Send Orders for Reprints to reprints@benthamscience.net



REVIEW ARTICLE

# PP2A Phosphatases Take a Giant Leap in the Post-Genomics Era

Malathi Bheri[1] and Girdhar K. Pandey[1,*]

[1]*Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi-110021, India*

ARTICLE HISTORY

Received: November 20, 2018
Revised: May 09, 2019
Accepted: May 09, 2019

*DOI:*
*10.2174/1389202920666190517110605*

**Abstract:** ***Background***: Protein phosphorylation is an important reversible post-translational modification, which regulates a number of critical cellular processes. Phosphatases and kinases work in a concerted manner to act as a “molecular switch” that turns-on or - off the regulatory processes driving the growth and development under normal circumstances, as well as responses to multiple stresses in plant system. The era of functional genomics has ushered huge amounts of information to the framework of plant systems. The comprehension of who’s who in the signaling pathways is becoming clearer and the investigations challenging the conventional functions of signaling components are on a rise. Protein phosphatases have emerged as key regulators in the signaling cascades. PP2A phosphatases due to their diverse holoenzyme compositions are difficult to comprehend.

***Conclusion***: In this review, we highlight the functional versatility of PP2A members, deciphered through the advances in the post-genomic era.

**Keywords:** Protein phosphorylation, protein phosphatases, Ser/Thr phosphatases, PP2A, regulatory B subunit, scaffolding A subunit, catalytic C subunit, genomics, proteomics, transcriptome profiling, stress signaling.

## 1. INTRODUCTION

The goal of functional genomics is to identify functions of genes and proteins and their interactions using genome-wide approaches in contrast to the gene-specific nature of classical molecular biology. The data sourced from cellular processes at gene, transcript and protein level lead to the development of models that define interactive and dynamic regulatory networks in the living systems [1]. The human genome [2, 3] and the genome of *Arabidopsis thaliana*, an important model system in plant research [4] are the landmarks that paved the way to understand the working of living systems on a large scale. The genomes of 236 angiosperm species have been completely sequenced [5]. The elucidation of signaling pathways has been made possible through the availability of technological resources and has changed the conventional perceptions. The technical advances in research have broadened our understanding of plant responses from the perspective of multiple stresses as well as growth and development.

The kinome and phosphatome, the complement of protein kinases and phosphatases, respectively account for approximately 2-4% of the protein-encoding genes in eukaryotes, such as humans, yeast, and plants [6]. The kinases and the phosphatases, bring about protein phosphorylation, an important reversible post-translational modification, responsible for the regulation of a number of critical cellular processes. The former transfers the γ-phosphoryl group of donor ATP to the acceptor protein side chains. while the latter removes the phosphate group from phosphoproteins through hydrolysis of phosphoric acid monoesters into a phosphate group and a molecule with a free hydroxyl group [7]. The two work in a concerted manner as a molecular switch to regulate a number of signaling pathways. Though proteins can be phosphorylated on nine amino acids, which include Tyrosine, Serine, Threonine, Cysteine, Arginine, Lysine, Aspartate, Glutamate and Histidine [8], it is reported that more than one-third of protein phosphorylation occurs on Serine, Threonine, and Tyrosine residues [9]. Recent mass spectrometry studies have shown that at least two-thirds of eukaryotic cellular proteins are phosphorylated [10, 11]. The proportions of phosphoserine (pS), phosphothreonine (pT), and phosphotyrosine (pY) sites are reported in the range of 82.7-85.0 %, 10.7-13.1 % and 4.2-4.3 % in Arabidopsis, while the proportions of pS, pT and pY sites are 84.8%, 12.3% and 2.9% in rice [12, 13]. The proportions of pS, pT and pY sites are reported to be 89.3%, 10.2% and 0.5% in soybean root hair phosphoproteome inoculated with the Rhizobium, *Bradyrhizobium japonicum* [14] whereas the proportions of pS, pT and pY sites are 86.0%, 12.7% and 1.3% in *Medicago truncatula* [15]. The proportions of pS, pT and pY sites are 86.4%, 11.8% and 1.8%, respectively in HeLa cell lines [10, 16]. Initial studies projected phosphatases as enzymes that were responsible for reversing the kinase driven phosphorylation processes and were referred to as housekeeping enzymes [17]. Over the years, phosphatases have emerged as major players that regulate the signaling pathways to ensure the proper functioning of developmental processes under normal and stressful environments. In this review, we present the progress made in the context of protein phosphatase-2A members.

*Address correspondence to this author at the Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi-110021, India; Tel: +91-011-24113106 X 7387; Fax: +91-011-24111208; E-mail: gkpandey@south.du.ac.in

1389-2029/19 $58.00+.00 ©2019 Bentham Science Publishers

Eukaryotic protein phosphatases are categorized into four gene families: Phosphoprotein Phosphatases (PPP), Metal-dependent Phosphatases (PPM), Protein Tyrosine Phosphatases (PTP) and Aspartate-dependent phosphatases [18, 19]. The Ser/Thr Phosphatases (STPs) include PPP and PPM families, while PTP includes Tyr specific and Dual-Specificity Phosphatases (DSPs) that dephosphorylate all three phosphoresidues, Ser, Thr and Tyr [20]. The PPPs are one of the most highly conserved proteins across eukaryotic species [21]. They are sub-classified as PP-1, -2A, -2B, -4, -5, -6, -7; PPM, SLP (*Shewanella*-like protein) phosphatase, aspartate-dependent phosphatases [TFIIF(transcription initiation factor IIF)-associating component of CTD (C-Terminal Domain) phosphatase/small CTD phosphatase], Rhizobiales-Like Phosphatases (RLPH), ApaH-Like (ALPH) Phosphatases and Protein Phosphatase with Kelch-Like repeat domains (PPKL) [22]. The PPM and PPP families vary in sequences and seem to have two different evolutionary origins, which converge in the highly related catalytic structures [23]. The PPM family includes PP2C, pyruvate dehydrogenase phosphatase and other $Mg^{2+}$-dependent Ser/Thr phosphatases [7, 24]. The PTP family has a catalytic motif CX5R, and dephosphorylates carbohydrates, mRNA and phosphoinositides [6, 25].

## 2. THE PP2A GENE FAMILY, REGULATION AND THE GENOMIC APPROACHES

PP2A phosphatases comprise three subunits, a ~65-kDa scaffolding subunit, "A", a regulatory subunit, "B" and a ~36-kDa catalytic subunit, "C". The C and A subunits make up the core enzyme while the B subunit imparts substrate specificity, cellular localization and enzymatic activity to the ABC trimer. This results in a number of holoenzymes exhibiting distinct properties and functions (Fig. **1**). The presence of isoforms of the A and C subunits has been reported in vertebrates as well as flowering plants. The gene families encoding B subunits are phylogenetically unrelated and are conserved in plants as well as animals. The A subunit comprises 15 HEAT (Huntingtin-Elongation-A subunit-TOR) repeats. The B subunit gene families are of three types: the B55 (PPP2R2/PR55; 55 kDa; encoding β-propeller proteins); the B56 (PPP2R5/B'/PR56/61; 54-74 kDa; encoding Huntingtin, EF3, PP2AA, TOR (HEAT) repeat proteins) and the B72 (PPP2R3/B"/PR72; 72-130 kDa; encoding EF hand-containing proteins) [26-28]. The *A. thaliana* genome has three subunit A isoforms, RCN1/PP2AA1, PP2AA2/PDF1 and PP2AA3/PDF2 [29, 30]. The genes encoding PP2A catalytic subunit are expressed ubiquitously, although their levels may vary [31]. The five C subunits are classified into two subfamilies, I and II, which show a sequence identity of more than 95% within the subfamilies and 80% between the two subfamilies [32]. The C I subfamily includes PP2A-C1 (At1g59830), PP2A-C2 (At1g10430), and PP2A-C5 (At1g69960) catalytic subunits while the C II subfamily includes PP2A-C3 (At2g42500) and PP2A-C4 (At5g58500) subunits [28]. The B subunit families include two B subunit isoforms (α and β), nine B' subunit isoforms (α, β, γ, *δ*, ε, ζ, η, θ, and κ), five B" (α, β, ɣ, *δ* and ε) subunit isoforms and TON2 (TONNEAU2) with a similarity to the human B" subunit PR72 [33-36]. The B' family is further divided into 3 subfamilies α, η and κ, where the η subfamily comprises the close homologs B'η, B'ɣ, B'θ and B'ζ [37].

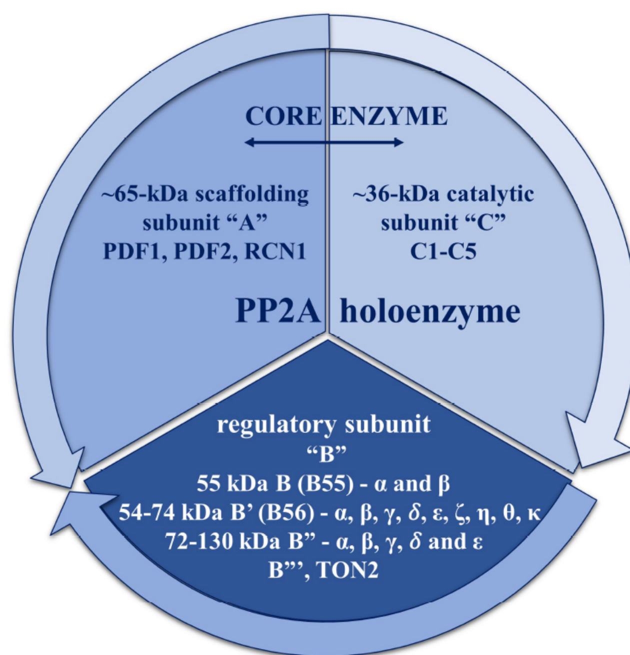


**Fig. (1).** The illustration shows the PP2A heterotrimeric composition with the holoenzyme comprising of three subunits, a ~65-kDa scaffolding subunit, "A", a regulatory subunit, "B" and a ~36-kDa catalytic subunit, "C". The C and A subunits make up the core enzyme while the B subunit imparts substrate specificity, cellular localization and enzymatic activity to the ABC trimer. The B subunit gene families are of three types: the B55 (55 kDa); the B56 (54-74 kDa) and the B72 (72-130 kDa). The B subunit families in *A. thaliana* include two B subunit isoforms (α and β), nine B' subunit isoforms (α, β, γ, *δ*, ε, ζ, η, θ, and κ), five B" (α, β, γ, *δ* and ε) subunit isoforms and TON2 (TONNEAU2). The B' family is further divided into 3 subfamilies α, η and κ, where the η subfamily comprises the close homologs B'η, B'γ, B'*θ* and B'ζ [37]. The *A. thaliana* genome encodes three subunit A isoforms: RCN1/PP2AA1, PP2AA2/PDF1 and PP2AA3/PDF2. The C subunit is classified into two sub-families: the C I subfamily (PP2A-C1, PP2A-C2 and PP2A-C5) and the C II subfamily (PP2A-C3 and PP2A-C4).

The PP2A subunit gene family lineages in flowering plants evolved much later than their mammalian counterparts [38]. The evolutionary history of PP2A gene families was studied through phylogenetic and synteny analyses in Arabidopsis. The expansion in this family in both plants and animals occurred through ancient whole-genome duplications and triplications, also known as, paleopolyploidization events followed by non-random gene loss. The expansion of B56 subunit gene family occurred through functional diversification in both plants and animals. Reduced expansion rates were observed in three distinct, non-expanding B subunit sub-clades, involved in cell division and beneficial microbial associations, indicating functional specialization in the non-expanding clades. The flowering plant B55 gene family forms three clades: (i) clade I, absent in Brassicaceae; (ii) clade II, absent in monocots and core eudicots; and (iii) clade III, comprising of the two Arabidopsis isoforms. The flowering plant B56 gene family forms five clades: (i) Arabidopsis B3, B4 and B7; (ii) B5 and B8; (iii) B6, B9 and B10; (iv) B11 and (v) BØ absent in Arabidopsis and other

Brassicaceae members. The B72 family forms two clades: (i) clade I including the FASS/TON2 clade including with mammalian B”γ and (ii) clade II including all non-FASS/TON2 plant B72 isoforms, related to mammalian B”α and B”β [38].

The Arabidopsis genome sequence was used to identify 112 Arabidopsis phosphatases with a majority of 69 PP2Cs, one PTP, 23 Ser/Thr phosphatases, 18 DSPs, and one LMW-PTP [39]. The NCBI non-redundant protein database was used to retrieve putative protein phosphatases in Arabidopsis and analyzed by two gene prediction programs: GENSCAN [40] and GENMARK [41]. The predicted amino acid sequences were searched through BLASTP of the non-redundant database and the predicted nucleic acid coding sequence was searched through BLASTN of the NCBI EST database from Arabidopsis. This was followed by an extensive search for protein phosphatases and phylogenetic analysis [39]. Later, the genomes of Arabidopsis, *Oryza sativa*, *Populus trichocarpa* and the green algae (*Chlamydomonas reinhardtii* and *Ostreococcus tauri*) were searched for the Arabidopsis phosphatome. Phylogenetic analysis revealed that the evolution of protein phosphatases of green algae occurred between that of animal and plant protein phosphatases. The Arabidopsis genome encodes 5 PP2A genes [18]. The availability of genome sequences in the public domain has been useful in the much needed systematic genome-wide comparative analyses of a number of gene families from an evolutionary perspective [42]. The expression profiling of genes is important for identifying their functional characteristics. The genome-wide expression profiling in the context of protein phosphatase gene family was carried out through whole genome *indica* rice microarrays for the vegetative, panicle and seed development stages as well as salt, cold and drought stress. The full complement of protein phosphatases comprising of 132-protein phosphatase-coding genes, was identified through *in silico* studies in the rice genome. The rice, tomato and hot pepper genomes encode 132, 113 and 102 PP-encoding genes, respectively [43, 44]. The entries for PP2A phosphatases in different plant systems (source: UniProt Knowledgebase, UniProtKB, 2018) have been presented in Table **1**. The PP2A encoding genes identified in rice [44] are presented in detail (Table **2** and Fig. **2a**) along with the nature of regulation under drought, salt and cold stress as well as panicle and seed developmental stages. Of these, PP2Cs were the largest class with 90 PP2Cs, 17 PP2As, 23 DSPs, 1 PTP and 1 LMWP [44].

## 3. PP2As: STRUCTURE AND REGULATION OF THE REGULATOR

PP2As regulate a number of cellular processes such as translation, transcription, cell proliferation, signal transduction, apoptosis, inflammation and differentiation [45, 46]. Malfunctions in enzyme activity have been linked with cancers and Alzheimer’s disease in humans [47-49]. The high identity level of the binding sites of okadaic acid in protein phosphatases 1 and 2A of *A. thaliana* with *H. sapiens* and their spatial structures indicate that interaction mechanisms with okadaic acid in animals and higher plants are common [50]. Upto 18 B subunits have been reported with the B’ family being the largest family with at least eight members in humans [51]. The crystallization studies of an AB’C PP2A holoenzyme revealed that the HEAT repeats of the scaffolding A subunit form a horseshoe-shaped fold, thereby, positioning the catalytic C and regulatory B’ subunits on the same side. The substrate specificity is decided by the B’ subunit, which forms pseudo-HEAT repeats and interacts with the C subunit present near the active site. The methylation of the C subunit at the C-terminal promotes B’ subunit recruitment by neutralization of charge repulsion as the methylated C subunit interacts with a highly negatively charged environment of the interface between A and B’ subunits [51]. The C subunit shares sequence homology with other Ser/Thr phosphatases (PP1, PP2B, PP4 and PP6). The C-terminal region spanning 294-309 amino acid residues of the C subunit has a conserved motif (TPDY307FL309), which is vital for the methylation of the carboxyl group on Leu309 that directs the recruitment and binding of the PP2A-B subunit to the PP2A A-C dimer [51]. More than 80 different combinations of the PP2A holoenzyme have been reported, which are responsible for the regulation of their activity and localization [52].

The amino acid sequences predicted for PP2AA2 and PP2AA3 show 86% identity to the RCN1 sequence in Arabidopsis. Also, these subunits were observed to differ in molecular weight by <0.1 kD with their predicted molecular weights of 65.49 kD - RCN1, 65.51 kD - PP2AA3, and 65.57 kD - PP2AA2 [30]. The gene encoding the maize PP2A regulatory subunit A, *ZmPP2AA1,* located on chromosome 6 (http://www.maizesequence.org/index.html), is 6.7 kb in length and contains 12 coding exons and 1 non-coding exon, similar to *AtPP2AAs*. The mRNA sequence of *ZmPP2AA1* (Acc. no. AY940682), comprises of 1765-bp and the predicted protein comprises of 583 amino-acid residues (calculated mol. weight = 65 kDa and theoretical isoelectric point = 4.93). The ZmPP2AA1 protein was observed to show 83.8%, 88.9%, and 87.6% similarity with AtPP2AA1/RCN1, AtPP2AA2 and AtPP2AA3, respectively. ZmPP2AA homologs, GMZM2G102858 and GMZM2G 122135, were also identified in maize. The ZmPP2AA1 protein shows approximately 93% similarity to GMZM 2G102858 protein and approximately 91% similarity with GMZM2G122135 protein. Similar to AtPP2AAs, the ZmPP2AA proteins contain the “HEAT” repeats. Phylogenetic analysis showed that the PP2AA proteins from the grass family: maize (GRMZM2G164352, GRMZM 2G102858, GRMZM2G122135); rice (Osl_30535, Os09g 0249700); barley (MLOC_2967); *Brachypodium* (BRAD I4G08720, BRADI4G08790), formed a separate clade from the three AtPP2AA proteins, (AT1G25490) AtPP2AA1, (AT3G25800) AtPP2AA2 and (AT1G13320) AtPP2AA3 [53].

The stability, enzymatic activity and organization of PP2A holoenzymes are controlled by five important PP2A regulators, target of rapamycin signaling Pathway Regulator-Like (TIPRL)-1, Leucine Carboxyl Methyl Transferase 1 (LCMT1), Phosphotyrosyl Phosphatase Activator (PTPA), PP2A Methyl Esterase 1 (PME-1), TAP46 (Type 2A Phosphatase-associated Protein of 46 kD) and α4 [54-57]. AtPTPA modulates the conformation of the C subunit in the AC dimer to enable the interaction of the C subunit with the B subunit resulting in the assembly of the PP2A holoenzyme. Protein-protein interaction studies showed that the

**Table 1. The table shows the number of protein phosphatases 2A in different plant systems (source: UniProt Knowledgebase, UniProtKB, 2018).**

| S. No. | Plant System | No. of Entries for PP2As |
|---|---|---|
| 1. | *Arabidopsis thaliana* (Mouse-ear cress) | 53 |
| 2. | *Oryza sativa* subsp. *japonica* (Rice) | 26 |
| 3. | *Triticum aestivum* (Wheat) | 67 |
| 4. | *Zea mays* (Maize) | 153 |
| 5. | *Cicer arietinum* (Chickpea) | 21 |
| 6. | *Pisum sativum* (Garden pea) | 2 |
| 7. | *Vicia faba* (Broad bean) | 6 |
| 8. | *Helianthus annuus* (Common sunflower) | 35 |
| 9. | *Populus trichocarpa* (Western balsam poplar) | 33 |
| 10. | *Solanum lycopersicum* (Tomato) | 13 |
| 11. | *Daucus carota* subsp. *sativus* (carrot) | 14 |
| 12. | *Nicotiana tabacum* (Common tobacco) | 66 |
| 13. | *Capsicum annuum* (Bell pepper) | 51 |
| 14. | *Glycine max* (Soybean) | 30 |

**Table 2. The table shows the details of the protein phosphatase 2A genes identified in the rice system and their regulation under abiotic stress. The genes along with their chromosomal location, TIGR locus ID, KOME accession no., size in bp and amino acid length, number of introns, events of alternative splicing, domains as well as their regulation under abiotic stress (drought, salt and cold stress), panicle (P1-6) and seed developmental stages (S1-5). "*" - Indicates genes present in segmental duplication. Abbreviations: TIGR (The Institute of Genomic Research); KOME (Knowledge based Molecular Biological Encyclopedia); AS - Alternative Splicing.**

| S. No. | Gene | Chromosome | TIGR Locus ID | KOME Accession | Size (bp) | Size (AA) | Introns | AS | Domain # | Regulation Under Abiotic Stress | Panicle Dev. Stages | Seed Dev. Stages |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *OsPP5* | 1 | LOC_Os01g24750 | AK100195 | 978 | 326 | 3 | - | a | - | - | - |
| 2. | *OsPP13* | 1 | LOC_Os01g49690 | AK068018 | 912 | 304 | 9 | - | a | - | - | - |
| 3. | *OsPP20* | 2 | LOC_Os02g12580.1* | AK072676 | 924 | 308 | 5 | - | a | - | - | - |
| 4. | *OsPP41* | 2 | LOC_Os02g57450 | AK120439 | 951 | 317 | 4 | 2 | a | - | ↓- P1-6 | ↓ - S1-5 |
| 5. | *OsPP44* | 3 | LOC_Os03g07150.1* | AK069884 | 945 | 315 | 10 | 2 | a | - | - | - |
| 6. | *OsPP47* | 3 | LOC_Os03g16110 | AK073140 | 969 | 323 | 4 | 3 | a | - | ↑ - P1-6 | ↑ - S1-5 |
| 7. | *OsPP54* | 3 | LOC_Os03g44500.1* | EST | 3012 | 1004 | 20 | - | a | ↑ | - | - |
| 8. | *OsPP56* | 3 | LOC_Os03g59060 | AK060885 | 924 | 308 | 10 | 2 | a | - | ↑-P1-5; P6 - ↓ | ↓ - S1-5 |
| 9. | *OsPP73* | 5 | LOC_Os05g05240 | EST | 2676 | 892 | 20 | 2 | a | - | - | - |
| 10. | *OsPP74* | 5 | LOC_Os05g11550 | AK101918 | 1452 | 484 | 12 | 2 | j | ↓ | ↓ - P1-6 | ↓ - S1-5 |
| 11. | *OsPP83* | 6 | LOC_Os06g06880 | AK064345 | 969 | 323 | 2 | - | A | ↓ | ↓ - P1-6 | ↓ - S1-5 |

**(Table 2) contd….**

| S. No. | Gene | Chromosome | TIGR Locus ID | KOME Accession | Size (bp) | Size (AA) | Introns | AS | Domain # | Regulation Under Abiotic Stress | Panicle Dev. Stages | Seed Dev. Stages |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12. | *OsPP88* | 6 | LOC_Os06g37660.1* | AK072676 | 921 | 307 | 5 | - | a | - | - | - |
| 13. | *OsPP101* | 8 | LOC_Os08g35440 | AK121378 | 924 | 308 | 2 | - | a | - | - | - |
| 14. | *OsPP103* | 8 | LOC_Os08g40200 | EST | 1287 | 429 | 4 | - | a | ↑ | ↓ - P1-6 | ↓ - S1-5 |
| 15. | *OsPP106* | 9 | LOC_Os09g11230 | AK073644 | 924 | 308 | 7 | - | a | - | ↑-P1, 5; ↓- P2,3,4,6 | ↓ - S1; ↑ - S2-5 |
| 16. | *OsPP112* | 10 | LOC_Os10g27050.1* | AK099604 | 945 | 315 | 10 | - | a | - | - | - |
| 17. | *OsPP132* | 12 | LOC_Os12g42310.1* | AK065064 | 3030 | 1010 | 20 | - | a | - | ↓ - P1-6 | ↓ - S1-5 |

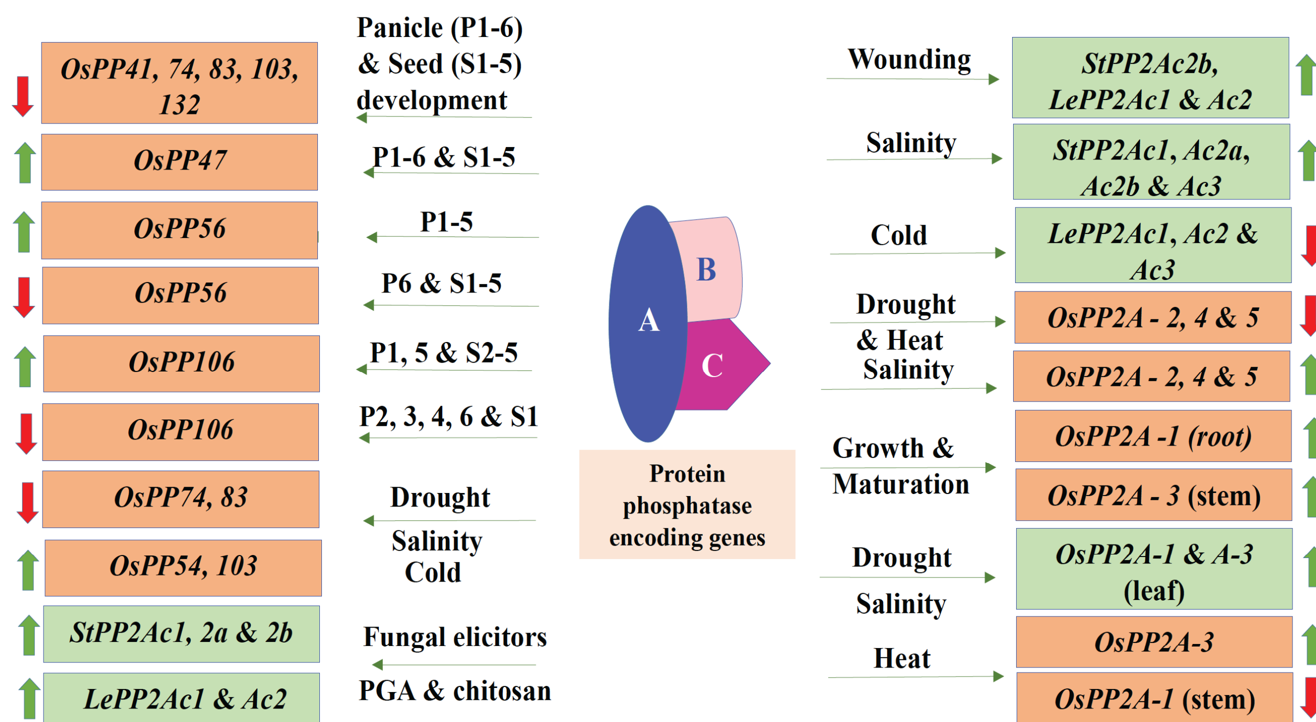


**Fig. 2(a).** The PP2A encoding genes identified in rice, tomato and potato, regulated under panicle and seed developmental stages as well as different types of stress: drought, salinity, heat and cold, wounding, fungal elicitors, are shown along with the nature of regulation. The green arrows represent upregulation while the red arrows represent downregulation. (*The color version of the figure is available in the electronic copy of the article*).

AtPTPA and PP2Ac interactions are dependent on subunit A, indicating the formation of a PP2A-A/AtPTPA/PP2A-C trimer. *AtPTPA* deficiency in *AtPTPA* knockout plants results in reduced interactions between B and C subunits, leading to reduced functional PP2A holoenzyme formation. Thus, AtPTPA is a critical factor for PP2A heterotrimer formation [58]. The crystallization studies of PME-1 and PP2A-PME-1 Complex were carried out. The structural analysis revealed that PME-1 directly binds to the PP2A active site, which led to conformational changes and PME-1 activation. Also, the PP2A inactivation occurs by the release of manganese ions that are essential for PP2A activity indicating the dual function of PME-1 in the regulation of PP2A activation, methylation and holoenzyme assembly [55].

The Brassinosteroid Insensitive 1 (BRI1) plant brassinosteroid (BR) receptor undergoes constitutive internalization. PP2A dephosphorylates BRI1 and the Arabidopsis *rcn1* mutant shows an increase in BRI1 levels and BR signaling. SBI1 encodes LCMT that methylates PP2A and controls its membrane-associated subcellular localization. The *sbi1* mutant shows preferential accumulation of BR-activated BRI1 but not of BAK1, indicating that BRs increase SBI1 levels, which methylate PP2A, enabling its association with activated BRI1, leading to receptor dephosphorylation and degradation, thereby, attenuating BR signaling [56]. Brassinosteroid promotes growth through dephosphorylation of BZR1 transcription factor by PP2As. B' subunits directly interact with and activate BZR1 through putative PEST domain containing the *bzr1-1D* mutation site. The dephosphorylation by PP2A is enhanced by the *bzr1-1D* mutation, decreased by

two intragenic *bzr1-1D* suppressor mutations and diminished by deletion of the PEST domain [59].

## 4. PP2A PHOSPHATASES AND THE RNA-CENTERED APPROACHES

The PP2A phosphatases are involved in the regulation of plant development and stress responses in plants. The expression of the MsPP2A β-subunit from *Medicago sativa* is induced by ABA [60]. *OsPP2A-1* and *OsPP2A-3*, closely related PP2A catalytic subunit genes isolated from rice, show ubiquitous expression with high levels in stems and flowers and low in leaves. *OsPP2A-1* in roots and *OsPP2A-3* in stems show an increase in expression at the maturation and young stages, respectively. An upregulation of both genes is observed in the leaves under drought and high salinity conditions, whereas under heat stress, *OsPP2A-1* is downregulated in stems while a ubiquitous expression of *OsPP2A-3* is observed [61]. *OsPP2A-2*, *OsPP2A-4* and *OsPP2A-5* show ubiquitous expression during plant development and are differentially regulated under salinity stress as well as a combination of drought and heat stresses [62]. The *LePP2Ac1*, *LePP2Ac2* and *LePP2Ac3* are potential regulators of cold stress responses in tomato plants while the *StPP2Ac1*, *StPP2Ac2a*, *StPP2Ac2b* and *StPP2Ac3* are involved in regulation of salt stress in potato. Also, *StPP2Ac2b*, *LePP2Ac1* and *LePP2Ac2* are involved in the regulation of wounding responses [63].

*ZmPP2AA1* is induced in roots by low phosphate (Pi) availability. Quantitative RT-PCR studies showed significant up-regulation of *ZmPP2AA1* under low phosphate (Pi) in comparison to sufficient phosphate conditions indicating that *ZmPP2AA1* gene responds to Pi deficiency with induced expression. The overexpression of *ZmPP2AA1* results in enhanced tolerance to Pi starvation in transgenic maize. The *ZmPP2AA1* OE maize lines showed curly growth and inhibition of Primary Roots (PRs) with agravitropic growth, increased Lateral Roots (LRs) density and length, as compared to wild type and *ZmPP2AA1* RNAi plants, independent of the Pi availability. The *ZmPP2AA1* OE lines promoted LR and Axial Root (AR) formation, under low Pi, resulting in a highly branched root architecture that enabled Pi acquisition. The *ZmPP2AA1* regulation may be linked with auxin signaling as the *ZmPP2AA1* OE lines also showed modulations in the free IAA levels in AR tips and sensitivity to IAA or NPA from exogenous sources, as well as enhanced yields under Pi deficiency [53].

Roots Curl in Naphthylphthalamic Acid1 (RCN1) is involved in auxin transport and ethylene responses in *A. thaliana* [64-67]. RCN1 is a positive transducer of ABA signaling in Arabidopsis. The *rcn1* mutants exhibit partial inhibition of ABA-induced stomatal closure and activation of anion channels as well as reduction in the probability of ABA-induced $[Ca^{2+}]_{cyt}$ increases. Also, the *rcn1* mutant showed partial ABA insensitivity towards the ABA-mediated inhibition of seed germination. RNA gel blot analyses indicated that the *rcn1* mutation decreased the ABA-induced *KIN1*, *KIN2* and *RD29A* transcript levels [68]. The loss-of-function mutants of *PP2AA2* and *PP2AA3* show normal phenotypes unlike *rcn1* mutant plants in spite of the high amino acid similarity of the three subunits. Protein expression studies for the *rcn1*, *pp2aa2-1* and *pp2aa3-1* mutant lines have shown that the expression patterns of the other A subunits are not altered by loss of one isoform, contrary to the compensatory upregulation model. The *rcn1pp2aa2* and *rcn1pp2aa3* double mutants show significant abnormal development as compared to the *pp2aa2pp2aa3* double mutant indicating that RCN1 is involved primarily in regulation of phosphatase activity while PP2AA2 and PP2AA3 functions are unmasked only in the absence of RCN1 [30]. The RCN1-containing PP2A complexes regulate root growth as the *rcn1* mutants show increased sensitivity to ionic, osmotic and oxidative stress and develop characteristic abnormalities in cell division patterns in the root apical meristem and reduced growth. The regulatory and coding sequences of RCN1 are required for the normal root tip development in *rcn1* mutants while the defective hypocotyl elongation is complemented by either *RCN1* or *PP2AA3* transgenes. RCN1-containing PP2A is involved in regulating post-embryonic root development by maintaining normal auxin distribution and stem cell function at the root apex [69]. The regulation of auxin signaling and root growth by PP2As has been presented (Fig. **2b**).

PP2AA and PINOID Ser/Thr kinase are involved in the transport-dependent auxin distribution in embryos and seedling roots. The *pp2aa* mutants show a basal-to-apical shift in PIN localization resulting in auxin depletion in the root and collapse of meristem, similar to alterations in PIN polarity in *PID* gain-of-function plants, indicating that PP2A and PID act antagonistically in the phosphorylation of PIN proteins. The expression pattern of PP2AA genes was analysed using *PP2AA1::GUS*, *PP2AA2::GUS*, *PP2AA3::GUS* and *PP2 AA1::PP2AA1:GFP* fusions. The three genes showed high and overlapping transcriptional activity in growing seedlings at 4 and 8 days after germination. The *PP2AA1* expression was observed in the whole root tip while *PP2AA2* expression was observed markedly in the elongation zone and columella root cap. The *PP2AA3* expression was limited to the columella root cap. The overlapping expression patterns of *PP2AA*s were in agreement with the global transcription data (http://www.weigelworld.org/resources/microarray/AtGenExpress). *P2AA1,2,3::GUS* embryos revealed transcriptional activity of all three genes from the 8-cell-stage onward. The expression of *PP2AA1::GUS* and *PP2AA1::PP2AA1:GFP* reporters were strong throughout the embryo in comparison to a weaker and similar *PP2AA2::GUS* expression. The *PP2AA3::GUS* expression was weakest of the three. Two different artificial microRNAs (amiRNAs) lines that target all three *PP2AA* genes showed identical defects but more severe than those observed in *pp2aa1pp2aa2* and *pp2aa1 pp2aa3* double mutants. The tamoxifen-inducible amiRNA lines showed a basal to-apical shift of PIN1, PIN2 and PIN4 polarity on tamoxifen treatment in contrast to the tamoxifen treated and untreated transgenic controls, in which no alterations in PIN polarity were observed [70]. PP2A-C3 and PP2A-C4, belonging to subfamily II, are involved in controlling embryo patterning and root development by the regulation of PIN1 polarity and auxin distribution. Through PIN1-GFP localization and *DR5pro:GFP* expression, it was demonstrated that PP2A-C3 and PP2A-C4 catalyse PIN dephosphorylation and subcellular distribution as the *c3c4* double

mutants lack a functional root and the embryos have disturbed patterning, PIN1 polarity and auxin distribution [32].

The activation of Nitrate Reductase (NR) in plants occurs through dephosphorylation after dark/light shifts, and is photosynthesis dependent. NR activation was strongly impaired by knockdown of all three A subunits by amiRNA and lowered in the *pp2aa2pp2aa3* double mutants while being unaffected in the *rcn1* mutant. The homozygous *Bβ* and heterozygous *Bα* mutants showed slower activation rate for NR as compared to WT plants indicating their involvement in the dephosphorylation of NR leading to its activation [71]. PP2A is involved in the regulation of 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), an important enzyme involved in isoprenoid biosynthesis, under both normal and stress conditions in Arabidopsis. B"α and B"β, which are $Ca^{2+}$ binding proteins with the EF-hand, interact with the isoforms of HMGR, HMGR1S and HMGR1L. PP2A was observed to be a post-translational negative regulator of HMGR activity and protein levels as well as a positive regulator of *HMG1* transcript levels. The enhanced HMGR activity observed *in vitro* and seedling establishment *in vivo*, occur in response to inhibition of PP2A. HMGR was regulated by the *B"β* gene at the post-transcriptional level in normal conditions while B"α was observed to negatively regulate root growth under salt stress. The ATH1 22k microarray data available at Genevestigator (https://www.genevestigator.com/gv/index.jsp) indicates that though the Arabidopsis *B"* transcript levels are steady in various organs during development, the *B"α* transcript level increases in response to salt, ABA, mildew attack and decreases in response to potyvirus attack [72].

*PP2A-B′γ* and *PP2A-B′ζ* show high promoter activities in rapidly growing tissues and are necessary for optimal plant growth under favourable conditions in Arabidopsis. The *pp2a-b′γ, pp2a-b′ζ1-1*and the *pp2a-b′γζ* plants acclimated to high light and enhanced temperature (800 μmol photons $m^{-2}s^{-1}$/28°C) were analyzed using Agilent Arabidopsis gene expression microarrays. The *pp2a-b′γ* plants did not exhibit significant transcript levels for salicylic acid (SA)-related defense genes in comparison to high light-acclimated WT plants whereas F-box and miscellaneous signaling genes were up-regulated. The *pp2a-b′ζ1-1* mutant plants showed a slight reduction in transcript levels of the flowering repressor *FLOWERING LOCUS C, FLC*, [73] contrary to the increased *FLC* transcript levels in the late flowering *pp2a-b′γ* mutant [74]. Differential transcript levels for 162 genes were observed in the *pp2a-b′γζ* mutant plants that were not expressed differentially in *pp2a-b′γ* and *pp2a-b′ζ1-1* mutants. The transcript profiles of ascorbate peroxidase, *APX2*, *HEAT SHOCK FACTOR A3 (HSFA3)* and a set of co-regulated heat shock proteins, HSPs (*HSP18.2*, *HSP21* and *HSP22*) involved in abiotic stress responses as well as those related to regulation of mitosis, responses to ionizing radiation and DNA modification were enhanced in *pp2a-b′γζ* double mutants as compared to wild-type plants under high light and elevated temperature. Also, *pp2a-b′γζ* double mutants did not show an upregulation in *JUB1* and *DREB2A* levels. Moreover, elevated amounts of antheraxanthin and *β*-carotene were observed in the double mutants. The *pp2a-b′γζ* double mutant plants displayed decreased growth under normal conditions but on acclimation to high light, elevated temperature and water deficit, they grow similar to wild type plants as well as show induction of photoprotective mechanisms and increased tolerance against abiotic stress. These transcriptomic changes indicate that PP2A-B′*γ* and PP2A-B′*ζ* are involved in plant developmental processes and photo-oxidative stress responses [75].

B′γ type regulatory subunit regulates disease resistance and the chloroplast integrity under moderately low light conditions in Arabidopsis. The disintegration of chloroplasts and the cell-death phenotype with hydrogen peroxide generation involving *Constitutive Expression of PR genes5* (*CPR5*), were observed which indicate constitutive activation of defense responses in the *pp2a-b′γ* mutants. Thus, B′γ acts as a negative regulator that prevents premature senescence and defense responses under normal conditions. The comparative transcriptome profiling of *pp2a-b′γ* and WT plants subjected to moderate light conditions for 4 weeks revealed that the *pp2a-b′γ* plants showed constitutive expression of defense-related genes with a significant upregulation in expression of: a) SA-related genes such as *Enhanced Disease Sensitivity1* (*EDS1A* and *EDS1B*), *Phytoalexin-Deficient4*, *Pathogenesis- Related1 (PR1), PR5* and *Isochorismate Synthase1*; b) resistance (R) genes encoding coiled-coil nucleotide-binding Leu-rich repeat receptors, intracellular Toll-interleukin 1 nucleotide-binding Leu-rich repeat receptors, and Leu-rich repeat class of disease resistance proteins; c) genes encoding cytosolic *h*-type thioredoxins, chitinases, glutathione S-transferases and other defense-related genes. The *pp2a-b′γ* plants also showed elevated expression of genes related to the biosynthesis of Jasmonic Acid (JA) and Ethylene (ET). Induction of two genes encoding 1-aminocyclopropane-1-carboxylate oxidases; three genes encoding allene oxide synthases, 12-oxophytodienoate reductase, lipoxygenase 2, *Plant Defensin1.2* and a Tyr aminotransferase in the *pp2a-b′γ* plants while the ROS markers (*At1g19020*, *At2g43510* and *At1g57630*) and cell death antagonists (*At5g47120* and *At3g16770*) showed slightly increased expression. However, the expression of anti-oxidant enzymes (chloroplast NADPH-dependent thioredoxin reductase, iron-superoxide dismutase, catalase 2 and glutathione peroxidase 7) were reduced in the *pp2a-b′γ* plants, whereas the expression of five genes encoding thioredoxin family proteins, two genes encoding chloroplastic monodehydroascorbate reductases, three encoding Met sulfoxide reductases and one encoding catalase 3 (*SEN2*) were higher in the *pp2a-b′γ* plants than in WT plants. Thus, the *pp2a-b′γ* plants show constitutive expression of ROS-, SA-, and JA/ET-responsive genes [76]. Peroxisomal targeting signal, PTS1, observed in the PP2A B subunit, B'*θ* of Arabidopsis and some of its close relatives, is instrumental in directing PP2A C2, C5, and A2 subunits to peroxisomes as they lack peroxisomal targeting signals. The *b'θ* knockout mutants exhibited impaired peroxisomal β-oxidation as the mutant seedlings showed a sugar-dependent phenotype. The *b'θ* mutant seedlings were also impaired in the transformation of protoauxins, Indole-3-butyric acid (IBA) and 2, 4-dichlorophenoxybutyric acid (2, 4-DB) to the bioactive auxins, indole-3-acetic acid (IAA) and 2, 4-dichlorophenoxy-acetic acid (2, 4-D), respectively and lack normal Triacylglycerol (TAG) mobilization. The expression of *B'θ*, observed to be lower in stratified seeds, stabilized in early ger-

mination and increased during senescence and in dessicated seeds, is coherent with the microarray data and the Genevestigator database. The *B'θ* expression was observed to be up-regulated in response to pathogens like *Pseudomonas syringae* pathovars, *Psm* and *Pst*, *Phytophthora infestans*, *Blumeria graminis*, *Phytophthora parasitica*, as well as the nematode, *Meloidogyne incognita*. The microarray data also showed up-regulation of *B'θ*, *C2*, and *C5* expression on flagellin 22 treatment in comparison to the down-regulation of *B'θ* expression in response to abiotic stresses [77]. B'ζ may be involved in energy metabolism as the *b'ζ* knockout seedlings showed retarded hypocotyl growth on sucrose-free medium. Also, it is highly expressed during senescence alongwith most of the fatty acid degradation enzymes indicating that both B'*θ* and B'ζ are involved in energy metabolism [78].

Similar to other B'η members, PP2A-B'*θ* is involved in negative regulation of plant innate immunity as observed by the decreased proliferation of the virulent *Pseudomonas syringae* in the *b'θ* mutants in comparison with the wild type plants. The *b'θ* mutants were found to show delayed flowering phenotype supported by high expression of the *FLOWERING LOCUS C, FLC* [78]. PP2A acts both as a positive and negative regulator of flowering with PP2A-B55 acting as a negative regulator whereas PP2A-B'γ acts as a positive regulator. PP2A-B'γ functions upstream of the main flowering inhibitor *FLC* by repressing it, as the *pp2a-b'γ* Arabidopsis mutant showed a late flowering phenotype and a significantly higher *FLC* transcript level than in wild type [74].

TAP46, an Arabidopsis homolog of *S. cerevisiae* TAP42 and mammalian α4, were found to interact through a yeast two-hybrid screen using Arabidopsis PP2Ac as bait. Thus, an interaction between PP2Ac and TAP46 as well as the existence of TOR (target-of-rapamycin) signaling pathway in plants is possible [79]. The expression of *TAP46* was observed to be induced particularly by chilling treatments but not by heat or anaerobic stress, similar to its homolog in rice [80], indicating that the gene could be involved in response to cold stress [79]. TAP46 interacts with PP2Ac, although it interacts with PP4 and PP6 as well with different affinities [81]. Recombinant *TAP46* protein was found to be phosphorylated by immunoprecipitated full-length and deletion forms of TOR under *in vitro* conditions, suggesting that it may be directly targeted by TOR kinase activities in plants. The cellular PP2A activities were observed to be modulated similarly in both *TAP46* and *TOR* RNAi plants. The characteristic phenotypes of TOR inactivation such as repressed global translation, activated autophagy and nitrogen mobilization processes were observed in *TAP46*- and *TOR* silenced plants during the initial stages of gene silencing as observed in Arabidopsis, yeast and mammals. The expression of nitrogen assimilatory genes, *NII1* (nitrite reductase), *NIA2* (nitrate reductase-2), *GS2* (chloroplast glutamine synthetase), and *GOGAT* (glutamate synthase) were significantly downregulated whereas the genes encoding cytosolic glutamine synthetase (*GS1*) and glutamate dehydrogenase (*GDH*), involved in ammonium assimilation, were upregulated, in the *TAP46* RNAi Arabidopsis. The NR activities of Nb *TAP46* VIGS *N. benthamiana* and the TRV:Nb-TOR *N. benthamiana* VIGS plants also showed reduced NR activities [81]. *TAP46* silencing in tobacco BY-2 cells resulted in chromatin bridge formation at anaphase, indicating its involvement in the segregation of sister chromatids. RNAi-induced down-regulation of *TAP46* induces PCD in Arabidopsis and Nb *TAP46*-regulated PCD and results in expression of several *PR* genes during cell death. Defense-related genes (*PR1a*, *PR1c*, *PR2*, *PR5*, *S25-PR6*, *HIN1*, *SAR8.2a*, *NTCP-23, p69d, SGT1*, *RAR1* and *SKP1)* were transcriptionally induced in the TRV: Nb-*TAP46* (N) plants (17 DAI) whereas tobacco homeobox genes (*NTH15*, *NTH20* and *NTH23*) were down-regulated. Therefore, Nb *TAP46*-mediated PCD enhances *PR* gene expression during HR cell death. *TAP46* may be a positive effector of TOR signaling in the regulation of cell growth and metabolic processes in plants [81]. *TAP46* is induced by ABA and is highly expressed in seeds. TAP46 positively regulates ABA signaling in Arabidopsis. Overexpression of *TAP46* increased ABA sensitivity and decrease in PP2A activity while *tap46* mutants show decreased ABA sensitivity and higher PP2A activity during seed germination in Arabidopsis. TAP46 and PP2A were observed to interact with the ABA-regulated transcription factor, ABA INSENSITIVE5 (ABI5) *in vivo*. TAP46 interacts and stabilizes ABI5 and prevents its dephosphorylation *in vivo*. Overexpression of *TAP46* results in increase of the free and phosphorylated ABI5 levels as well as an increase in the transcript levels of ABI5-regulated genes, *RD29A*, *RD29B* and *NCED3,* during seed development and seed germination [82].

PP2A holoenzyme assembly depends on Arabidopsis Phosphotyrosyl Phosphatase Activator (AtPTPA), a PTPA ortholog in Arabidopsis. Though it is expressed in most tissues, it is highly expressed in developing lateral root and reproductive tissues. The artificial microRNA (amiRNA) technique was used to study the down-regulation of the expression of *AtPTPA* in Arabidopsis and the effects of AtPTPA deficiency in plants. PP2Ac is methylated by suppressor of brassinosteroid insensitive1, Sbi1, a methylating enzyme. AtPTPA deficiency resulted in almost complete loss of Leu-309 methylation in PP2Ac as compared to the reduced PP2A activity than in *sbi1* mutant plants. The complete loss of PP2Ac methylation in the *sbi1* mutant results in 30% decrease in PP2A activity. Thus, the interaction of AtPTPA with PP2Ac may be required prior to methylation of PP2Ac by SBI1 in plants. Reduced *AtPTPA* expression results in decreased PP2A activity, decreased methylation in PP2A-C subunits, defective plant development, altered responses to ABA, ethylene and sodium chloride [58]. The catalytic subunit 5 of PP2A, PP2A-C5, regulates salt tolerance in Arabidopsis as *pp2a-c5–1* mutants showed salt hypersensitivity whereas *PP2A-C5*-overexpression lines were more salt tolerant [83]. The double mutants of *pp2a-c5* and *sos1–1*, *sos2–2* and *sos3–1* were hypersensitive to salt stress during seedling growth and root growth than their parental single mutants, indicating that PP2A-C5 acts independent of the SOS pathway. The C5-Com1 and C5-Com2, *PP2A-C5* OE lines in the *pp2a-c5-1* mutant background, showed higher *PP2A-C5* transcript levels than WT plants, but lower than in case of *PP2A-C5-OE* plants in the WT background. RT-PCR studies showed that the *PP2A-C5* transcripts were upregulated on salt exposure. The highest *PP2A-C5* transcript level was observed at 150 mM NaCl concentration in the *PP2A-C5*-OE plants. The *pp2a-c5-1* mutant plants showed shorter roots and smaller leaves under salt stress

whereas the *pp2a-c5-1* mutants expressing the *P35S:: PP2A-C5* transgene showed longer roots, similar to WT plants. This indicates that the *PP2A-C5* gene is involved in the salt sensitivity in the *pp2a-c5-1* mutant. The AtCLCa, AtCLCb, AtCLCc and AtCLCg, vacuolar membrane Chloride Channel (CLC) proteins, interact with PP2A-C5 as determined from the yeast two-hybrid analysis. The *AtCLCc* overexpression in Arabidopsis resulted in increased salt tolerance and $Cl^-$ accumulation in transgenic plants indicating that PP2A-C5-regulated salt tolerance may involve up-regulation of CLC function [84]. The soybean $Cl^-/H^+$ antiporter, GmCLC1, enhances salt tolerance by regulating chloride ion accumulation in soybean, poplars, Arabidopsis and yeast [85-87].

PP2Ac-2 catalytic subunit is a negative regulator of ABA-dependent gene expression as the *PP2AC-2* mutants are hypersensitive to ABA, whereas the *PP2Ac-2* overexpression lines are less sensitive to ABA than wild type. The *pp2ac-2abi1-1* double mutants show partial suppression of ABA insensitivity, with PP2Ac-2 acting either downstream of or at the level of ABI1 in ABA signaling. The transcriptome profiling of *pp2ac-2* and WT plants exposed to ABA was carried out and compared with whole-genome Arabidopsis long-oligonucleotide microarrays. ABA-treated *pp2ac-2* plants showed a total of 57 differentially expressed genes, with 20 upregulated genes and 37 downregulated genes (false discovery rate < 10% and a fold change > 1.5). More than 50% of the upregulated genes were found to be upregulated by ABA as well and 90% are downregulated by exposure to norfluorazon, an ABA biosynthesis inhibitor, according to the meta-analysis of the upregulated genes using Genevestigator (http://www.genevestigator.ethz.ch). The *pp2ac-2* mutants show elevated expression of ABA-regulated genes. The ABA-Response Element (ABRE) was the most overrepresented sequence in the promoters of differentially expressed genes as identified using promoter (http://bbc.botany.utoronto.ca) and motif analysis (TAIR). The PP2A activity showed fluctuations with decrease and increase of activity after ABA exposure in ABA-treated *pp2ac-2* and *PP2A*-OE plants. The *PP2Ac-2* expression and activity are antagonistically regulated by ABA through the restoration of the PP2A activity post ABA-exposure, indicating that ABA signaling requires the early release of PP2A repression, which allows ABA sensitivity to be reset post-induction [88].

TaPP2Ac-1, the catalytic subunit of PP2A isolated from the drought-tolerant wheat cultivar ‘Hanxuan10’, may be involved in drought stress responses as tobacco plants transformed with *pCAPE2-TaPP2Ac-1* constructs were observed to be tolerant to water deficit [89]. TaPP2AbB"-α, a novel regulatory subunit B identified in wheat, located in the cell membrane, cytoplasm and nucleus, interacts with both TaPP2Aa and TaPP2Ac. The expression level of *TaPP2AbB"-α* was observed to be upregulated in response to NaCl, Polyethylene Glycol (PEG), cold and ABA stresses. Root system was observed to be more developed in *TaPP2AbB"-α*-OE Arabidopsis plants than the control plants under normal conditions as well as on exposure to NaCl and mannitol suggesting that it may be involved in root growth and development [90].

PP2Ac proteins belonging to several plant species cluster into two subfamilies, I and II. Two tomato genes encode catalytic subunits of PP2A, *LePP2Ac1* and *LePP2Ac2*, belonging to subfamily I of *PP2Ac* genes. *LePP2Ac1* gene is rapidly induced in resistant tomato leaves in response to an avirulent strain of *Pseudomonas syringae* pv. *tomato*. The subfamily I of *PP2Ac* genes, suppressed using Virus-Induced Gene Silencing (VIGS), led to a fall in PP2A activity, constitutive expression of Pathogenesis-Related (*PR*) genes and localized cell death in stems and leaves. The plants were observed to be more resistant to a virulent strain of *P. syringae* pv. *tabaci*, exhibiting an increased Hypersensitive Response (HR) to effector proteins from the bacterial pathogen, *P. syringae* as well as the fungal pathogen, *Cladosporium fulvum*. Therefore, the catalytic subunits of PP2Ac subfamily I function as negative regulators of both AvrPto/Pto- and Avr9/Cf9-dependent signaling indicating that the signaling components common to the R genes recognizing both fungal and bacterial pathogens are targets of the PP2Ac [91].

## 5. PP2A AND THE PROTEOMIC PERSPECTIVE

Mass Spectrometry (nLCMS/MS) on tryptic peptides of immunoprecipitated PIN1:GFP protein isolated from seedling roots, led to the identification of seven different PIN1 peptides belonging to the large hydrophilic loop, one of which occurred in a non-phosphorylated and phosphorylated state. The *in vitro* and the *in vivo* phosphorylation assays showed that PID acts as a positive regulator whereas PP2A acts as a negative regulator of PIN phosphorylation [70]. Yeast two-hybrid, yeast three-hybrid and *in vivo* co-immunoprecipitation assays showed that FyPP1 (Phytochrome-associated serine/threonine protein phosphatase1), FyPP3, SAL (SAPS Domain-Like) and PP2AA proteins (RCN1 as well as PP2AA3) physically interact to form a PP6-type holoenzyme complex and interact with PIN proteins. Arabidopsis PP6-type phosphatase holoenzyme acts antagonistic to PID by regulating PIN phosphorylation to direct auxin polarity and plant development [92].

SnRK2-type protein kinases, PP2A-type protein phosphatases and proteins involved in lipid and galactolipid metabolism act as OST1-Interacting Proteins (OIPs). The PP2A-type protein phosphatase regulatory subunits, PP2AA and PP2AB’, interact with ABA-activated SnRK2-type protein kinases as *pp2a* double mutant combinations exhibited ABA hyposensitivity during seed germination and stomatal closure and hypersensitivity to ABA in root growth assays. The *in vivo* protein complex isolations of OST1-HF (6xHis-3xFLAG) tag and Liquid Chromatography (LC)-tandem mass spectrometry (MS/MS) experiments led to the identification of 120,299 peptides from The Arabidopsis Information Resource 10 (TAIR10) Protein Database. Five PP2A subunits and two putative subunits were identified, of which, four were detected only at low abundance. The co-IP of HF-PP2AB’β with mVenus-OST1 followed by phosphopeptide enrichment and LC-MS/MS analyses revealed that PP2AB’β showed phosphorylation at Ser-16, which is conserved in six of nine PP2AB’ subunits. The Ser-14 residue of PP2AB’γ, PP2AB’ζ and PP2AB’κ have a consensus SnRK2-type protein kinase target site indicating that SnRK2-type protein kinases may target PP2AB’ subunits. BiFC and co-IP studies showed the networking between SnRK2-type protein kinases

and PP2A- A and B' subunit proteins. Protein complexes of PP2AA1-3 with PP2AC subunits were observed in the cytoplasm while the PP2AB subunits showed differential distribution within the cell. The single PP2AA- and PP2AC-subunit fusion proteins were localized in the cytoplasm as well as nucleus and a low interaction of RCN1 with PP2AC5 was observed. The interaction of PP2AA2 was strongest with PP2AC3 while in case of PP2AA3, the interaction was strongest with PP2AC3-PP2AC5. These interactions were confirmed with yeast two-hybrid analyses, however, no interaction was observed between PP2AA3 and PP2AC4. Thus, the network of PP2AA and PP2AC subunits is in line with their partial overlap in ABA-mediated responses [93]. The phosphoproteome profiling of maize adventitious roots under low Pi stress showed that the PP2A catalytic subunit isoform 2 (PP2Ac2) exhibited dynamic temporal patterns under low Pi conditions while differential accumulation of PP2A subunits was absent [94].

Proteomic analysis using two-dimensional (2D) gels showed higher levels of 11 proteins in the soluble leaf extracts of *pp2a-b′γ* plants in comparison with WT plants. These include proteins related to: (a) Met-salvage pathway [S-adenosyl-L-homocysteine hydrolase (SAHH), adenosine kinase (ADK)], (b) amino acid metabolism, (c) the non-oxidative route of pentose phosphate pathway, (d) defense and stress [myrosinase thioglucoside glucohydrolase 1 (TGG1), carbonic anhydrase 1 (CA1), AtGSTF2, chloroplastic CSD2]; (e) metabolic enzymes [cytoplasmic Gln synthase 1 (AtGLN1;1), chloroplastic transketolase (TKL)]. The *pp2a-b′γ* plants showed decreased steady-state levels of peroxiredoxin Q and chloroplastic glutathione peroxidase 7. The phosphoprotein identification was done by analyzing the acquired MS/MS spectra against the Arabidopsis database, using the Ser and Thr phosphorylation modification in Mascot searches. The phosphopeptides, Gln synthase 2 (GS2), phosphoribulokinase, Ala glyoxylate aminotransferase (AGT1), SAHH1, TGG1, FBA and TKL, were detected. Though the transcript levels of Reactive Oxygen Species (ROS), SA, and JA/ ET-responsive genes were enhanced, however, the SA, JA levels were not affected indicating that *PR5* is epistatic to PP2A- B′γ in defense signaling, with B′γ operating downstream of hormonal responses. The decreased DNA methylation levels with the increased levels of components of the Met-salvage pathway, SAHH and ADK, indicate that PP2A-B′γ-dependent signaling events are involved in the regulation of defense signaling at the DNA level. Also, the *pp2a-b′γ* mutants show increased resistance to both a hemibiotroph, *Botrytis cinerea* and a necrotroph, *Pseudomonas syringae* pv tomato DC3000 [76]. The phosphoproteome of *pp2a-b′γ* mutant leaves analyzed using total leaf soluble extracts showed strong phosphorylation of calreticulin 1 (CRT1: At1g56340) indicating that PP2a-B′γ is involved in its dephosphorylation in wild type plants. Since calreticulins are involved in the Unfolded Protein Response (UPR), the *pp2a-b′γ* mutants undergo regulatory imbalances leading to ER-stress and premature yellowing of leaves [95]. Using immunoblotting, Data-dependent Acquisition (DDA) and Selected Reaction Monitoring (SRM) MS based approaches, it has been observed that PP2A-B’γ negatively regulates the abundance of alternative oxidase isoforms, AOX1A and AOX1D, in leaf mitochondria, evident from increased levels of AOX1A and AOX1D in *pp2a-b’γ* mutants, thereby, influencing foliar $H_2O_2$ metabolism and ROS homeostasis [96].

The role of PP2A-B′γ in controlling day length-dependent responses to intracellular oxidative stress was examined using catalase deficient mutant, *cat2,* which under Long Day (LD) conditions, shows reduced growth and Pathogenesis-Related (PR) responses. The formation of lesions observed in the *cat2pp2a-b′γ* double mutant under Short Day (SD) conditions was SA dependent. The *Phytochrome A* (*PHYA*) transcript levels were found to be decreased in the *pp2a-b′γ* mutant plants in comparison to WT plants. The phosphoproteome of *cat2*, *pp2a-b′γ, cat2 pp2a-b′γ* mutants and Col-0 WT, grown in SD conditions was analyzed using total soluble and membrane fractions. Differentially regulated proteins such as those involved in amino acid metabolism, SA-dependent proteins and redox homeostasis were identified in the *cat2pp2a-b′γ* mutant. These proteins include SAHH, GLN1;1, serine:glyoxylate aminotransferase (SGAT) /hydroxypyruvate reductase, pathogenesis-related proteins (PR2, PR5), ACC oxidase 2 (ACO2), Copper/Zinc superoxide dismutase 2 (CSD2), Glutathione S-transferase F2 (GSTF2), Protein disulfide isomerase 2 (PDI2), calreticulins (CRT1 and CRT2), *Arabidopsis thaliana* monodehydroascorbate reductase2 (AtMDAR2). Therefore, PP2A-B′γ is involved in the suppression of lesions resulting from oxidative stress and related defense responses in SD [97]. MS/MS analysis led to the identification of nine phosphopeptides from eight enzymes involved in TAG metabolism uniquely in the *b’θ*-1 mutant. The peptides such as acyl-CoA synthetase (LACS5), ketoacyl-CoA thiolase (KAT1), were phosphorylated at Ser or Thr residues except for one, indicating potential targets of PP2A [77].

## 6. PP2A AND METABOLIC ADJUSTMENTS

The metabolic alterations responsible for the lesions observed in the *cat2pp2a-b′γ* mutant plants under SD conditions have been identified through non-targeted metabolite profiling by gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) of up to 100 metabolites. The hierarchical clustering of metabolites showed a prominent effect on the metabolite profiles observed in the *cat2pp2a-b′γ* double mutant, whereas *cat2* and *pp2a-b′γ* mutations did not show any major effect. The accumulation of a large sub-cluster of compounds was observed in the *cat2pp2a-b′γ* double mutant, which were detected at low levels or were absent in the *cat2* single mutant under SD conditions. These metabolites were observed to accumulate in the *cat2* single mutant plants but not in *cat2sid2* double mutant plants, under LD conditions. Fifty-three metabolites including SA, nicotinic acid and gluconic acid, were identified in the double mutant that were significantly different from those in the wild-type seedlings. The double mutation in the *cat2pp2a-b′γ* plants showed a marked effect on many amino acids such as isoleucine, ornithine, threonine, lysine, β-alanine, GABA, phenylalanine, tyrosine, tryptophan and arginine. HPLC analysis of plants under SD conditions showed that the SA and camalexin levels in the double mutant were found to be similar to those observed in *cat2* in LD conditions, in comparison to their levels in *cat 2* mutant and the WT plants. Thus, the *pp2a-b′γ* mutation results in metabolic alterations

**Table 3. The table shows the PP2A subunits with the mutants used for their functional characterization, the concerned plant system and their functions.**

| S. No. | PP2A Subunit | Mutant Plants Used | Plant System | Function | References |
|---|---|---|---|---|---|
| 1. | ZmPP2AA1 | *ZmPP2AA1* OE , *ZmPP2AA1* RNAi | Maize | Root development, auxin signaling, low Pi responses | [53] |
| 2. | RCN1 | *rcn1* | Arabidopsis | Positive Transducer of ABA Signaling | [68] |
| 3. | RCN1 | *pp2aa2-1, pp2aa3-1; rcn1pp2aa2, rcn1pp2aa3, pp2aa2pp2aa3* double mutants | Arabidopsis | PP2A regulation, root growth and stress response | [30] |
| 4. | RCN1 | *rcn1 pp2aa2* and *rcn1 pp2aa3 double mutants, pp2aa2 pp2aa3 double mutants, rcn1, pp2aa2 and pp2aa3 single mutants, rcn1 RYA-32, rcn1 AYA-6, rcn1 AYA16* | Arabidopsis | postembryonic root development | [69] |
| 5. | PP2AA1, AA2, AA3 | *pp2aa1 pp2aa2, pp2aa1 pp2aa3 double-mutant; rcn1 pp2aa2* and *rcn1 pp2aa3, pp2aa1 pp2aa3 pid mutant seedlings; pp2aa1 pp2aa3 35S::PID plants; amiRNA lines* | Arabidopsis | transport-dependent auxin distribution in embryos and seedling roots, PID kinase and PP2A act antagonistically on reversible phosphorylation of PIN proteins | [70] |
| 6. | *OsPP2A-1* to *PP2A-5* | - | *Oryza sativa* | developmental stages, drought, salinity and heat stress | [61, 62] |
| 7. | PP2A-C3, -C4 | *c3, c4, c3 c4/+* and *c3/+c4* | Arabidopsis | PIN dephosphorylation and subcellular distribution, embryo patterning and root development | [32] |
| 8. | *LePP2Ac1*, *Ac2*, *Ac3*, *StPP2Ac1*, *2a*, *2b*, *c3* | - | Tomato, Potato | cold, salt and wounding stress responses | [63] |
| 9. | *LePP2Ac1*, *Ac2* | Silencing of *PP2Ac* using PVX or PVX::*PP2Ac* | *N. benthamiana* | negative regulator of both AvrPto/Pto- and Avr9/Cf9-dependent signaling | [91] |
| 10. | PP2A-C5 | *pp2a-c5, PP2A-C5-OE, double mutants of p2a-c5 and sos1–1, sos2–2 and sos3–1* | Arabidopsis | Interacts with vacuolar chloride channel proteins, up-regulation of CLC function, increased salt tolerance in plants | [83, 84] |
| 11. | B55 | *rcn1; pp2aa2pp2aa3* double mutants*; b55αb55β b55β, b55α, bsl2, bsl1, bsl3, PAPP5-OX1, papp5-1* | Arabidopsis | Interacts with nitrate reductase, NR dephosphorylation | [71] |
| 12. | PP2A-B55, B’γ | *pp2a-b55, pp2a-bα, pp2a-bβ, pp2a-b’γ, pp2a-b’γ-*complemented, *elf6* (early flowering control), *edm2* (late flowering control) | Arabidopsis | PP2A-B55 - negative regulator of flowering, PP2A-B’γ - positive regulator of flowering | [74] |

**(Table 3) contd….**

| S. No. | PP2A Subunit | Mutant Plants Used | Plant System | Function | References |
|---|---|---|---|---|---|
| 13. | PP2A-B′γ, B′ζ | *pp2a-b′γ, pp2a-b′ζ; pp2a-b′γζ* double mutants | Arabidopsis | required for optimal growth under favourable conditions; induction of photoprotective mechanisms and enhanced tolerance against abiotic stress; acclimation strategies upon environmental perturbations | [75] |
| 14. | PP2A-B'γ | *pp2a-b'γ,pp2a-b′ζ; pp2a-b′γζ* double mutants | Arabidopsis | Interacts with Aconitase 3, negatively regulates AOX1A and AOX1D; foliar $H_2O_2$ metabolism and ROS homeostasis | [96] |
| 15. | B'*θ* subunit of PP2A | *b'θ, pex 14* mutants | Arabidopsis | Interacts with A2, C2, and C5 Subunits, peroxisomal β-oxidation, protoauxin transformation, triacylglycerol mobilization; flowering; increases during senescence and in dessicated seeds | [77] |
| 16. | B'ζ | *b' ζ, b'η, b'γ* mutants | Arabidopsis | energy metabolism, highly expressed during senescence | [78] |
| | B'ζ, B'η, B'*θ*, B'γ | | Arabidopsis | regulation of innate immunity | |
| | B'η, B'*θ*, B'γ | | Arabidopsis | regulation of flowering time | |
| 17. | PP2A-B′*γ* | *pp2a-b′γ* mutant | Arabidopsis | negative regulator that prevents premature senescence and defense responses under normal conditions | [76] |
| 18. | PP2A-B′*γ* | *pp2a-b′γ* mutant | Arabidopsis | dephosphorylation of calreticulin 1 | [95] |
| 19. | PP2A-B′*γ* | *pp2a-b′γ, cat2, sid2, npr1 and cat2pp2a-b′γ* | Arabidopsis | controlling day length-dependent responses to intracellular oxidative stress; repression of SA-dependent PR responses under oxidative stress in SD | [97] |
| 20. | PP2A-B'γ, B'ζ | *pp2a-b'γ, pp2a-b'ζ; pp2a-b'γ ζ* double mutants | Arabidopsis | regulation of plant tolerance to aphid infestation | [73] |
| 21. | B"α, B"β | *rcn1, b″α-1, b″α-2, b″α-3, b″β-1* mutants; *B″β*-OE | Arabidopsis | Interacts with HMGR1S and HMGR1L, post-translational negative regulator of HMGR; positive regulator of *HMG1* transcript levels | [72] |
| 22. | TAP46 | *tap46-1, TAP46-OE* | Arabidopsis | Interacts with PP2A-associated protein, ABI5, negatively regulates PP2A activity, positive regulator in ABA signaling. | [82] |
| 23. | TAP46 | *TOR* RNAi Arabidopsis, DEX-Inducible *Tap46* RNAi Arabidopsis, Nb *Tap46* VIGS plants and Nb *Tap46* RNAi, *NahG*-OE *N. benthamiana, TRV:NbTap46* Lines, DEX-Inducible Nb *Tap46* RNAi Lines in BY-2 Cells | *N. benthamiana,* Arabidopsis, *tobacco (N. tabacum) BY-2 cells* | Interacts with PP2Ac, PP4 and PP6 , TOR signaling pathway | [81] |
| 24. | TaPP2Ac-1 | *pCAPE2-TaPP2Ac-1* | Tobacco | drought stress responses | [89] |
| 25. | TaPP2AbB"-α | *TaPP2AbB"-α* OE lines | Wheat, Arabidopsis | Interacts with TaPP2Aa and TaPP2Ac multi-stress responses | [90] |

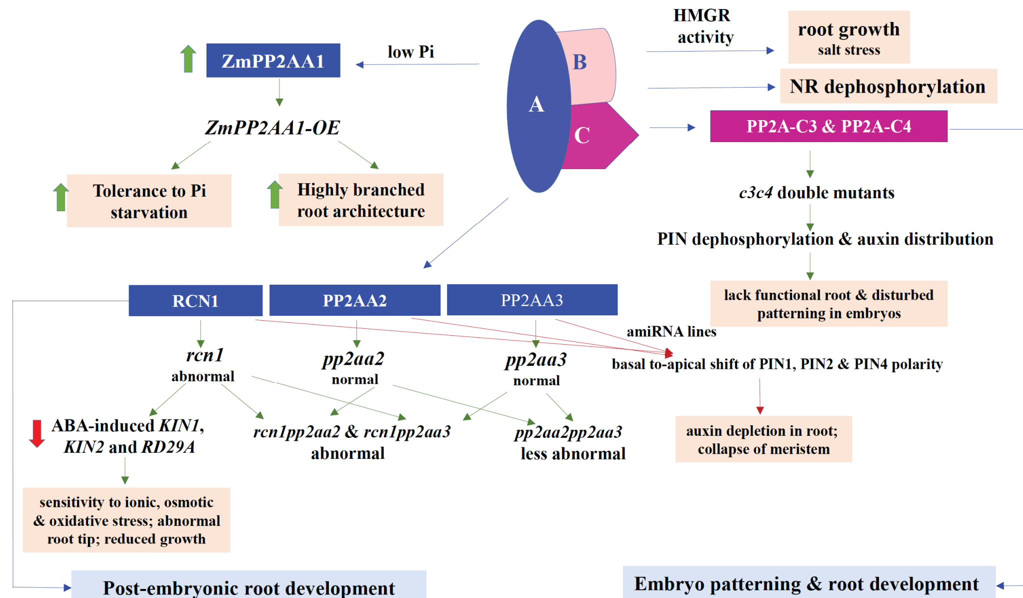


**Fig. 2(b).** The representation shown here depicts the regulation of auxin signaling and root growth by PP2As. ZmPP2AA1 is induced in roots by low phosphate (Pi) availability. The *ZmPP2AA1* OE Lines Promote LR and Axial Root (AR) formation resulting in a highly branched root architecture under low Pi that enabled Pi acquisition. The *ZmPP2AA1*-OE lines showed modulations in the free IAA levels and sensitivity to IAA or NPA from exogenous sources, as well as increased yields under Pi deficiency. RCN1-containing PP2A is involved in regulating post-embryonic root development by maintaining normal auxin distribution and stem cell function at the root apex. The loss-of-function mutants of *PP2AA2* and *PP2AA3* show normal phenotypes unlike the *rcn1* mutant plants. The *rcn1pp2aa2* and *rcn1pp2aa3* double mutants show abnormal development as compared to the *pp2aa2pp2aa3* double mutant. PP2A-C3 and PP2A-C4 are involved in controlling embryo patterning and root development by the regulation of PIN1 polarity and auxin distribution. The *c3c4* double mutants lack a functional root and the embryos have disturbed patterning, PIN1 polarity and auxin distribution. PP2As are involved in Nitrate Reductase (NR) activation as well as in the regulation of 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) activity.

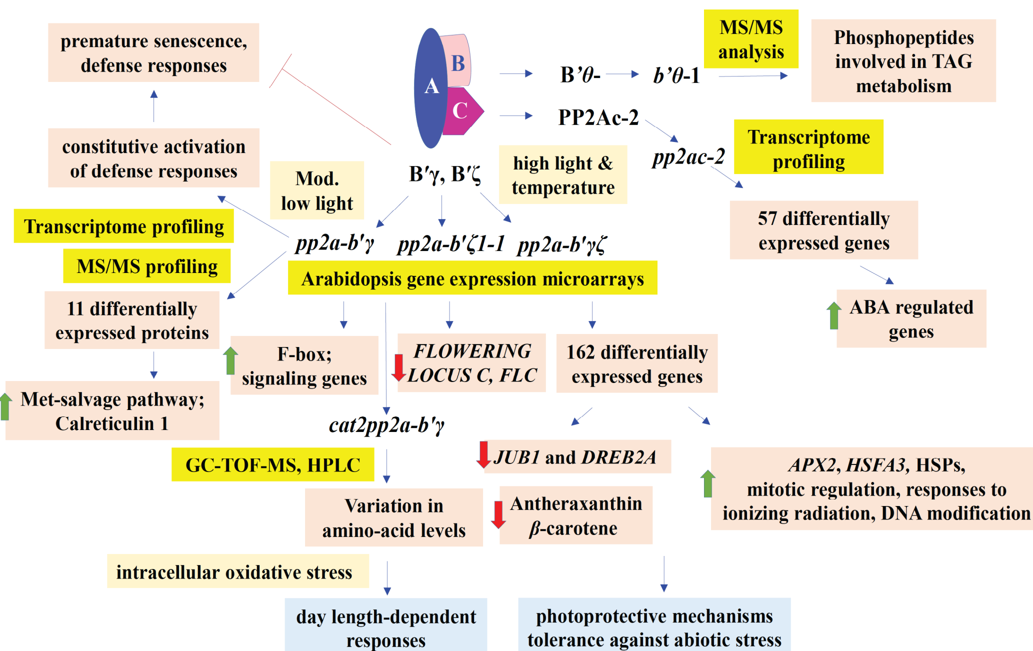


**Fig. 2(c).** The representation shown here depicts the methodologies such as gene expression microarrays, transcriptomics, proteomics, GC-TOF-MS, HPLC, used to expand the understanding of signaling by the B and C subunits of PP2As. PP2Ac-2 catalytic subunit is a negative regulator of ABA-dependent gene expression. Transcriptome profile of ABA-treated *pp2ac-2* plants showed 57 differentially expressed genes (20 upregulated and 37 downregulated genes). PP2A-B′γ and PP2A-B′ζ are necessary for optimal plant growth under favourable conditions in Arabidopsis. Differential transcript levels for 162 genes were observed in the *pp2a-b′γζ* mutant plants that were not expressed in *pp2a-b′γ* and *pp2a-b′ζ1-1* mutants. B′γ type regulatory subunit regulates disease resistance. The transcriptome profiling of *pp2a-b′γ* revealed that the *pp2a-b′γ* plants showed constitutive expression of defense-related genes. PP2A-B′γ-dependent signaling is involved in the regulation of defense signaling and in controlling day length-dependent responses to intracellular oxidative stress. The *pp2a-b′γ* plants showed higher levels of 11 proteins identified through proteomic analysis. The *B'θ* expression is involved in TAG metabolism as nine phosphopeptides from eight enzymes were unique to the *b'θ*-1 mutant as analyzed from LC-tandem Mass Spectrometry (MS/MS) analysis.

under intracellular oxidative stress in SD conditions, which are observed in LD conditions, in the presence of PP2A-B′γ depending on the isochorismate pathway of SA synthesis [97]. PP2A-B'γ has been observed to physically interact with the cytoplasmic metabolic enzyme, Aconitase 3, involved in mitochondrial respiration, oxidative stress responses and regulation of cell death in plants. The relative proportion of phosphorylated ACONITASE 3 unique peptide was higher in the leaves of *pp2a-b'γ* mutants than in the wild-type indicating that PP2A-B'γ is involved in controlling the phosphorylation of Aconitase 3 in the cytoplasm [96].

## CONCLUSION

Protein phosphatases in plant systems have received less attention as compared to their animal counterparts. In the post-genomic era, crop plants have been explored towards the development of stress tolerant varieties for the benefit of mankind. In this regard, regulatory molecules, which can be utilized to develop such varieties, need to be understood in the background of other networks operating in the plant systems as well to monitor crosstalk between different pathways. In such complex scenarios, the availability of high-throughput methodologies spanning the genomic, proteomic and metabolomic approaches, have opened up the possibilities to evaluate multiplicity of regulatory networks. The functional versatility of PP2As, (Table **3**), has benefited greatly by the integration of omics approaches into the conventional, yet essential, pharmacological, biochemical, genetic and molecular methods (Fig. **2c**). Traversing a signal through a cascade of components like receptors, secondary messengers, sensors, their interacting proteins, transcription factors, which transduce the signal and finally bring about a response by change in the gene expression and/or direct change in cellular physiology, is a huge challenge. PP2As and their networking with signaling components as well as other phosphatases need to be examined to fully understand their functional dimensions. However, the complete potential of omics approaches is still to be explored with respect to protein phosphatases and given the diversity of PP2A holoenzymes, leaves much to be discovered.

## LIST OF ABBREVIATIONS

Ser = Serine
Thr = Threonine
Tyr = Tyrosine
pS = phosphoserine
pT = phosphothreonine
pY = phosphotyrosine
STPs = Ser/Thr Phosphatases
PPP = Phosphoprotein Phosphatases
PPM = Metal-Dependent Phosphatases
PTP = Protein Tyrosine Phosphatases
DSPs = Dual-Specificity Phosphatases
SLP phosphatases = *Shewanella*-Like Protein Phosphatases
RLPH = Rhizobiales-Like Phosphatases
ALPH = ApaH-Like Phosphatases
PPKL = Protein Phosphatase with Kelch-like Repeat Domains
TON2 = TONNEAU2
TOR = Target of Rapamycin
HEAT = Huntingtin-Elongation-A subunit-TOR
TIPRL-1 = Target of Rapamycin Signaling Pathway Regulator-like-1
LCMT1 = Leucine Carboxyl Methyl Transferase 1
PTPA = Phosphotyrosyl Phosphatase Activator
PME-1 = PP2A Methyl Esterase 1
TAP46 = Type 2A Phosphatase-Associated Protein of 46 kD
PR = Primary Root
LR = Lateral Root
AR = Axial Root
Pi = Low Phosphate
RCN1 = Roots Curl in Naphthylphthalamic ACID1
$[Ca^{2+}]_{cyt}$ = Cytoplasmic $Ca^{2+}$Concentration
amiRNAs = Artificial microRNAs
NR = Nitrate Reductase
HMGR = 3-hydroxy-3-methylglutaryl CoA Reductase
HSFA3 = Heat Shock Factor A3
CPR5 = Constitutive Expression of *PR* genes5
EDS1 = Enhanced Disease Sensitivity1
JA = Jasmonic Acid
ET = Ethylene
TAG = Triacylglycerol
SBI1 = Suppressor of Brassinosteroid Insensitive1
CLC = Vacuolar Membrane Chloride Channel
FyPP1 = Phytochrome-Associated Serine/Threonine Protein Phosphatase1
SAL = Saps Domain-Like
OIPs = OST1-Interacting Proteins
LC-MS/MS = Liquid Chromatography-Tandem Mass Spectrometry
TAIR10 = The Arabidopsis Information Resource 10
SAHH = S-adenosyl-L-homocysteine Hydrolase
ADK = Adenosine Kinase
SGAT = Serine: Glyoxylate Aminotransferase
PR = Pathogenesis-related Proteins
ACO2 = ACC Oxidase 2

| | | |
|---|---|---|
| CSD2 | = | Copper/Zinc Superoxide Dismutase 2 |
| GSTF2 | = | Glutathione S-Transferase F2 |
| PDI | = | Protein Disulfide Isomerase |
| CRT | = | Calreticulin |
| AtMDAR2 | = | *Arabidopsis thaliana* Monodehydroascorbate Reductase2 |
| LACS5 | = | Long Chain acyl-CoA Synthetase 5 |
| KAT1 | = | Ketoacyl-CoA thiolase 1 |
| LD | = | Long Day |
| SD | = | Short Day |

## CONSENT FOR PUBLICATION

Not applicable.

## FUNDING

Research work in GKP's lab is supported by Department of Biotechnology (DBT), Department of Science and Technology (DST-PURSE grant), University Grant Commission (UGC-DRSIII grant), India.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Declared none.

## REFERENCES

[1] Bunnik, E.M.; Le Roch, K.G. An introduction to functional genomics and systems biology. *Adv. Wound Care (New Rochelle),* **2013**, *2*(9), 490-498.

[2] Lander, E.S.; Linton, L.M.; Birren, B.; Nusbaum, C.; Zody, M.C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; Funke, R.; Gage, D.; Harris, K.; Heaford, A.; Howland, J.; Kann, L.; Lehoczky, J.; LeVine, R.; McEwan, P.; McKernan, K.; Meldrim, J.; Mesirov, J.P.; Miranda, C.; Morris, W.; Naylor, J.; Raymond, C.; Rosetti, M.; Santos, R.; Sheridan, A.; Sougnez, C.; Stange-Thomann, Y.; Stojanovic, N.; Subramanian, A.; Wyman, D.; Rogers, J.; Sulston, J.; Ainscough, R.; Beck, S.; Bentley, D.; Burton, J.; Clee, C.; Carter, N.; Coulson, A.; Deadman, R.; Deloukas, P.; Dunham, A.; Dunham, I.; Durbin, R.; French, L.; Grafham, D.; Gregory, S.; Hubbard, T.; Humphray, S.; Hunt, A.; Jones, M.; Lloyd, C.; McMurray, A.; Matthews, L.; Mercer, S.; Milne, S.; Mullikin, J.C.; Mungall, A.; Plumb, R.; Ross, M.; Shownkeen, R.; Sims, S.; Waterston, R.H.; Wilson, R.K.; Hillier, L.W.; McPherson, J.D.; Marra, M.A.; Mardis, E.R.; Fulton, L.A.; Chinwalla, A.T.; Pepin, K.H.; Gish, W.R.; Chissoe, S.L.; Wendl, M.C.; Delehaunty, K.D.; Miner, T.L.; Delehaunty, A.; Kramer, J.B.; Cook, L.L.; Fulton, R.S.; Johnson, D.L.; Minx, P.J.; Clifton, S.W.; Hawkins, T.; Branscomb, E.; Predki, P.; Richardson, P.; Wenning, S.; Slezak, T.; Doggett, N.; Cheng, J.F.; Olsen, A.; Lucas, S.; Elkin, C.; Uberbacher, E.; Frazier, M.; Gibbs, R.A.; Muzny, D.M.; Scherer, S.E.; Bouck, J.B.; Sodergren, E.J.; Worley, K.C.; Rives, C.M.; Gorrell, J.H.; Metzker, M.L.; Naylor, S.L.; Kucherlapati, R.S.; Nelson, D.L.; Weinstock, G.M.; Sakaki, Y.; Fujiyama, A.; Hattori, M.; Yada, T.; Toyoda, A.; Itoh, T.; Kawagoe, C.; Watanabe, H.; Totoki, Y.; Taylor, T.; Weissenbach, J.; Heilig, R.; Saurin, W.; Artiguenave, F.; Brottier, P.; Bruls, T.; Pelletier, E.; Robert, C.; Wincker, P.; Smith, D.R.; Doucette-Stamm, L.; Rubenfield, M.; Weinstock, K.; Lee, H.M.; Dubois, J.; Rosenthal, A.; Platzer, M.; Nyakatura, G.; Taudien, S.; Rump, A.; Yang, H.; Yu, J.; Wang, J.; Huang, G.; Gu, J.; Hood, L.; Rowen, L.; Madan, A.; Qin, S.; Davis, R.W.; Federspiel, N.A.; Abola, AP.; Proctor, M.J.; Myers, R.M.; Schmutz, J.; Dickson, M.; Grimwood, J.; Cox, D.R.; Olson, M.V.; Kaul, R.; Raymond, C.; Shimizu, N.; Kawasaki, K.; Minoshima, S.; Evans, G.A.; Athanasiou, M.; Schultz, R.; Roe, B.A.; Chen, F.; Pan, H.; Ramser, J.; Lehrach, H.; Reinhardt, R.; McCombie, W.R.; de la Bastide, M.; Dedhia, N.; Blöcker, H.; Hornischer, K.; Nordsiek, G.; Agarwala, R.; Aravind, L.; Bailey, J.A.; Bateman, A.; Batzoglou, S.; Birney, E.; Bork, P.; Brown, D.G.; Burge, C.B.; Cerutti, L.; Chen, H.C.; Church, D.; Clamp, M.; Copley, R.R.; Doerks, T.; Eddy, S.R.; Eichler, E.E.; Furey, T.S.; Galagan, J.; Gilbert, J.G.; Harmon, C.; Hayashizaki, Y.; Haussler, D.; Hermjakob, H.; Hokamp, K.; Jang, W.; Johnson, L.S.; Jones, T.A.; Kasif, S.; Kaspryzk, A.; Kennedy, S.; Kent, W.J.; Kitts, P.; Koonin, E.V.; Korf, I.; Kulp, D.; Lancet, D.; Lowe, T.M.; McLysaght, A.; Mikkelsen, T.; Moran, J.V.; Mulder, N.; Pollara, V.J.; Ponting, C.P.; Schuler, G.; Schultz, J.; Slater, G.; Smit, A.F.; Stupka, E.; Szustakowki, J.; Thierry-Mieg, D.; Thierry-Mieg, J.; Wagner, L.; Wallis, J.; Wheeler, R.; Williams, A.; Wolf, Y.I.; Wolfe, K.H.; Yang, S.P.; Yeh, R.F.; Collins, F.; Guyer, M.S.; Peterson, J.; Felsenfeld, A.; Wetterstrand, K.A.; Patrinos, A.; Morgan, M.J.; de Jong, P.; Catanese, J.J.; Osoegawa, K.; Shizuya, H.; Choi, S.; Chen, Y.J.; Szustakowki, J. Initial sequencing and analysis of the human genome. *Nature,* **2001**, *409*(6822), 860-921.

[3] Venter, J.C.; Adams, M.D.; Myers, E.W.; Li, P.W.; Mural, R.J.; Sutton, G.G.; Smith, H.O.; Yandell, M.; Evans, C.A.; Holt, R.A.; Gocayne, J.D.; Amanatides, P.; Ballew, R.M.; Huson, D.H.; Wortman, J.R.; Zhang, Q.; Kodira, C.D.; Zheng, X.H.; Chen, L.; Skupski, M.; Subramanian, G.; Thomas, P.D.; Zhang, J.; Gabor, M.G.L.; Nelson, C.; Broder, S.; Clark, A.G.; Nadeau, J.; McKusick, V.A.; Zinder, N.; Levine, A.J.; Roberts, R.J.; Simon, M.; Slayman, C.; Hunkapiller, M.; Bolanos, R.; Delcher, A.; Dew, I.; Fasulo, D.; Flanigan, M.; Florea, L.; Halpern, A.; Hannenhalli, S;. Kravitz, S.; Levy, S.; Mobarry, C.; Reinert, K.; Remington, K.; Abu-Threideh, J.; Beasley, E.; Biddick, K.; Bonazzi, V.; Brandon, R.; Cargill, M.; Chandramouliswaran, I.; Charlab, R.; Chaturvedi, K.; Deng, Z.; Di Francesco, V.; Dunn, P.; Eilbeck, K.; Evangelista, C.; Gabrielian, A.E.; Gan, W.; Ge, W.; Gong, F.; Gu, Z.; Guan, P.; Heiman, T.J.; Higgins, M.E.; Ji, R.R.; Ke, Z.; Ketchum, K.A.; Lai, Z.; Lei, Y.; Li, Z.; Li, J.; Liang, Y.; Lin, X.; Lu, F.; Merkulov, G.V.; Milshina, N.; Moore, H.M.; Naik, A.K.; Narayan, V.A.; Neelam, B.; Nusskern, D.; Rusch, D.B.; Salzberg, S.; Shao, W.; Shue, B.; Sun, J.; Wang, Z.; Wang, A.; Wang, X.; Wang, J.; Wei, M.; Wides, R.; Xiao, C.; Yan, C.; Yao, A.; Ye, J.; Zhan, M.; Zhang, W.; Zhang, H.; Zhao, Q.; Zheng, L.; Zhong, F.; Zhong, W.; Zhu, S.; Zhao, S.; Gilbert, D.; Baumhueter, S.; Spier, G.; Carter, C.; Cravchik, A.; Woodage, T.; Ali, F.; An, H.; Awe, A.; Baldwin, D.; Baden, H.; Barnstead, M.; Barrow, I.; Beeson, K.; Busam, D.; Carver, A.; Center, A.; Cheng, M.L.; Curry, L.; Danaher, S.; Davenport, L.; Desilets, R.; Dietz, S.; Dodson, K.; Doup, L.; Ferriera, S.; Garg, N.; Gluecksmann, A.; Hart, B.; Haynes, J.; Haynes, C.; Heiner, C.; Hladun, S.; Hostin, D.; Houck, J.; Howland, T.; Ibegwam, C.; Johnson, J.; Kalush, F.; Kline, L.; Koduru, S.; Love, A.; Mann, F.; May, D.; McCawley, S.; McIntosh, T.; McMullen, I.; Moy, M.; Moy, L.; Murphy, B.; Nelson, K.; Pfannkoch, C.; Pratts, E.; Puri, V.; Qureshi, H.; Reardon, M.; Rodriguez, R.; Rogers, Y.H.; Romblad, D.; Ruhfel, B.; Scott, R.; Sitter, C.; Smallwood, M.; Stewart, E.; Strong, R.; Suh, E.; Thomas, R.; Tint, N.N.; Tse, S.; V.C.; Wang, G.; Wetter, J.; Williams, S.; Williams, M.; Windsor, S.; Winn-Deen, E.; Wolfe, K.; Zaveri, J.; Zaveri, K.; Abril, J.F.; Guigó, R.; Campbell, M.J.; Sjolander, K.V.; Karlak, B.; Kejariwal, A.; Mi, H.; Lazareva, B.; Hatton, T.; Narechania, A.; Diemer, K.; Muruganujan, A.; Guo, N.; Sato, S.; Bafna, V.; Istrail, S.; Lippert, R.; Schwartz, R.; Walenz, B.; Yooseph, S.; Allen, D.; Basu, A.; Baxendale, J.; Blick, L.; Caminha, M.; Carnes-Stine, J.; Caulk, P.; Chiang, Y.H.; Coyne, M.; Dahlke, C.; Mays, A.; Dombroski, M.; Donnelly, M.; Ely, D.; Esparham, S.; Fosler, C.; Gire, H.; Glanowski, S.; Glasser, K.; Glodek, A.; Gorokhov, M.; Graham, K.; Gropman, B.; Harris, M.;

Heil, J.; Henderson, S.; Hoover, J.; Jennings, D.; Jordan, C.; Jordan, J.; Kasha, J.; Kagan, L.; Kraft, C.; Levitsky, A.; Lewis, M.; Liu, X.; Lopez, J.; Ma, D.; Majoros, W.; McDaniel, J.; Murphy, S.; Newman, M.; Nguyen, T.; Nguyen, N.; Nodell, M.; Pan, S.; Peck, J.; Peterson, M.; Rowe, W.; Sanders, R.; Scott, J.; Simpson, M.; Smith, T.; Sprague, A.; Stockwell, T.; Turner, R.; Venter, E.; Wang, M.; Wen, M.; Wu, D.; Wu, M.; Xia, A.; Zandieh, A.; Zhu, X. The sequence of the human genome. *Science,* **2001**, *291*(5507), 1304-1351.

[4] Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **2000**, *408*(6814), 796-815.

[5] Chen, F.; Dong, W.; Zhang, J.; Guo, X.; Chen, J.; Wang, Z.; Lin, Z.; Tang, H.; Zhang, L. The sequenced angiosperm genomes and genome databases. *Front. Plant Sci.* **2018**, *9*, 418.

[6] Moorhead, G.B.G.; De Wever, V.; Templeton, G.; Kerk, D. Evolution of protein phosphatases in plants and animals. *Biochem. J.* **2009**, *417*(2), 401-409.

[7] Barford, D. Molecular mechanisms of the protein serine/threonine phosphatases. *Trends Biochem. Sci.,* **1996**, *21*(11), 407-412.

[8] Hunter, T. Protein phosphorylation: what does the future hold? In: *Life Sciences for the 21st Century*; Ehud Keinan, I.S.; Sela, M.; Eds.; Wiley, Hoboken, NJ, **2004**; pp 191-223.

[9] Hubbard, M.J.; Cohen, P. On target with a new mechanism for the regulation of protein phosphorylation. *Trends Biochem. Sci.,* **1993**, *18*(5), 172-177.

[10] Olsen, J.V.; Vermeulen, M.; Santamaria, A.; Kumar, C.; Miller, M. L.; Jensen, L.J.; Gnad, F.; Cox, J.; Jensen, T.S.; Nigg, E.A.; Brunak, S.; Mann, M. Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. *Sci. Signal.* **2010**, *3*(104), ra3.

[11] Sharma, K.; D'Souza, R.C.J.; Tyanova, S.; Schaab, C.; Wiśniewski, J.R.; Cox, J.; Mann, M. Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. *Cell Rep.,* **2014**, *8*(5), 1583-1594.

[12] Nakagami, H.; Sugiyama, N.; Mochida, K.; Daudi, A.; Yoshida, Y.; Toyoda, T.; Tomita, M.; Ishihama, Y.; Shirasu, K. Large-scale comparative phosphoproteomics identifies conserved phosphorylation sites in plants. *Plant Physiol.,* **2010**, *153*(3), 1161-1174.

[13] Sugiyama, N.; Nakagami, H.; Mochida, K.; Daudi, A.; Tomita, M.; Shirasu, K.; Ishihama, Y. Large-scale phosphorylation mapping reveals the extent of tyrosine phosphorylation in arabidopsis. *Mol. Syst. Biol.,* **2008**, *4*(1), 193.

[14] Nguyen, T.H.N.; Brechenmacher, L.; Aldrich, J.T.; Clauss, T.R.; Gritsenko, M.A.; Hixson, K.K.; Libault, M.; Tanaka, K.; Yang, F.; Yao, Q.; Pasa-Tolić, L.; Xu, D.; Nguyen, H.T.; Stacey, G. Quantitative phosphoproteomic analysis of soybean root hairs inoculated with *Bradyrhizobium japonicum*. *Mol. Cell. Proteomics,* **2012**, *11*(11), 1140-1155.

[15] Grimsrud, P.A.; den Os, D.; Wenger, C.D.; Swaney, D.L.; Schwartz, D.; Sussman, M.R.; Ane, J.-M.; Coon, J.J. Large-scale phosphoprotein analysis in *Medicago truncatula* roots provides insight into *in vivo* kinase activity in legumes. *Plant Physiol.,* **2010**, *152*(1), 19-28.

[16] Olsen, J.V.; Blagoev, B.; Gnad, F.; Macek, B.; Kumar, C.; Mortensen, P.; Mann, M. Global, *in vivo*, and site-specific phosphorylation dynamics in signaling networks. *Cell* **2006**, *127*(3), 635-648.

[17] Tonks, N.K. Protein tyrosine phosphatases-from housekeeping enzymes to master regulators of signal transduction. *FEBS J.,* **2013**, *280*(2), 346-378.

[18] Kerk, D.; Templeton, G.; Moorhead, G.B.G. Evolutionary radiation pattern of novel protein phosphatases revealed by analysis of protein data from the completely sequenced genomes of humans, green algae, and higher plants. *Plant Physiol.,* **2008**, *146*(2), 351-367.

[19] Uhrig, R.G.; Labandera, A.-M.; Moorhead, G.B. Arabidopsis PPP family of serine/threonine protein phosphatases: many targets but few engines. *Trends Plant Sci.,* **2013**, *18*(9), 505-513.

[20] Keyse, S.M. An emerging family of dual specificity MAP kinase phosphatases. *Biochim. Biophys. Acta,* **1995**, *1265*(2-3), 152-160.

[21] Brautigan, D.L. Protein Ser/Thr phosphatases - the ugly ducklings of cell signalling. *FEBS J.,* **2013**, *280*(2), 324-325.

[22] Andreeva, A.V; Kutuzov, M.A. Widespread presence of "Bacterial-like" PPP phosphatases in eukaryotes. *BMC Evol. Biol.,* **2004**, *4*(1), 47.

[23] Das, A.K.; Helps, N.R.; Cohen, P.T.; Barford, D. Crystal structure of the protein serine/threonine phosphatase 2C at 2.0 A resolution. *EMBO J.,* **1996**, *15*(24), 6798-6809.

[24] Ingebritsen, T.S.; Foulkes, J.G.; Cohen, P. The protein phosphatases involved in cellular regulation. 2. glycogen metabolism. *Eur. J. Biochem.,* **1983**, *132*, 263-274.

[25] Tonks, N.K. Protein tyrosine phosphatases: from genes, to function, to disease. *Nat. Rev. Mol. Cell Biol.,* **2006**, *7*(11), 833-846.

[26] Mayer-Jaekel, R.E.; Hemmings, B.A. Protein phosphatase 2A - a "Ménage à Trois." In: *Trends in Cell Biology*. Elsevier Current Trends: **1994**; Vol. *4*(8), pp. 287-291.

[27] Groves, M.R.; Hanlon, N.; Turowski, P.; Hemmings, B.A.; Barford, D. The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs. *Cell,* **1999**, *96*(1), 99-110.

[28] Farkas, I.; Dombrádi, V.; Miskei, M.; Szabados, L.; Koncz, C. Arabidopsis PPP family of serine/threonine phosphatases. *Trends Plant Sci.,* **2007**, *12*(4), 169-176.

[29] Slabas, A.R.; Fordham-Skelton, A.P.; Fletcher, D.; Martinez-Rivas, J.M.; Swinhoe, R.; Croy, R.R.D.; Evans, I.M. Characterisation of CDNA and genomic clones encoding homologues of the 65 KDa regulatory subunit of protein phosphatase 2A in *Arabidopsis thaliana*. *Plant Mol. Biol.,* **1994**, *26*(4), 1125-1138.

[30] Zhou, H.-W.; Nussbaumer, C.; Chao, Y.; DeLong, A. Disparate roles for the regulatory a subunit isoforms in arabidopsis protein phosphatase 2A. *Plant Cell,* **2004**, *16*(3), 709-722.

[31] Ariño, J.; Pérez-Callejón, E.; Cunillera, N.; Camps, M.; Posas, F.; Ferrer, A. Protein phosphatases in higher plants: multiplicity of type 2A phosphatases in *Arabidopsis thaliana*. *Plant Mol. Biol.,* **1993**, *21*(3), 475-485.

[32] Ballesteros, I.; Domínguez, T.; Sauer, M.; Paredes, P.; Duprat, A.; Rojo, E.; Sanmartín, M.; Sánchez-Serrano, J.J. Specialized functions of the PP2A subfamily II catalytic subunits PP2A-C3 and PP2A-C4 in the distribution of auxin fluxes and development in arabidopsis. *Plant J.,* **2013**, *73*(5), 862-872.

[33] Corum, J.W.; Hartung, A.J.; Stamey, R.T.; Rundle, S.J. Characterization of DNA sequences encoding a novel isoform of the 55 KDa B regulatory subunit of the type 2A protein serine/threonine phosphatase of *Arabidopsis thaliana*. *Plant Mol. Biol.,* **1996**, *31*(2), 419-427.

[34] Janssens, V.; Goris, J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem. J.,* **2001**, *353*(Pt 3), 417-439.

[35] White, R.J.; Davis, M.; Esmon, C.A.; Myrick, T.L.; Cochran, D.S.; Rundle, S.J. Functional analysis of the B′ subunit of arabidopsis protein phosphatase type 2A. *Plant Sci.,* **2002**, *162*(2), 201-209.

[36] Camilleri, C.; Azimzadeh, J.; Pastuglia, M.; Bellini, C.; Grandjean, O.; Bouchez, D. The arabidopsis TONNEAU2 gene encodes a putative novel protein phosphatase 2A regulatory subunit essential for the control of the cortical cytoskeleton. *Plant Cell,* **2002**, *14*(4), 833-845.

[37] Terol, J.; Bargues, M.; Carrasco, P.; Pérez-Alonso, M.; Paricio, N. Molecular characterization and evolution of the protein phosphatase 2A B' regulatory subunit family in plants. *Plant Physiol.,* **2002**, *129*(2), 808-822.

[38] Booker, M.A.; DeLong, A. Atypical protein phosphatase 2A gene families do not expand *via* paleopolyploidization. *Plant Physiol.,* **2017**, *173*(2), 1283-1300.

[39] Kerk, D.; Bulgrien, J.; Smith, D.W.; Barsam, B.; Veretnik, S.; Gribskov, M. The complement of protein phosphatase catalytic subunits encoded in the genome of arabidopsis. *Plant Physiol.,* **2002**, *129*(2), 908-925.

[40] Burge, C.; Karlin, S. Prediction of complete gene structures in human genomic DNA. *J. Mol. Biol.,* **1997**, *268*(1), 78-94.

[41] Lukashin, A.V; Borodovsky, M. GeneMark. Hmm: new solutions for gene finding. *Nucleic Acids Res.,* **1998**, *26*(4), 1107-1115.

[42] Xue, T.; Wang, D.; Zhang, S.; Ehlting, J.; Ni, F.; Jakab, S.; Zheng, C.; Zhong, Y. Genome-wide and expression analysis of protein phosphatase 2C in rice and arabidopsis. *BMC Genomics,* **2008**, *9*, 550.

[43] Kim, S.; Park, M.; Yeom, S.I.; Kim, Y.M.; Lee, J.M.; Lee, H.A.; Seo, E.; Choi, J.; Cheong, K.; Kim, K.T.; Jung, K.; Lee, G.W.; Oh, S.K.; Bae, C.; Kim, S.B.; Lee, H.Y.; Kim, S.Y.; Kim, M.S.; Kang, B.C.; Jo, Y.D.; Yang, H.B.; Jeong, H.J.; Kang, W.H.; Kwon, J.K.; Shin, C.; Lim, J.Y.; Park, J.H.; Huh, J.H.; Kim, J.S.; Kim, B.D.; Cohen, O.; Paran, I.; Suh, M.C.; Lee, S.B.; Kim, Y.K.; Shin, Y.; Noh, S.J.; Park, J.; Seo, Y.S.; Kwon, S.Y.; Kim, H.A.; Park, J.M.; Kim, H.J.; Choi, S.B.; Bosland, P.W.; Reeves, G.; Jo, S.H.; Lee, B.W.; Cho, H.T.; Choi, H.S.; Lee, M.S.; Yu, Y.; Do Choi, Y.; Park, B.S.; van Deynze, A.; Ashrafi, H.; Hill, T.; Kim, W.T.; Pai, H.S.; Ahn, H.K.; Yeam, I.; Giovannoni, J.J.; Rose, J.K.; Sørensen, I.; Lee, S.J.; Kim, R.W.; Choi, I.Y.; Choi, B.S.; Lim, J.S.; Lee, Y.H.; Choi, D. Genome sequence of the hot pepper provides insights into the evolution of pungency in capsicum species. *Nat. Genet.* **2014**, *46*(3), 270-278.

[44] Singh, A.; Giri, J.; Kapoor, S.; Tyagi, A.K.; Pandey, G.K. Protein phosphatase complement in rice: genome-wide identification and transcriptional analysis under abiotic stress conditions and reproductive development. *BMC Genomics,* **2010**, *11*(1), 435.

[45] Van Hoof, C.; Goris, J. Phosphatases in apoptosis: to be or not to be, PP2A is in the heart of the question. *Biochim. Biophys. Acta,* **2003**, *1640*(2-3), 97-104.

[46] Codreanu, S.G.; Adams, D.G.; Dawson, E.S.; Wadzinski, B.E.; Liebler, D.C. Inhibition of protein phosphatase 2A activity by selective electrophile alkylation damage. *Biochemistry,* **2006**, *45*(33), 10020-10029.

[47] Turowski, P.; Fernandez, A.; Favre, B.; Lamb, N.J.; Hemmings, B.A. Differential methylation and altered conformation of cytoplasmic and nuclear forms of protein phosphatase 2A during cell cycle progression. *J. Cell Biol.,* **1995**, *129*(2), 397-410.

[48] Perrotti, D.; Neviani, P. Protein phosphatase 2A: a target for anticancer therapy. *Lancet Oncol.,* **2013**, *14*(6), e229-e238.

[49] Sontag, J.-M.; Sontag, E. Protein phosphatase 2A dysfunction in alzheimer's disease. *Front. Mol. Neurosci.,* **2014**, *7*, 16.

[50] Samofalova, D.A.; Karpov, P.A.; Nuporko, A.Y.; Blume, Y.B. Reconstruction of the spatial structure of plant phosphatases types 1 and 2A in complexes with okadaic acid. *Cytol. Genet.,* **2011**, *45*(3), 153-162.

[51] Cho, U.S.; Xu, W. Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. *Nature,* **2007**, *445*(7123), 53-57.

[52] Magnusdottir, A.; Stenmark, P.; Flodin, S.; Nyman, T.; Kotenyova, T.; Gräslund, S.; Ogg, D.; Nordlund, P. The structure of the PP2A regulatory subunit B56γ: the remaining piece of the PP2A jigsaw puzzle. *Proteins Struct. Funct. Bioinforma.,* **2009**, *74*(1), 212-221.

[53] Wang, J.; Pei, L.; Jin, Z.; Zhang, K.; Zhang, J. Overexpression of the protein phosphatase 2A regulatory subunit a gene ZmPP2AA1 improves low phosphate tolerance by remodeling the root system architecture of maize. *PLoS One,* **2017**, *12*(4), e0176538.

[54] Yang, J.; Roe, S.M.; Prickett, T.D.; Brautigan, D.L.; Barford, D. The structure of Tap42/A4 reveals a tetratricopeptide repeat-like fold and provides insights into PP2A regulation. *Biochemistry,* **2007**, *46*(30), 88078815.

[55] Xing, Y.; Li, Z.; Chen, Y.; Stock, J.B.; Jeffrey, P.D.; Shi, Y. Structural mechanism of demethylation and inactivation of protein phosphatase 2A. *Cell,* **2008**, *133*(1), 154-163.

[56] Wu, G.; Wang, X.; Li, X.; Kamiya, Y.; Otegui, M.S.; Chory, J. Methylation of a phosphatase specifies dephosphorylation and degradation of activated brassinosteroid receptors. *Sci. Signal.,* **2011**, *4*(172), ra29.

[57] Sents, W.; Ivanova, E.; Lambrecht, C.; Haesen, D.; Janssens, V. The biogenesis of active 2A Holoenzymes: a tightly regulated process creating protein phosphatase phosphatase specificity. *FEBS J.,* **2013**, *280*(2), 644-661.

[58] Chen, J.; Hu, R.; Zhu, Y.; Shen, G.; Zhang, H. Arabidopsis phosphotyrosyl phosphatase activator is essential for protein phosphatase 2A holoenzyme assembly and plays important roles in hormone signaling, salt stress response, and plant development. *Plant Physiol.,* **2014**, *166*(3), 1519-1534.

[59] Tang, W.; Yuan, M.; Wang, R.; Yang, Y.; Wang, C.; Oses-Prieto, J. A.; Kim, T.W.; Zhou, H.W.; Deng, Z.; Gampala, S.S.; Gendron, J.M.; Jonassen, E.M.; Lillo, C.; DeLong, A.; Burlingame, A.L.; Sun, Y.; Wang, Z.Y. PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. *Nat. Cell Biol.,* **2011**, *13*(2), 124-131.

[60] Csordás T.É.; Vissi, E.; Kovács, I.; Szöke, A.; Ariño, J.; Gergely, P.; Dudits, D.; Dombrádi, V. Protein phosphatase 2A holoenzyme and its subunits from *Medicago sativa*. *Plant Mol. Biol.,* **2000**, *43*(4), 527-536.

[61] Yu, R.; Zhou, Y.; Xu, Z.-F.; Chye, M.-L.; Kong, R.Y.C. Two genes encoding protein phosphatase 2A catalytic subunits are differentially expressed in rice. *Plant Mol. Biol.,* **2003**, *51*(3), 295-311.

[62] Yu, R.M.K.; Wong, M.M.L.; Jack, R.W.; Kong, R.Y.C. Structure, evolution and expression of a second subfamily of protein phosphatase 2A catalytic subunit genes in the rice plant (*Oryza sativa* L.). *Planta,* **2005**, *222*(5), 757-768.

[63] País, S.M.; González, M.A.; Téllez-Iñón, M.T.; Capiati, D.A. Characterization of potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) protein phosphatases type 2A catalytic subunits and their involvement in stress responses. *Planta,* **2009**, *230*(1), 13-25.

[64] Garbers, C.; DeLong, A.; Deruére, J.; Bernasconi, P.; Söll, D. A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in arabidopsis. *EMBO J.,* **1996**, *15*(9), 2115-2124.

[65] Deruère, J.; Jackson, K.; Garbers, C.; Söll, D.; Delong, A. The RCN1-encoded a subunit of protein phosphatase 2A increases phosphatase activity *in vivo*. *Plant J.,* **1999**, *20*(4), 389-399.

[66] Larsen, P.B.; Cancel, J.D. Enhanced ethylene responsiveness in the arabidopsis Eer1 mutant results from a loss-of-function mutation in the protein phosphatase 2A A regulatory subunit, RCN1. *Plant J.,* **2003**, *34*(5), 709-718.

[67] Rashotte, A.M.; DeLong, A.; Muday, G.K. Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response, and lateral root growth. *Plant Cell,* **2001**, *13*(7), 1683-1697.

[68] Kwak, J.M.; Moon, J.-H.; Murata, Y.; Kuchitsu, K.; Leonhardt, N.; DeLong, A.; Schroeder, J.I. Disruption of a guard cell-expressed protein phosphatase 2A regulatory subunit, RCN1, confers abscisic acid insensitivity in arabidopsis. *Plant Cell,* **2002**, *14*(11), 2849-2861.

[69] Blakeslee, J.J.; Zhou, H.-W.; Heath, J.T.; Skottke, K.R.; Barrios, J.A.R.; Liu, S.-Y.; DeLong, A. Specificity of RCN1-mediated protein phosphatase 2A regulation in meristem organization and stress response in roots. *Plant Physiol.,* **2007**, *146*(2), 539-553.

[70] Michniewicz, M.; Zago, M.K.; Abas, L.; Weijers, D.; Schweighofer, A.; Meskiene, I.; Heisler, M.G.; Ohno, C.; Zhang, J.; Huang, F.; Schwab, R.; Weigel, D.; Meyerowitz, E.M.; Luschnig, C.; Offringa, R.; Friml, J. Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell,* **2007**, *130*(6), 1044-1056.

[71] Heidari, B.; Matre, P.; Nemie-Feyissa, D.; Meyer, C.; Rognli, O. A.; Moller, S.G.; Lillo, C. Protein phosphatase 2A B55 and A regulatory subunits interact with nitrate reductase and are essential for nitrate reductase activation. *Plant Physiol.,* **2011**, *156*(1), 165-172.

[72] Leivar, P.; Antolín-Llovera, M.; Ferrero, S.; Closa, M.; Arró, M.; Ferrer, A.; Boronat, A.; Campos, N. Multilevel control of *Arabidopsis* 3-hydroxy-3-methylglutaryl coenzyme A reductase by protein phosphatase 2A. *Plant Cell,* **2011**, *23*(4), 1494-1511.

[73] Rasool, B.; Karpinska, B.; Konert, G.; Durian, G.; Denessiouk, K.; Kangasj Ã.S.; Foyer, C.H. Effects of light and the regulatory b-subunit composition of protein phosphatase 2A on the susceptibility of *Arabidopsis thaliana* to Aphid (*Myzus persicae*) infestation. *Front. Plant Sci.,* **2014**, *5*, 405.

[74] Heidari, B.; Nemie-Feyissa, D.; Kangasjärvi, S.; Lillo, C. Antago-

nistic regulation of flowering time through distinct regulatory subunits of protein phosphatase 2A. *PLoS One,* **2013**, *8*(7), e67987.

[75] Konert, G.; Rahikainen, M.; Trotta, A.; Durian, G.; Salojärvi, J.; Khorobrykh, S.; Tyystjärvi, E.; Kangasjärvi, S. Subunits B′ $\gamma$ and B′ $\zeta$ of protein phosphatase 2A regulate photo-oxidative stress responses and growth in *Arabidopsis thaliana*. *Plant. Cell Environ.,* **2015**, *38*(12), 2641-2651.

[76] Trotta, A.; Wrzaczek, M.; Scharte, J.; Tikkanen, M.; Konert, G.; Rahikainen, M.; Holmstrom, M.; Hiltunen, H.-M.; Rips, S.; Sipari, N.; Mulo, P.; Weis, E.; von Schaewen, A.; Aro, E.M.; Kangasjärvi, S. Regulatory subunit B' of protein phosphatase 2A prevents unnecessary defense reactions under low light in arabidopsis. *Plant Physiol.,* **2011**, *156*(3), 1464-1480.

[77] Kataya, A.; Heidari, B.; Hagen, L.; Kommedal, R.; Slupphaug, G.; Lillo, C. Protein phosphatase 2A holoenzyme is targeted to peroxisomes by piggybacking and positively affects peroxisomal β-oxidation. *Plant Physiol.,* **2015**, *167*(2), 493-506.

[78] Kataya, A.; Heidari, B.; Lillo, C. Protein phosphatase 2A regulatory subunits affecting plant innate immunity, energy metabolism, and flowering time – joint functions among B'η subfamily members. *Plant Signal. Behav.* **2015**, *10*(5), e1026024.

[79] Harris, D. M.; Myrick, T. L.; Rundle, S. J. The arabidopsis homolog of yeast TAP42 and mammalian alpha4 binds to the catalytic subunit of protein phosphatase 2A and is induced by chilling. *Plant Physiol.,* **1999**, *121*(2), 609-617.

[80] Binh, le T.; Oono, K. Molecular cloning and characterization of genes related to chilling tolerance in Rice. *Plant Physiol.,* **1992**, *99*(3), 1146-1150.

[81] Ahn, C.S.; Han, J.-A.; Lee, H.-S.; Lee, S.; Pai, H.-S. The PP2A regulatory subunit Tap46, a component of the TOR signaling pathway, modulates growth and metabolism in plants. *Plant Cell* **2011**, *23*(1), 185-209.

[82] Hu, R.; Zhu, Y.; Shen, G.; Zhang, H. TAP46 plays a positive role in the Abscisic Acid Insensitive5-Regulated gene expression in arabidopsis. *Plant Physiol.,* **2014**, *164*(2), 721-734.

[83] Hu, R.; Zhu, Y.; Shen, G.; Zhang, H. Overexpression of the PP2A-C5 gene confers increased salt tolerance in *Arabidopsis thaliana*. *Plant Signal. Behav.,* **2017**, *12*(2), e1276687.

[84] Hu, R.; Zhu, Y.; Wei, J.; Chen, J.; Shi, H.; Shen, G.; Zhang, H. Overexpression of PP2A-C5 that encodes the catalytic subunit 5 of protein phosphatase 2A in arabidopsis confers better root and shoot development under salt conditions. *Plant Cell Environ.,* **2017**, *40*(1), 150-164.

[85] Sun, W.; Deng, D.; Yang, L.; Zheng, X.; Yu, J.; Pan, H.; Zhuge, Q. Overexpression of the chloride channel gene (GmCLC1) from soybean increases salt tolerance in transgenic *Populus deltoides × P. Euramericana* "Nanlin895". *Plant Omics,* **2013**, *6*(5), 347-354.

[86] Wei, P.; Wang, L.; Liu, A.; Yu, B.; Lam, H.-M. GmCLC1 confers enhanced salt tolerance through regulating chloride accumulation in soybean. *Front. Plant Sci.,* **2016**, *7*, 1082.

[87] Jossier, M.; Kroniewicz, L.; Dalmas, F.; Le Thiec, D.; Ephritikhine, G.; Thomine, S.; Barbier-Brygoo, H.; Vavasseur, A.; Filleur, S.; Leonhardt, N. The arabidopsis vacuolar anion transporter, atclcc, is involved in the regulation of stomatal movements and contributes to salt tolerance. *Plant J.,* **2010**, *64*(4), 563-576.

[88] Pernas, M.; García-Casado, G.; Rojo, E.; Solano, R.; Sánchez-Serrano, J. J. A protein phosphatase 2A catalytic subunit is a negative regulator of abscisic acid signalling1. *Plant J.,* **2007**, *51*(5), 763-778.

[89] Xu, C.; Jing, R.; Mao, X.; Jia, X.; Chang, X. A wheat (*Triticum aestivum*) protein phosphatase 2A catalytic subunit gene provides enhanced drought tolerance in tobacco. *Ann. Bot.,* **2007**, *99*(3), 439-450.

[90] Liu, D.; Li, A.; Mao, X.; Jing, R. Cloning and characterization of TaPP2AbB"-α, a member of the PP2A regulatory subunit in wheat. *PLoS One,* **2014**, *9*(4), e94430.

[91] He, X.; Anderson, J.C.; Del Pozo, O.; Gu, Y.Q.; Tang, X.; Martin, G.B. Silencing of subfamily I of protein phosphatase 2A catalytic subunits results in activation of plant defense responses and localized cell death. *Plant J.,* **2004**, *38*(4), 563-577.

[92] Dai, M.; Zhang, C.; Kania, U.; Chen, F.; Xue, Q.; Mccray, T.; Li, G.; Qin, G.; Wakeley, M.; Terzaghi, W.; Wan, J.; Zhao, Y.; Xu, J.; Friml, J.; Deng, X.W.; Wang, H. A PP6-type phosphatase holoenzyme directly regulates PIN phosphorylation and auxin efflux in arabidopsis. *Plant Cell,* **2012**, *24*(6), 2497-2514.

[93] Waadt, R.; Manalansan, B.; Rauniyar, N.; Munemasa, S.; Booker, M.A.; Brandt, B.; Waadt, C.; Nusinow, D.A.; Kay, S.A.; Kunz, H.-H.; Schumacher, K.; DeLong, A.; Yates, J.R.; Schroeder, J.I. Identification of open stomata1-interacting proteins reveals interactions with sucrose non-fermenting1-related protein kinases2 and with type 2A protein phosphatases that function in abscisic acid responses. *Plant Physiol.,* **2015**, *169*(1), 760-779.

[94] Li, K.; Xu, C.; Fan, W.; Zhang, H.; Hou, J.; Yang, A.; Zhang, K. Phosphoproteome and proteome analyses reveal low-phosphate mediated plasticity of root developmental and metabolic regulation in maize (*Zea mays* L.). *Plant Physiol. Biochem.,* **2014**, *83*, 232-242.

[95] Trotta, A.; Konert, G.; Rahikainen, M.; Aro, E.M; Kangasjärvi, S. Knock-down of protein phosphatase 2a subunit b'γ promotes phosphorylation of calreticulin 1 in *Arabidopsis thaliana*. *Plant Signal. Behav.,* **2011**, *6*(11), 1665-1668.

[96] Konert, G.; Trotta, A.; Kouvonen, P.; Rahikainen, M.; Durian, G.; Blokhina, O.; Fagerstedt, K.; Muth, D.; Corthals, G.L.; Kangasjärvi, S. Protein phosphatase 2A (PP2A) regulatory subunit B′γ interacts with cytoplasmic ACONITASE 3 and modulates the abundance of AOX1A and AOX1D in *Arabidopsis thaliana*. *New Phytol.,* **2015**, *205*(3), 1250-1263.

[97] Li, S.; Mhamdi, A.; Trotta, A.; Kangasjärvi, S.; Noctor, G. The protein phosphatase subunit PP2A-B′γ is required to suppress day length-dependent pathogenesis responses triggered by intracellular oxidative stress. *New Phytol.,* **2014**, *202*(1), 145-160.