

Role of Protein Tyrosine Phosphatases in Plants

Alka Shankar, Nisha Agrawal, Manisha Sharma, Amita Pandey and Girdhar K. Pandey*

Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaulakuan, New Delhi-110021, India



G. K. Pandey

Abstract: Reversible protein phosphorylation is a crucial regulatory mechanism that controls many biological processes in eukaryotes. In plants, phosphorylation events primarily occur on serine (Ser) and threonine (Thr) residues, while in certain cases, it was also discovered on tyrosine (Tyr) residues. In contrary to plants, extensive reports on Tyr phosphorylation regulating a large numbers of biological processes exist in animals. Despite of such prodigious function in animals, Tyr phosphorylation is a least studied mechanism of protein regulation in plants. Recently, various chemical analytical procedures have strengthened the view that Tyr phosphorylation is equally prevalent in plants as in animals. However, regardless of Tyr phosphorylation events occurring in plants, no evidence could be found for the existence of gene encoding for Tyr phosphorylation i.e. the typical Tyr kinases. Various methodologies have suggested that plant responses to stress signals and developmental processes involved modifications in protein Tyr phosphorylation. Correspondingly, various reports have established the role of PTPs (Protein Tyrosine Phosphatases) in the dephosphorylation and inactivation of mitogen activated protein kinases (MAPKs) hence, in the regulation of MAPK signaling cascade. Besides this, many dual specificity protein phosphatases (DSPs) are also known to bind starch and regulate starch metabolism through reversible phosphorylation. Here, we are emphasizing the significant progress on protein Tyr phosphatases to understand the role of these enzymes in the regulation of post-translational modification in plant physiology and development.

Keywords: Carbohydrate binding module, Laforin, Kinases, Protein tyrosine phosphatase, Tyrosine phosphorylation, Signal transduction, Stress.

INTRODUCTION

Protein phosphorylation is a crucial post-translational event, first reported almost 50 years ago by Edwin Krebs and Edmond Fischer [1, 2]. Protein phosphorylation is known to alter the three dimensional structure, activity, cellular localization, and the stability of a protein, which in turn acts as an ‘on and off’ switch in numerous pathways regulating growth, differentiation, and oncogenesis [3-5]. Almost more than 70% of all the known proteins of eukaryotes are reversibly phosphorylated and hence, the importance of reversible protein phosphorylation to cellular regulation cannot be overstated [6]. Phosphorylation usually takes place at nine amino acids (Ser, Thr, Tyr, Cys, Arg, Lys, Asp, Glu and His) but most extensively phosphorylated amino acids are Ser, Thr and Tyr. Phosphoserines are the most abundant group of phosphoproteins followed by phosphothreonine and phosphotyrosine. In eukaryotes, phosphorylation on Ser, Thr, and Tyr is nearly 86.4%, 11.8% and 1.8%, respectively [2]. Although, the percentage of Tyr phosphorylation is relatively less as compared to the Ser and Thr residues, it is still considered as a crucial event in cells and is known to play significant role in various signaling and regulatory processes in

eukaryotes (especially animals). Tyr phosphorylation is a fundamental mechanism for numerous important aspects of eukaryotic physiology [7]. However, it is true that the balance between protein tyrosine kinase (PTK) and protein tyrosine phosphatase (PTP) activity is the key for successful regulation of Tyr phosphorylation, as the numbers of PTKs are more than PTPs in the cell [8]. This is evident from the fact that, first PTP was identified and cloned ten years after the identification of first PTK [9].

PROTEIN TYROSINE PHOSPHATASES: STRUCTURAL FEATURES AND CLASSIFICATION

Although, different types of protein phosphatases have been discovered in plants [10, 11] and the role of many such phosphatases in various pathways are also known but our understanding of the protein Tyr phosphatase structure and function has largely been elucidated from animal and yeast systems where these enzymes were first discovered [12, 13]. PTPs are a family of enzymes whose structural diversity and complexity rival those of the PTKs. The PTKs are well known to share sequence similarity with protein serine/threonine kinases; however, the enzymes belonging to PTPs share no sequence similarity with protein serine/threonine phosphatases [14]. PTPs are characterized by the presence of active site signature motif (H/V)C(X)5R(S/T) in the conserved catalytic domain. This domain harbors the cysteine residue involved in the formation of phosphoenzyme reaction intermediate [15]. Structural do-

*Address correspondence to this author at the Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaulakuan, New Delhi-110021, India; Tel: +91-11-24116615; Fax: +91-11-24115270; E-mail: gkpandey@south.du.ac.in

mains like src homology 2 (SH2), PSD-95/Dlg/ZO-1 (PDZ), extracellular domains, etc. are also present in the PTP [16]. The three dimensional structure of over a dozen PTPs of animal origin has been determined, while not much work has yet been done in plants. The first crystal structure elucidation of protein phosphatase was that of PTP isolated from human placenta [17]. The crystal structure not only provides insight into the structural basis for catalysis but also provides insight into the substrate specificity. The PTPs so far characterized are known to possess both α helices and β sheets. The structure showed the presence of β sheet flanked by α helices on both sides [18]. Both the classes of PTPs (Tyr specific and dual-specificity) despite of having great difference in the amino acid sequences and the substrate specificity, showed high similarity in their crystal structure [19]. The active site of the PTP is located inside the crevices on the protein's surface. The depth of active site cleft is more for Tyr specific phosphatases as compared to dual specificity phosphatases. Study on crystal structure of PTPs revealed that much deeper active site cleft in the Tyr-specific phosphatases selects exclusively pTyr-containing substrates, while the more shallow, active site cleft for the DSPs may contain both pTyr and pSer/pThr residues [20-23]. In addition, from structural study, we now recognised that the structure of the P-loop, including different residues (Cys and Arg, Ser/Thr, and Asp residues) are conserved in the PTPs, DSPs and LMWPTPs and thus, utilize a common strategy for phosphate monoester hydrolysis. In catalysis mechanism, Cys residue act as nucleophile and attack on phosphate ester to form a thiophosphate intermediate. The formation of thiophosphate is supported by a conserved aspartic acid (Asp181), which acts as a general acid for stabilization of the negative charge on the leaving thiolate group [19]. For the hydrolysis of intermediate product, Asp residue now serves as a general base by recruiting water molecule. Furthermore, Arg residue (Arg221) functions to facilitate the breakdown of the thiophosphate intermediate [24].

Earlier PTPs were categorized into three broad classes namely receptor-like PTPs, intracellular PTPs, and dual-specificity PTPs. These enzymes are unique among the PTPs in their capability to utilize phosphoserine and phosphothreonine as substrates in addition to phosphotyrosine [25]. While, on the basis of amino acid residues in their catalytic domains, PTPs are grouped into four sub-families i.e., type 1, 2, and 3 Cys-based PTPs, and the Asp-based phosphatases [26, 27]. The type-1 sub-family is the largest sub-family. This sub-family contains Cys in their catalytic domain. This is further subdivided into two sub-groups, based on the substrate specificity i.e., tyrosine specific classical PTPs and dual specificity phosphatases. The type-2 PTPs are low molecular weight PTPs and are tyrosine specific. Type-2 PTPs are frequently found in prokaryotes [26, 27]. The third sub-group of PTPs includes cell cycle regulators and show specificity towards both Tyr and Thr residues. Type-3 PTPs are known to dephosphorylate cyclin-dependent kinases (CDKs) causing inhibition of cell cycle progression [28]. Fourth sub-group of phosphatases are aspartic acid based PTPs, which comprises a heterogeneous group of phosphatases, that can be either Ser or Tyr specific, for example, EYA (Eyes Absents) phosphatase and HAD (Haloacid Dehalogenase) family of phosphatases in humans [29-31]. A

widely used way to classify Tyr phosphatases is to categorize them into two main classes, one being Tyr specific PTPs and the other as dual specificity phosphatases (DSP) [32]. Both of these classes of Tyr phosphatases are well characterized in animals and microbes but not many of them are studied in plants.

Similarly, PTKs are an important family of regulatory enzymes in higher eukaryotes. The protein Ser/Thr kinases and protein Tyr kinases (PTKs) act on Ser/Thr and Tyr residues respectively, and are distinct from dual-specificity PTKs (DsPTKs). DsPTKs phosphorylates Ser, Thr, and Tyr. PTKs and DsPTKs share a common catalytic domain of around 250 amino acids containing 11 conserved subdomains (I to XI) [33]. At the N-terminal extremity of catalytic domain, subdomain II has a conserved lysine residue, known to be involved in ATP binding. The subdomain VII, located in the middle of the catalytic region contain aspartic acid residue, which is crucial for the catalytic activity of the kinase. Subdomain VI and VIII confers Ser/Thr and Tyr specificity, respectively. Consensus DL(R/A)A(A/R)N is specific for Tyr kinases whereas, the consensus DLKXXN is Ser/Thr-specific [7]. So far, a typical PTK could not be reported in plants; however, several DsPTKs have been identified through bioinformatics analysis [34].

IDENTIFICATION OF PROTEIN TYROSINE PHOSPHATASES

The role of PTP in various cellular processes has recently been elucidated in organisms like *Homo sapiens*, *Strongylocentrotus purpuratus*, *Plasmodium falciparum* and others [24, 35]. Even though, several lines of evidences proved the presence of PTPs in various plant species, the complete PTPome has only been explored in Arabidopsis and rice [10, 11]. Tyr phosphorylation in plants was first described by Torruella *et al.* (1986), where they observed Tyr kinase activity in etiolated pea plants [36]. Since then, wide ranges of experimental tools have been employed to explore the presence of Tyr phosphorylation in plants [37-39]. Radioactive labeling has been extensively used for the detection of Tyr phosphorylation. The initial detection of phosphor-Tyr was based on radioactive labeling of ^{32}P -ortho-phosphate of proteins present in the cells, followed by electrophoresis to separate the proteins and then partial acid hydrolysis. It was succeeded by thin layer chromatography and autoradiography of the hydrolysates, which led to the detection of phosphor-Ser, Phosphor-Thr, and phosphor-Tyr [40]. Due to limited occurrence of Phosphor-Tyr in comparison to phosphor-Ser and phosphor-Thr in the cell, its detection was quite difficult using conventional electrophoretic techniques [41]. An alternative approach for the detection of Tyr-phosphorylation makes use of monoclonal antibodies [42]. Phosphor-Tyr specific monoclonal antibodies are used for detecting phosphorylated-Tyr residues by using western blot technique. In this technique, antibodies are raised against immunizing rabbits with synthetic phosphopeptides representing the amino acid sequences, adjacent to the phosphorylation site of the target protein. This antibody largely recognized proteins containing phosphotyrosine. Afterwards, by using western blot technique multiple phosphoproteins could be resolved by their molecular weight. The presence of Tyr-phosphorylation in *Pisum sativum* was detected using this technique [35].

This technique has also been used to detect the presence of Tyr phosphorylated proteins in other plants such as *Arachis hypogaea* and *Cocos nucifera* [34, 41, 42].

The specific components of the signal transduction pathways can be deciphered through loss-of-function of one of these elements. This can be done using pharmacological approaches, which can inactivate single component in intact cells of the organisms. This approach uses PTKs and PTPs inhibitors such as genistein and lavendustin, respectively [45, 46]. Because of the advancement of tools and techniques, PTPs in plants could also be identified after ten years of the discovery of PTP in animals. The first plant PTP, AtPTP1 was characterized in Arabidopsis followed by dual specificity phosphatase by Luan and co-workers [13, 46, 48]. Subsequently, Singh *et al.* (2010) classified PTPs as well as DSPs gene families for the first time in crop plant rice (*Oryza sativa*) [11]. Presence of Tyr phosphatase like gene has also been identified in other plants like tomato and *Pinus sylvestris* [49, 50]. Recent advances in the analytical techniques such as mass spectrometry and affinity purification have led to discovery of several tyrosine phosphatases in plants.

PROTEIN TYROSINE PHOSPHATASE GENE FAMILY IN PLANTS

As mentioned earlier, Tyr phosphatases have been widely studied in animals and they play critical roles in regulating a large number of cellular pathways. Luan and co-workers have identified the first plant Tyr phosphatase, AtPTP1 in Arabidopsis [13]. Later, orthologues of AtPTP1 were also reported in other plants species [51]. The first dual specificity phosphatase AtDsPTP1 was identified in Arabidopsis in 1998 [47]. AtDsPTP1 resembles animal MKPs and it can dephosphorylate both phospho-tyrosine and phospho-serine or phospho-threonine. AtPTP1 and AtDsPTP1 shared homology within their respective signature motifs in the catalytic core. The overall protein sequence of Tyr-specific PTP and DsPTP share little homology but these phosphatases contain the CX5R motif in their catalytic domain: (V/I)HCXAGXGR(S/T) [47]. Similarly, in animals, the role of DsPTPs in the inactivation and dephosphorylation of Tyr kinases and MAPKs has been widely reported [52, 53]. Subsequently, whole genome sequencing of Arabidopsis facilitated identification of many more genes, encoding proteins having catalytic core motif of PTPs [10]. Out of around 24 members of putative PTPs from Arabidopsis, one encodes a Tyr specific PTP, 22 genes encode dual-specificity protein Tyr phosphatases (DSPs or DsPTPs) and a single gene of low molecular weight PTP (LMWPTP) [10]. Furthermore, by using complete human genome as reference, some novel PTPs have been identified from the genomes of *Arabidopsis thaliana*, *Chlamydomonas reinhardtii*, *Ostreococcus tauri*, *Oryza sativa*, and *Populus trichocarpa* [54]. From this study a single member of PTP was identified from all the four species. Also, a single member of low molecular weight PTP (LMWPTP) was identified in *A. thaliana*, *O. sativa*, and *C. reinhardtii*. Moreover, 2 LMWPTP were present in *P. trichocarpa* [54]. Later on, genome-wide study from rice revealed that rice comprised of 23 DSPs, a single member of PTP and LMWPTP [11, 55]. Additionally, 29 PTPs have been identified from maize on the basis of sequence similarity of

protein phosphatases from rice and Arabidopsis [56]. Interestingly, genomes of Arabidopsis, rice, and maize contain much lesser PTPs than the human genome, comprised of more than 100 PTPs including 60 DSPs. On the other hand, the number of protein kinases in Arabidopsis is twice the number found in human. This observation leads us to a conclusion that either the protein Tyr-specific phosphatases (PTPs) and DSPs are directed at more sites or the protein Tyr component is limited in plant phosphoproteoms.

FUNCTIONAL ROLE OF PROTEIN TYR PHOSPHORYLATION AND DSP IN PLANTS

Protein Tyr phosphorylation plays a major role in many signaling pathways. In animal cells, many physiological roles of Tyr phosphorylation such as regulation of growth, differentiation, or oncogenesis have been studied in detail [5]. On the other hand, Tyr phosphorylated proteins have been detected in higher plants and evidence for regulation by Tyr phosphorylation in plant physiological processes is beginning to emerge. This section of the review provides an overview of Tyr phosphorylation in signaling processes and their role in physiological responses in plant cells. These include regulation at the level of expression, localization, substrate specificity, and activity of these enzymes.

In Response to Abiotic Stress

The growth and development of land plants is greatly affected by a variety of abiotic factors like dehydration, low temperature, salinity, heat, wounding, and pathogen infection. Consequently, plants exposed to diverse stress conditions, activate a complex set of distinct signaling pathways from perception of stress signal to amplification, transmission, and finally generation of responses, which helps in stress adaptation [57-59]. Protein phosphorylation and dephosphorylation, catalyzed by protein kinases and phosphatases play an important role in the co-ordination of various signal transduction pathways [5, 60]. One of the immediate and important responses under a particular condition is change in the gene expression that might be linked to regulation of that particular response. In Arabidopsis, the expression of *AtPTP1*, a protein Tyr phosphatase, was found to be manifold increased under salinity stress while reduced significantly in cold stress [13]. Recently, it was found that *AtDsPTP1* (Dual-specificity protein phosphatase 1) mutant grew better than wild type (WT) under osmotic stress and hence acts as a negative regulator of osmotic stress (Fig. 1). Also, *AtDsPTP1* regulated the expression of many genes associated with ABA biosynthesis and ABA-catabolism related genes such as *NCED3* and *CYP707A4* and hence positively regulates ABA accumulation [61]. Another report in chestnut showed *CsDSP4* to be highly induced in the stems of chestnut seedlings exposed to low temperatures [62]. *OsPFA-DSP1* (fungi atypical dual-specificity phosphatase from rice) is known to function as a negative regulator of drought stress in rice (Fig. 1). Overexpression of this DSP protein results into increased sensitivity of plants towards drought stress [63]. Arabidopsis, *MAPK phosphatase 1* (*MKPI*) overexpression line is tolerant to salt stress. It is also proven that mutants lacking *AtMKPI* are hypersensitive to salinity [64]. Furthermore, *AtMKPI*, is essential for UV resistance in Arabidopsis, as disruption of this gene showed hypersensitive phenotype on exposure to UV rays.

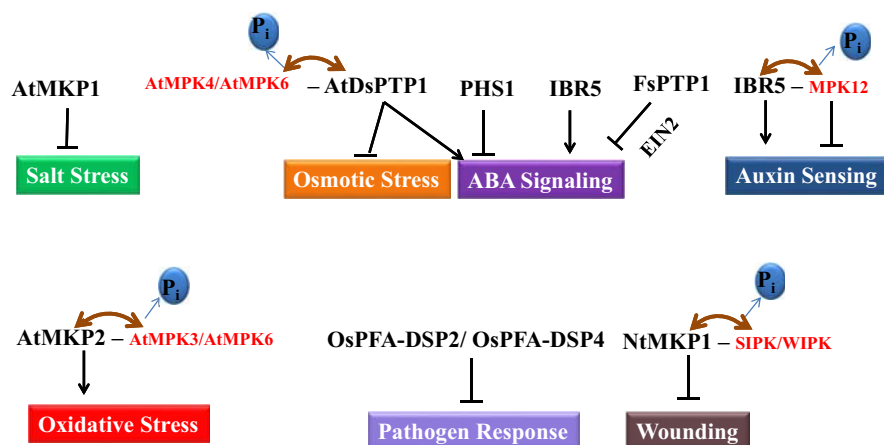


Fig. (1). Overview of responses regulated by plant Tyr phosphatases.

Several genes encoding plant Tyr phosphatase are responsible for the regulation of different biological processes such as osmotic, oxidative, salt stresses, ABA signaling, auxin sensing, wounding, and pathogen response. Mitogen-activated protein kinase (MAPK) dephosphorylation by phosphatase is represented in red color while phosphatase is presented in black. The positive (⇓) and negative (⊥) regulatory role reflect the physiological function of the phosphatase. Brown double-headed arrow represents the phosphorylation and dephosphorylation event.

Additionally, *MKP1* is also known to be a key regulator of MAP kinases, *MPK3* and *MPK6* in response to UV stress [65, 66]. This has been known that *AtMKP2* dephosphorylates *AtMPK3* and *AtMPK6*; both of these proteins are positive regulator of oxidative stress signaling pathways and *AtMKP2*-silenced plants exhibit enhanced sensitivity to ozone stress (Fig. 1) [67]. Activation and deactivation occur rapidly and this transient signaling is controlled by MAPKs and MKPs. Both PTPs and DSPs play a vital role in deactivation of MAPKs and hence counteract the responses mediated by MAPKs.

The phytohormone abscisic acid (ABA) regulates plant development and responses to many environmental constraints, shoot and root growth, stomatal closure, storage protein synthesis, and seed dormancy [68, 69]. The foremost components of the ABA signaling cascade includes calcium, G-proteins, kinases, phosphatases, phospholipase C and D, and redox signals produced by NADPH oxidase [70, 71]. The significance of the phosphorylation events in ABA signal transduction came into picture with the discovery of mutants defective in ABA responses. For example, *ABI1* and *ABI2* are type-2C protein phosphatases acting as negative regulators in several ABA responses [72, 73]. Under water deficit condition, ABA induces stomatal closure by reducing the turgor pressure of guard cells. It has been demonstrated that the regulation of stomatal closure is achieved by the role of PTPs. This phenomenon of stomatal closure is induced by H_2O_2 , darkness, Ca^{2+} and ABA. All these factors may lead to alterations in intracellular calcium homeostasis, which then changes the K^+ channel activity in plasma membrane as well as on tonoplast [74, 75]. The knockdown mutation in *AtPHS1* gene, a DsPTP, leads to hypersensitivity to ABA during early development and stomatal closure, which suggest *PHS1* as a negative regulator of ABA signaling pathway (Fig. 1) [76]. It was shown that several ABA-dependent responses are inhibited by phenylarsine oxide (PAO), a specific inhibitor of PTP [77]. Inhibitors of PTKs and PTPs activity interferes with the expression of ABA responsive genes such as *RAB18* (responsive to ABA18) and it blocks

the ABA regulated phenomenon such as stomatal closure [78]. The other effect of PAO has been reported in the inhibition of ABA-induced *RAB16* gene expression in barley (*Hordeum vulgare*) aleurone protoplasts [79]. Moreover, PAO prevents stomatal closure in *Commelina communis* by reopening them, which might get closed either by the induction of ABA, high external Ca^{2+} , darkness, and hydrogen peroxide [77]. Furthermore, PAO enhances the inhibition of germination in Arabidopsis suggesting PTP function as a negative regulator of the ABA signaling [80]. In conclusion, the above-mentioned aspects provides important insights related to Tyr phosphorylation signaling during abiotic stress conditions.

In Response to Hormone and Development

Plant hormones are well-known to assist responses to stress and adaptation. Phytohormones have a diverse effect on plant growth and development. They influence nearly whole plant lifecycle starting from seed germination to maturation. It is well-known fact that ABA and gibberellins play critical roles in seed dormancy and germination [81, 82]. Tyr dephosphorylation is known as a key regulatory mechanism in post-germination arrest of seeds, mediated by ABA. By using an inhibitor of Tyr phosphatases, PAO, it was realized that Tyr dephosphorylation might function as a negative regulator of MAPK during the post-germination arrest of development mediated by ABA [7, 78, 80]. Constitutive expression of *FsPTP1* (a tyrosine-specific phosphatase from *Fagus sylvatica*) in Arabidopsis caused plants to be insensitive towards ABA with simultaneous induction of *EIN2* (ethylene insensitive2), a key gene of ethylene signaling. Additionally, the expression of ABA marker genes (*RAB18* and *RD29*) was reduced and expression of the *EIN2* gene was higher in *FsPTP1* overexpression lines. Further, it has been suggested that negative effect of *FsPTP1* in ABA signaling might be regulated by the modulation of ethylene signaling (Fig. 1) [83]. Additionally, Yoo *et al.* (2008) have reported the role of *MKK9-MPK3/MPK6* cascade in the regulation of *ethylene-insensitive3 (EIN3)*-mediated tran-

scription in ethylene signaling and shown to act downstream of the ethylene receptors [84].

The phytohormone auxin is considered as one of the key regulator of plant growth and development. It was observed that phytohormone auxin (synthetic auxin; 2,4-D) and cytokinin (kinetin) caused a higher degree of Tyr phosphorylation in *Arabidopsis* hypocotyls [85]. On the basis of sequence similarity, *IBR5*, encoding a putative DSP like protein in *Arabidopsis* was found as a positive regulator of both auxin and ABA [86]. *ibr5* null mutants were less sensitive to auxin and auxin transport inhibitors. Similarly, seedlings of these mutants were comparable in phenotype with other auxin-response mutants, with long root and short hypocotyl in light, fewer lateral roots, increased leaf serration, defective vascular patterning, and reduced accumulation of an auxin-inducible reporter (*DR5-GUS*) [86, 87]. The double mutant of *ibr5* with an auxin receptor mutant, *tir1*, improves auxin resistance compared to that of either parent [87]. All these facts clearly suggest that *IBR5* promotes auxin responses independently and downstream of TIR1-mediated degradation of Aux/IAA-proteins. Also, it has been found that *IBR5* interacted *in vitro* and *in vivo* with *MPK12* and *IBR5* dephosphorylated *MPK12* [88]. On the basis of plant genetic studies, it was speculated that *MPK12* act as a negative regulator in *IBR5*-regulated auxin signaling, as suppression of *MPK12* in transgenic plants results in the upregulation of auxin-responsive genes and exhibited auxin-hypersensitive root growth phenotype (Fig. 1) [88]. Recently, it was revealed that *IBR5* alternatively spliced to generate two isoforms i.e. *IBR5.1* and *IBR5.3*, which may have diverse as well as overlapping functions in growth and development of plant [89].

Brassinosteroid (BR) is a plant steroid hormone, involved in growth regulation. It stimulates cell elongation and its deficiency causes plants to grow as dwarfs in light and have a light-grown phenotype in the dark. Most of the BR mutants showed reduced fertility, delayed senescence and altered vascular development [90]. It was observed that many brassinosteroid increased the level of phosphorylation on Tyr residues. Western blotting with monoclonal antibodies in *Pisum sativum* showed that brassinolide (BL) induced the phosphorylation of leaf proteins on Tyr residues [91]. It is a well-known fact that *BRI1* (*BRASSINOSTEROID INSENSITIVE 1*), which is the receptor of BRs, is a leucine-rich repeat kinase, located in the plasma membrane and interacts with the cytosolic protein *BAK1* (*BRI1-ASSOCIATED RECEPTOR KINASE 1*) in BR signaling [92, 93]. Recent studies revealed that both *BRI1* and *BAK1* can autophosphorylate on Tyr, therefore also designated as dual specificity protein Tyr kinases (DsPTKs) [94, 95]. The autophosphorylation of *BRI1* and *BAK1* are necessary for root growth inhibition induced by BRs [94]. Furthermore, it was seen that autophosphorylation/dephosphorylation of the GSK3-like kinase, *BRASSINOSTEROID INSENSITIVE2* (*BIN2*) on Tyr 200 is a critical switch in downstream regulation of BR signaling [96]. Moreover, upon BR perception, *BRI1* phosphorylates the *BRI1 KINASE INHIBITOR 1* (*BKI1*) on Tyr-211, and release *BKI1* into the cytosol thus facilitating the formation of an active signaling complex [97]. All these results clearly suggest the involvement of Tyr phosphorylation/dephosphorylation in the plant developmental processes controlled by auxins and BRs.

Leaf senescence is the final stage of leaf development and regulated by numerous environmental and endogenous signals, such as age, developmental cues, and plant growth regulators [98, 99]. *AtMKP2* mutant plants displayed an early senescence compared to wild type and *AtMKP2* over-expressing plants. However, overexpression of *AtMKP2* did not prolong the plant life span [100]. Also, a MAPK cascade that involved *MKK9* and *MPK6*, was shown to play an important role in regulation of leaf senescence in *Arabidopsis* [101]. Loss-of-function of *MKK9* exhibited delayed leaf senescence, while overexpression of *MKK9* causes premature senescence in leaves and in whole *Arabidopsis* plants. Additionally, *in vitro* phosphorylation revealed that *MPK6* is a direct and downstream target of *MKK9* [101]. Loss-of-function mutant of *MPK6* also showed delayed leaf senescence. These evidences suggested that PTPs also play an important role in regulation of leaf senescence.

In higher plants embryo development and seed germination are also controlled by Tyr phosphorylation. Associations between protein Tyr phosphorylation and plant embryogenesis have also been shown in *Daucus carota* and *Coccoloba nucifera* [102, 103]. A subset of well-known PTK inhibitors, including genistein, prevented the establishment of zygotic polarity in *Fucus*, by inhibition of germination [104]. Rudrabhatla et al. (2006) revealed that many genes of DsPTKs family were expressed particularly during seed germination and early seedling stages in *Arabidopsis* [35]. Tyr phosphorylation also regulates the dynamics and organization of microtubules in the plant cell. Enhanced Tyr phosphorylation after treatment with sodium orthovanadate (PTP inhibitor) induced intense root growth and led to shortened elongation zone [105]. Similar targets of Tyr phosphorylation, other than MAPKs are profilin and actin [106, 107]. The kinases that phosphorylate profilin and actin may be Tyr kinase or dual specificity kinase and this question is still remains to be answered.

In animals, loss-of-function mutations in *PTEN* causes tumor, hence termed as a tumor suppressor gene [108]. *PTEN* having a PTP catalytic core motif is a DsPTP that show significant similarity with the cytoskeleton-interacting protein tensin [109, 110]. *AtPTEN1* is an *Arabidopsis* DsPTP similar to *PTEN* (Phosphatase and tensin homolog, a tumor suppressing phosphatase in human) and is required for pollen maturation following cell division [111, 112]. *AtPTEN1* expresses exclusively in pollen grains and is necessary for pollen development. RNA interference mediated silencing of *AtPTEN1* caused pollen cell death after mitosis [111]. Presence of different Tyr-phosphorylated proteins at different embryo developmental stages, revealed the strong correlation between protein Tyr phosphorylation and plant development

Expression Profile of Rice DSPs in Abiotic Stress, Nutrition Deficiency and Hormone Treatment

From literature survey of plant DSPs, it was evident that most of the work has been carried out on dicot model plant *Arabidopsis*, but knowledge related to these phosphatases is miniscule in crop plant rice. Several reports proposed the involvement of these phosphatases in regulation of stress signaling and tolerance, and development in *Arabidopsis*. It

is quite obvious that these phosphatases might also be involved in related functions in rice. So, we carried out expression analysis of these putative DSPs under abiotic stress, nutrition deficiency and hormone treatment in plants. The expression analysis of rice DSPs hints toward the possible functional role of this family under these conditions.

By using Genevestigator database, we have analyzed the whole DSP gene family, and found that some of the rice DSPs were differentially expressed during different stress conditions in rice. Expression of *OsPP3* (*LOC_Os01g20940*), which encodes rice PHS1, was significantly upregulated under drought and cold stresses in leaf and root tissue and no expression detected under heat stress. *OsPP6* (*LOC_Os01g29469*) and *OsPP126* (*LOC_Os12g05660*) exhibited downregulation under all the three conditions. *OsPP14* (*LOC_Os01g53710*) showed high expression in drought and low expression in cold and heat stresses. *OsPP39* (*LOC_Os02g53160*), *OsPP77* (*LOC_Os05g44910*) and *OsPP85* (*LOC_Os06g20340*) exhibited high expression under cold and drought stresses (Fig. 2a). Moreover, *Arabidopsis* *STARCH EXCESS4* (*AtSEX4*), a close ortholog of rice *OsPP42*, was significantly regulated under cold, salt, and heat stress treatments [113]. Also, microarray expression data from *Solanum tuberosum*, exhibited that a dual specificity protein tyrosine phosphatase 1 was downregulated under salinity stress [113].

One of the major constrains for plant growth and development is mineral nutrient deficiency, which affects agricultural yield worldwide [115]. From microarray analysis for K^+ and Ca^{2+} deficiency conditions in rice, several of the rice DSPs were not found to be affected under K^+ and Ca^{2+} mineral deficiency [115, 116]. However, *OsPP3* (*LOC_Os01g20940*) was found to be upregulated under low phosphorus and low ammonium conditions, whereas downregulated in low potassium, low iron and low phosphate conditions. *OsPP14* (*LOC_Os01g53710*) showed low expression under K^+ deficiency while, exhibited high expression under Fe^{2+} deficiency (Fig. 2b). *OsPP39* (*LOC_Os02g53160*) showed downregulation and *OsPP105* (*LOC_Os09g05020*) showed high expression under low phosphorus. *OsPP42* (*LOC_Os03g01750*), *OsPP82* (*LOC_Os06g05870*), *OsPP100* (*LOC_Os08g29160*) genes showed downregulation under low Fe^{2+} and low phosphate conditions (Fig. 2b). Rice DSP *OsPP6* (*LOC_Os01g29469*) was significantly downregulated after the treatment with phytohormone NAA (naphthaleneacetic acid), GA_3 (gibberellic acid), KT (kinetin), and trans-zeatin. The regulation of *OsPP14* (*LOC_Os01g53710*) and *OsPP39* (*LOC_Os02g53160*) genes was highly reduced upon treatment with trans-zeatin. *OsPP3* (*LOC_Os01g20940*) and *OsPP82* (*LOC_Os06g05870*) showed up-regulation after treatment with NAA and GA_3 (Fig. 2c).

It is widely known that plants can sense, process, respond to environmental stimuli and activate related-gene expression to adapt different stress conditions. The expression analysis, of rice DSP genes revealed differential expression pattern of some of the DSPs like *OsPP3*, *OsPP6*, *OsPP14*, *OsPP39*, *OsPP126*, etc. under stress conditions. The biological function of this group of PTPs in plants, especially in rice, has not yet been elucidated. Thus, it can be speculated

that these genes might contribute towards stress tolerance in plants and may be an important regulator for plant adaptation under abiotic stress, nutrition deficiency and hormone signaling. Moreover, this expression analysis provides a platform towards the *in planta* functional characterization of genes.

In Response to Biotic Stress

In the environment, plants encounter a variety of biotic stresses including bacteria, viruses, fungi, parasitic worms and insects, which strongly affect the plant growth and development and hence the productivity [117]. To counteract the biotic stresses, plants have developed active defense mechanism to protect themselves and one of the mechanisms, they adopt is transcriptional activation of a large number of genes to induce defense responses [118]. Reactive oxygen species (ROS) play an important role in controlling many biological processes, such as gene expression, activation of transcription factors, redox balance, programmed cell death (PCD) and regulation of the mitogen-activated protein kinase (MAPK) pathways [119, 120]. H_2O_2 is an important component of ROS and prevent the growth, penetration and proliferation of pathogen by stimulating program cell death (PCD) [121, 122]. Studies have shown that two orthologous genes from rice and Arabidopsis, *OsPFA-DSP2* and *AtPFA-DSP4* act as negative regulator of the pathogen responses in transgenic plants through H_2O_2 -mediated pathway, respectively (Fig. 1) [123]. The *OsPFA-DSP2* overexpressing lines showed increased sensitivity to *Magnaporthe grisea*, and inhibited the accumulation of H_2O_2 as well as suppressed the expression of pathogenesis-related (PR) genes after fungal infection. Also, Arabidopsis plants overexpressing *AtPFA-DSP4*, exhibited sensitivity to *Pseudomonas syringae*, and decreased accumulation of H_2O_2 .

Presently, MAPKs have been characterized in higher plants and the importance of Tyr phosphorylation in plant cells cannot be ignored. MAPKs play a vital role in the signal transduction pathways in higher plants [124]. It is well known that PTPs plays a major role in regulation of MAPK cascade and their expression is often induced by various stress signals [124]. For example, Arabidopsis *MPK6* is activated by various pathogenic stimuli and abiotic stress signals [126, 127]. Also, the activation of MAPK is associated with Tyr phosphorylation [128]. For instance, AtMPK4 in Arabidopsis phosphorylated at Tyr residue in order to get activated. If AtPTP1 dephosphorylates this Tyr residue then it leads to complete loss of kinase activity [13]. The role of MAPK cascades in plant defense is well established but very little is known about plant Tyr phosphatases. *MKP1* is associated with pathogenic responses in plants. *Nicotiana tabacum* *MAPK phosphatase1* (*NtMKP1*) over-expression showed abolished salicylic acid-induced protein kinase (SIPK) mediated cell death. SIPK is known as the ortholog of Arabidopsis *MPK6*. Moreover, induction of *NtMKP1* inhibits wound induced MAPK (WIPK), an ortholog of AtMPK3 [129, 130]. A fungal elicitor induced Tyr phosphorylation of the 47-kD myelin basic protein kinase (MBP) was verified by immunoblots and immunoprecipitation analysis with anti-phospho-Tyr antibody in tobacco cells [131]. Additionally, *Arabidopsis thaliana* Columbia ecotype introgressed *mpk1* mutant plants exhibited constitutive defense responses such as inhibited growth, enhanced

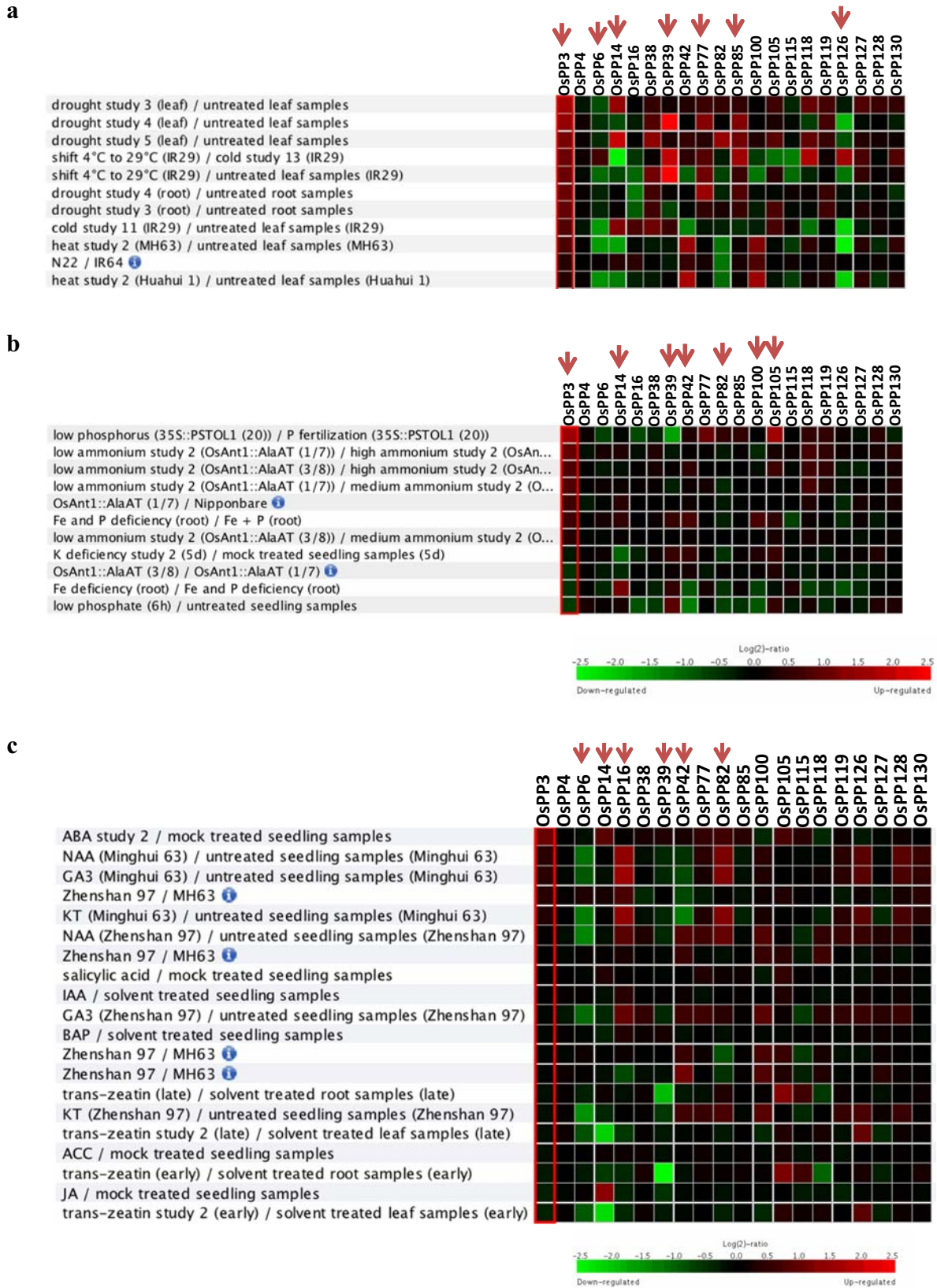


Fig. (2). Expression profile of rice DSPs under abiotic stress (a), nutrition deficiency (b), and hormones (c).

On the left side tissue types, conditions and treatment controls are mentioned. Gene names are listed on the top of heat map. Green color indicates the down regulation and red color is for up regulation of the transcript level of individual DSP genes. Expression profile of selected genes is shown on the basis of differential expression. Red arrow above the heat map indicates the genes, which are differentially expressed.

expression of defense response markers such as PR genes, accumulation of salicylic acid and resistance to the bacterial pathogen *Pseudomonas syringae* [132]. Mutations in the *SNC1* (*SUPPRESSOR OF npr1-1 CONSTITUTIVE1*) also reduced the growth defects and PR gene accumulation in *mkp1* mutants. Recent studies have revealed that *AtMKP1* negatively regulates *MPK6*-mediated pathogen-associated molecular patterns (PAMP) responses and resistance against bacteria, as *mkp1/mpk6* double mutants showed suppressed defense responses [133].

Apart from its role in BR signaling, *BAK1* has been shown to play an important role in plant defense mechanisms. The phosphorylation of BAK1 at Tyr-610 residue affects the expression of several defense-related genes [96]. Mass spectrometry (MS) analysis of BIK1 autophosphorylation and transphosphorylation by BAK1 identified three BIK1 tyrosine phosphorylation sites Y150, Y234, and Y250 [134]. Transgenic complementation assays suggested that, this Tyr phosphorylation was important in BIK1-mediated plant innate immunity because mutation of Tyr to phenylalanine (Phe) failed to complement the *bik1* mutant deficiency in immunity [134]. *BAK1/SERK3* binds to *FLS2*, the flagellin receptor, and positively regulates FLS2-mediated innate immunity in Arabidopsis [135]. The above-mentioned data clearly indicate the involvement of Tyr phosphorylation in regulation of defense responses mediated by MPKs and PTPs.

In Starch Metabolism

Starch belongs to a group of most abundant water-insoluble biomolecules on earth. Starch in plants and glycogen in animals are the major carbohydrates reserve for most of the organisms. Glucose residues of both, starch and glycogen are linked via α -1,4-glycosidic bonds and branched via α -1,6-glycosidic linkages. Amylopectin is a major component of starch (containing 70% or more of starch) and branching in amylopectin allows formation of insoluble semicrystalline nature of starch granules [136, 137]. In plants, starch synthesis occurs during daytime as a result of photosynthesis along with sucrose. Since, plants cannot transport starch in its native form, these are first converted to sucrose at night by combination of enzymes [136]. In most of the plants, sucrose is an essential carbon source for energy and growth.

Plants have two glucan dikinases, which phosphorylate starch, i.e. glucan water dikinase (GWD) and phosphoglucan water dikinase (PWD) [138-140]. Both enzymes add beta-phosphate groups from ATP to glucose residues within starch polymers. The addition of charged and hydrophilic phosphate groups may interrupt the organization of the starch granule matrix by unwinding the amylopectin double helices and make it a better substrate for exoamylolytic attack. Glucan hydrolyzing enzymes, such as exoamylases, endoamylase, and debranching enzymes are involved in the subsequent degradation of starch [137]. For the complete degradation of starch, removal of phosphate group added by GWD/PWD is essential (Fig. 3) [138-140]. The products obtained from the degradation i.e., maltose and glucose are exported from the chloroplast to the cytosol for metabolism processes. GWD phosphorylates the C-6 positions of gluco-

syl residues, whereas PWD phosphorylates the C-3 positions [141]. Plants contain two glucan phosphatases for dephosphorylation of starch and promote degradation by amylases (Fig. 3) [142-144]. Glucan phosphatases are members of the PTP family. Mutation in a glucan phosphatase, *STARCH EXCESS4* (*SEX4*), also known as *PTPKIS* (*protein-tyrosine phosphatase kinase interaction sequence*) and *DSP4* (*dual-specificity phosphatase 4*) results in stunted plant growth and delayed flowering, due to accumulation of starch [142, 145]. Arabidopsis plants having disrupted *DSP4* are impaired in starch degradation during the night-time and thus, increased accumulation of starch [146]. The starch-binding capacity of *DSP4* is regulated by light through redox and pH [145]. *SEX4* dephosphorylates the starch and as a result, allowed it to be further hydrolyzed by β -amylase3 (*BAM3*) and isoamylase3 (*ISA3*). Some of the recent studies have shown that *SEX4* preferentially dephosphorylates the C6-position and another glucan phosphatases, *LIKE SEX FOUR2* (*LSF2* or *DSP5*) specifically dephosphorylates the C3-position of starch glucose [144, 147]. *SEX4/DSP4* and *LSF2/DSP5* proteins both contain a chloroplast targeting peptide, a DSP domain, and a unique C-terminal (CT) motif [142, 144, 148, 149]. Chloroplast targeting peptide facilitate the localization of proteins to the site of starch metabolism i.e. chloroplast. Among all the plant DSPs, CT motif was detected in *SEX4*, which is needed for protein stability and function [149]. Moreover, *SEX4* contains a carbohydrate-binding module (CBM) that is common in starch-interacting enzymes and modify substrate surfaces to facilitate enzymatic hydrolysis [150, 151].

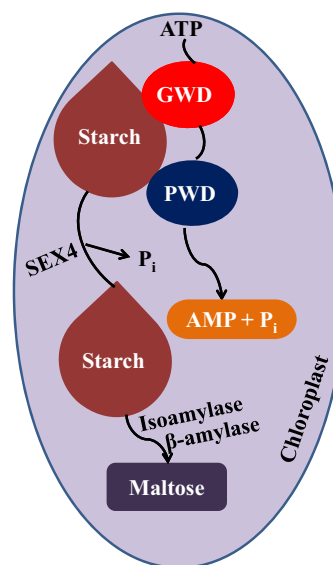


Fig. (3). The proposed pathway of starch degradation in chloroplast.

Starch dikinase (GWD/PWD) phosphorylates starch polymer and add extra phosphate group to the polymer. The hydrolysis of starch is catalysed by β -amylases (*BAM3*) and Isoamylase (*ISA*). On the other hand phosphate group is released by *SEX4* to allow complete degradation.

With the help of bioinformatics analysis, it has been known that lafora DSP of humans, fungal DSP and DSPs of some plants share a wide spread ancient carbohydrate binding domain (CBD) [148]. These DSPs plays important role

in glycogen metabolism in animals and starch metabolism in plants. Two of the DSPs of *Arabidopsis At3g52180 (DSP4)* and *At3g01510 (DSP5)* have been shown to display sequence and functional homology with the human laforin, which is a DSP containing carbohydrate binding motif and any mutation in this protein results in lafora disease (LD), a neurodegenerative disorder with epilepsy in humans [148]. Both in animals and plants, laforin (dual specificity proteins) regulate carbohydrate accumulation indicating that both of them play an essential role in storage form of carbohydrate (starch or glycogen) metabolism. Hence, in spite of the significant differences in the enzymes directly involved in starch metabolism in plants and glycogen metabolism in mammals, both SEX4 and Laforin plays a key role in regulation of glucan metabolism.

CONCLUSION AND FUTURE PERSPECTIVE

Despite of the identification of large number of protein Tyr kinases and phosphatases in the regulation of number of responses in animal system, the role of Tyr phosphorylation in plants is still under explored. The most likely reason for this is absence of a typical Tyr kinases as well as phosphatases in plants. However, plants possess few PTP and DSP like enzymes but so far no PTK type enzyme has been identified in plants. Recently, several genome-wide studies and large scale proteomic analysis have facilitated identification of phospho-Tyr proteins in plants. Protein Tyr phosphorylation plays important role in many plant processes and majorly found in the regulation of developmental, abiotic and biotic stress responses (as summerized in Fig. 1, 2). However, the impact of protein Tyr phosphorylation on the regulation of large number of physiological processes in plant is not completely understood and revelations are just beginning to emerge. Recent genetic, molecular, and biochemical studies have shed light on some aspect of their functions and the mode of action. Nonetheless, more plant Tyr-phosphorylated proteins still require to be identified. Improved analytic technologies such as mass spectrometry (MS) can be employed to identify more relevant phosphorylation sites in proteins. Genetic studies can be used to decipher the role of a specific enzyme by using knockdown and knockouts with the complementation substitution of kinases and phosphatases causing visible change in phenotype [152]. Similarly, the impact on the phosphorylation level of a particular protein by the overexpression of a phosphatase can be analyzed. Subsequently, various domain prediction tools could be used to study set of Tyr-directed protein kinases and linked to their substrate. With detail *in planta* functional role of these Tyr phosphatases, some of these can be utilized for development of future crop by genetic intervention to enhance the crop productivity and quality, especially the seed quality and enhancement of yield under stress conditions.

LIST OF ABBREVIATIONS

ABA	=	Abcisic acid
DSP	=	Dual specific phosphatase
MAPK	=	Mitogen-activated protein kinase
PAO	=	Phenyl arsine oxide
PTK	=	Protein tyrosine kinase
PTP	=	Protein tyrosine phosphatase

ROS	=	Reactive oxygen species
SA	=	Salicylic acid
SEX4	=	Starch excess 4
SH2	=	Src homology 2
Ser	=	Serine
Thr	=	Threonine
Tyr	=	Tyrosine

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

GKP is thankful to Department of Biotechnology (DBT) and Department of Science and Technology (DST), India for supporting research work. AS acknowledge Council for Scientific and Industrial Research (CSIR), India for research fellowship.

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