physcomitrella patens shows presence of 5 PpPIN genes. Their nomenclature and locus ID provided in the table1. PpPIN3-1 and PpPIN3-2 has ORF length of 2142 nucleotide with 5 introns in their genomic sequences (figure 1). PpPIN3 have ORF length of 2097 nucleotide where as PpPIN6-1 have ORF length of 1227 nucleotide each of having 5 introns (figure 1). PpPIN6-2 have ORF length of 342 nucleotide having only 1 intron (figure 1). Amino acid sequence alignment shows, they are more conserved in N-terminal region, where as C-terminal region is less conserved with regard to N-terminal region, middle region shows dynamicity in amino acid sequence alignment (figure 2). Middle variable region is very much conserved in terms of T-P-R motif which are highlighted red in figure 2. The T-P-R motif is very much conserved in Arabidopsis, rice and Physcomitrella patens. This T-P-R motifs are potential phosphorylation site of PIN protein (Sorenson et al., 2012). Protein phosphorylation is very essential to carry out proper functional role in cell. Protein phosphorylation is usually carried out by kinases (mitogen activated protein kinase, cyclin dependent protein kinase etc.) (Sinha et al., 2011). The T-P-R motif are potential phosphorylation site of MAPK4 and MAPK6. The presence of conserve T-P-R motif explains evolution of protein phosphorylation is conserved.

Phylogenetic analysis shows PIN genes are clustered into five separate groups (figure 3). PpPIN proteins are present separately in group II and group IV. PpPIN6-1 and PpPIN6-2 are very closer to AtPIN6. PpPIN3, PpPIN3-1 and PpPIN3-2 are closer to AtPIN8 of group I and OsPIN5 of group III. Development of plant body strongly depends on intercellular transport and distribution of phytohormone auxin (Barbez et al., 2012). Domain annotation using TMMOD (The Hidden Markov Model for Transmembrane Protein Topology Prediction) shows presence of transmembrane domain and auxin efflux carrier domain (figure 4) (Kahsay et al., 2005). Auxin efflux carrier genes are characterized by presence of auxin efflux carrier domain and presence of N and C-terminal conserved region and central hydrophilic region (Wang et al., 2009). PIN genes also grouped into different group by presence of long or short transmembrane domain. This study shows, PpPIN3-1 and PpPIN3-2 are having long transmembrane domain, where as PpPIN3, PpPIN6-1 are having short transmembrane domain (figure 4). In PpPIN6-2 transmembrane domain is absent which may be due to event of deletion. Mravec et al. (2009) reported that Arabidopsis thaliana PIN5 encodes functional auxin transport that is required for auxin mediated plant development. PIN5 does not have direct role in cell-to-cell transport but regulates intracellular homeostasis and metabolism. Study carried out by Lewis et al. (2011) shows PIN3 plays important role in auxin transport. PIN3 mutant shows reduction in density and number of lateral root formation where as PIN3 and PIN7 double mutant shows much stronger reduction in lateral root formation. AtPIN8 plays role in development of male gametophyte of Arabidopsis and has crucial role in pollen development and its functionality (Ding et al., 2012). Ectopic expression in sporophytic tissue shows role of AtPIN8 in regulation of auxin homeostasis and metabolism. PIN8 co-localizes with PIN5 and found in endoplasmic reticulum (ER) where it acts as auxin transporter. Genetic analysis shows

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PIN5 and PIN8 regulates auxin homeostasis, sporophyte and gametophyte development as well as growth of pollen tube. Phylogenetic analysis of PpPIN shows, PpPIN3, PpPIN3-1, PpPIN3-2 grouped in between AtPIN8 and OsPIN5 which plays important role in auxin transport as well as development of gametophyte, sporophyte and pollen tube. P. patens has gametophytic generation and sporophytic generation. So, presence of PpPIN3, PpPIN3-1, PpPIN3-2 gene in between PIN5 and PIN8 strongly suggest they play important role auxin homeostasis, development of gamete, spore and pollen tube. So it can be concluded that presence of three PpPIN3 gene in P. patens plays significant role in auxin homeostasis, maintenance of gametophytic as well as sporophytic generation as well as formation of root like structure and hence its life cycle.

Conclusion

P. patens is a non vascular plant which shows presence of auxin efflux carrier protein. Earlier it was thought that bryophytes don't have rooting system and hence there may not be significant role of auxin in it growth, development and life cycle. But, presence of auxin efflux carrier gene clearly demonstrates, auxin homeostasis and distribution is important in P. patens for its proper growth and development. Evolution of auxin and its role evolved from the era of bryophytes which dominates during the Paleozoic era of evolutionary history.

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References

Altschul, S.F., Madden, T.L. et al (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402.

Barbez, E., Kubes, M. et al (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. Nature, 485:119-122, doi:10.1038/nature11001.

Blakeslee, J.J., Bandyopadhyay, A., et al (2004). Relocalization of the PIN1 auxin efflux facilitator plays a role in phototropic responses. Plant Physiol. 134, 28–31.

Blilou, I., Xu, J., et al (2005). The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 433, 39–44.

Cuming, A. (2011). "Molecular bryology: mosses in the genomic era". Field Bryology 103: 9–13.

Ding, Z., Wang, B., et al (2012). ER-localized auxin transporter PIn8 regulates auxin homeostasis and male gametophyte development in Arabidopsis. Nature Communications | 3:941 | DoI: 10.1038/ncomms1941.

Duff, R.J., and Nickrent, D.L., (1999). Phylogenetic relationships of land plants using mitochondrial small-subunit rDNA sequences. Am. J. Bot. vol. 86 no. 3 372-386.

Ga¨lweiler L., et al. (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science 282, 2226–2230.

Ishida, T., Adachi, S., et al (2010). Auxin modulates the transition from the mitotic cycle to the endocycle in Arabidopsis Development, 137(1):63-71. doi: 10.1242/dev.035840.

Ishizaki, K., Chiyoda, S., Yamato, K.T., and Kohchi, T., (2008). Agrobacterium-Mediated Transformation of the Haploid Liverwort Marchantia polymorpha L., an Emerging Model for Plant Biology. Plant Cell Physiol. 49(7): 1084–1091, doi:10.1093/pcp/pcn085.

Kahsay, R,, Liao, L., Gao, G., (2005). An improved hidden markov model for transmembrane protein topology prediction and its application to complete genomes. Bioinformatics, 21:1853-1858.

Larkin, M.A., Blackshields, G., et al (2007). ClustalW and ClustalX version2. Bioinformatics 23(21): 2947-2948. doi:10.1093/bioinformatics/btm404.

Lewis, D.R., Negi, S., et al (2011). Ethylene inhibits lateral root development, increases IAA transport and expression of PIN3 and PIN7 auxin efflux carriers. Development 138, 3485-3495, doi:10.1242/dev.065102.

Leyser, O., (2005). The fall and rise of apical dominance. Curr. Opin. Genet. Dev. 15, 468–471.

Mravec, J., Sku, P.A., Bailly, A.L., et al (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature, Vol 459, doi:10.1038/nature08066.

Mohanta, T.K., (2012. Advances in Ginkgo biloba research: genomics and metabolomics perspectives. Afr. J. Biotechnol. Vol. 11(93): 15936-15944.

Mohanta, T.K., Occhipinti A, Zebelo SA et al (2012). Ginkgo biloba responds to herbivory by activating early signaling and direct defenses. PLoS One, 7(3): e32822, doi:101371/ journal.pone.0032822.

Mravec, J., Sku, P.A., Bailly, A.L. et al (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature, Vol 459, doi:10.1038/nature08066.

Palme, K., Dovzhenko, A., and Ditengou, F.A., (2006). Auxin transport and gravitational research: perspectives. Protoplasma, 229, 175–181.

Received Manuscript 03-05-2013 Accepted Manuscript- 18-06-2013

Paponov, I.A., Teale, W.D., et al (2005). The PIN auxin efflux facilitators: evolutionary and functional perspectives. Trends in Plant Science. Vol. 10(4):170-177.

Pasternak, T., Potters, G., et al (2005). Complementary interactions between oxidative stress and auxins control plant growth responses at plant, organ, and cellular level. J. Exp. Bot. 56(418): 1991-2001.doi: 10.1093/jxb/eri196

Petrasek, J., and Friml, J., (2009). Auxin transport routes in plant development. Development 136, 2675–2688.

Rahman, A., (2013). Auxin: a regulator of cold stress response. Physiologia Plantarum, Special Issue: Abiotic Stress, Vol. 147(1): 28–35.

Rensing, S.A., Lang, D., Zimmer, A.D., et al (2007). The Physcomitrella Genome Reveals Evolutionary Insights into the Conquest of Land by Plants, Vol. 319 no. 5859 pp. 64-69.

Reski, R., Faust, M., Wang, X.H., Wehe, M., & Abel, W.O., (1994). "Genome analysis of the moss Physcomitrella patens (Hedw.) B.S.G.". Molecular and General Genetics 244 (4): 352–359. doi:10.1007/BF00286686.PMID 8078460.

Sinha, A.K., Jaggi, M., et al (2011) Mitogen activated protein kinase signaling in plant under abiotic stress. Plant signaling and behavior, 6(2): 196-203.

Sorenson, C., Lenman, M., Schopper, S., Vilg, J.V., Ljugdahl, T., Grotlis, M., Tamas, M.J., Peck, S.C., Andreaasson., (2012). Determination of primary sequence specificity of Arabidopsis MAP kinase MPK3 and MPK6 leads to identification of new substrates. Biochemical journal, 446:271-278.

Wang, R.J., Hu, H., Wang, G.H., Li, Ji., Chen, J.Y., Wu, P., (2009). Expression of PIN genes in rice (Oryza sativa L.): Tissue specificity and regulation by hormones. Molecular Plant, 2(4): 823-831.

Zhao, Y., (2010). Auxin biosynthesis and its role in plant development. Annu. Rev. Plant Biol. 61, 49–64

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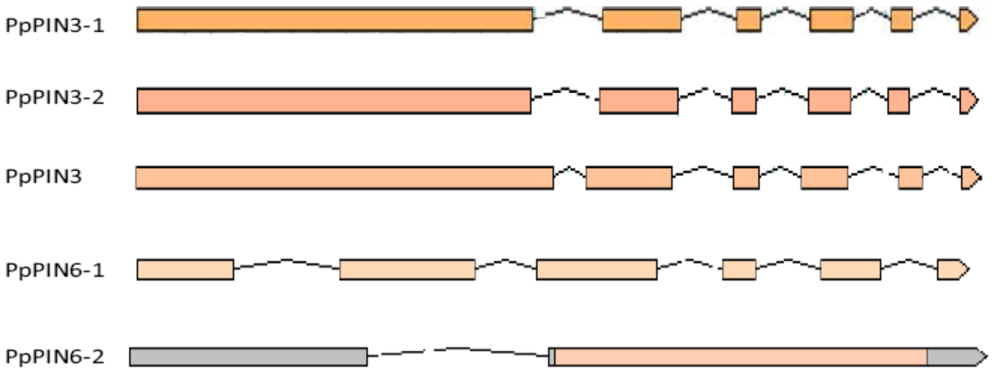
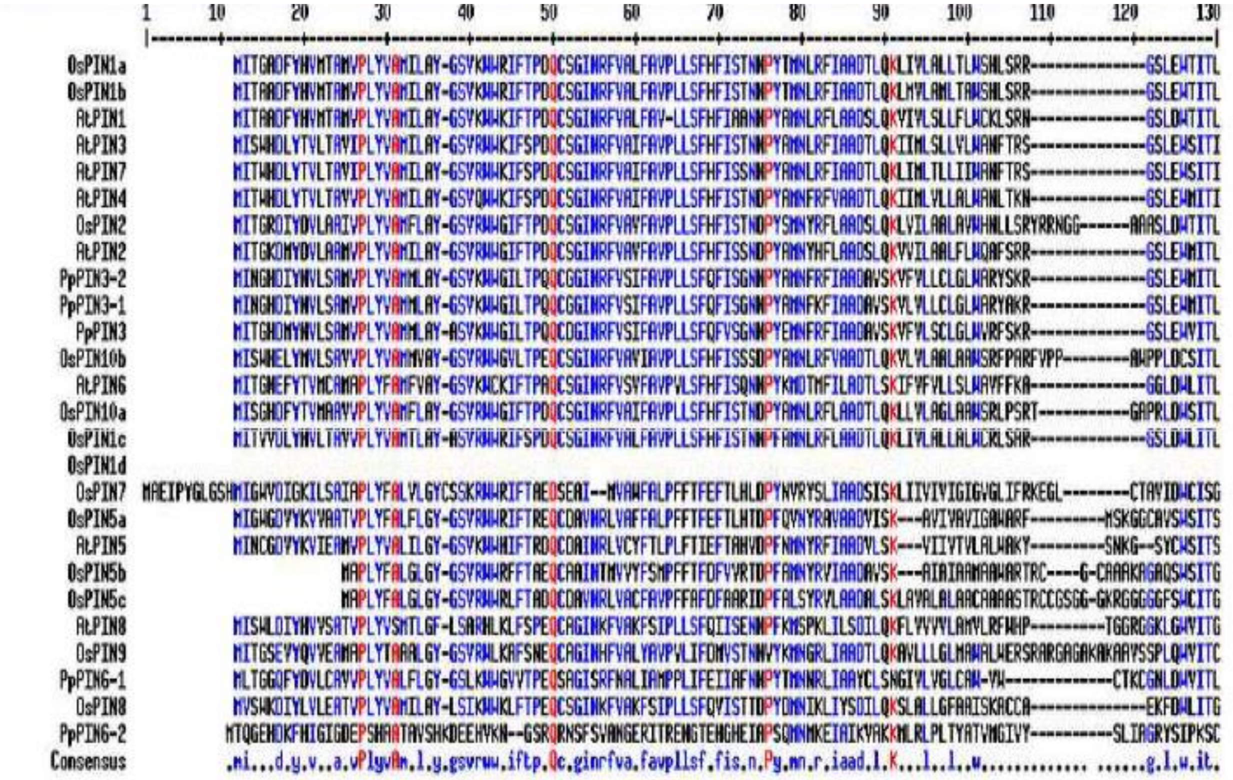


Figure1. Genomic organization of PpPIN gene showing exonic and intronic region.



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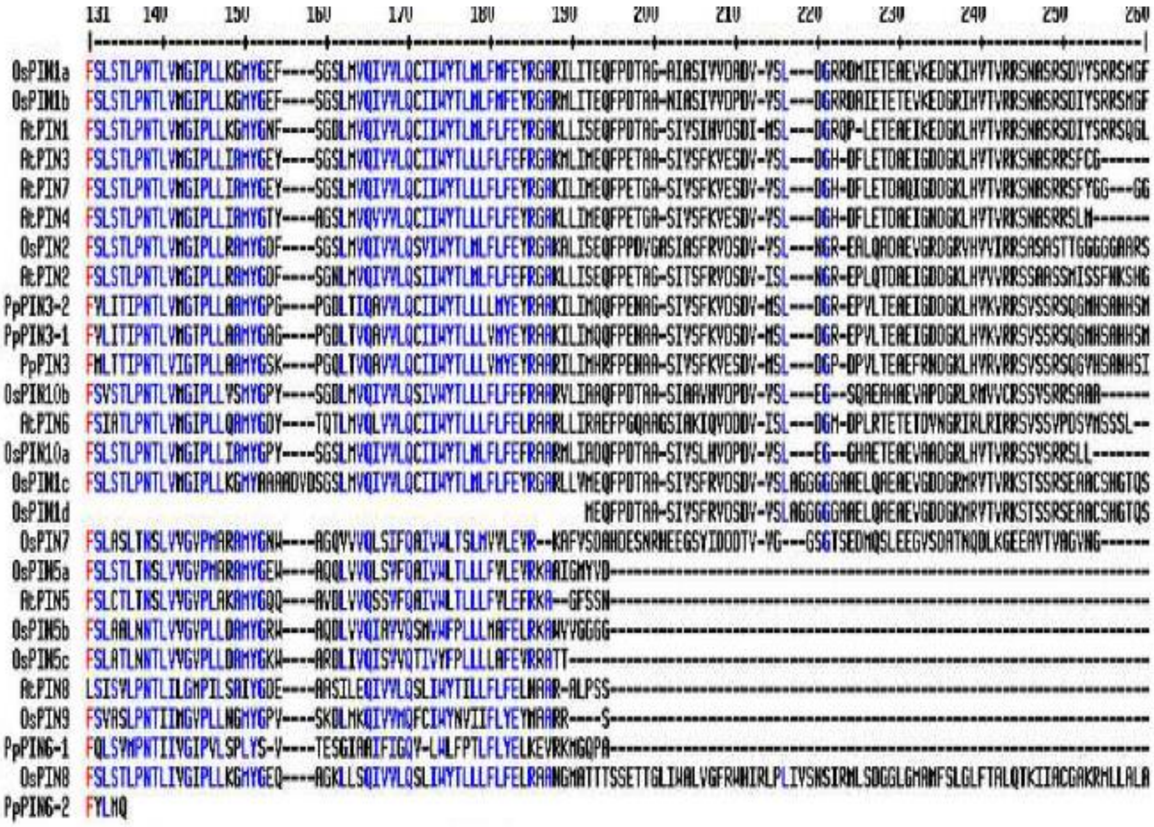
131
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150
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190
200
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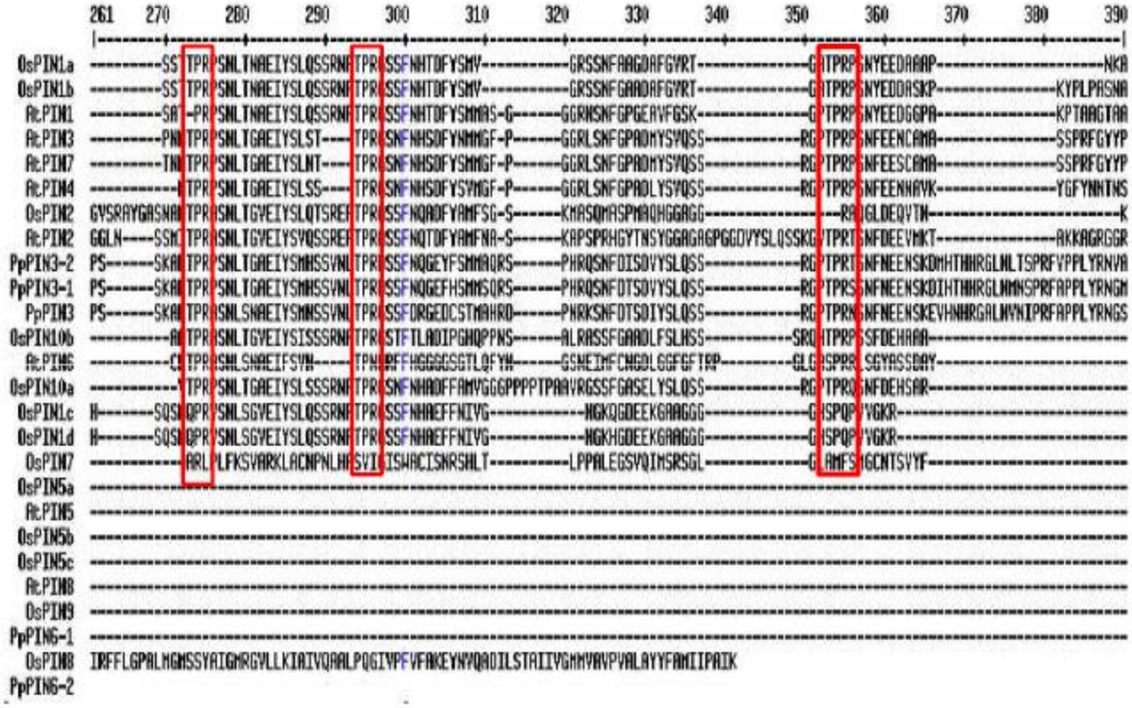
220
230
240
250
260
OsPIN1a
OsPIN1b
AtPIN1
AtPIN3
AtPIN7
AtPIN4
OsPIN2
AtPIN2
PpPIN3-2
PpPIN3-1
PpPIN3
OsPIN10b
AtPIN6
OsPIN10a
OsPIN1c
OsPIN1d
OsPIN7
OsPIN5a
AtPIN5
OsPIN5b
OsPIN5c
AtPIN8
OsPIN9
PpPIN6-1
OsPIN8
PpPIN6-2


261
270
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330
340
350
360
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380
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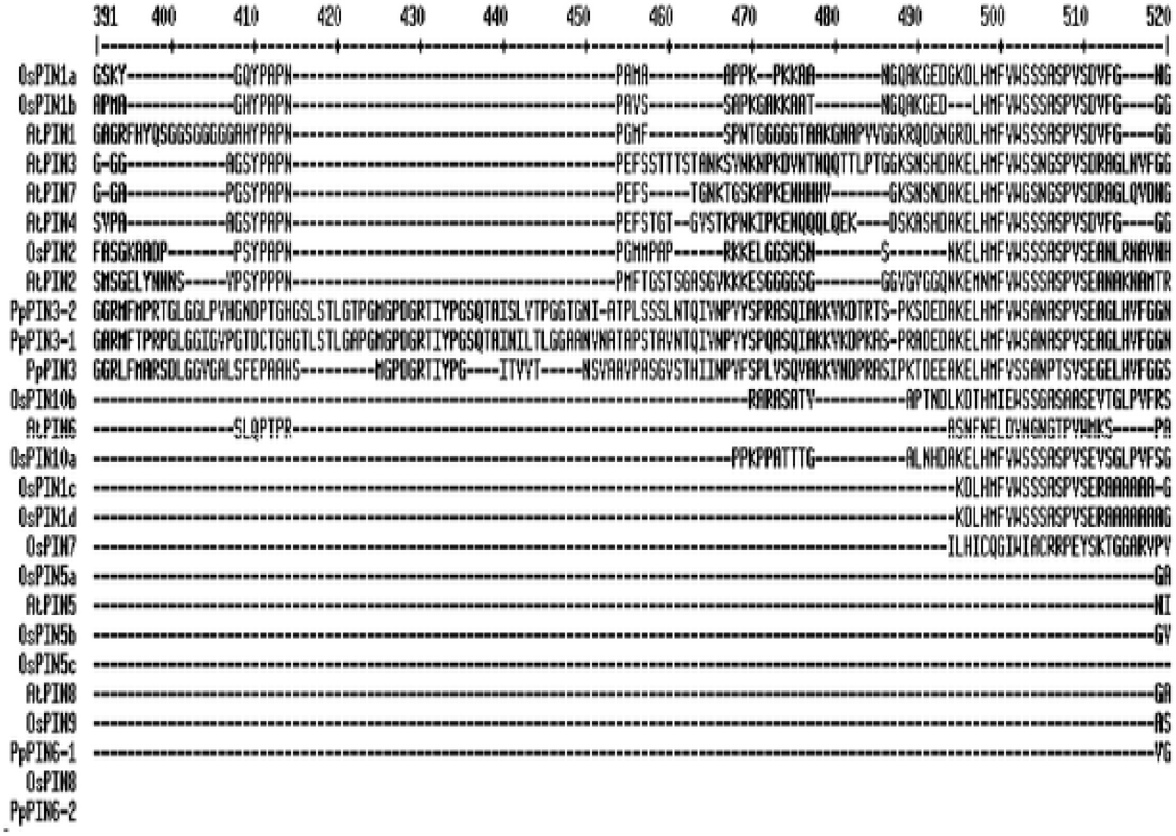
OsPIN1a
OsPIN1b
AtPIN1
AtPIN3
AtPIN7
AtPIN4
OsPIN2
AtPIN2
PpPIN3-2
PpPIN3-1
PpPIN3
OsPIN10b
AtPIN6
OsPIN10a
OsPIN1c
OsPIN1d
OsPIN7
OsPIN5a
AtPIN5
OsPIN5b
OsPIN5c
AtPIN8
OsPIN9
PpPIN6-1
OsPIN8
PpPIN6-2



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         391     400       410       420       430       440       450       460       470       480       490       500       510      520
         |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
 OsPIN1a GSKY-------------GQYPAPN---------------------------------------PAMA---------APPK--PKKAA--------NGQAKGEDGKDLHMFVWSSSASPVSDVFG----NG
 OsPIN1b APMA-------------GHYPAPN---------------------------------------PAVS---------SAPKGAKKAAT--------NGQAKGED---LHMFVWSSSASPVSDVFG----GG
  AtPIN1 GAGRFHYQSGGSGGGGGAHYPAPN---------------------------------------PGMF---------SPNTGGGGGTAAKGNAPVVGGKRQDGNGRDLHMFVWSSSASPVSDVFG----GG
  AtPIN3 G-GG------------AGSYPAPN---------------------------------------PEFSSTTTSTANKSYNKNPKDVNTHQQTTLPTGGKSNSHDAKELHMFVWSSNGSPVSDRAGLNVFGG
  AtPIN7 G-GA------------PGSYPAPN---------------------------------------PEFS-----TGNKTGSKAPKENHHHY-------GKSNSNDAKELHMFVWGSNGSPVSDRAGLQVDNG
  AtPIN4 SYPA------------AGSYPAPN---------------------------------------PEFSTGT--GYSTKPNKIPKENQQQLQEK----DSKASHDAKELHMFVWSSSASPVSDVFG----GG
  OsPIN2 FASGKAADP--------PSYPAPN---------------------------------------PGMMPAP------RKKELGGSHSN--------$-------NKELHMFVWSSSASPVSEANLRNAVNH
  AtPIN2 SMSGELYNNNS-----YPSYPPPN---------------------------------------PMFTGSTSGASGVKKKESGGGGSG--------GGVGYGGQNKEMNMFVWSSSASPVSEANAKNAMTR
PpPIN3-2 GGRMFMPRTGLGGLPVHGNDPTGHGSLSTLGTPGMGPDGRTIYPGSQTAISLYTPGGTGNI-ATPLSSSLNTQIYNPYYSPRASQIAKKYKDTRTS-PKSDEDAKELHMFVWSANASPVSEAGLHVFGGN
PpPIN3-1 GARMFTPRPGLGGIGVPGTDCTGHGTLSTLGAPGMGPDGRTIYPGSQTAINILTLGGAANVNATAPSTAVNTQIYNPYYSPQASQIAKKYKDPKAS-PRADEDAKELHMFVWSANASPVSEAGLHVFGGN
  PpPIN3 GGRLFMARSDLGGVGALSFEPAAHS---------MGPDGRTIYPG----ITVVT-----NSVAAVPASGVSTHIINPVFSPLVSQVAKKYNDPRASIPKTDEEAKELHMFVSSANPTSVSEGELHVFGGS
OsPIN10b -------------------------------------------------------------------------------RARASATY-----------APTNDLKDTHMIEWSSGASAASEVTGLPVFRS
  AtPIN6 -----------------SLQPTPR-------------------------------------------------------------------------------ASNFNELDVNGNGTPVWMKS-----PA
OsPIN10a -----------------------------------------------------------------------------PPKPPATTTG-----------ALNHDAKELHMFVWSSSASPVSEVSGLPVFSG
 OsPIN1c --------------------------------------------------------------------------------------------------------KDLHMFVWSSSASPVSERAAAAAA-G
 OsPIN1d --------------------------------------------------------------------------------------------------------KDLHMFVWSSSASPVSERAAAAAAAG
  OsPIN7 -------------------------------------------------------------------------------------------------------ILHICQGIWIACRRPEYSKTGGARVPV
 OsPIN5a --------------------------------------------------------------------------------------------------------------------------------GA
  AtPIN5 --------------------------------------------------------------------------------------------------------------------------------NI
 OsPIN5b --------------------------------------------------------------------------------------------------------------------------------GV
 OsPIN5c ----------------------------------------------------------------------------------------------------------------------------------
  AtPIN8 --------------------------------------------------------------------------------------------------------------------------------GA
  OsPIN9 --------------------------------------------------------------------------------------------------------------------------------AS
PpPIN6-1 --------------------------------------------------------------------------------------------------------------------------------VG
  OsPIN8
PpPIN6-2
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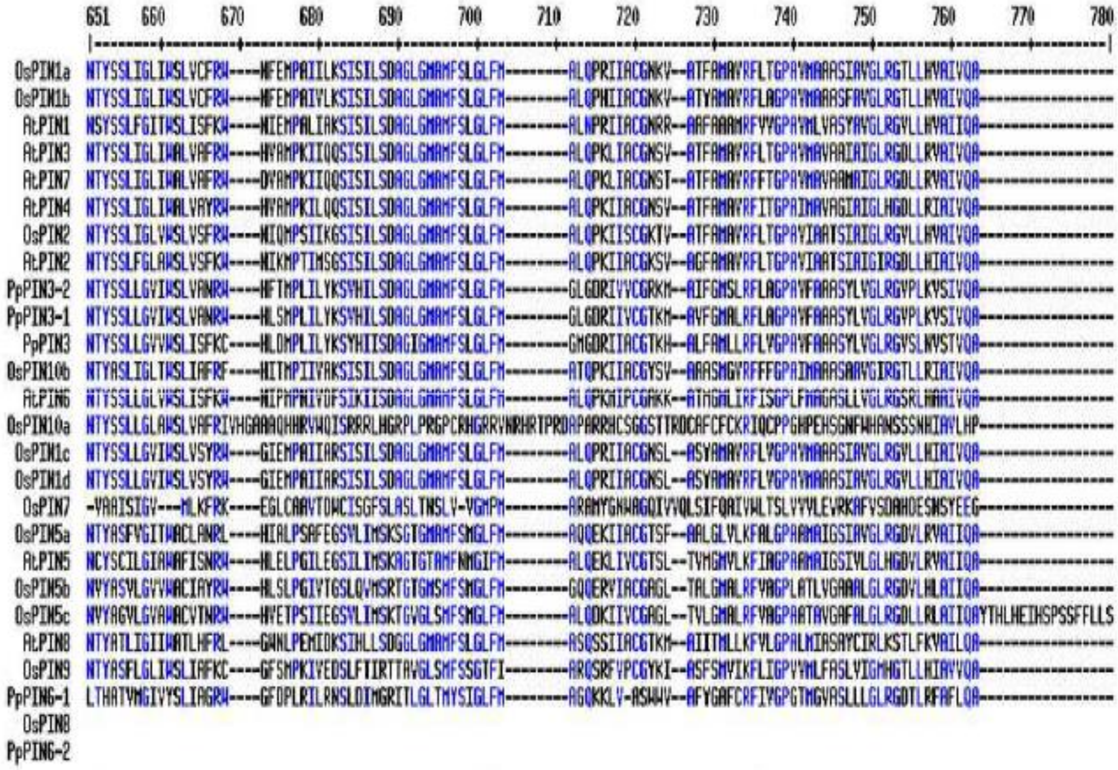

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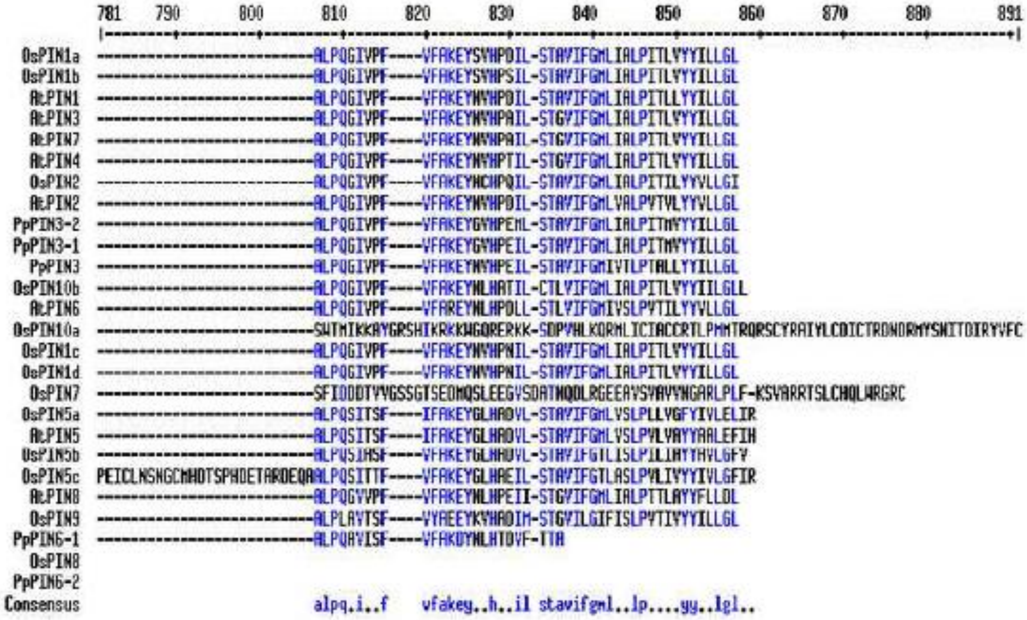

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            781  790       800       810       820       830       840       850       860       870       880       891
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
  OsPIN1a   --------------------------ALPQGIVPF----VFAKEYSVHPDIL-STAVIFGMLIALPITLVYYILLGL
  OsPIN1b   --------------------------ALPQGIVPF----VFAKEYSVHPSIL-STAVIFGMLIALPITLVYYILLGL
   AtPIN1   --------------------------ALPQGIVPF----VFAKEYNVHPDIL-STAVIFGMLIALPITLLYYILLGL
   AtPIN3   --------------------------ALPQGIVPF----VFAKEYNVHPAIL-STGVIFGMLIALPITLVYYILLGL
   AtPIN7   --------------------------ALPQGIVPF----VFAKEYNVHPAIL-STGVIFGMLIALPITLVYYILLGL
   AtPIN4   --------------------------ALPQGIVPF----VFAKEYNVHPTIL-STGVIFGMLIALPITLVYYILLGL
   OsPIN2   --------------------------ALPQGIVPF----VFAKEYNCHPQIL-STAVIFGMLIALPITILYYVLLGI
   AtPIN2   --------------------------ALPQGIVPF----VFAKEYNVHPDIL-STAVIFGMLVALPVTVLYYVLLGL
  PpPIN3-2  --------------------------ALPQGIVPF----VFAKEYGVHPEML-STAVIFGMLIALPITMVYYILLGL
  PpPIN3-1  --------------------------ALPQGIVPF----VFAKEYGVHPEIL-STAVIFGMLIALPITMVYYILLGL
   PpPIN3   --------------------------ALPQGIVPF----VFAKEYNVHPEIL-STAVIFGMIVTLPTALLYYILLGL
  OsPIN10b  --------------------------ALPQGIVPF----VFAKEYNLHATIL-CTLVIFGMLIALPITLVYYIILGLL
   AtPIN6   --------------------------ALPQGIVPF----VFAREYNLHPDLL-STLVIFGMIVSLPVTILYYVLLGL
  OsPIN10a  --------------------------SWTMIKKAYGRSHIKRKKWGQRERKK-SDPVHLKQRMLICIACCRTLPMMTRQRSCYRAIYLCDICTRDNDRMYSNITDIRYVFC
  OsPIN1c   --------------------------ALPQGIVPF----VFAKEYNVHPNIL-STAVIFGMLIALPITLVYYILLGL
  OsPIN1d   --------------------------ALPQGIVPF----VFAKEYNVHPNIL-STAVIFGMLIALPITLVYYILLGL
   OsPIN7   --------------------------SFIDDDTVVGSSGTSEDMQSLEEGVSDATMQDLRGEEAVSVAVVNGARLPLF-KSVARRTSLCHQLWRGRC
  OsPIN5a   --------------------------ALPQSITSF----IFAKEYGLHADVL-STAVIFGMLVSLPLLVGFYIVLELIR
   AtPIN5   --------------------------ALPQSITSF----IFAKEYGLHADVL-STAVIFGMLVSLPVLVAYYAALEFIH
  OsPIN5b   --------------------------ALPQSIASF----VFAKEYGLHADVL-STAVIFGTLISLPILIAYYAVLGFV
  OsPIN5c   PEICLNSNGCMHDTSPHDETARDEQAALPQSITTF----VFAKEYGLHAEIL-STAVIFGTLASLPVLIVYYIVLGFIR
   AtPIN8   --------------------------ALPQGVVPF----VFAKEYNLHPEII-STGVIFGMLIALPTTLAYYFLLDL
   OsPIN9   --------------------------ALPLAVTSF----VYAEEYKVHADIM-STGVILGIFISLPVTIVYYILLGL
  PpPIN6-1  --------------------------ALPQAVISF----VFAKDYNLHTDVF-TTA
   OsPIN8
  PpPIN6-2
 Consensus                            alpq.i..f    vfakey..h..il stavifgml..lp....yy..lgl..
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Figure 2.
Amino acid sequence alignment of *Physcomitrella patens* PIN genes with *Arabidopsis thaliana* and *Oryza sativa* PIN gene , shows close homology and presence of conserved motif T-P-R. T-P-R motif is potential target phosphorylation site of MAPK3 and MAPK6.

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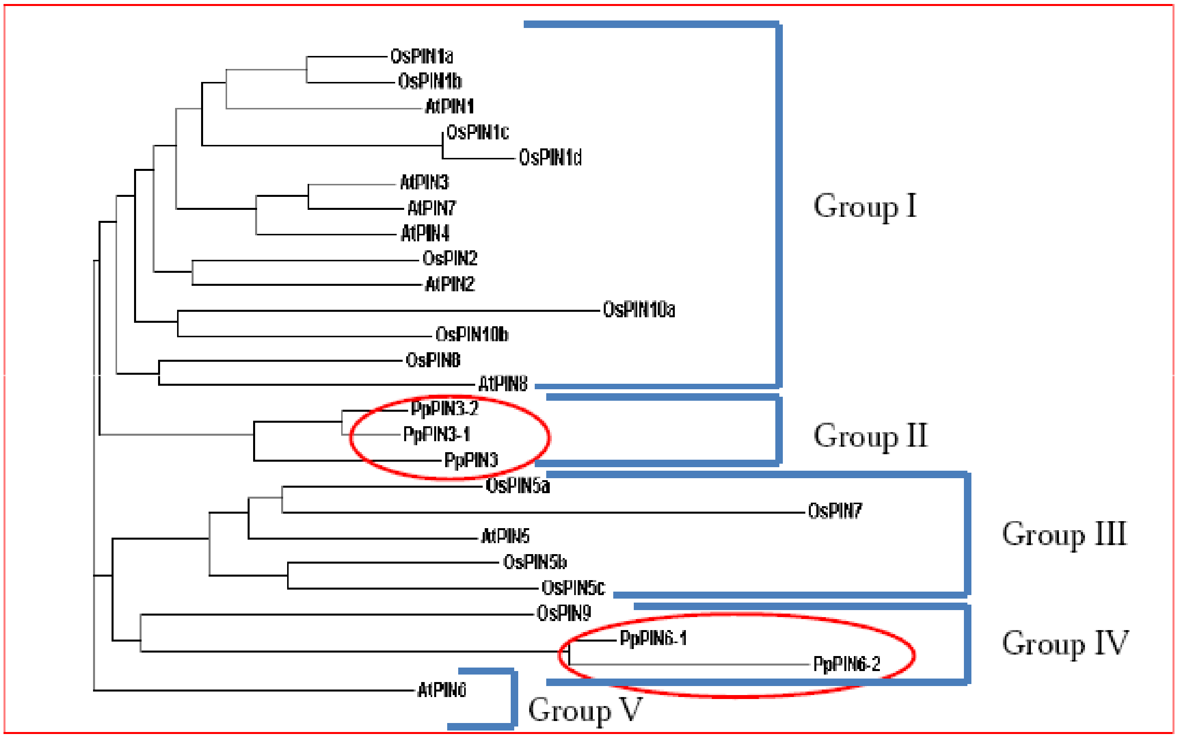
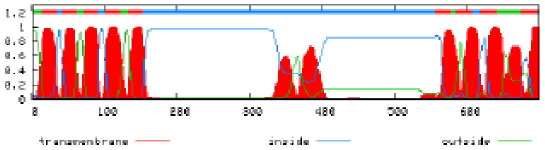
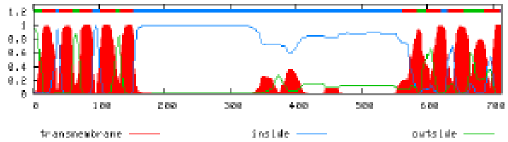


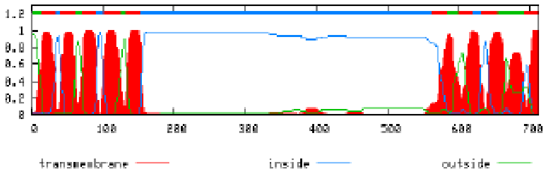
Figure 3.
Phylogenetic analysis of *Physcomitrella patens* PIN genes with *Arabidopsis thaliana* and *Oryza sativa* PIN gene. *P. patens* PIN3, PIN3-1 and PIN3-2 genes clustered together between *Arabidopsis* and *Oryza* PIN genes.



PpPIN3



PpPIN3-1



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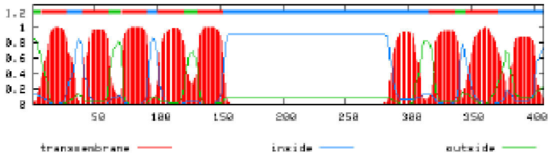

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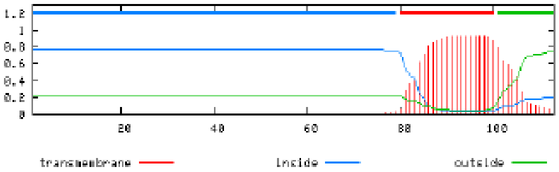

PpPIN6-2

Figure 4. Transmembrane domain prediction of PpPIN gene using "The Hidden Markov Model for Transmembrane Protein topology Prediction" .

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