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

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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.
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 phosphoserines, 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 (Shevannella-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 PPT 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- 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 (PP2P2R/PR55; 55 kDa; encoding β-propeller proteins); the B56 (PP2P2R/B/PR56/61; 54-74 kDa; encoding Huntingtin, EF3, PP2AA, TOR (HEAT) repeat proteins) and the B72 (PP2P2R/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 gene 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, γ, δ and ε) subunit isoforms and TON2 (TONEA2U) 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’γ, 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 (TONEA2U). 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) B8 absent in Arabidopsis and other...
Brassicaceae members. The B72 family forms two clades: (i) clade I including the FASS/TON2 clade including with mammalian Bγ and (ii) clade II including all non-FASS/TON2 plant B72 isoforms, related to mammalian Bα and Bβ [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 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 transcription, translation, 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 defined 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 kDa with their predicted molecular weights of 65.49 kDa - RCN1, 65.51 kDa - PP2A3, and 65.57 kDa - PP2AA2 [30]. The gene encoding the maize PP2A regulatory subunit A, ZmPP2A1A1, 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 AtPP2As. The mRNA sequence of ZmPP2A1A1 (Acc. no. AT940682), 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 ZmPP2A1 protein was observed to show 83.8%, 88.9%, and 87.6% similarity with AtPP2AA1/RCN1, AtPP2AA2 and AtPP2AA3, respectively. ZmPP2AA homologs, GMZM2G102858 and GMZM2G122135, were also identified in maize. The ZmPP2AA1 protein shows approximately 93% similarity to GMZM2G102858 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 (GMZM2G164352, GMZM2G122135) and rice (Osl_30535, Os09g 0249700); barley (MLOC_2967; Brachypodium (BRAD I4G08720, BRADIG087890), 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 (PM-E1), TAP46 (Type 2A Phosphatase-associate 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; 6-↓        | ↓- 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      | ↓ + P1-6                       | ↓ + S1-5           | -                |
| 14.  | OsPP103 | 8          | LOC_Os08g40200   | EST            | 1287      | 429       | 4       | -  | a      | ↑                            | ↓ + P1-5; ↓ - P2,3,4,6 | ↓ - S1; ↓ - S2-5  |
| 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 *bzl1-1D* mutation site. The dephosphorylation by PP2A is enhanced by the *bzl1-1D* mutation, decreased by
two intragenic bzk1-1D 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 rcnl mutants exhibit partial inhibition of ABA-induced stomatal closure and activation of anion channels as well as reduction in the probability of ABA-induced [Ca2+]cyt increases. Also, the rcnl mutant showed partial ABA insensitivity towards ABA-mediated inhibition of seed germination. RNA gel blot analyses indicated that the rcnl 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 rcnl mutant plants in spite of the high amino acid similarity of the three subunits. Protein expression studies for the rcnl, 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 rcnlpp2aa2 and rcnlpp2aa3 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 rcnl 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 rcnl 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 basilar-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 PP2AA1::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 PP2AA3 were in agreement with the global transcription data (http://www.weigelworld.org/resources/microarray/AtGenEx press). PP2A1,2,3::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 pp2aa1pp2aa3 double mutants. The tamoxifen-inducible amiRNA lines showed a basilar-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 DR3pro: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 pp2a-2pp2a-3 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 Ca2+ 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′α 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′δ show high promoter activities in rapidly growing tissues and are necessary for optimal plant growth under favourable conditions in Arabidopsis. The pp2a-2-b′γ, pp2a-2-b′δ1-1 and the pp2a-2-b′γδ plants acclimated to high light and enhanced temperature (800 μmol photons m⁻²s⁻¹/28°C) were analyzed using Agilent Arabidopsis gene expression microarrays. The pp2a-2-b′γδ 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-2-b′γδ1-1 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-2-b′γ mutant [74]. Differential transcript levels for 162 genes were observed in the pp2a-2-b′γδ mutant plants that were not expressed differentially in pp2a-2-b′γ and pp2a-2-b′γδ1-1 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-2-b′γδ double mutants as compared to wild-type plants under high light and elevated temperature. Also, pp2a-2-b′γδ 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-2-b′γδ 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′δ 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-2-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-2-b′γ and WT plants subjected to moderate light conditions for 4 weeks revealed that the pp2a-2-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 cysteolic h-type thioredoxins, chitinases, glutathione S-transferases and other defense-related genes. The pp2a-2-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-aminoacyclopropane-1-carboxylate oxidases; three genes encoding allene oxide synthases, 12-oxophytodienoate reductase, lipoxygenase 2, Plant Defensin1.2 and a Tyraminotransferase in the pp2a-2-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 antioxidant enzymes (chloroplast NADPH-dependent thioredoxin reductase, iron-superoxide dismutase, catalase 2 and glutathione peroxidase) were reduced in the pp2a-2-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-2-b′γ plants than in WT plants. Thus, the pp2a-2-b′γ plants show constitutive expression of ROS-, SA-, and JA/ET-responsive genes [76]. Peroxisosomal 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 protoxins, 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-dichlorophenoxyacetic 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’\(\theta\) expression was observed to be up-regulated in response to pathogens like *Pseudomonas syringae* pathovars, *Psm* and *Pst*, *Phytophthora infestans*, *Blumearia graminis*, *Phytophthora parasitica*, as well as the nematode, *Meloidogyne incognita*. The microarray data also showed up-regulation of B’\(\theta\), C2, and C5 expression on flag-ellin 22 treatment in comparison to the down-regulation of B’\(\theta\) expression in response to abiotic stresses [77]. B’\(\zeta\) may be involved in energy metabolism as the b’\(\zeta\) knockout seedlings showed retarded hypocotyl growth on sucrose-free me- dium. Also, it is highly expressed during senescence along- with most of the fatty acid degradation enzymes indicating that both B’\(\theta\) and B’\(\zeta\) are involved in energy metabolism [78].

Similar to other B’\(\eta\) members, PP2A-B’\(\theta\) is involved in negative regulation of plant innate immunity as observed by the decreased proliferation of the virulent *Pseudomonas syringae* in the b’\(\theta\) mutants in comparison with the wild type plants. The b’\(\theta\) mutants were found to show delayed flowering phenotype supported by high expression of the FLOW-ERING 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’\(\gamma\) acts as a positive regulator. PP2A-B’\(\gamma\) functions upstream of the main flowering inhibitor FLC by repressing it, as the pp2a-b’\(\gamma\) Arabidopsis mutant showed a late flowering phenotype and a significa- tively higher FLC transcript level than in wild type [74].

TAP46, an Arabidopsis homolog of *S. cerevisiae* TAP42 and mammalian \(\alpha\), 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 PP2A, although it interacts with PP4 and PP6 as well with different affinities [81]. Recombinant TAP46 protein was found to be phospho-orylated 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 charac-teristic phenotypes of TOR inactivation such as repressed global translation, activated autophagy and nitrogen mobiliza-tion 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 nitro-gen 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 (*GHD*), in-volved 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, SARR2a, NTCP-23, p69d, SGT1, RAR1 and SKP1) were transcriptionally induced in the TRV: Nb-TAP46 (N) plants (17 DA1) 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 posi-tive 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 (ABIS5) in vivo. TAP46 interacts and stabilizes ABIS5 and prevents its dephosphorylation in vivo. Overexpression of TAP46 results in increase of the free and phospho-orylated ABIS5 levels as well as an increase in the transcript levels of ABIS5-regulated genes, RD29A, RD29B and NCE63, during seed development and seed germination [82].

PP2A holoenzyme assembly depends on Arabidopsis Phosphotyrosyl Phosphatase Activator (AIPTPA), 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 AIPTPA in Arabidopsis and the effects of AIPTPA deficiency in plants. PP2Ac is methylated by suppressor of brassinosteroid insensitive1, Sbi1, a methylating enzyme. AIPTPA deficiency resulted in almost complete loss of Leu-309 methylation in PP2Ac as compared to the re-duced 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 AIPTPA with PP2Ac may be required prior to methylation of PP2Ac by Sbi1 in plants. Reduced AIPTPA 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 as in *pp2a-c5–1* mutants showed salt hy-persensitivity 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 tran-script 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-c-5-1 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-c-5-1 mutant. The AtCLCa, AtCLCb, AtCLCc and AtCLCd, 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 CI 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-2a/h1-1 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 norflurazon, 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 (TAILR). 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]. TaPP2AB2B-a, 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 TaPP2AbB2-a 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 TaPP2AbB2-a-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/Cf-9-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-PP2ABβ with mVenus-OST1 followed by phosphopeptide enrichment and LC-MS/MS analyses revealed that PP2ABβ 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’k 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 PP2AC 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 (AGLN1;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 phosphopetidides, 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 oxidative isoforms, AOX1A and AOX1D, in leaf mitochondria, evident from increased levels of AOX1A and AOX1D in pp2a-b’γ mutants, thereby, influencing foliar H₂O₂ 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) /hydroxyprolyl 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 (PD1), 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’γ-1 mutant. The peptides such as acyl-CoA synthetase (LACS5), ketocacyl-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 subcluster 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 cat2sidi2 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, SIPP2Ac1, 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      | pp2ac5, 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; b55b55b55 b55b55, b55a, bsl2, bsl1, bsl3, PAPP5-OX1, papp5-1 | Arabidopsis  | Interacts with nitrate reductase, NR dephosphorylation | [71] |
| 12.    | PP2A-B55, B’γ | pp2a-b55, pp2a-ba, pp2a-bf, 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$_2$O$_2$ 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, protonatonin transformation, triacylglycerol mobilization; flowering; increases during senescence and in dessicated seeds | [77] |
| 16.   | B’ζ | b’ζ, b’θ, 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 | [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’γζ double mutants | Arabidopsis | regulation of plant tolerance to aphid infestation | [73] |
| 21.   | B’α, B”β | ren1, 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 HMGR1 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, Nah-G-OE N. benthamiana, TRV:NbTap46 Lines, DEX-Inducible Nb Tap46 RNAi Lines in BY-2 Cells | Arabidopsis | N. benthamiana, Arabidopsis, tobacco (N. tabacum) BY-2 cells | [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 rcni mutant plants. The rcni/pp2aa2 and rcni/pp2aa3 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. PP2Ac2 catalytic subunit is a negative regulator of ABA-dependent gene expression. Transcriptome profile of ABA-treated pp2ac2 plants showed 57 differentially expressed genes (20 upregulated and 37 downregulated genes). PP2A-Bγ and PP2A-Bζ are necessary for optimal plant growth under favourable conditions in Arabidopsis. Differential transcript levels for 162 genes were observed in the pp2a-bγζ mutant plants that were not expressed in pp2a-bγ and pp2a-bζ-1 mutants. Bγ type regulatory subunit regulates disease resistance. The transcriptome profiling of pp2a-bγ revealed that the pp2a-bγ plants showed constitutive expression of defense-related genes. PP2A-Bγ-dependent signaling is involved in the regulation of defense signaling and in controlling day length-dependent responses to intracellular oxidative stress. The pp2a-bγζ plants showed higher levels of 11 proteins identified through proteomic analysis. The Bθ expression is involved in TAG metabolism as nine phosphopeptides from eight enzymes were unique to the bθ-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-Bγ depending on the isochorismate pathway of SA synthesis [97]. PP2A-Bγ 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-bγ mutants than in the wild-type indicating that PP2A-Bγ 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**

| Abbreviation | Full Form |
|--------------|-----------|
| 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 | Shenwanna-Like Protein Phophatases |
| 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         | Phosphorylosyl 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 Acid |
| [Ca2+]cys1   | Cytoplasmic Ca2+ 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 |
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CONFLICT OF INTEREST

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

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