Chapter 5
Type 2C Protein Phosphatases in Plant Signaling Pathways under Abiotic Stress

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5.1 Introduction

Protein phosphatases (PPs) form a superfamily of highly conserved enzymes from simple prokaryotes to advanced eukaryotes. These PPs are counterparts of protein kinases (PKs), and together with signal receptors, they form delicate systems for a wide range of environmental signal perception and transduction, thus playing a crucial role to the survival and development of embryophytes or land plants. Type 2C protein phosphatases (PP2Cs) are representatives of a unique class of enzymes, namely, the phosphoprotein metallophosphatase, classified by the Mg^{2+}-/Mn^{2+}-dependent characteristics (Fuchs et al. 2013). In higher plants (e.g., Arabidopsis and rice), PP2C family consists of more than 80 members, which can be divided into ten or more subgroups (A–K) with diverse functions (Singh et al. 2010; Xue et al. 2008). In this chapter, updated reviews of clades A and B PP2Cs, which have important functions under unfavorable abiotic stress conditions, particularly involved in abscisic acid (ABA)-dependent signaling pathway and mitogen-activated protein kinase (MAPK) cascade, will be our attention. Biological roles and molecular functions of PP2Cs in the signaling pathways under abiotic stresses, as well as

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PP2C-based genetic engineering approaches for crop improvement, are the major key points to be summarized below.

5.2   Regulatory Targets of PP2Cs in Plant Stress Signaling Pathways

5.2.1   Core ABA Signaling Module

The phytohormone ABA has always been recognized as the key factor in regulating plant response to disadvantageous environmental conditions. PP2Cs are known as important regulators of ABA signaling. However, it was not until the breakthrough discovery of novel ABA receptors (ABARs) in Arabidopsis genome that the crucial roles of these proteins in ABA signaling pathway were revealed (Fujii et al. 2009; Ma et al. 2009; Park et al. 2009). Since then, PP2Cs have been given more and more attention from plant scientists all over the world. Fuchs et al. (2013) categorized this plant protein family in Arabidopsis into 12 clades, in which 9 PP2Cs involved in ABA signaling module belong to clade A. These PP2Cs include ABA-insensitive (ABI) 1, ABI2 (Kuhn et al. 2006; Merlot et al. 2001; Saez et al. 2006), ABA-hypersensitive germination (AHG) 1 (Nishimura et al. 2007), AHG3/PP2CA (Kuhn et al. 2006; Yoshida et al. 2006), hypersensitive/homology to ABA (HAB) 1, HAB2 (Kuhn et al. 2006; Robert et al. 2006; Saez et al. 2004, 2006), highly ABA-induced (HAI) 1, Arabidopsis K^+ transporter 1 (AKT1)-interacting PP2C 1 (AIP1)/HAI2, and HAI3 (Fujita et al. 2009).

In majority of higher plants including Arabidopsis, there are three core protein classes that participate in the ABA signaling module in response to abiotic stress conditions, which are (1) the novel ABARs, pyrabactin resistance (PYR)/pyrabactin-like (PYL)/regulatory components of the ABA receptor (RCAR); (2) the negative regulators, clade A PP2Cs; and (3) the positive regulators, sucrose non-fermenting (SNF) 1-related protein kinases type 2 (SnRK2s) (de Zelicourt et al. 2016). Under normal condition, in the absence of ABA, PP2Cs continuously inactivate SnRK2s including SnRK2.2, SnRK2.3, and SnRK2.6 (or Open Stomata 1, OST1) by dephosphorylating their activation loop and hence preventing the kinases to phosphorylate their downstream targets (Soon et al. 2012). When plants face with environmental negative factors such as drought, salt, and cold, ABA level increases in the cytosol to initiate adaptation responses of plants (Tuteja and Sopory 2008). ABA now enters the open ligand-binding pocket of ABARs, induces the gate and latch loops to close due to the conformational change of highly conserved β-loops, and thus provides a binding surface for PP2Cs (Melcher et al. 2009). The ABA-ABAR complex interacts with the tryptophan (Trp) residue inside the PP2Cs, which leads to the inhibition of PP2C active site (Park et al. 2009). At this stage, SnRK2s are liberated from the dephosphorylation activity of PP2Cs and become activated through autophosphorylation and can then regulate a wide range of Arabidopsis downstream effectors

through phosphorylation, which includes transcription factors (TFs) such as the ABA-responsive element (ABRE)-binding proteins (AREBs)/ABRE-binding factors (ABFs), ABI3 and ABI5 (Fujii and Zhu 2009; Furihata et al. 2006; Sirichandra et al. 2010), as well as plasma membrane proteins that function in controlling stomatal aperture such as slow anion channel-associated 1 (SLAC1) (Brandt et al. 2012; Geiger et al. 2009; Lee et al. 2009), quick anion channel 1/aluminum-activated malate transporter 12 (QUAC1/ALMT12) (Imes et al. 2013), the potassium channel KAT1 (Sato et al. 2009), NADPH oxidase respiratory burst oxidase homolog F, and the anion/proton exchanger CLCa (Sirichandra et al. 2009; Wege et al. 2014).

5.2.2 Chromatin Remodeling Complex

The complexities of plant response to adversities have been illustrated by various tiers of regulation. Localization analysis demonstrated the presence of Arabidopsis HAB1 in both nucleus and cytosol (Saez et al. 2008), suggesting the capacity of PP2C to interact with various ABA-signaling partners in different steps. Amino acid sequence analysis of several Arabidopsis PP2Cs revealed that at least ABI1, ABI2, HAB1, and PP2CA contain short nuclear localization signal at the end of the C-terminals (Himmelbach et al. 2002; Moes et al. 2008). Further experiments in Arabidopsis have found the nuclear-localized interaction between PP2Cs and SWI3B, which is a core subunit of the SWItch (SWI)/SNF chromatin remodeling complexes (Saez et al. 2008).

In eukaryotes in general and plants in particular, adaptation to environmental stresses requires delicate alteration in gene expression. The process of expressing a gene involves series of steps, in which the utmost requirement is the accessibility of the regulatory proteins to the gene, which requires extensive chromatin modification involving two major mechanisms—either posttranslational modification of histones or ATP-dependent reorganization of histone-DNA interactions (Han et al. 2015). Chromatin remodeling complexes are often called SWI/SNF-related ATP-dependent chromatin remodeling complexes. Their major roles in chromatin remodeling relate to the utilization of energy derived from hydrolysis of adenosine triphosphate (ATP) molecules for alteration of nucleosome occupancy or position (Archacki et al. 2016). Based on the conserved domains, chromatin remodeling ATPases have been categorized into four major subfamilies (i.e., inositol-requiring 80 (INO80)/sick with RSC/Rat1 (SWR1), chromodomain-helicase-DNA (CHD), imitation switch (ISWI), and SWI/SNF) (Han et al. 2015). Each family has a specific domain, such as chromo- or bromo-domain, or plant homeo-domain (PHD), allowing them to act in various circumstances. Among those, the yeast SWI/SNF complex was the first to be described. The complex is composed of the ATPase Swi2/Snf2 as the major catalytic subunit, and the central core consists of three additional polypeptides, Swi3, Snf5, and Swp73, which are essential for assembling and functionality of the complex (Saez et al. 2008). The ATPases of the SWI/SNF complexes were further divided into three types, which are encoded by all land plants

and share significant similarities with the metazoan counterparts: BRAHMA (BRM), SPLAYED (SYD), and MINUSCULE (MINU) (Han et al. 2015).

HAB1-SWI1B is the first PP2C interaction with the SWI/SNF chromatin remodeling complex to be discovered in Arabidopsis, emphasizing the consistent role of PP2C as a negative regulator of ABA signaling under various tiers of gene expressing regulation (Saez et al. 2008). Strong interaction between HAB1 catalytic domain and SWI3B was revealed by a yeast two-hybrid assay (Saez et al. 2008). Swi3p, Rsc8p, Moira (also known as SWIRM), and ZZ zinc finger domains of SWI3B protein were required for the interaction as deletion of either of them results in abolition of HAB1-SWI3B interaction (Saez et al. 2008). Moreover, nuclear localization signals are present at the C-terminal of the HAB1, ABI2, and PP2CA, illustrating the capacity of nuclear protein interactions of PP2C and SWI3. Meanwhile, the *swi3b* mutants displayed reduced ABA sensitivity and ABA-dependent gene expression, as well as the HAB1-SWI3B interaction (Saez et al. 2008). These findings strongly implied the role of SWI3B as a positive regulator of ABA signaling and the ability of PP2Cs, HAB1 in particular, to regulate the putative SWI/SNF complex to repress activities of some ABA-dependent promoters.

Nevertheless, a recent study on ATPase BRM in Arabidopsis has revealed a direct linkage of the chromatin remodeling to ABA signaling pathway (Peirats-Llobet et al. 2016). Bimolecular fluorescence complementation (BiFC) assay images confirmed positive BRM physical interactions with either OST1/SnRK2.2/2.3/2.6 or HAB1/PP2CA clade A PP2Cs in nucleus at both N- and C-terminals (Peirats-Llobet et al. 2016). Further mass spectrometry experiments demonstrated the capacity of phosphorylation/dephosphorylation of the C-terminal region of BRM by OST1/PP2CA *in vitro*. The major phosphorylation sites were identified to be around the AT hook and bromo-domain of BRM (Peirats-Llobet et al. 2016). Such regions have been reported to play crucial roles in BRM functionality, enabling interactions with linker and nucleosomal DNA as well as the histone octamer (Farrona et al. 2007). However, no phosphorylation sites have been detected in other regions of BRM required for ATPase activities, including the active site of ATP hydrolysis and the Snf2-ATP coupling (SnAC) domain, both of which utilize energy from ATP hydrolysis into nucleosome rearrangement. Hence, such "hotspots" of phosphorylation in the C-terminal, starting from the AT-hook domain and the bromo-domain, are considered as the core regulatory sites via phosphorylation/dephosphorylation activities. The Arabidopsis *brm* loss-of-function mutants exhibited ABA-hypersensitive phenotypes, further indicating that SnRK2 phosphorylation releases the repression of BRM in ABA signaling. In contrast, consistent role of PP2C phosphatases as negative regulators of ABA responses, such as PP2CA and HAB1, is emphasized as the dephosphorylation of BRM returning its activities in the absence of ABA and enabling plant growth in normal condition (Peirats-Llobet et al. 2016).

It was shown that *A. thaliana brm* knock-out mutant shared the same ABA-hypersensitive germination phenotype as the *swi3c* mutant, one of the plant's four SWI3 homologues, suggesting the notion that both BRM and SWI3C belong to the same complex (Sarnowska et al. 2016). While both BRM and SWI3B exhibit direct

interactions to at least one PP2C in Arabidopsis (i.e., HAB1), the *swi3b* defective mutant was found to have opposite phenotype to *brm* mutant, whereby the former displayed lower ABA sensitivity during germination and reduced expression of the ABA-responsive genes, such as *responsive to ABA 18* (*RAB18*) and *response to desiccation 29B* (*RD29B*) (Saez et al. 2008). In other words, BRM and SWI3B are negative and positive regulators of the ABA signaling pathway, respectively. In the Asensi-Fabado et al. 2017 review, Asensi-Fabado suggested two possibilities, which are either SWI3B competing with BRM for HAB1 binding thereby dephosphorylating BRM or SWI3B being associated with a different complex with distinct function to the BRM/SWI3C complex (Sarnowski et al. 2005; Sarnowska et al. 2016). The exact mode by which either BRM or SWI3B regulates the target loci remains to be elucidated, but nucleosome repositioning involvement might be the best explanation.

5.2.3   MAPK Cascades

MAPK signaling pathway is one of the most well-studied signaling mechanisms that is evolutionary conserved throughout eukaryotic organisms such as plants, insects, yeast, and mammals (Hamel et al. 2012). In plant kingdom, MAPK cascades consist of proteins from a large family with the ability to sense and transduce stress signals for appropriate responses during plant adaptation (Danquah et al. 2014). A MAPK cascade comprises at least three protein kinases at three levels, which are MAPK kinase kinases (MAPKKK/MKKK/MEKK, MAP3K), MAPK kinases (MAPKK/MKK/MEK, MAP2K), and MAPK (MPK). These proteins stimulate each other in a sequential manner through phosphorylation (Colcombet and Hirt 2008). The large number of MAPK pathway components in plants allows them to form thousands of different MAPK cascades. During the first step, activated MAPKKK phosphorylates two threonine (Thr/T)/or serine (Ser/S) residues located within the activation loop of MAPKK. MAPK is then activated in the next step via dual phosphorylation along T-X-Tyrosine (Tyr/Y) motif by the activated MAPKK (Hamel et al. 2012). This consecutive activation results in the phosphorylation of specific targets and the regulation of TF activities as well as the expression of different sets of genes that function in response to various environmental stresses (Popescu et al. 2009; Taj et al. 2010). In 2008, Colcombet and Hirt suggest that there may be a fourth level of kinases, MAP4Ks (MAP3K kinases), as mediators to link upstream signaling steps to core MAPK modules. Data from different studies have shown the involvement of MAPKs in signal transduction of plant adaptions to divergent stimuli, both biotic and abiotic (de Zelicourt et al. 2016).

Clade B PP2Cs have been categorized as regulators of MAPK activities and consist of six genes in Arabidopsis, namely, phosphatases type 2C (AP2Cs) which are orthologous to *Medicago sativa* protein phosphatases 2C (MP2Cs) (Fuchs et al. 2013). Although most of research focuses on the role of clade B PP2Cs in biotic stress responses, this group of proteins also displays potential association with

abiotic stress. Four members of this cluster (AP2C1–4) have been identified to contain the MAPK interaction motif known as kinase interaction motif (KIM), which regulates interaction activities of MAPKs with MAPK phosphatases (MKPs, phosphatase enzymes responsible for downregulation of MAPK signaling), MAP2K, or TFs in animals and plants (Fuchs et al. 2013). All MAPKs, except for the most distant group D, carry and are activated through a T-glutamic acid (Glu/E)-Y phosphorylation motif (Ichimura et al. 2002). AP2C/MP2C deactivates MAPK activities through the dephosphorylation of the pT (phosphorylated threonine residue) in the pTEpY activation loop of MAPK (Schweighofer et al. 2007; Umbrasaite et al. 2010). All four AP2Cs contain KIM domain and interact with Arabidopsis MPK3, MPK4, and MPK6, the three MAPKs which are involved in various stress signaling pathways (Moustafa et al. 2008; Hoang et al. 2012), suggesting a potential role of clade B PP2Cs in plant adaptation.

In addition to clade B PP2Cs, members of clade A are also believed to be involved in the regulation of MAPK cascades in plants due to close relation between MAPKs and ABA. Recently, Mitula et al. (2015) have successfully identified a member of Arabidopsis MAPK cascade, MKKK18, which is regulated by ABI1, a clade A PP2C. MKKK18 functions in an MAPK module comprised of MKKK18-MKK3-MPK1/2/7/14 (Danquah et al. 2015) and acts as a positive regulator of stomata density and ABA-induced stomatal closure (Mitula et al. 2015). In Arabidopsis, ABI1 was also found to interact with MAPK6 (Leung et al. 2006), suggesting that this clade A PP2C, and possibly some other members in the cluster, might also be involved in different tiers to regulate MAPK cascades in plant stress adaptation (Fig. 5.1).

5.2.4   Other Targets

Aside from their inhibition with the calcium-independent kinase SnRK2 family, PP2Cs are also found to interact with other kinase families such as calcium-dependent protein kinases (CDPKs/CPKs) or SnRK3s/calcineurin B-like protein (CBL)-interacting protein kinases (CIPKs). However, the effects of these interactions are quite different. Zhao et al. (2011) have reported the phosphorylation activity of Arabidopsis CPK12 with ABI2, a clade A PP2C, which results in stimulating catalytic activity of this phosphatase, suggesting that CPK12 could be negatively involve in ABA signaling pathways. Interestingly, two clade A PP2Cs, ABI1 and ABI2, have been reported to inhibit Arabidopsis CIPK26 in a similar manner in which they inactivate SnRK2s (Lyzenga et al. 2013, 2017). These findings may indicate an antagonistic correlation between these two kinase families, yet more studies should be conducted to clarify this assumption.

In Arabidopsis, ABI1, along with PP2CA, was also found to interact with and dephosphorylate SnRK1.1, a member of the subgroup 1 of SnRK-type protein kinases that is involved in sugar responses under stress controlled through ABA signaling (Rodrigues et al. 2013). In addition to this finding, Chen et al. (2016) also
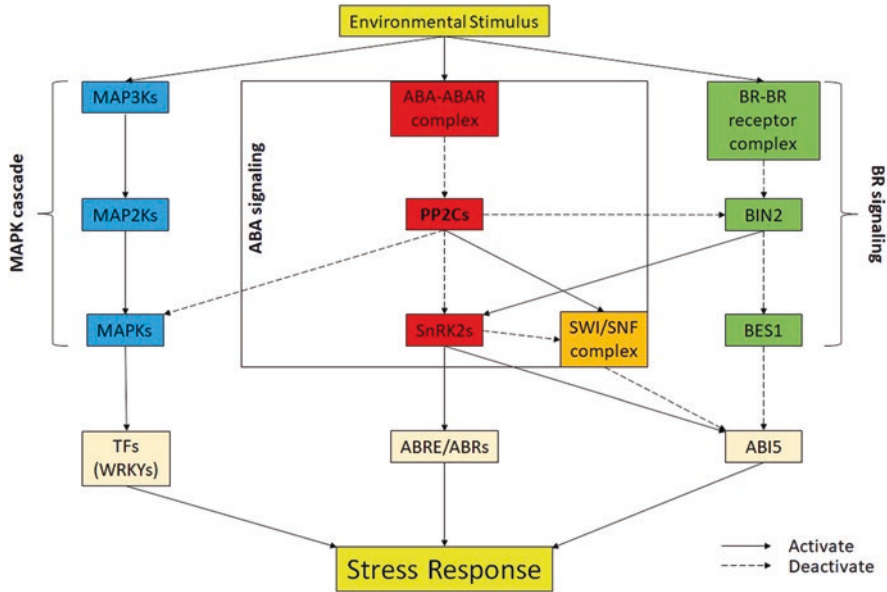
Fig. 5.1 Suggested model of regulatory functions of type 2C protein phosphatases (PP2Cs) in different pathways relating to abscisic acid (ABA)- and brassinosteroid (BR) signaling pathways and mitogen-activated protein kinase (MAPK) cascade in plant response to abiotic stress conditions such as drought, salinity, and cold. Under normal condition, PP2Cs regulate components of different signaling pathways through dephosphorylation. Upon exposing to stress, plants receive and transmit signal and initiate ABA-ABA receptor (ABAR) binding which will inhibit the active site of PP2Cs and in turn alter activities of downstream components including various kinase enzymes, resulting in appropriate responses (Ojolo et al. 2018; Opdenakker et al. 2012; Planas-Riverola et al. 2019; Singh et al. 2016)

reported the direct interaction between Arabidopsis ABI2 and the receptor-like kinase FERONIA (FER), suggesting the coordinated function of ABA in different pathways during plant stress responses.

Interestingly, Wang et al. (2018) reported the regulatory activities of Arabidopsis ABI1 and ABI2 in brassinosteroid (BR) signaling pathway through the BR-negative regulator brassinosteroid insensitive 2 (BIN2) kinase, which can serve as a bridge for constructing the crosstalk between two phytohormones, ABA and BR, under abiotic stress. Moreover, Arabidopsis PP2Cs, including ABI1 and ABI2, also interact with salicylic acid (SA) in an antagonistic manner with respect to ABA (Manohar et al. 2017). These findings indicate the cooperative correlation between/among phytohormones in balancing plant growth and development during stress conditions.

In general, PP2Cs have appeared as essential factors of ABA signaling pathway in regulating plant reactions to abiotic stresses. Through interaction and modulation of different target proteins, this protein family has provided significant roles in adaptive response of several plant species. Latest findings have further revealed some novel and unique functions of PP2Cs in ABA signaling pathway and uncovered a whole new area for future research.

5.3 Current Studies on PP2Cs in Plant under Abiotic Stress

5.3.1 Clade A

Due to the important role of PP2Cs in ABA signaling pathway, several investigations under different abiotic stress conditions have been reported to aid further understanding of their molecular function. A common characteristic that is shared between different clade A PP2Cs is that their expression levels are induced by ABA and stressful conditions (Fujita et al. 2009, 2011). Expression analyses in different plant species, such as Arabidopsis (Xue et al. 2008), rice (Singh et al. 2010), tomato (Sun et al. 2011), maize (Wei and Pan 2014), Chinese cabbage (Kong et al. 2018), and banana (Hu et al. 2017), have consolidated this fact. Many studies also discovered the fact that *PP2C* genes from different species have been found to exhibit overlapping expression profile under various abiotic stress treatments, including drought, salt, cold, and heat stresses (Cao et al. 2016; Li et al. 2018; Singh et al. 2010; Xue et al. 2008; Yang et al. 2018). These findings may indicate that plants respond to different abiotic stresses through a resembling molecular mechanism, in which PP2Cs can be important cross-talking factors between/among different signaling pathways.

Among the nine clade A PP2C members that involve in ABA signaling pathway identified in Arabidopsis, ABI1 and ABI2 are two best-studied proteins, which control the full range of ABA responses under abiotic stresses and during development (Fuchs et al. 2013; Singh and Pandey 2012). These two PP2Cs were found to physically interact with various cytosolic and nuclear-localized proteins. Such interactions are commonly seen in various PP2Cs such as interaction with homeodomain TF (e.g., HB6, CIPK24) or the more selective, specific interaction with preprotein of fibrillin and CIPK8 (Fuchs et al. 2013). In addition to being the regulators of activities of SnRK2s, ABI1 and its homolog, ABI2, have been identified to be associated with other regulatory activities in plant response to environmental changes, such as the CBL1/CBL9-CIPK23 pathway in stomata aperture regulation (Mao et al. 2016) and nitrate sensing (Léran et al. 2015), and the proteasome degradation through the ubiquitin/26 s proteasome system (UPS) (Ludwików 2015).

Besides ABI1 and ABI2, the three "HAI" PP2Cs, HAI1, AKT1-interacting PP2C1/HAI2, and HAI3, also show interesting behaviors under abiotic stress conditions. Under polyethylene glycol (PEG) treatment condition, *HAI* PP2C mutants showed enhancing proline and osmoregulatory solute accumulation, whereas these features were not apparently seen in Arabidopsis carrying mutation in other clade A PP2Cs (Bhaskara et al. 2012). While Arabidopsis PP2C *HAI* single mutants did not produce ABA-responsive phenotype (Yoshida et al. 2006), double and triple mutation in genes encoding HAI PP2C showed different ABA sensitivity levels at different stages of plant development. During germination stage, Arabidopsis *HAI* double and triple mutants were found to be ABA insensitive, which is in contrast with the hypersensitive phenotypes of other clade A *PP2C* mutants (Yoshida et al. 2006). However, when entering post-germination stage, mutants of various *HAI*

genes in this cluster showed similar hypersensitivity characteristics to ABA (Bhaskara et al. 2012). Furthermore, the *pp2ca-1hai1-1* (or *ahg3-1hai1-1*) double mutant plants displayed enhanced ABA-mediated growth inhibition, increased ABA-responsive gene induction, and diminished water loss compared with the *pp2ca-*1 single mutants (Antoni et al. 2012), suggesting that *hai1-1* mutation enhanced the ABA sensitivity of *pp2ca-1/ahg3-1* mutant. These results indicate that HAI PP2Cs may have a greater role in ABA-independent pathway rather than in ABA-dependent pathway in response to drought stress.

Several *in planta* studies have been conducted to further understand the functional roles of PP2Cs under abiotic stresses. This protein family has shown to possess potential candidates for producing abiotic stress-tolerant transgenic plants. Singh et al. (2015) reported that transgenic Arabidopsis overexpressing rice clade A PP2C *OsPP108* confers high tolerance under salt, mannitol, and drought stresses. Similarly, transgenic Arabidopsis ectopically expressing *Brachypodium distachyon BdPP2CA6* displayed enhanced stomatal closure and salinity tolerance (Zhang et al. 2017a). Singh et al. (2016) also summarized studies of other PP2Cs on their expression level and *in planta* functional roles in response to stress conditions (Table 5.1).

5.3.2   Clade B

Among clade B PP2Cs, AP2C1 and AP2C3 are the two best-characterized members and were found to regulate stomatal developmental pathway in Arabidopsis (Schweighofer et al. 2007; Umbrasaite et al. 2010). In the absence of AP2C1 and AP2C3, stomatal closure was impaired, indicating the involvement of these two proteins in regulating water loss rate of plants, especially under adverse conditions. In 2007, Schweighofer et al. discovered that AP2C1 controlled wound-induced MAPK activities and stress-induced ethylene responses. The early expression of *AP2C1* at the site of wounding indicates its involvement in order to antagonize effect of the aforementioned stress conditions. Moreover, AP2C1 also negatively controls production of jasmonate, a phytohormone that is believed to play a role in plant responses to abiotic stresses such as drought, salt, and heat. Results from investigation revealed that *ap2c1* knock-out plants showed enhanced jasmonate production upon wounding and better tolerance to herbivory effects (Schweighofer et al. 2007). On the other hand, AP2C3, which is closely related to AP2C1, shows unique expression that differs from those of other members of the family. Distinct expression pattern in stomata and stomatal lineage cells along with the ability to interact with/downregulate signaling activity of MAPKs consolidates the participation of AP2C3 in regulating MPK3 and MPK6 during stomatal development (Umbrasaite et al. 2010). Recently, AP2C1 was also found to negatively regulate CIPK9 under K⁺ deficiency condition, which is also a kind of abiotic stress experienced by the plants (Singh et al. 2018).

Table 5.1 Summary of recent studies on identification of PP2Cs that mediate plant response to abiotic stress conditions

Plant species	Gene	Type of study	Findings	References
Arabidopsis *thaliana*	Clade D—*AtPP2C*s	RT-qPCR	Expression of *AtPP2C*s was significantly influenced by alkali and salt stresses, suggesting possible involvement or direct interaction	Chen et al. (2018)
Soybean (*Glycine soja*)	Clade D—*GsPP2C*s	RT-qPCR	Similar expression pattern in clade D—*PP2C*s of *G. soja* and in clade D—*PP2C*s of *A. thaliana*, suggesting conserved functions of clade D—PP2Cs between plant species	
Rice (*Oryza sativa*)	Clade F—*OsPP18*	Molecular, genetic, and physiological analyses	Overall expression of *OsPP18* led to osmotic and oxidative stress tolerance. *ospp18* mutants and suppressed *OsPP18*-RNAi exhibit drought-hypersensitive phenotypes, with lower reactive oxygen species (ROS)-scavenging gene expression, suggesting potential role of OsPP18 in drought tolerance mediation	You et al. (2014)
	Clade A—*OsPP108*	Molecular and genetic analyses using heterologous system	Arabidopsis ectopically expressing *OsPP108* showed enhanced abscisic acid (ABA) insensitivity and high tolerance to salt, mannitol, and drought stresses at various stages of development	Singh et al. (2015)
Brachypodium distachyon	Various clades—*BdPP2C*s	Transcriptome/RT-qPCR	50–80% of *BdPP2C* genes displayed upregulation in response to abiotic stresses (cold, heat, PEG and NaCl treatments), suggesting possible involvement of BdPP2Cs in *B. distachyon* resistance to abiotic stresses	Cao et al. (2016)

Table 5.1 (continued)

Plant species	Gene	Type of study	Findings	References
Barrel clover (*Medicago truncatula*)	Various clades— *MtPP2Cs*	Microarray/RT-qPCR	Most of *MtPP2C* genes showed differential expression patterns in response to abiotic stresses (i.e., cold, drought, and ABA stress), aiding the identification of stress-related *MtPP2C* genes	Yang et al. (2018)
Wheat (*Triticum aestivum*)	Clade F—*TaPP2C1*	Expression, molecular, biochemical, and physiological analyses using heterologous system	Ectopic expression of *TaPP2C1* in tobacco resulted in reduced ABA sensitivity and increased salt tolerance of the transgenic seedlings	Hu et al. (2015)
Tomato (*Solanum lycopersicum*)	Clade A— *SlPP2C1*	Expression, molecular, and biochemical analyses using homologous system	*SlPP2C1*-RNAi tomato plants displayed hypersensitivity to ABA and increased drought stress tolerance	Zhang et al. (2017b)

5.3.3 Other Clades

Apart from clades A and B, there is limited information about function of other clades in plants under abiotic stress conditions. However, some lines of evidence also indicate the involvement of these proteins in plant adaptation. For example, Chen et al. (2018) found that expression level of members of clade D PP2Cs was significantly altered upon alkali and salt stress treatments in soybean and Arabidopsis, suggesting the direct or indirect association of this class of PP2Cs in stress signaling pathways.

5.4 Conclusion

Plant genomes code for larger number of PP2Cs than other groups of organisms including yeast, mouse, or human, which indicates the important role of this protein family in various cellular processes in plants. Recent analyses of plant PP2Cs have revealed the novel regulatory modes and functions of this class of protein phosphatases in different signaling pathways. Due to the large number of members in this gene family, many functions and activities of PP2Cs in plants remain unknown. However, results obtained from performed studies and analyses on PP2Cs have proven them to be potential targets for further investigation to thoroughly understand their role in mediating plant adaptation to environmental stimuli and serve as base to develop appropriated methods for overcoming stress conditions.

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