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REVIEW ARTICLE

Protein phosphatases: a genomic outlook to understand their function in plants

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Abstract Protein phosphatases are the vital regulatory components of various signal transduction pathways in eukaryotes. Signaling pathways triggered during stress and development have been regulated by different classes of protein phosphatases in plants. Recently, genome-wide expressional analysis in *Arabidopsis* and crop plant such as rice revealed differential expression pattern for several protein phosphatases under different abiotic stresses, in various tissues and at different developmental stages. This expression pattern could be extrapolated to the possible function of protein phosphatases in abiotic stress signaling and tolerance, and during plant development. Here, we discuss organisation and expression patterns of members of the protein phosphatase gene family, and their potential functional role in plants.

Keywords Protein phosphatase · Expression · Abiotic stress · Development · Signal transduction

Introduction

Reversible protein phosphorylation is one of the major events in various signal transduction networks and regulates a number of biological processes in eukaryotes. The balance in the phospho-regulation is critical to maintain a normal cell survival state. Protein kinases and phosphatases are the key players, which maintain the phospho-regulation in normal conditions and modulate this balance in adverse conditions as per the requirement of the cell. The protein kinases have been investigated extensively in terms of their structure and evolution in a number of eukaryotes (Manning et al. 2002a, b; Caenepeel et al. 2004; Champion et al. 2004; Kerk et al. 2008) and most of the kinases share a highly conserved functional domain. In contrast, protein phosphatases display a great degree of diversity and harbour different catalytically important signature motifs and domains (Luan 2003; Moorhead et al. 2007; Lee et al. 2010). Based on the amino acid residue they dephosphorylate, protein phosphatases have been classified into two major categories namely serine/threonine phosphatases and tyrosine phosphatases. Cloning, sequence analysis, biochemical and genomic analysis revealed that ser/thr phosphatases can be divided into two major families: phosphoprotein phosphatases (PPP), which includes PP1, PP2A, PP2B phosphatases and other distantly related phosphatases (PP4, PP5, PP6 and PP7) with unique domains of unknown function whereas PPM includes PP2C group of phosphatases and other Mg^{2+} dependent phosphatases (Luan 2003; Schweighofer et al. 2004; Moorhead et al. 2007; Lee et al. 2010; Singh et al. 2010). However PP2B, which is a Ca^{2+} dependent phosphatase and is also known as calcineurin A (CNA), has not been identified so far in plants (Moorhead et al. 2007; Kerk et al. 2008; Singh et al. 2010). Similarly, the presence of a signature motif CX_5R characterised protein tyrosine phosphatases (PTPs), which are also composed of two groups namely-tyrosine specific phosphatases (PTP), which specifically act on phosphotyrosine and dual specificity phosphatases (DSPs), which can dephosphorylate phosphotyrosine as well as phosphoserine/phosphothreonine (Stone and Dixon 1994; Tonks and Neel 1996).

In the post-genomic era, availability of complete sequence of a number of plant genomes, shared databases and softwares and analysis tools have made it feasible to carry out genome-wide analysis to decipher the genomic and functional diversity among various gene families, as demonstrated by the identification of many gene families in plants (Kerk et al. 2002; Jain et al. 2007; Agarwal et al.

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2007; Arora et al. 2007; Li et al. 2007; Xue et al. 2008; Singh et al. 2010, 2012). Whole complements of protein phosphatase encoding genes of important plant species such as model eudicot plant *Arabidopsis* and monocot crop plant rice (Kerk et al. 2002; Singh et al. 2010) have been identified and their structural and expression analyses have been carried out.

Here, we provide an overview of the genomic organisation of protein phosphatase gene family in plants, their phylogenetic and evolutionary relationship, expression analysis and discuss their role in important processes such as abiotic stress signaling and plant development.

Protein phosphatase gene family in plants

Examination of the *Arabidopsis* genome has revealed the existence of 126 protein phosphatases belonging to the major classes of protein phosphatase i.e. 26 members of PPP family, 76 of PPM family, which comprised of PP2C class of phosphatases and 24 members of protein tyrosine phosphatases including a single PTP, 22 DSPs and a single gene of low molecular weight PTP (LMWPTP) (Kerk et al. 2008). However, a later study of protein phosphatases claimed 80 PP2C genes in the *Arabidopsis* genome (Xue et al. 2008). We carried out a genome wide survey of protein phosphatases in rice genome and found 132 protein phosphatase encoding genes, which like *Arabidopsis* could be distributed into different categories (Singh et al. 2010). As in other plants, PP2C is the major class of protein phosphatase in rice and includes 90 members and is subdivided into 11 subfamilies, followed by DSP and PP2A, which are comprised of 23 and 17 members, respectively. Further, similar to *Arabidopsis*, PTP and LMWP class contain one member each. As mentioned earlier, PP2B phosphatase was not identified in any of these studies. It is surprising that *Arabidopsis* and rice genome contain much higher number of protein kinases than the humans (Dardick et al. 2007; de la Fuente van Bentem and Hirt 2007), but protein tyrosine phosphatases (PTPs) in plants are much less than in humans where more than 100 PTPs are present including approximately 60 DSPs (Kerk et al. 2008). This suggests that either tyrosine phosphorylation components are less or a single PTP/DSP may act on multiple sites in a signal transduction pathway in plants. Moreover, lesser number of protein tyrosine kinases have been reported in plants than animals, which also possibly accounts for the lesser number of protein tyrosine phosphatases in plants compared to animal system. Although, whole protein phosphatase complement is yet to be reported in other major plant species, PP2A subfamily has been identified in the genome of solanaceae plants tomato and potato where five and six isoforms, respectively have been reported (He et al. 2004; Pais et al. 2009). Sequence analysis and evolutionary studies have revealed that various phosphatase classes are highly conserved in plants (Kerk et al. 2002, 2008; Xue et al. 2008; Singh et al. 2010). Most of the phosphatase members of different classes from rice and *Arabidopsis* aligned together to form a phylogenetic clade and indicated towards their common ancestory and similar evolutionary lineage (Singh et al. 2010). Additionally, a high degree of chromosomal duplication (both segmental and tandem) has been observed for protein phosphatases, both in *Arabidopsis* and rice, which might have been a potent evolutionary force for the vast expansion of this gene family in plants (Xue et al. 2008; Singh et al. 2010). It has been shown that chromosomal duplication of protein phosphatase genes in rice, apart from genomic diversity has also lead to the functional diversification of this gene family. Several segmental and tandem duplicated protein phosphatase genes (*OsPPs*) have retained their function during the course of evolution as indicated by their expression profiles under different abiotic stresses and during different stages of development (Singh et al. 2010). Importantly, many duplicated phosphatases have attained a novel function and exhibited neo-functionalization, whereas few others have lost their function during the evolution and exhibited pseudo-functionalization (Singh et al. 2010).

Expression analysis under abiotic stresses

Protein phosphatases are important signal transduction components, and induction of their gene expression by various stress stimuli is crucial for the plants to regulate the stress triggered signaling pathways. Therefore, knowledge of their transcript level will help to understand the signaling networks at critical stages. In our recent genome level expression analysis by microarray, a large proportion of rice protein phosphatase genes (46 out of 132) was found to be differentially expressed under different abiotic stresses such as drought, cold and high salinity (Singh et al. 2010). The spatial and temporal expression might reflect the involvement of protein phosphatases in some specialized function. More genes (66 %) were up-regulated than down-regulated under one or multiple abiotic stresses. Most of the differentially expressed genes belonged to PP2C class, which is also the largest phosphatase group in plants. This suggests that PP2C genes are involved in multiple cellular processes, especially those influenced by abiotic stresses. Drought stress emerged as the major stress responsible for perturbation in expression level for most of the differentially expressed genes. Several *OsPP* genes were specifically up- and down-regulated under drought stress and an overlap in the expression was observed under drought and salt stresses together for a subset of *OsPP* genes. Previously, it has been well established that same

gene can be induced or activated by multiple triggers in different signal transduction pathways (Shinozaki and Yamaguchi-Shinozaki 2000; Knight and Knight 2001). This type of overlapping expression could be because of a common component such as calcium acting as a "Hub" in various stress triggered pathways and results in the cross-talk of signaling networks. A high degree of cross talk in the signaling network triggered by various abiotic stresses has been observed in plants (Knight and Knight 2001). Interestingly, expression of several members of rice PP2C group A was highly induced under abiotic stresses and this was consistent with another study where this group of rice phosphatases was found to be induced by ABA, salt, mannitol and cold treatment (Xue et al. 2008; Singh et al. 2010). In *Arabidopsis* also, the expression of nine members of PP2C group was elevated by ABA, which is known as a stress hormone and mediates abiotic stresses in plants (Xue et al. 2008). Also, five members of this *Arabidopsis* PP2C group (ABI1, ABI2, HAB1, HAB2 and AtPP2CA) have been well studied and characterised as the negative regulators of ABA mediated signaling (Merlot et al. 2001; Tahtiharju and Palva 2001; Saez et al. 2004; Yoshida et al. 2006). These findings fit appropriately in the recently developed model of ABA signaling, which emerged after the discovery of novel ABA receptors in plants (Ma et al. 2009; Park et al. 2009; Fujii et al. 2009). In this breakthrough discovery, it has been established that 14 members of *Arabidopsis* PYR/PYL family of START protein act as ABA receptors and they interact with PP2C protein phosphatase to remove the inhibitions of a protein kinase such as SnRK2 in the presence of ABA and regulate the downstream ABA signaling to generate stress and development mediated responses in plants (Fujii et al. 2009; Umezawa et al. 2009). Also, this novel paradigm has brought a new impetus in plant stress biology and the protein phosphatases have been recognised as the critical enzymes to genetically engineer the plants for imparting stress tolerance. Although PP2C is the most studied class of phosphatases, other important classes such as PP2A have also been implicated in abiotic stress signaling in different plant species. Various isoforms of tomato and potato PP2A were highly induced by cold and salt stresses, as determined by the northern blot analysis (Pais et al. 2009). Recently, a dual specificity protein tyrosine phosphatase OsPFA-DSP1 has been characterised in rice as well as in tobacco (Liu et al. 2012). Expression level of OsPFA-DSP1 was elevated under different stress treatments such as NaCl, ABA and PEG. In addition, OsPFA-DSP1 has been found to negatively regulate the drought stress responses in transgenic plants. Thus, a substantial proportion of large protein phosphatase gene family appears to be significantly involved in abiotic stress and ABA mediated signaling in plants and regulates stress responses.

Expression analysis during development

Plant development is a complex process and regulated at different steps by the networks of signaling cascades. Protein phosphorylation and dephosphorylation have been recognised as regulatory mechanism in various aspects of plant growth and development. Recent studies in rice and *Arabidopsis* have shown that transcript levels of a number of protein phosphatase encoding genes were differentially regulated in different tissues and at various stages of development (Xue et al. 2008; Singh et al. 2010). In *Arabidopsis*, by different expression detection techniques such as microarray, MPSS and EST datasets, high expression level was observed for a large set of 49 PP2C genes in various tissues and at different developmental stages including vegetative tissues such as leaf, root and reproductive stages such as inflorescence and siliques (Xue et al. 2008). In rice, microarray based expression profiling has been done for the protein phosphatases at the whole genome level during a wide spectrum of vegetative (leaf, root and seedling) and reproductive developmental (panicle and seed) stages (Singh et al. 2010). High percentage of rice protein phosphatases (~63 %, including all the classes) was found to be differentially expressed during developmental stages P1-P6 (from floral transition stage to mature pollen) and seed stages S1–S5 (early globular embryo to dormancy and desiccation tolerance) and approximately equal number of protein phosphatases were up- and down-regulated with a significant change in expression level. Overlap was also observed in the expression of *OsPPs* during panicle and seed developmental stages, and a subset of genes was commonly expressed during the two phases of reproductive development, while very few were exclusively expressed during panicle and seed stages. Similar to abiotic stresses, during development also PP2C class had major share of differentially regulated genes. These expression based findings emphasize the significant role of protein phosphatase in reproductive development of plants. Interestingly, an overlapping expression was detected for a few *OsPP* genes during developmental stages and abiotic stresses. All the protein phosphatase genes, which were commonly up-regulated in stresses and developmental stages belonged to PP2C class whereas down-regulated genes included PP2C, PP2A and DSPs (Singh et al. 2010). Genes with overlapping expression in seed development and abiotic stresses, suggested an interconnection between abiotic stresses and the plant development, which has also been established by prior studies in plants (Schroeder et al. 2001; Vij et al. 2008; Singh et al. 2010). During later stages of seed development and seed dormancy, generally a programmed dehydration event is known to trigger various cellular and physiological changes in the seed (Hetherington 2001; Schroeder et al. 2001; Agarwal et al. 2007). Previously, two rice genes

OsPP2A-1 and *OsPP2A-3*, encoding catalytic subunit of PP2A were found to exhibit an overlapping expression under drought and high salinity stresses and in root, leaf and stem tissues during various stages of development (Yu et al. 2003). Drought and salt stress resulted in high transcript level of both the genes in leaves while heat stress affected the transcript level negatively for *OsPP2A-1* in stems and resulted in induction of *OsPP2A-3* in all the organs. This overlapping expression could be regulated by phytohormone ABA, which is the major component for acquisition of dehydration trigger and regulation of seed germination in plants (Pei and Kuchitsu 2005; Yamaguchi-Shinozaki and Shinozaki 2006; Holdsworth et al. 2008; McCourt and Creelman 2008; Nakashima et al. 2009). This notion has been supported by the presence of ABRE elements in the promoters of protein phosphatase genes, both in rice and *Arabidopsis* (Xue et al. 2008; Singh et al. 2010), which are responsible for the regulation of ABA mediated signaling and responses. These observations further support the existence of recently elucidated ABA-PYR/PYL-PP2C-SnRK2 model, as several members of SnRK2 kinase family have also been implicated in ABA mediated abiotic stress signaling, seed development and dormancy (Boudsocq et al. 2004; Fujii et al. 2007; Nakashima et al. 2009; Fujii and Zhu 2009).

Functional role in abiotic stress signaling and plant development

Extensive functional characterization studies of protein phosphatases in plants have established PP2C group of phosphatases as the critical components of ABA signaling and implicated them in the regulation of important ABA mediated cellular processes. Two of the most studied protein phosphatases of *Arabidopsis*, ABI1 and ABI2 (PP2Cs) have been characterised as the main components of ABA signaling under abiotic stresses and during development, which function to regulate ABA responses negatively (Leung et al. 1994; Meyer et al. 1994; Merlot et al. 2001; Saez et al. 2006; Yoshida et al. 2006). The dominant mutant *abi1-1* and *abi2-1* harbour Gly to Asp substitution near Mg^{2+} binding site, which lead to reduction in phosphatase activity. These mutations resulted in ABA insensitivity, impaired seed dormancy, defects in stomata movement and ultimately poor drought tolerance (Meyer et al. 1994; Bertauche et al. 1996; Leung et al. 1994, 1997; Rodriguez et al. 1998; Schweighofer and Meskiene 2008). Studies of the interactors of ABI1 and ABI2, have provided the clue about their mechanism of operation in ABA mediated responses. It has been proposed that the phosphatases interaction complex include a protein kinase CIPK15 and Ca^{2+} binding protein CBL1 (Guo et al. 2002; Lee et al. 2010). In yeast two-hybrid assays, SOS2/CIPK24 and other members of this class of kinases interact with ABI1/ABI2 through a protein kinase interaction motif (PKI) within the catalytic phosphatase region (Schweighofer and Meskiene 2008). This interaction was disrupted in *abi2-1* mutants due to mutation in PKI motif and led to enhanced salt tolerance in plants (Ohta et al. 2003). It was observed that mutant plants of CIPK15 and CBL1 display hypersensitivity to ABA during seed germination and seedling growth, whereas *abi1* and *abi2* mutations supressed this sensitive phenotype (Guo et al. 2002; Lee et al. 2010) indicating that this complex is responsible for negative regulation of ABA signaling in calcium dependent manner. ABI1 also interacts with another protein kinase SnRK2E/SnRK2.6/OST1, which is also ABA and osmotic stress activated protein kinase, and *abi1-1* mutation results in decreased interaction (Mustilli et al. 2002; Yoshida et al. 2002; Yoshida et al. 2006). Mutant plants of both *srk2e/ost1* and *abi1-1* exhibited a wilty phenotype, indicating that ABI1 regulates the activation of SnRK2E. Similarly, interaction has also been established for ABI1 with ATHB6; an ABA and drought inducible transcription factor and with phospholipase D (PLD) derived phosphatidic acid (PA) (Himmelbach et al. 2002; Zhang et al. 2004). ATHB6 was found to be the negative regulator of ABA signaling and ABI1 acts upstream of this transcription factor in ABA signaling and interaction of this phosphatase with PLD derived PA has been found responsible for tethering ABI1 to plasma membrane (Himmelbach et al. 2002; Zhang et al. 2004). Based on these findings, a model has been proposed, which suggests that ABI1 translocates to the nucleus and interacts and activates ATHB6 to regulate ABA signaling negatively. On the other hand, PLD derived PA prevents ABI1 translocation to the nucleus by tethering it to the plasma membrane (Lee et al. 2010). Similarly, closely related members of *Arabidopsis* PP2C family; HAB1 and HAB2 are proposed to be the negative regulators of ABA signaling (Rodriguez 1998; Leonhardt et al. 2004; Saez et al. 2004; Yoshida et al. 2006). From the mutant analysis of HAB1, it was inferred that HAB1 mutants showed hypersensitivity to ABA during seed germination and enhanced stomata closure, whereas overexpression of HAB1 resulted in ABA insensitivity and impaired stomata closure, ABA resistant root growth. Moreover, *abi1-2/hab1-1* and *abi1-3/hab1-1* double mutants exhibited enhanced responsiveness to ABA, suggesting an overlapping function of ABI1 and HAB1, which together might act in negative regulatory loop of ABA signaling (Saez et al. 2006). Another member of *Arabidopsis* group-A, PP2C; AtPP2CA or AHG3, has been found to be highly induced under ABA, cold salt and drought stresses (Sheen 1998) and was found to be a strong negative regulator of ABA signaling mainly regulating seed germination and stomatal closure (Kuhn et al. 2006; Yoshida et al. 2006). Apart from PP2C, PHS1 a dual-specificity protein phosphatase from *Arabidopsis* has also been implicated in ABA signaling, since the

mutant of PHS1 revealed ABA hypersensitivity during seed germination and reduction in stomatal aperture (Quettier et al. 2006). Apart from ABA signaling, *Arabidopsis* PP2C genes have been involved in other pathways to regulate plant growth and development. Kinase associated protein phosphatase (KAPP) and poltergeist (POL) are two different types of PP2C phosphatases, which negatively regulate CLAVATA1, which mediates SAM (shoot apical meristem) development and also regulates flower development in *Arabidopsis* (Williams et al. 1997; Stone et al. 1998; Yu et al. 2000; Lee et al. 2010). CLAVATA1 is a receptor like kinase (RLK) and KAPP can bind to the kinase domain of RLKs through forkhead-associated domain (FHA) and leads to a *clv1* mutant like phenotype (Stone et al. 1998; Shah et al. 2002), whereas reduced KAPP expression reversed this phenotype. POL regulates the CLV1 pathway by modulating the activity of WUS, a target protein in CLAVATA pathway (Yu et al. 2000). Protein phosphatases also interact with the mitogen activated protein kinases (MAPKs) and regulate several important signaling cascades. Studies have revealed that PTPs and DSPs are the main phosphatases, which dephosphorylate MAPKs and hence, regulate MAPK signaling pathways (Keyse 1995, 1998; Wurgler-Murphy and Saito 1997; Zhan et al. 1997). In *Arabidopsis,* it has been shown that PTP1 can deactivate MAPK4 by de-phosphorylation (Huang et al. 2000). Moreover, the activation of MAPKs appeared to be stronger in *ptp1* mutant plants whereas reverse was observed for PTP1 over-expressing plants (Lee et al. 2010). In addition, a few PP2Cs have also been implicated in the MAPK signaling. MP2C (PP2C) from alfalfa could deactivate stress induced MAPK (SIMK) by dephosphorylation of threonine residue (Meskiene et al. 1998, 2003). AP2C1, another PP2C from *Arabidopsis* has been found to deactivate stress responsive MAPK4 and MAPK6 and regulate plant defense responses (Schweighofer

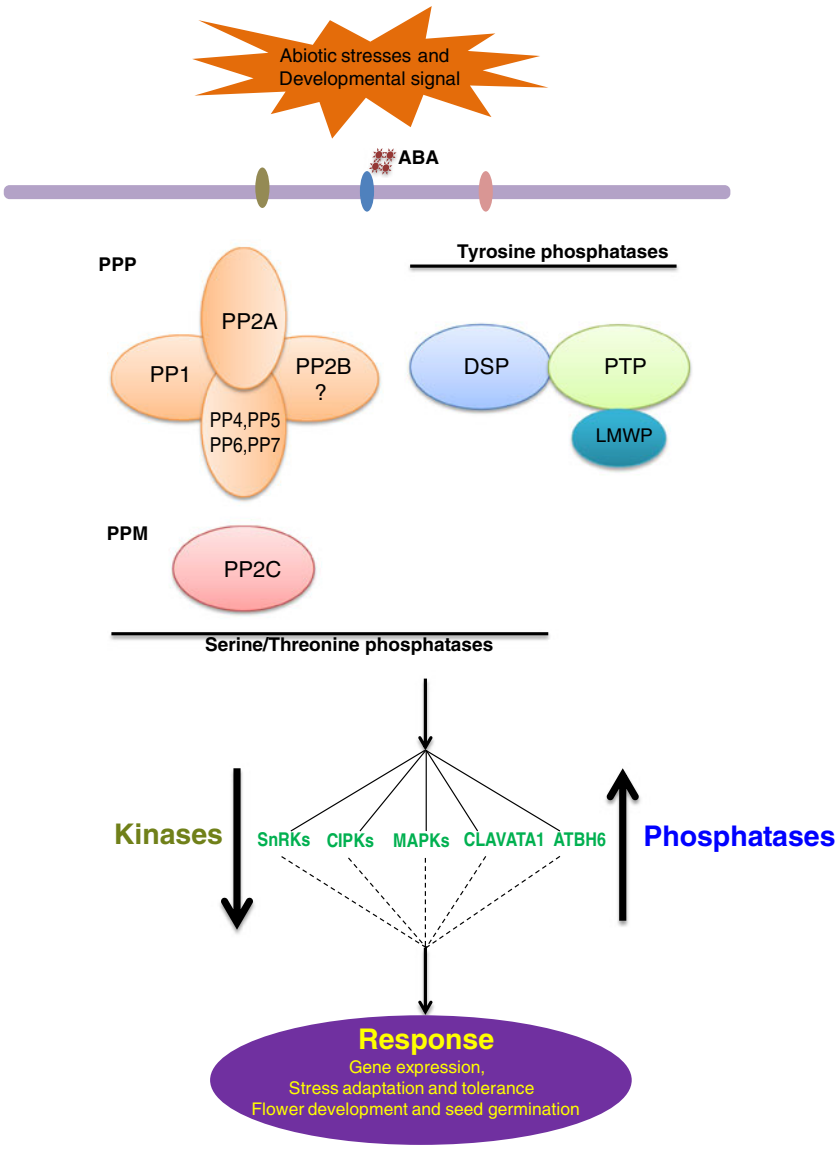

Fig. 1 A hypothetical model depicting different types of protein phosphatases in a plant cell and their involvement in the signal transduction pathways triggered by abiotic stresses and developmental signals. Most of the abiotic stresses and developmental events are mediated by phytohormone ABA, which is sensed by novel ABA receptors at the plasma membrane. In response to stresses and developmental triggers, the expression of members from different phosphatase classes is differentially regulated. Protein phosphatases interact with several signaling components such as ser/thr protein kinases i.e. *SnRK2s*, CBL-interacting protein kinases (*CIPKs*), mitogen activated protein kinases (*MAPKs*), receptor like kinases such as *CLAVATA1* and transcription factors such as *ATBH6* in different signaling pathways and regulate their activity. These components act upon other downstream signaling elements to generate a cellular response by modulation of expression of stress genes leading to stress adaptation and tolerance, and regulation of developmental processes such as flower development and seed germination

et al. 2007). AtPP2C5, which is a MAPK phosphatase, directly interacts with the stress induced MAPK3, MAPK4 and MAPK6 in the nucleus and positively regulates seed germination, stomatal closure and ABA-mediated gene expression (Brock et al. 2010). Similarly, AP2C3 acts upon these MAPKs and regulates stomata development in *Arabidopsis* (Umbrasaite et al. 2010). A model showing the involvement of different types of protein phosphatases in signal transduction pathways triggered by abiotic stress and developmental signals is presented in Fig. 1.

Thus, recent expression based information and prior studies show the regulation of various signaling pathways by protein phosphatases in plants and emphasize on their important role in abiotic stress responses and adaptation, plant growth and development. Further molecular dissection of signal transduction pathways involving protein phosphatases might provide information for engineering crop plants to tolerate high degree of abiotic stress and thereby lead to better agricultural productivity.

Future perspectives

Protein phosphatases have been identified at the level of whole genome, especially in model plants *Arabidopsis* and rice, and their expression profiles under various abiotic stress conditions and developmental stages are now available in public databases. Various molecular, cellular, biochemical and genetic approaches can be adopted for the detailed functional characterization of several novel phosphatase candidate gene(s). And it would be very interesting to find out whether protein phosphatases mediate stress and developmental signaling through interaction with ABA receptors or other signaling molecules like calcium and other phytohormones such as cytokinin, auxin, GA, ethylene. Once the role of a particular candidate phosphatase is functionaly known, it is important to establish a link towards upstream and downstream of this phosphatase in signaling pathway. Hence, downstream interacting partners (such as proposed SnRK2) can be identified for the candidate phosphatases by yeast two-hybrid library screenings and a novel signaling pathway can be reconstituted. In the hunt for downstream target of phosphatases, it is important to identify the physiological substrates, which will relate their function in a particular physiological or gene expression process. Promoter characterization studies can be undertaken for developmentally regulated phosphatases. Moreover, localization of various protein phosphatases to various organelles at subcellular level will provide the clue about their site of action and type of process they regulate. Ultimately, by genetic approach, the loss-of-function mutant and gain-of-function transgenic plants can be generated to evaluate the functional role of phosphatses *in planta*.

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