Molecular players of auxin transport systems: advances in genomic and molecular events*

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1. Introduction

Auxin is one of the most important plant hormone that plays diverse roles in plants, starting from the cell division, and cell elongation to morphogenesis. Auxins are transported to different parts of the plant by different sophisticated transporter molecules known as ‘auxin transporters’. There are four auxin transporter families that have been reported so far in the plant kingdom which includes AUX/LAX (AUXIN-RESISTANT1–LIKE), PIN (PIN-FORMED, auxin efflux carriers), ABCB (ATP-binding cassette-B (ABCB)/P-glycoprotein (PGP)) and PILS (PIN-Likes). Auxin influx and efflux carriers are distributed in a polar fashion in the plasma membrane whereas ABCB and PILS are present in a non-polar fashion. Other than AUX/LAX, other auxin transporters harbor N-and C-terminal conserved domains along with a variable hydrophilic loop in the transmembrane domain. The AUX/LAX, ABCB and PIN transporters mediate long distance auxin transport whereas PILS and PIN5 protein involved in intracellular auxin homeostasis.

2. Auxin transporters

The phytohormone auxin is a non-polar solute and hence the transport of auxin across the membrane depends upon its physio-chemical properties. Auxin (IAA) is a weak acid and present as IAA+ (protonated) in its native state. The pH of apoplastic cellular environment of IAA molecule is ranges in between 5 to 5.5 (Gout et al. 1992; Pin Ng et al. 2015) due to the presence of plasma membrane bound H+ ATPases and at this pH, 83% of the IAA molecules remain in anionic (IAA−, dissociated) and 17% (IAA+, associated) in cationic form (Zažimalová et al. 2010). The negative charge (anion) of the IAA− molecule prevents it to pass through the lipophilic plasma membrane and it only allows the protonated (cation) IAA+ by passive diffusion (Zažimalová et al. 2010; Pin Ng et al. 2015). However, around 83% of the anionic IAA− cannot pass though the plasma membrane and thus it requires auxin influx carriers to transport these molecules inside the cells. Further, after entering the cytosol, IAA encounters with the alkaline environment (pH 7 to 7.5) of the cell (Gout et al. 1992; Zažimalová et al. 2010; Pin Ng et al. 2015). The IAA remains in the anionic (IAA−) form in the alkaline environment which makes it difficult to pass out of the cell, making the cell a weak anionic chamber. To overcome this paradox, asymmetrical localization of auxin transporter molecules is required in different parts of the cell, to efflux IAA− out of the cell. To facilitate the transport of IAA− molecule, cells require specialized transporter molecules e.g. auxin efflux carrier (PIN) and ATP-binding cassette-B (ABCB)/P-glycoprotein (PGP)
transporters (Friml et al. 2004; Terasaka et al. 2005). The cell-to-cell movement of auxin requires both influx and efflux carrier proteins in the plasma membrane and also in the intracellular spaces. The rate of non-polar auxin transport is about 5–20 cm/h whereas the rate of polar auxin transport is 5–20 mm/h (Michniewicz et al. 2007). To date, three major auxin transporters families have been reported which includes auxin influx carrier (AUXIN-RESISTANT1–LIKES (AUX1/LAX), PIN-FORMED (commonly known as an auxin efflux carriers, or PIN), and an ATP-binding cassette-B (ABCB)/P-glycoprotein (PGP). Recently identified auxin transporters from the intracellular spaces have been named as PIN-LIKES (PILS) (Barbez et al. 2012; Mohanta et al. 2015).

3. Auxin influx carrier (AUX/LAX)

The cellular movement of auxin is facilitated by the combined activities of the auxin influx and efflux carriers. The existence of auxin influx carrier came from the reports of Rubery and Sheldrake in 1974 (Rubery and Sheldrake 1974) where saturable auxin uptake in crown gall suspension cells of Parthenocissus tricuspidata was observed. Further, evidence about the presence of auxin transporters came when uptake of Indole-3-acetic acid (IAA) by sealed zucchini membrane vesicles was noticed and this was seen as an active process driven by the proton motive forces with the help of an auxin influx carrier, which was expected to be a proton symporter (Lomax et al. 1985). This hypothesis was later supported by Sabater and Rubery (1987). It has been seen that AUX/LAX auxin transporter homologs are present throughout the plant kingdom and they may have evolved before the evolution of the land plants as AUX/LAX-like sequences were also reported in several unicellular and colony forming Chlorophyta as well (De Smet et al. 2011; Swarup and Péret 2012).

3.1. Genomics, structure, polarity, and localization of AUX/LAX

The AUX1 gene belongs to a small gene family and consists of four members in Arabidopsis thaliana, which includes AUX1 and like-AUX1 (LAX) (LAX1, LAX2, and LAX3). These are plant-specific proteins within the amino acid/auxin permease super-family (Young et al. 1999; Peret et al. 2012). The genome of Oryza sativa (rice) encodes for five AUX/LAX transporters in comparison to three AUX/LAX transporters in A. thaliana (Chai and Subudhi 2016). AUX/LAX proteins harbor membrane-spanning transmembrane domains and present in the plasma membrane as well as in sub-cellular compartments (Figure 1, Table 1, Fig. S1). The AUX/LAX proteins present in the plasma membrane have nine (OsLAX5) and eleven (OsLAX3) transmembrane helices, respectively while as others (AtAUX1, AtLAX1, AtLAX2, AtLAX3, OsLAX1, OsLAX2, and OsLAX4) harbor ten transmembrane helices. The N- and C-terminal domains are present in the extracellular spaces whereas the transmembrane helices are embedded in the phospholipid bilayer. The AtAUX1 contains 485 amino acids out of which around 219 amino acids reside within the transmembrane helices. The AUX/LAX proteins share a significant sequence similarity and also contain conserved motifs (Fig. S2). A few of the conserved motifs of AUX/LAX proteins are W-H-G-S-x-D-A-W-F-S-C-A-S-N-Q-V-A-Q-V-L-L-T-L-P-Y-S-F, Q-L-G-M-x-S-G-I, F-Y-G-x-L-G-S-W-T-A-Y-L-I-S-V-L-Y-x-E-Y-R, N-H-V-I-Q-W-F-E-V-L-D-G-I-L-G, G-L-x-F-N-C-T-F-L-L-F-G-x-V-I-Q-L-I-x-C-A-S-N-I-Y-Y-I-N-D, D-K-R-T-W-T-Y-I-F-G-A-C-C-A-T-T-V-F-I-P-S-H-N-Y-R-I-W-S-F-L-G-L-x-M-T-T-Y-T-A-W-Y, Y-F-T-G-A-T-N-I-L-Y-T-F-G-G-H-A-V-T-V-E-I-M-H-Y-A-M-W, T-L-T-x-P-S-A, Y-W-A-F-G-D-x-L-x-H-S-N-A-x2-L-L-P, R-D-x-A-V-I-V-L-M-L-I/V-H-Q-F-I-T-F-G-F-A-C-T-P-L-Y-F-V-W-E-K, and R-L-P-V/I-V-x-P-I-W-F-x-A-I-I-F-P-F-G-P.

Figure 1. Pictorial representation of auxin transporters in the cell. PIN proteins are localized to the plasma membrane in a polarized fashion, whereas, AUX/LAX and ABCB are present in the plasma membrane and also in the sub-cellular compartments. PILS proteins are confined to the endoplasmic reticulum.
The presence of conserved motifs in AUX/LAX signifies their conserved roles in auxin signaling events. The details regarding the functionalities of these conserved motifs are provided in the supplementary file (Table S1).

The localization of AUX1 is either non-polar or polar, which depends on the cell or the tissue types. For instance, it is present in the apical position in protophloem, whereas in the lateral root caps, it occupies the basal position. However, no such polarity patterns were seen in the columella cells (Bennett et al. 1996; Swarup et al. 2001). Besides this, AUX1 also plays an important role in maintaining the cell polarity in root hairs. The AUX1 is not only present in the plasma membrane, but also in the sub-cellular compartments such as endosomes and the Golgi complex (Figure 1) (Kleine-Vehn et al. 2006). The plasma membrane localization of AUX1 requires endoplasmic reticulum (ER) chaperon, AUX_RESISTANT 4 (AXR4) (Dharmasiri et al.2006). Auxin transport inhibitor disrupts the polar distribution of AUX1. Brefeldin A, a fungal toxin is the classic example of auxin response factor (ARF) inhibitor that inhibits the internal trafficking of AUX1 and leads to the disappearance of PIN proteins from the plasma membrane (Shevell et al. 1994; Kleine-Vehn et al. 2006). However, this process is fully reversible and it leads to constitutive cycling of PIN proteins between plasma membrane and endosomes (Geldner and Palme 2001).

### 3.2. Regulation and function of AUX/LAX

A few studies have demonstrated that organ-level signals are required for the regulation of AUX1-mediated auxin transport. For instance, Li et al. (2011) reported that shoots of *A. thaliana* that are supplied with ammonium cation inhibit the initiation of lateral roots (Li et al. 2011). This resulted due to the fact that AUX1 is required for the formation of lateral root, and the shoot supplied with ammonium suppressed the expression of AUX1 gene in the vascular tissue (Li et al. 2011). In contrast, application of *A. thaliana* shoots with iron triggers the initiation of lateral roots by inducing the expression

| Protein | Protein domain             | Sub-cellular localization                      |
|---------|----------------------------|-----------------------------------------------|
| AUX/LAX | AtAUX/LAX                  | Integral membrane protein Plasma membrane     |
|         | AtLAX1                     | Integral membrane protein Plasma membrane     |
|         | AtLAX2                     | Integral membrane protein Plasma membrane     |
|         | AtLAX3                     | Integral membrane protein Plasma membrane     |
| PIN     | AtPIN1                     | Integral membrane protein Plasma membrane     |
|         | AtPIN2                     | Integral membrane protein Plasma membrane     |
|         | AtPIN3                     | Integral membrane protein Plasma membrane     |
|         | AtPIN4                     | Integral membrane protein Plasma membrane     |
|         | AtPIN5                     | Integral membrane protein Plasma membrane     |
|         | AtPIN6                     | Integral membrane protein Plasma membrane     |
|         | AtPIN7                     | Integral membrane protein Plasma membrane     |
|         | AtPIN8                     | Integral membrane protein Plasma membrane     |
| ABCB    | AtABCB1                    | Integral membrane protein Plasma membrane     |
|         | AtABCB2                    | Integral membrane protein Mitochondrial       |
|         | AtABCB3                    | Integral membrane protein Plasma membrane     |
|         | AtABCB4                    | Integral membrane protein Plasma membrane     |
|         | AtABCB5                    | Integral membrane protein Plasma membrane     |
|         | AtABCB6                    | Integral membrane protein Plasma membrane     |
|         | AtABCB7                    | Integral membrane protein Plasma membrane     |
|         | AtABCB8                    | Integral membrane protein Plasma membrane     |
|         | AtABCB10                   | Integral membrane protein Plasma membrane     |
|         | AtABCB11                   | Integral membrane protein Plasma membrane     |
|         | AtABCB12                   | Integral membrane protein Plasma membrane     |
|         | AtABCB13                   | Integral membrane protein Plasma membrane     |
|         | AtABCB14                   | Integral membrane protein Plasma membrane     |
|         | AtABCB15                   | Integral membrane protein Plasma membrane     |
|         | AtABCB16                   | Integral membrane protein Plasma membrane     |
|         | AtABCB17                   | Integral membrane protein Plasma membrane     |
|         | AtABCB18                   | Integral membrane protein Plasma membrane     |
|         | AtABCB19                   | Integral membrane protein Plasma membrane     |
|         | AtABCB20                   | Integral membrane protein Plasma membrane     |
|         | AtABCB21                   | Integral membrane protein Plasma membrane     |
|         | AtABCB22                   | Integral membrane protein Plasma membrane     |
| PILS    | AtPILS1                    | Integral membrane protein Endoplasmic reticulum |
|         | AtPILS2                    | Integral membrane protein Endoplasmic reticulum |
|         | AtPILS3                    | Integral membrane protein Endoplasmic reticulum |
|         | AtPILS4                    | Integral membrane protein Endoplasmic reticulum |
|         | AtPILS5                    | Integral membrane protein Endoplasmic reticulum |
|         | AtPILS6                    | Integral membrane protein Endoplasmic reticulum |
|         | AtPILS7                    | Integral membrane protein Endoplasmic reticulum |
| OsPILS  | OsPILS1                    | Integral membrane protein Endoplasmic reticulum |
|         | OsPILS2                    | Integral membrane protein Endoplasmic reticulum |
|         | OsPILS5                    | Integral membrane protein Endoplasmic reticulum |
|         | OsPILS6                    | Integral membrane protein Endoplasmic reticulum |
|         | OsPIL5a                    | Integral membrane protein Endoplasmic reticulum |
|         | OsPIL5b                    | Integral membrane protein Endoplasmic reticulum |
|         | OsPIL57a                   | Integral membrane protein Endoplasmic reticulum |
|         | OsPIL57b                   | Integral membrane protein Plasma membrane & Endoplasmic reticulum |

I-N-S-x-V-G-x-L-V-F-T-V-Y-I-I-P-x-L-A-H (Fig. S2).
of AUX1 gene (Giehl et al. 2012). AUX1 is required for long-distance auxin transport, from the shoot tip in a basipetal fashion towards the roots, through vascular bundle whereas LAX is involved in the maintenance of the local auxin gradients (Swarup et al. 2002; Swarup et al. 2004; Swarup et al. 2008).

The AUX/LAX encodes a putative auxin carrier domain and a mutation in these proteins manifests in auxin-related developmental defects (Table 2) (Bennett et al. 1996; Swarup et al. 2004; Swarup et al. 2008). Characterization of aux1 mutant revealed that they are sensitive towards the application of different auxins, however root gravitropic defect of aux1 can be rescued only by the application of 1-naphthylacetic acid (1-NAA) (Yamamoto and Yamamoto 1998; Utsuno et al. 1998; Benková et al. 2003). It has been seen that aux1 mutants of Arabidopsis thaliana produced 50% fewer lateral roots than the control (Hobbie and Estelle 1995). It is interesting to note that although AUX1 is very crucial for the development of root hair, its expression is only seen in the neighboring cells but not in the root hairs (Jones et al. 2001; Porco et al. 2016; Yu et al. 2016).

Table 2. Auxin transporter genes and their functions.

| Genes   | Functional role                                                                 | References                                                                 |
|---------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| AUX1    | Cell elongation, gametophyte development, embryogenesis, embryonic root cell organization | (Ugaritecha-Chirino et al. 2010; Panoli et al. 2015; Robert et al. 2015; Street et al. 2016) |
| ATLX1   | Vascular pattern, xylem differentiation, gametophyte development, phylotaxis    | (Bainbridge et al. 2008; Fabregas et al. 2015; Panoli et al. 2015)          |
| ATLX2   | Serration in leaf margin, vascular pattern in cotyledons, phylotaxis           | (Bainbridge et al. 2008; Peret et al. 2012; Kasprzewska et al. 2015)        |
| ATLX3   | Lateral root emergence, hook formation of hypocotyle, auxin homeostasis, phylotaxis | (Bainbridge et al. 2008; Swarup et al. 2008; Mellor et al. 2015; Porco et al. 2016; Yu et al. 2016) |
| PttLAX1-3| Development of vascular cambium                                               | (Schrader et al. 2003)                                                    |
| PsLAX   | Root gravitropism                                                             | (Hoyerová et al. 2008)                                                    |
| MtLAX1-S| Early nodule formation                                                        | (de Billy et al. 2001; Schnabel and Frugoli 2004)                         |
| MtLAX3  | Plant growth, development, root and nodule development                        | (Revalská et al. 2015)                                                    |
| CsAUX1  | Root gravitropism                                                             | (Kamada et al. 2003)                                                     |
| LcAUX1  | Development of etiolated hypocotyl                                             | (Oliveros-Valenzuela et al. 2007)                                        |
| CgLAX1  | Nodule formation                                                              | (Péret et al. 2007)                                                       |
| CgLAX3  | Nodule formation                                                              | (Péret et al. 2007)                                                       |
| OsAUX1  | Primary root and root hair elongation in Cd stress, lateral root initiation   | (Yu et al., 2015, Zhao et al., 2015)                                       |
| ZmAUX1  | Root development                                                              | (Hochholdinger et al. 2000)                                               |
| PINs    | Embryogenesis, phylloxy, vein formation, development of lateral organ, & vascular development | (Müller et al. 1998; Benková et al. 2003; Reinhardt et al. 2003; Weijers et al. 2005; Scarpella et al. 2006) |
| APIN2   | Root gravitropism, organogenesis                                              | (Chen et al. 1998; Luschnig et al. 1998; Müller et al. 1998; Utsuno et al. 1998; Benková et al. 2003) |
| APIN3   | Phototropism, gravitropism, and organ development                            | (Friml, Wfiniewska, et al. 2002b, Benková et al. 2003)                    |
| APIN4   | Embryogenesis, root patterning                                                | (Friml et al. 2003; Weijers et al. 2005; Dhonaskhe et al. 2007)            |
| APIN5   | Intracellular auxin homeostasis                                               | (Mravec et al. 2009)                                                      |
| APIN6   | Auxin transport activity                                                      | (Benková et al. 2003; Petrášek et al. 2006)                                |
| APIN7   | Root development, embryogenesis                                               | (Benková et al. 2003; Friml et al. 2003)                                   |
| MPIN1   | Inhibition of primary root, increased lateral root, enhanced phototropism and geotropism | (An et al. 2016)                                                          |
| NPIN4   | Auxillary bud growth                                                          | (Xie et al. 2017)                                                         |
| NsPIN1  | Adventitious root emergence & tillering, shorter plant height                | (Ku et al. 2005; Chen et al. 2012)                                         |
| ABCBs   |                                                                      |                                                                          |
| AAABC1, AtABC19       | Dwarfism                                                                     | (Noth et al. 2001)                                                        |
| AAABC4  | Root hair development                                                         | (Cho et al. 2007)                                                         |
| AAABC14 | Vascular development                                                         | (Kaneda et al. 2011)                                                      |
| AAABC19 | Root gravitropism, post embryonic organ separation                           | (Zhao et al. 2013; Cho et al. 2014)                                       |
| OsABC14 | Iron homeostasis                                                             | (Xu et al. 2014)                                                          |
| PILS    |                                                                      |                                                                          |
| AtPL5S3 | Ectopic expression line shows dwarf and or bushy plant, defect in flower development, sterility in T1 generation, homeotic transformation of flower organ to flower buds, triplication of gynoecium, unfused carpel, enhanced hypocotyls growth, higher lateral root | (Barbez et al. 2012)                                                      |
| AtPL5S2 | Lateral root development                                                     | (Barbez et al. 2012)                                                      |
| AtPL5S3 | Ectopic expression line shows dwarf and or bushy plant, defect in flower development, sterility in T1 generation, homeotic transformation of flower organ to flower buds, triplication of gynoecium, unfused carpel. Reduced root hair length | (Barbez et al. 2012)                                                      |
| AtPL5S  | Reduced hypocotyls growth, gravitropism, hyposensitive root growth           | (Barbez et al. 2012)                                                      |
Although, the expression of AUX1 was not seen in the root hair cells, still aux1 mutant had relatively short root hairs. This abnormal phenotype of aux1 mutant later restores to the wild type upon application of exogenous auxin. Therefore, it clearly demonstrates its role in root hair development (Jones et al. 2009). In the mutant of werewolf/myb23-that lacked non-hair cells, expression of AUX1 was not detected in the epidermis. These mutants had shorter root hairs that could be restored to the wild-type by auxin treatment. Therefore, it is clear that the non-hair cells, have a direct impact on the development of root hairs and also affect the auxin concentration in root hair cells (Jones et al. 2009). In a simulation study it was found that, expression of AUX1 in non-hair cells increases the auxin concentration by more than ten times compared to the adjacent hair cells, suggesting the role of AUX1 in regulation of high auxin balance between non-hair and hair cells to facilitates the growth and development of roots (Jones et al. 2009; Swarup and Péret 2012).

Except AUX1, no other members of the AUX/LAX family participate in the root gravitropic responses (Peret et al. 2012). LAX2 and LAX3 are only expressed in columella cells. The lax2 and lax3 single mutant do not exhibit any root gravitropic defects and lax2 aux1 double mutant does not show any defects that are sever than aux1 single mutants (Peret et al. 2012). However, LAX3 plays a significant role in the regulation of lateral root development (Swarup et al. 2008). The lax3 mutant has a reduced number of lateral roots. Swarup et al. (2008) reported that, LAX3 is expressed in the epidermis and cortical cells, most specifically, in front of the lateral root primordia (Table 2) (Swarup et al. 2008). Earlier Benková et al. (2003) reported that auxin maxima is located in the lateral root primordia and hence it is possible that LAX3 expression can have a significant impact in formation of auxin maxima (Benková et al. 2003). Besides this, several cell wall remodeling genes are also co-expressed with LAX3 (Swarup et al. 2008). Auxin from the lateral root primordia enters the cortical cells and induces the expression of the LAX3 gene. LAX3 protein in the plasma membrane facilitates auxin uptake and reinforces its expression within the same cell. Consequently, a higher level of auxin accumulates in the cortical cells, which ultimately induces the expression of cell wall remodeling enzymes. Proteins of these family members are targeted towards the plasma membrane, however, LAX2 and LAX3 failed in getting localized to the plasma membrane that express AUX1 in tissues (Peret et al. 2012). This suggests that there are some molecular factors present in the auxin influx carrier or there might be some cell tissue-specific regulators that are operational during the intracellular trafficking of different members of the AUX/LAX family. Peret et al. (2012) suggested that there might be specific molecular chaperones that are required for the regulation and trafficking of specific AUX/LAX proteins (Dharmasiri et al. 2006; Peret et al. 2012).

4. Auxin efflux carrier (PIN)

4.1. Genomics, structure, polarity, and localization of PINs

The auxin efflux carrier (PIN) is an important protein that coordinates and channels auxin transport. Upon influx of auxin by AUX/LAX, it is highly important to efflux it out so as it reaches the next cell and this is where the role of the auxin efflux carrier becomes important. The auxin efflux carrier is found in almost all land plants, including bryophytes, Lycopodiopsidae, monocot and eudicots (Křeček et al. 2009; Mohanta and Mohanta 2013; Singh et al. 2015). In A. thaliana, there are eight members of the PIN gene family that divided into four sub-groups while the crop plant O. sativa encodes 12 PIN genes in its genome (Křeček et al. 2009; Wang et al. 2009). The A. thaliana PIN sub-groups are PIN1 and 2; PIN3, 4 and 7; PIN6; and PIN5 and 8. In O. sativa the PIN genes are named as OsPIN1a-d, OsPIN2, OsPIN5a-c, OsPIN8, OsPIN9 and OsPIN10a-b (Wang et al. 2009). In terms of evolutionary plant lineage, PIN genes are grouped into seven groups (Křeček et al. 2009). Their diverse molecular phylogeny reflects its functional diversification within the PIN sub-groups during the evolution. The length of the PIN protein sequences ranges from 351 to 647 amino acid residues. The exon-intron position of A. thaliana PINs shows AtPIN1, AtPIN4 and AtPIN7 are close to each other. Unlike AtPIN1, AtPIN1 shares much closer relation with AtPIN3, AtPIN4, and AtPIN7. Although, genetic architecture of the AtPIN1 gene correlates more with the AtPIN3, AtPIN4, and AtPIN7 than with AtPIN2, but still, it is sub-grouped with AtPIN2 at the protein level. This suggests that the structural conservation at protein level is more important than the sequence conservation at the genomic level. The homology for A. thaliana PIN genes has been identified in most of the land plants and the numbers of PIN genes vary greatly among different species.

PIN proteins are membrane bound and have five to ten membrane-spanning transmembrane helices. The number of transmembrane helices present in different PIN proteins are as follows; five in OsPIN8 and OsPIN10a, seven in OsPIN5a, OsPIN5b and OsPIN5c, eight in AtPIN8, nine in AtPIN1, AtPIN2, AtPIN3, AtPIN5, AtPIN6, AtPIN7, OsPIN1c, and OsPIN2 and ten in AtPIN4, OsPIN1a, OsPIN1b, OsPIN9 and OsPIN10b. The N- and C-terminal domains of the PIN protein are connected by a central hydrophilic loop. Depending upon the length of this hydrophilic loop, PIN proteins are classified as a short or long-domain PIN protein. The PIN protein whose hydrophilic loop contains more than 50 amino acids is refereed as a long domain PIN protein, whereas those having less than 50 amino acid residues are refereed as a short domain PIN proteins. PIN1-4, PIN6 and PIN7 and their close orthologs have a long hydrophilic loop whereas PIN5, PIN8 and their close orthologs have a short hydrophilic loop. Multiple sequence alignment shows the presence of several conserved motifs in the N-and C-terminal region as well as in the dynamic hydrophilic region. The PINs possess conserved P-L-Y-x-A, D-Q-C-S-G-I-N-R, A-V-P-x-L-x-F, A-A-D-x-L-x-K, L-D-x1-x-T-x-F-x-S-x-L-P-N-T, V-M-G-I-P-I-L-x-M-x-Y, L-M-x-Q-x-V-x-W-L-Q, I/V-W-Y-x1-x-F-L-x-E, Q-x-F-P, V-D-x-D-x-V-x-S-L, P-x-R-x-S-N-L-x-E-I-Y-S and T-P-R motifs in the N-terminal end (Fig. S3). In C-terminal domain they possess conserved P-V-x-D, P-x-S-V-M-x-R-L-I-L, V-x-R-K-L-x-x-N-P-N-T-Y-x-S-L-x-G, M-P-x-x-I-x-S, L-G-M-x-M-F-S-x-G-x-F-x-A-x-Q, A-I-V-Q-A-A-L-P, F-V-F-x2-E-Y, L-S-T-x-x-I, and L-P-I-T-x-Y-I-x-L-G motifs (Fig. S3). The short PINs have conserved P-L-Y, E-Q-C-x2-V-I-N, and N/D-P-F/Y motifs at the N-terminal end and G-x2-W-A, G-x-G-x2-M-F, A-I-x-Q-A, A-L-P-Q, F-I/V-F-A-K-E-Y, and S-T-V-I motifs at the C-terminal end (Fig. S4). Previous studies have also reported the presence of conserved motifs in PIN proteins (Mohanta et al. 2014;
The long PINs are localized to the plasma membrane in a polarized fashion, whereas the short PINs are predominantly localized in the sub-cellular compartments like endoplasmic reticulum (ER) (Figure 1) (Křeček et al. 2009; Mravec et al. 2009; Ganguly et al. 2010; Bosco et al. 2012; Ding et al. 2012). The long PINs are localized asymmetrically in the plasma membrane for directional flow of auxin and to create auxin gradients (Figure 1). PIN5 protein is consistently localized to ER whereas PIN8 has a dynamic localization and can be found in ER as well as in the plasma membrane (Ganguly et al. 2010). This dynamic nature of the short hydrophilic loop involved in the localization of PIN8 to the plasma membrane varies with the cell type (Ganguly et al. 2010). Molecular cues in the short hydrophilic loop of PIN protein could be the possible reason for this. Similarly, the long hydrophilic loop might also have diverse molecular signatures for trafficking of PIN proteins to the plasma membrane, clathrin-mediated endocytosis, and also in various phosphorylation, and ubiquitylation events (Grunewald and Friml 2010; Kleine-Vehn et al. 2011; Ganguly et al. 2012; Leitner et al. 2012).

PIN1, PIN3, PIN4, and PIN7 are localized to the basal side of the cell facilitating basipetal auxin flow towards the root cells (Friml, Benková, et al. 2002a; Friml, Wiśniewska, et al. 2002b; Bílou et al. 2005). Localization of PIN1 in the plasma membrane of the leaf primordia which arise from the shoot apical meristem establishes auxin maxima for the inception of developmental events in leaf (Reinhardt et al. 2003). Apical localization of PIN2 in root epidermal cells promotes auxin transport from the root tip acropetally towards the upper end (Luschnig et al. 1998; Muller 1998). Basal localization of PIN2 in the root cortex along with the lateral localization of PIN3 in the pericycle directs auxin flow back to the root meristem (Bílou et al. 2005). In the root columella, PIN3 and PIN7 are redistributed due to the gravity vector which causes gravitropic bending of roots in the plants (Friml, Wiśniewska, et al. 2002b; Kleine-Vehn et al. 2010). During phototropism, the activity of PIN3 decreases on the illuminated side of the hypocotyl to facilitate auxin transport to the non-illuminating side (Ding et al. 2011).

4.2. Regulation and function of PINs

The study of tissue-specific expression of O. sativa PIN genes revealed that OsPIN genes were constitutively expressed in stems, leaves and young panicles (Wang et al. 2009). OsPIN1a and OsPIN1b were highly expressed in the aforementioned tissues, while low expression of OsPIN1c was seen in young panicles and leaves. The OsPIN2 has weak expression in stem, leaves, and young panicles whereas expression of OsPIN5a was higher than OsPIN5b in young panicles (Wang et al. 2009). Higher expression of OsPIN9 was seen in the base of the stem and root than other tissues. OsPIN10a was highly expressed in all tissues except the roots whereas, OsPIN10b was relatively highly expressed in the leaves (Wang et al. 2009). A GUS driven assay revealed, OsPIN1a was expressed in root cap whereas, OsPIN1b, OsPIN1c and OsPIN9 were predominantly expressed in the stelar. OsPIN1b, OsPIN1c, OsPlN5a and OsPIN5b were detected in the meristems. OsPIN1c exclusively expressed in the root primordia (Wang et al. 2009). OsPIN1a, OsPIN1c and OsPIN10b were expressed in flower veins of hull and anthers whereas, OsPIN1a had high expression in root primordia and the vascular tissue (Wang et al. 2009). The expression of OsPIN genes was also modulated in O. sativa in the presence of exogenous auxin and cytokinin (Singh et al. 2015). The expression pattern of OsPIN5c is very negligible in auxin and cytokinin treated root tissues. Significant transcript accumulation of OsPIN1b, OsPIN2 and OsPIN9 occurs in auxin and cytokinin treated O. sativa. In 7 days old seedlings treated with auxin, the expression of OsPIN2 is up-regulated by four folds. OsPIN1b and OsPIN9 were significantly up-regulated in 7 days rice seedling upon treatment with cytokinin while auxin treatment up-regulates their expression at 14 and 21 days time period (Singh et al. 2015). Firml et al. (2003) reported the expression of PIN genes during the embryogenesis of A. thaliana (Friml et al. 2003). AtPIN1 and AtPIN3 expressed in apical and columella cells, respectively (Friml et al. 2003). The localization of AtPIN4 protein was detected in the hypophysis and provascular initials of root meristem. The expression pattern of AtPIN7 resembled to that of AtPIN1 (Friml et al. 2003). To understand the correlation between the AtPIN1 and AtPIN7 during early embryogenesis, detection of AtPIN1 protein was seen from one to sixteen-cell stage, in all newly formed cell boundaries. No polarity patterns were seen at this stage, but polarity of AtPIN1 was detected at thirty two cell stage. It was localized to provascular cells facing towards the basal embryo pole. AtPIN7 later shifted to the quiescent center cells on the basal side. AtPIN7 was present in the apical and basal cells during the post-zygotic division in the endomembrane (Friml et al. 2003). AtPIN7 was found in the apical position of the suspensor cell at thirty-two cell stage thus making it as a polarity marker and later its position shifted to the basal side of the suspensor cell post 32-cell stage, followed by its appearance in the boundary wall. This concluded that the accumulation of AtPIN1 in the proembryo cells and shifting/ reversal of AtPIN7 polarity are directly correlated with the apical-to-basal reversal of auxin gradient (Jiri Friml et al. 2003). Besides this, analysis of pin7 mutant revealed its participation in auxin distribution and the embryos with pin7 mutation failed to establish the apical-basal auxin gradient (Jiri Friml et al. 2003). Mutational analysis revealed that PIN1 and PIN4 are involved in organogenesis; PIN2 and PIN3 participate in root gravitropism; PIN1 and PIN3 actively participate in phototropism and PIN1, PIN3, PIN4 and PIN7 are involved in embryogenesis (Table 2) (Paponov et al. 2005). A. thaliana pin1 mutant (PIN-FORMED) lacks an organ in the inflorescence which results in its deformed structure (Okada et al. 1991). Similarly, loss of PIN2 leads to defects in root gravitropism and growth of root hairs, suggesting their crucial role in diverse developmental processes (Chen et al. 1998; Luschnig et al. 1998; Muller 1998; Utsuno et al. 1998).

5. ATP-binding cassette transporters (ABC B)

5.1. Genomics, structure, polarity, and localization of ABC Bs

The POLYGLYCOPROTEIN (PGP) / MULTIDRUG RESISTANCE (MDR)/ATP-binding cassette transporters of B class (ABC B) proteins belong to the super family of ABCB transporters and most of the plant ABCB proteins characterized have been found to be auxin transporters (Noh et al. 2001; Luschnig 2002; Terasaka et al. 2005; Geisler and Murphy 2006). There are twenty one known ABCB members out of which ABCB1, ABCB4 and ABCB19 are
involved in auxin transport (Table 2) (Noh et al. 2001; Multani et al. 2003; Geisler et al. 2005; Terasaka et al. 2005; Geisler and Murphy 2006; Cho et al. 2007). However, a recent report revealed that ABCB14 and ABCB15 also participate in polar auxin transport (Kaneda et al. 2011). Another study suggests that ABCB1, ABCB4 and ABCB19 have lower auxin exporting capacities compared to their counterpart PIN proteins (Cho and Cho 2013). The model plant A. thaliana has twenty one ABCB proteins whereas O. sativa has twenty-two, Sorghum bicolor has twenty-four and Zea mays has thirty-five (Chai and Subudhi 2016). All auxin-transporting PIN proteins have been found in the same clade during the phyllogenetic analysis, whereas ABCBS fall into three distinct clades (Cho and Cho 2013). The O. sativa OsABCBs contain proteins ranging from 524 (OsABCB17) to 1482 (OsABCB12) amino acids. The molecular weights of OsABCBS varies from 56 (OsABCB17) to 158 (OsABCB12) kDa and the isoelectric point ranges from 5.7 (OsABCB21) to 9.3 (OsABCB11) (Chai and Subudhi 2016). The diverse molecular weights and isoelectric points of ABCBs proteins allow them to participate in polar movement across the cell. Besides this, such diverse molecular properties of ABCBs might allow them to interact with cell polarity complex to complete their function. ABCB localize to the plasma membrane and are dynamically distributed there, this might be due to their dynamic molecular weights, and isoelectric points (Figure 1). Except OsABCB8 and OsABCB22, the majority of the OsABCBs are predicted to be localizing to the plasma membrane (Chai and Subudhi 2016). OsABCB12 and OsABCB17 localize to the chloroplast. The OsABCBs have 4-13 membrane-spanning transmembrane helices and based on their topological character of transmembrane domain, they are divided into two groups (Chai and Subudhi 2016). Most OsABCBs have two transmembrane helices at the N- and C-terminal ends and are linked by a central loop of variable length whereas OsABCB10, OsABCB16 and OsABCB17 have one cluster transmembrane helices (Chai and Subudhi 2016). The transmembrane helices of N- and C-termini are conserved and the loops are highly variable (Chai and Subudhi 2016). The transmembrane domain of the ABCB transporter has a nucleotide binding domain as well (Geisler and Murphy 2006). The ABCB19 is confined to the detergent-resistant membrane (DRM) region of the plasma membrane where glucosyl-ceramide and sitosterol are abundant (Titapiwatanakun et al. 2009). ABCB19 defines the membrane structure and provides a platform for the stable localization of PIN1 (Titapiwatanakun et al. 2009). Multiple sequence alignment shows the presence of conserved motifs in ABCB proteins. The most conserved motifs present at the N-terminal end of ABCB proteins were, A-x-V-G-x2-G-x-G-K-S, E-R-F-Y-D-P, V-x-Q-E-P-x-L, I-x-E-N, V-I/G-E-X-G-x2-L-S-G-G-Q-K-Q-R-I-x-L-A-R, P-x-I-L-L-L-D-E-A-T-S-A-L-D and G-Q-x-Q-R-I-A-x-A-R motifs are the characteristic conserved sequences of ABCB transporter family (Guillemette et al. 2004). These two motifs are the characteristic features of the ABC-ATPase protein and they collectively constitute the nucleotide binding motif. These conserved motifs might play a crucial role in transporting of auxin molecules across the plasma membrane. In animals, mutation in ABCB protein leads to several serious genetic diseases and over expression leads to multi-drug resistance in bacteria, viruses and cancer (Stolarczyk et al. 2011). The P-glycoprotein ABCB proteins contain a linker region having consensus phosphorylation site, suggesting its functional regulatory role (Davies and Coleman 2000). The ABC transporter gets their energy from breakdown of ATP and act like ATPases as well. Study led by Aryal et al. (2015) described that ABC transporters are regulated through protein phosphorylation event (Aryal et al. 2015). In addition to the role of auxin transport, ABC transporter protein AtABC25 involved is in abscisic acid transport as well (Kuromori et al. 2010).

5.2. Regulation and function of ABCBs

The ABC super-family consists of many universal transporters associated with the movement and transport of various small molecules, nutrients and xenobiotics. A comparative study between the bacterial and murine ABC transporter with the plant ABCB transporter revealed an exceptionally high degree of structural conservation. Although a remarkable structural conservation was found between them, the ABCBS exhibits limited substrate specificity in certain organisms but are promiscuous in others. In some cells, ABCB shows polarity while in others they do not (Geisler et al. 2005; Terasaka et al. 2005; Blakeslee et al. 2007; Cho et al. 2007; Wu et al. 2007; Mravec et al. 2008; Cho et al. 2012). Unlike PIN and AUX/LAX, the ABCB proteins serve as either facultative auxin influx/exflux carrier (ABCB21) or auxin carrier with fixed direction (ABCB1, ABCB4 and ABCB19) (Table 2) (Geisler and Murphy 2006; Cho et al. 2012; Kamimoto et al. 2012). The facultative or directional auxin flow by ABCBs depends on the cellular auxin level (Geisler and Murphy 2006; Cho et al. 2012; Kamimoto et al. 2012). ABCB is predominantly non-polar and could determine the amount of auxin concentration available for PIN mediated polar auxin transport (Mravec et al. 2008).

Although auxin transport is required for both short and long-distance delivery of auxin, the long distance transport from the shoot tip to the root is conducted by ABCB1 and ABCB19 along with PIN1 and PIN7 (Gälweiler 1998; Friml et al. 2003; Blakeslee et al. 2007). The polarity of ABCB localization might be associated with the secondary cell wall and early stages of cytokinesis rather than the cell plate formation (Blakeslee et al. 2007; Titapiwatanakun et al. 2009). The expressions of ABCB1 and PIN7 are predominantly restricted to the shoot apex and both are persistent in their act during auxin loading to the vascular stream (Zažimalová et al. 2010). The expression of ABCB19 and PIN1 occurs throughout the plant and they maintain the auxin flow from the shoot apex to the root apex (Zažimalová et al. 2010). Acropetal auxin flow from the base of the root to the root tip is directed by ABCB1 and PIN. Auxin gets distributed to the epidermal cells (basipetal flow) and lateral root cap by the action of ABCB1, ABCB4, ABCB19, AUX1, PIN1, PIN2, PIN3, and
PIN4. Along with other auxin transporters, ABCB1 and ABCB19 function in loading, while ABCB1, and ABCB4 along with PIN2 continue the auxin stream along the epidermal cells, cortical cells, and root cap to drive the cell division, cell elongation and root hair development (Gälweiler 1998; Muller 1998; Swarup et al. 2001; Friml, Benková, et al. 2002a; Geisler et al. 2005; Terasaka et al. 2005; Blakeslee et al. 2007; Mravec et al. 2008). Although ABCB19 and PIN1 are the principal mediators of polar auxin transport along the axis (Gälweiler 1998; Blakeslee et al. 2007), the retention of auxin in the stream of vascular transport is mediated by the combined activities of ABCB19 and PIN3 which localized to the bundle sheath cells (Friml et al. 2002b, Blakeslee et al. 2007). The ABCB19 that localizes in the endodermis and pericycle might have similar functions in the root (Blakeslee et al. 2007). The long-distance auxin flow, upwards from the root apex in the epidermal cells above the elongation zone is mediated by ABCB transporters (Geisler et al. 2005; Terasaka et al. 2005; Lewis et al. 2007; Titapiwatanakun et al. 2009; Zažimalová et al. 2010). ABCB4 regulates auxin homeostasis in root trichoblasts (Cho et al. 2007; Yang and Murphy 2009). The ABCB4 has import activity at low auxin concentrations. As the level of auxin increases, its function is reversed and changes to export activity (Yang and Murphy 2009). Hence the function of ABCB4 is to maintain auxin homeostasis when AUX1 is not present and it is confined to regions such as root hair and elongating cell zones where auxin levels are usually stable.

Besides auxin transport, ABCB proteins also participate in other diverse functions. OsABCB14 functions in both auxin transport and iron homeostasis (Xu et al. 2014). Other ABCBs are involved in calcium homeostasis, aluminium detoxification, stomatal response to CO₂ and secondary metabolite transport (Sasaki et al. 2002; Shitan et al. 2003; Lee et al. 2008). Several studies reported about the role of direct interaction between AtABCB and PIN towards the junction with auxin as well (Lee et al. 2008). The abcb mutants have a defective cell elongation and long-distance transport of auxin. However, only limited defect is present in embryo development and organogenesis (Mravec et al. 2008). The abcb mutants have a defective cell elongation and long-distance transport of auxin. However, only limited defect is present in embryo development and organogenesis (Noh et al. 2001; Geisler et al. 2003; Terasaka et al. 2005; Blakeslee et al. 2007). Lee et al. (2008) reported that ABCB14 is a malate transporter and competitive transport occurs in conjunction with auxin as well (Lee et al. 2008). The abcb mutants exhibit reduced malate-inhibitable auxin transport in the shoot (Zažimalová et al. 2010).

A protein known as TWISTED DWARF1 (TWD1) directly interacts with ABCB1 and ABCB19. The phenotype of twd1 loss-of-function mutant is similar to the abc1/abc19 mutants (Murphy et al. 2002; Geisler et al. 2003). This shows that, TWD1 is an activator of membrane localized ABCB complexes and alters conformational changes of ABCB proteins (Geisler et al. 2003; Bouchard et al. 2006; Bailly et al. 2008). The localization of ABCB1, ABCB4 and ABCB19 in the plasma membrane was severely compromised in twd1 mutant (Wu et al. 2010). TWD1 protein is localized to the plasma membrane and endoplasmic reticulum. This suggests that ER-localized TWD1 protein serve for trafficking of ABCB protein and plasma membrane bound TWD1 modulate the activity of ABCB protein. TWD1 protein directly binds to the PINOID (PID) and TWD1-PID interactions regulate ABCB1-mediated auxin transport (Henrichs et al. 2012). In the absence of PWD1, PID phosphorylates the serine residue at position 634 in the ABCB linker domain and increases ABCB1-mediated auxin signaling (Henrichs et al. 2012). When the serine amino acid at position 634 is replaced by alanine (A), it leads to a defect in phosphorylation and when substituted by glutamate (E), it results in mimicking the phosphorylation. The substitution by A leads in reduced auxin export activities whereas substitution by E enhances auxin export activities (Henrichs et al. 2012). It has been reported that, ABCB19 is the substrate of PHOT1, and PHOT1-dependent phosphorylation of ABCB19 inhibits auxin-efflux activity, which triggers production of higher auxin levels above the hypocotyl apex (Christie et al. 2011). Later these auxin is channeled into PIN3 to the shoot elongation zone (Christie et al. 2011). Furthermore, the endogenous plant flavonoids that emerged in the early land plants obstruct with the principal mechanism of ABCB transporters while PIN proteins are indirectly affected by flavonoids (Rausher 2006; Peer and Murphy 2007; Santelia et al. 2008). This suggests that plant flavonoids might have a selective force in the evolution of plasma membrane mediated auxin efflux. The mdr1 mutant exhibits epinasty of the cotyledons and the first true leaves (Noh et al. 2001). Besides this, it also produced waviness in the hypocotyls and roots (Noh et al. 2001). Defects in ABCB4 produces longer root hairs than the wild type, whereas abcb14 mutant produces slightly altered vascular development in the florescence stem (Noh et al. 2001; Cho et al. 2007). The abc1 abcb19 double mutant shows dwarfism in A. thaliana (Noh et al. 2001). The abc19 and abc1 abcb19 mutants display reduced root-directed auxin transport relative to pin1.

Recently, Chai et al. (2016) identified and analyzed the expression profiles of ABCB genes from O. sativa in response to phytohormone stimuli and abiotic stresses (Chai and Subudhi 2016). The authors reported twenty-two putative OsABCB genes from the rice genome. Most of them were regulated by drought, salt and hormonal stimuli (Chai and Subudhi 2016). Among them, four were exclusively expressed in the leaves, whereas only a few of them were up-regulated (OsABCB5, OsABCB12, OsABCB18, and OsABCB19) in certain tissues. Most of these auxin-regulated genes were down-regulated due to the drought and salinity stresses, whereas only a few of them were up-regulated (OsABCB5-7, OsABCB9-13, and OsABCB15-20) in certain tissues. OsABCB5, OsABCB12, OsABCB18, and OsABCB19 were up-regulated under drought and salinity stresses. In moderate drought conditions, the number of differentially expressed OsABCB genes in roots and leaves were seventeen and eighteen, respectively, whereas under severe drought stress, root expressed twenty-four OsABCB genes whereas leaves expressed only fifteen. Nevertheless, more genes were differentially expressed in roots than in leaves (fifteen vs. ten, respectively) (Chai and Subudhi 2016). The transcriptional regulation of OsABCB genes due to the drought and salinity stress indicates that they might be involved in the adaptation to abiotic stresses. Upon auxin treatment, twenty of the twenty-two OsABCB genes were expressed differently. Among them, four were exclusively expressed in the leaves, nine in the roots and ten in both leaves and roots (Chai and Subudhi 2016). Most of these auxin-regulated OsABCB genes were up-regulated and only four were down-regulated at various time points. All OsABCB genes were modulated
due to abscisic acid (ABA) treatment. The modulation of OsABCB genes by auxin and abscisic acid treatments reflect their possible role in a hormonal cross talk, which enables plant to adapt various stress conditions.

6. PIN-likes (PILS)

6.1. Genomics, structure, polarity, and localization of PILS

Recently, a new family of auxin transporters has been reported known as PIN-likes (PILS) protein family (Barbez et al. 2012; Feraru et al. 2012; Mohanta et al. 2015). The topologies of PILS proteins are highly similar to the PIN proteins; hence they were named as PIN-likes (PILS) protein. Unlike PIN proteins, PILS also contain Interpro auxin carrier domain (Barbez et al. 2012; Feraru et al. 2012; Mohanta et al. 2015).

*A. thaliana* and *O. sativa* contain seven PILS genes (Barbez et al. 2012; Mohanta et al. 2015). The PILS proteins in *A. thaliana* range in size from 390 (AtPILS3) to 472 (AtPILS1) amino acids (Feraru et al. 2012), whereas those in *O. sativa* range from 414 (OsPILS1) to 1280 (OsPILS5) amino acids (Mohanta et al. 2015). The molecular weight of OsPILS ranges from 44.076 (OsPILS1) to 140.721 (OsPILS5) kDa, whereas the isoelectric point lies between 6.91 (OsPILS5) to 8.38 (OsPILS1). The diverse range of molecular weight and isoelectric points of different PILS protein enables them to localize to different parts of the cell. AtPILS3, AtPILS4, and AtPILS5 genes contain nine introns whereas AtPILS1 contains eleven, AtPILS6 contains eight and AtPILS7 contains seven introns and AtPILS2 is intronless. Similarly, from seven OsPILS genes, four (OsPILS1, OsPILS6a, OsPILS6b, and OsPILS7a) of them have ten introns, while OsPILS5 has seven, OsPILS7b has nine and OsPILS2 has only one intron. The orthologs of the PILS2 gene either have one intron or it may be intronless. Phylogenetic analyses of OsPILS and AtPILS have revealed that the orthologs of *A. thaliana* AtPILS3 and AtPILS4 are absent in *O. sativa* (Mohanta et al. 2015).

Unlike PIN proteins, the PILS are also membrane bound proteins that are present inside as well as outside of the membrane of sub-cellular compartments (Figure 1, Table 2) (Mohanta et al. 2015). Except OsPILS5, all other PILS proteins contain ten transmembrane helices. In addition, OsPILS5 has only four transmembrane helices and the major part of the OsPILS5 is present outside of the membrane. The PILS proteins are characterized by the presence of two hydrophobic transmembrane domains at the N-and C-terminal end (Feraru et al. 2012). The hydrophobic regions are organized into five transmembrane helices which are flanked by a short hydrophilic loop directed towards the cytosol (Feraru et al. 2012). Amino acid sequence analysis reveals the presence of a conserved N-x-G-N motif at the N-terminal end (Mohanta et al. 2015). The C-terminal of OsPILS has conserved A-P-L and G-G-N-L-G-x-G consensus sequences (Mohanta et al. 2015). The central hydrophilic loop is very dynamic and devoid of any conserved consensus sequence. Instead, it contains a conserved threonine amino acid in the hydrophilic loop (Mohanta et al. 2015). However, PIN protein does not have any conserved motifs in the loop (Křeček et al. 2009). A comparative study between PIN and PILS protein, has indicated that they share only 10-18% sequence identity (Feraru et al. 2012). These authors classified the PILS proteins based on the presence of generic phosphorylation site (non-kinase specific, viz serine, threonine and tyrosine), and kinase-specific phosphorylation site. Further, they grouped them as, (1) with less than ten phosphorylation sites (PILS5 and PILS7), (2) carrying between ten and fifteen sites (PILS2, and PILS6), and (3) which has more than fifteen phosphorylation sites (PILS1, PILS3 and PILS4). The grouping of PILS based on the phosphorylation site indicates their functional diversification and shows that phosphorylation based mechanisms might play a crucial role in their functional diversity. Although the hydrophilic loop is variable in nature, but the presence of a conserved threonine residue in the hydrophilic loop is the most likely target phosphorylation site for upstream kinases (Mohanta et al. 2015). Except AtPILS4, all PILS proteins of *A. thaliana* localize to the membrane of sub-cellular compartment endoplasmic reticulum (Barbez et al. 2012). Studies with the N-and C-terminal fusion proteins of AtPILS4 revealed their absence from ER or the plasma membrane. Similar to the AtPILS, OsPILS proteins of *O. sativa* also localize to the sub-cellular compartments. The OsPILS, OsPILS5, OsPILS6b and OsPILS7a localize to the endoplasmic reticulum whereas OsPILS1 and OsPILS6a localizes to the vacuole and OsPILS7b localize to the plasma membrane and in endoplasmic reticulum (Table 2) (Mohanta et al. 2015).

6.2. Regulation and function of PILS

Barbez et al. (2012) reported that AtPILS genes are broadly expressed in various tissues, including seedlings, rosette leaves, stems, cauline leaves, flowers and siliquae (Barbez et al. 2012). Except AtPILS1, all of the AtPILS express in seedlings, whereas the expression of AtPILS1, and AtPILS5 was very low in rosette leaves. Lower expression levels were also detected in stems for AtPILS1, AtPILS2, AtPILS3 and AtPILS5. In cauline leaves, the expression of AtPIL1 was very less and in siliquae low expression was observed for AtPILS1, AtPILS5 and AtPILS7 (Barbez et al. 2012). Barbez et al. (2012) demonstrated that the expressions of AtPILS2-AtPILS7 were transcriptionally up-regulated by auxin treatment in wild-type seedlings (Barbez et al. 2012). More specifically, AtPILS2, AtPILS3 and AtPILS5 were highly up-regulated suggesting their role in auxin-mediated signaling process. Rice seedlings treated with 5 µM IAA shows differential expression of OsPILS genes. From the transcript analysis it was found that all OsPILS genes were up-regulated in leaf tissues, whereas same genes were down regulated in the 21 days old root tissues (Mohanta et al. 2015). This suggests that there is significant impact of OsPILS genes in plant development on 21 days (Mohanta et al. 2015). However, a significant variation in expressions of OsPILS genes was also observed in cytokinin treated leaf and root tissues (Mohanta et al. 2015).

The phytohormone mediated differential expression of AtPILS led Barbez et al. (2012) to deeply investigate their functional role in auxin signaling, by expressing AtPILS1 and AtPILS3 using viral 35S constitutive promoter (Barbez et al. 2012). The ectopic expression of AtPILS1 or AtPILS3 produces dwarf and/or bushy plants with severe deformity in flower development (Table 2) (Barbez et al. 2012). The flowers displayed severe patterning defects with homeotic transformation of flower organs into new flower buds, unfused carpels and triplication of the gynoecium. The T1 generation of the over expressed AtPILS1 and AtPIL3 were
sterile. However, the over expressed p35S::AtPIL5 lines produced moderately fertile flowers. AtPILS2 and AtPILS5 were abundantly expressed at the seedling stages; therefore mutant analysis was performed on these genes. They found enhanced hypocotyl growth in pils2 pils5 double mutants. However, dark grown p35S::PILS5::GFP expressing plants had reduced hypocotyls. The PILS2 and PILS5 exhibits an overlapping expression in the root transition zone and PILS5 gain of function experiments manifest in agravitropic growth of hypocotyls (Barbez et al. 2012). The pils2 single mutant and the pils2 pils5 double mutant makes longer roots than the wild-type seedlings, whereas over expression of AtPILSS5 results in relatively short roots (Barbez et al. 2012). The pils2, and pils5 single mutant and the pils2 pils5 double mutant display a higher lateral root density, whereas the PILS5 gain-of-function mutant had reduced lateral roots. To understand the role of the auxin response element (AuxRE) DR5 in the pils mutant, pils2-2 and pils5-2 knockout mutants were expressed with pDR5rev::GFP (Barbez et al. 2012). Such plants display higher signal in lateral roots, but no alteration in the DR5 activity was seen in the main root tip. However, moderately expressing p35S::PILS5::GFP seedlings exhibit reduced auxin responses in the root tip and lateral root. It was observed that pils2 pils5 loss-of-function mutant show hypersensitive root growth whereas PILS5 gain-of-function mutant produces hyposensitive root growth (Barbez et al. 2012). The pils2 pils5 double mutant had relatively higher auxin export activity which revealed a reduced auxin holding capacity in pils2 pils5 loss-of-function mutants. In BY cell line study it was reported that the ratios of auxin-glutamate (Glu) to auxin-aspartate (Asp) conjugate shifted towards the free auxin in pils2 and pils5 loss-of-function mutants. The p35S::PILSS5::GFP expressing line shows reduced auxin response in the root tip. A significant shift was found in the auxin-Glu and auxin-Asp to free auxin ratios in p35S::PILS5::GFP seedlings which indicates higher rate of auxin conjugation and hence conjugation-based auxin metabolism (Barbez et al. 2012). Study led by Béziat et al. (2017) reported the role of PILS protein in light mediated reduction in nuclear auxin signaling for transition of plant growth (Béziat et al. 2017). PILS2 and PILS5 lead to asymmetric gene expression during apical hook formation (Béziat et al. 2017). pils2 single mutant show delayed onset of apical hook development whereas pils1 pils2 double mutant show strong delay in apical hook formation (Béziat et al. 2017). However, pils1 pils2 pils3 triple mutant do not show apical hook opening that might be due to functional divergence of distinct PILS genes (Béziat et al. 2017). In addition, PILS proteins also regulate the reduction of nuclear auxin signaling during apical hook formation (Béziat et al. 2017).

7. Conclusion and future perspective

The role of auxin signaling is of pivotal importance in plant growth, development, tropism and gravitropic responses. However, it has not been elucidated how PINs and ABCB proteins localize in the plasma membrane in a polar and non-polar fashion, respectively. Also localization of PILS to the endo-membrane is not clear. Understanding the molecular mechanisms that govern their polarity patterns and also their non-polar distribution is important. It is also important to study the role of various conserved motifs in different auxin transporter proteins to find out the possible auxin binding domains. It is also necessary to find the signaling sequences of AUX/LAX, PIN, ABCB and PILS proteins. The role of important signal sequences, including palmitoylation and myristoylation event cannot be ruled out for the localization of auxin transporters in the plasma membrane. Because palmitoylation and myristoylation events play a crucial role in membrane attachment and protein trafficking.

Acknowledgement

The authors would like to extend their sincere appreciation to the deanship of Scientific Research at King Saud University for its funding of research group NO (RGP-271).

Disclosure statement

No potential conflict of interest was reported by the authors.

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