Published Online on 13 December 2017

Proc Indian Natn Sci Acad **83** No. 4 December 2017 pp. 845-863

DOI: 10.16943/ptinsa/2017/49223

Review Article

Phosphoinositides and Phospholipase C Signalling in Plant Stress Response - A Revisit

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(Received on 28 August 2017; Revised on 20 September 2017; Accepted on 06 October 2017)

Eukaryotic membrane lipids create hydrophobic physical barriers which control information and substance exchange between the outside and inside of cells and also between different cellular compartments. Membrane lipids like Phosphoinositides (PIs) also exert regulatory effect on cells in addition to its membrane trafficking property. Apart from that, phosphoinositide specific phospholipase C (PI-PLC), the enzyme acting upon phosphoinositides, also plays a significant role in exerting such regulatory effect on cell functions. PI-PLCs belong to an important class of enzymes and involved in lipid signalling. The functional mechanism of PI-PLCs is well studied in animal system. Certainly, it is evident that phosphoinositides and phospholipase C both play key roles in plant growth, development and stress tolerance. However, an exact mechanism pertaining to the action of the role of PI-PLC is still under elucidation. In this communication, an attempt has been made to highlight the mechanism of regulation in plant PI-PLCs and to revisit the area of functioning of PIs and PI-PLCs in response to biotic and in particular abiotic stresses in plants.

Keywords: Phosphoinositide; Phospholipase C; Salinity; Cold; Drought

Introduction

Cellular membranes act as physical barriers which separate the internal cellular environment from the external surroundings and play a crucial role under any type of external pressure or stress. Cell membranes are the primary sites for the perception of external stimuli and associated with an array of succeeding steps of signalling cascade, such as effector protein activation, secondary messenger generation and cellular metabolism modification. Although, proteins and genes in response to stress are studied the most, it is also evident that membrane lipids including their derivatives play a significant role in plant signalling network in response to environmental stresses (Heilmann, 2016). Though eukaryotic cells have structural complexities in their membrane system, they have developed to contain particular types of membrane lipids which apply regulatory controls on membrane-associated procedures (Lee, 2004). Such types of lipids apply their ability by influencing the bio-physical properties of the membrane or act as a ligand for membrane-linked proteins (Lundbaek *et al.*, 2010, Eyster 2007). Unlike other membrane lipids which participate in a structural role, phosphoinositides (PIs) are regulatory membrane lipids and are present in minor abundance in plants. Although PIs were discovered since three decades, their function in plant growth, development and stress tolerance are still under elucidation. However, at present, it is evident that PIs have crucial functions in plant development and stress tolerance (Heilmann and Heilmann, 2015; Bose and Im, 2012; Singh *et al.*, 2015) Furthermore, phosphoinositide specific phospholipases have emerged as key enzymes that carryout the initial step of phospholipid breakdown and generate multiple lipid derived secondary messengers (Heilmann and Heilmann, 2015). Two types of phospholipase activities have been recognized in plants, which include phosphoinositide specific phospholipase C, which primarily hydrolyzes phosphoinositides and the non-specific phospholipase C which hydrolyzes common

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membrane phospholipids, such as phosphatydilcholine (PC) and phosphatidylethanolamine (PE). Phosphoinositide-specific phospholipase C (PLC) belongs to a class of membrane-associated enzyme which plays a vital role in eukaryotic cell physiology and stress tolerance, especially in signal transduction pathway. In this review, we have revisited the area which gives an overview of PIs, PLCs, their structure, function and role in plant stress tolerance.

The condensation of *myo*-inositol and cytidine-diphosphodiacylglycerol catalyzed by phosphatydylinositol synthase (EC. 2.7.8.11) generates phosphatidylinositol (PtdIns) which is the precursor of all phosphoinositides (Heilmann, 2010). Phosphoinositides are derived from the PtdIns after phosphorylation of the head group of inositol. The inositol ring of PtdIns can be phosphorylated by specific lipid kinases at different positions which have only been seen in eukaryotes (Heilmann, 2010). A total of six phosphoinositides were identified in plants which are structurally related and were generated by phosphorylations of PtdIns at consecutive steps (Heilmann 2009). For example, phosphorylation of PtdIns forms phosphatidylinositol-4-phosphate (PtdIns4P) by PI 4-kinases, and PtdIns4P forms phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] by PI4P 5-kinases. Phosphoinositides show distinct irregularity between membrane and cytosol, where a majority is present in the cytosolic leaflet (Heilmann, 2010). PIs can work as ligands for partner proteins in eukaryotic cells. For example, PIs can attach to integral membrane proteins and can control their activity, as confirmed for ion channels and ATPase in some plants (Heilmann, 2009). PI binding also engages cytosolic protein to the cell membrane and regulates their biochemical activity (Heilmann, 2010). Partner protein-specific binding also takes place through phosphoinositides inositolpolyphosphate head groups. Pleckstrin homology (PH)-domains, Phox homology (PX)-domains and Fab1p-YOPB-Vps27p-EEA1 (FYVE)-domains are the examples of PI-binding domains, which binds to different PIs (Lemmon, 2003). Eukaryotic genome encodes a huge number of specific PI-binding domains (Figure 1) demonstrating involvement of PIs in different cellular processes (Lemmon, 2003).

Effect of Phosphoinositides on Membrane Properties

Apart from communications with partner proteins, the accretion of PIs can exert effects on the membranes biophysical properties. It has been established that membrane lipids with a high level of unsaturated fatty acid moieties will accrue in high curvature areas. The occurrence of such lipid favours the configuration of membrane vesicles. A supportive model for description of the influence of structure of lipid on membrane properties is the geometric estimation of the shape of lipid. According to this model, lipids having equivalent diameters of head group will be considered cylindrical (e.g. phosphatidylcholine), lipids with small head groups are conical (e.g. phosphatidic acid) and lipids with large head groups are inverse-conical (e.g. phosphoinositide) (Koijman *et al.*, 2003). Phosphoinositides are entirely linked with the cytosolic leaflets of the bilayer membrane and PIs may dictate increased membrane curvature area. Thus, PIs would make possible vesicle budding in the direction of cytosol during endocytosis or help in fusion of secretory vesicles with plasma membrane during exocytosis (Heilmann, 2010). Physiological studies in plant cells support the idea that PIs are involved in vesicle fusion and budding during membrane trafficking (Heimann, 2010). As phosphoinositides can arbitrate the conscription of vesicle coat proteins which will additionally stabilize membrane curvature, it remains unanswered whether PIs influence plasma membrane curvature principally through protein recruitment or through bio-physical effects or both.

Phosphoinositides and Phospholipase C

Phospholipase C cleaves the polar head group from inositol phospholipids and is called as phosphoinoside specific PLC. Being controlled by receptors on the cell surface, these enzymes hydrolyse the highly-phosphorylated lipid phosphatidylinositol 4,5 trisphosphate (PtdIns (4,5) P_3). Cleavage of PtdIns (4, 5) P_2 generates the intracellular signal products of inositol 1,4,5-trisphosphate (InsP3) and diacylglycerol (DAG). InsP_3 is a universal calcium (Ca^{2+}) mobilising second messenger and DAG activates protein kinase C (Singh *et al.*, 2015, Kanehara *et al.*, 2015, Peters *et al.*, 2014**).** PI-PLC has been identified in a vast number of eukaryotes ranging from yeast to mammals and a simplified form of this enzyme has also been

found to occur in bacterial systems (Kadamur *et al.*, 2013).

Of the earliest studies with this class of enzymes by Hokins (Hokin and Hokin, 1953) and then by Michel (2001), led to the recognition of PLC as a key enzyme in phosphoinositide metabolism and calcium signalling. Streb et al 1983, conclusively linked PLC with the release of intracellular calcium stores. During the early 1980s and the 90s three mammalian PLC sub-types β, γ, and δ were determined. Simultaneously, researchers identified a number of PLC regulators especially the GTP binding (G) α2 related sub-units and the protein tyrosine kinase. In the mammalian system, the role and regulation of PI-PLC has been well documented (Tuteja and Sopory, 2008). The present animal model of PI-PLC mode of action is the cleavage of the membrane phospholipid PtdIns (4,5) P_2 into InsP3 and DAG which are linked to the activation of protein kinase C (PKC) by DAG and the release on intracellular Ca^{2+} mediated by InsP3 sensitive channels (Delage *et al.*, 2013). However, the plant PI-PLC system has to have a different mode of action as plants lack sufficient amount of PtdIns 4, 5-P_2 in the membranes and definitive InsP3 receptors are yet to be demonstrated (Krinke *et al.*, 2007). Further, no plant PKC orthologs have been identified till date (Pokotylo *et al.*, 2014). Despite these, it is clear that PI-PLC in plants do play significant role during various environmental stresses as well at various developmental stages (Dowd and Gilroy, 2010, Ruelland *et al.*, 2014; Zhang *et al.*, 2014; Singh and Pandey, 2016; Li *et al.*, 2017).

Plant phospholipases are classified into phospholipase D (PLD), phospholipase C (PLC), phospholipase A1 (PLA1), and phospholipase A2 (PLA2) based on the site of glycerophospholipid hydrolysis. Each of the mentioned phopholipases has further differences in structure, substrate selectivity, cofactor requirements, and reaction conditions and is grouped in sub-families (Tasma *et al.*, 2008).

Structure of PI-PLC

Schematic representation of the different domains of bacteria, animal and plant PI-PLC has been shown in Fig. 1.

PI-PLCs are soluble multi-domain proteins ranging from ~85 kDa to ~150 kDa. Four β, two γ,

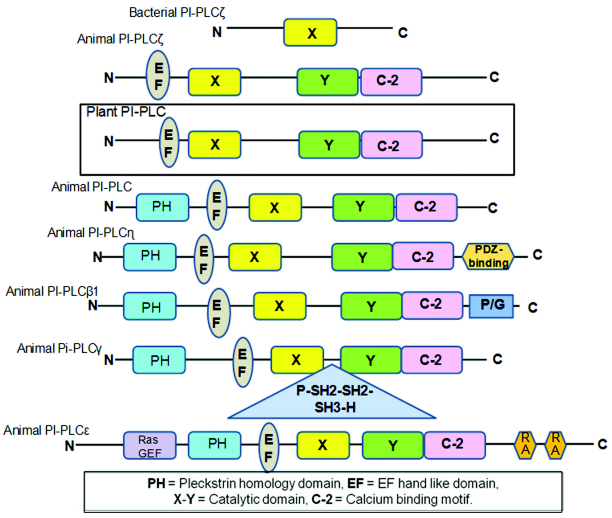


Fig. 1: Schematic representation of different domains of animal and plant PI-PLC. Bacterial PI-PLC shows the simplest form in comparison to the increasing complexity exhibited by animal and plant PI-PLC. The additional domains in animal PLCs have different functions.

four δ isoforms and numerous spliced variants have been described in mammals. PI-PLC occurring in yeasts, slime moulds, filamentous fungi and plants closely resemble the δ form. A total of 13 PI-PLC enzymes have been noted in mammals representing six isoforms (Munnik and Testerink, 2009). In plants, a great number of different PI-PLCs have been identified. Nine PI-PLC members have been identified in the Arabidopsis genome (AtPLC1-AtPLC9). Interestingly, AtPLC8 and AtPLC9 have large deletions in the catalytic domain reducing their catalytic activities. Four and 6 PLCs have been identified in the rice and tobacco genomes respectively (Bunney and Katan, 2011; Tasma *et al.*, 2008; Singh *et al.*, 2013). 12 putative PLCs genes are identified in the soybean genome on chromosomes 2, 11, 14 and 18 (Wang *et al.*, 2015).

In every PI-PLC, the catalytic X and Y domains are flanked by regulatory sequences (Tuteja and Sopory, 2008). Among the animal PLCs, PLC ζ is structurally the simplest isoform. It consists of a catalytic X-Y domain, flanked by N-terminal EF-hand and a C terminal C2 domain (Tuteja and Sopory, 2008). The other PLCs consist of additional domains. All of the PLCs consist of a N-terminal pleckstrin homology (PH) domain involved in membrane targeting and protein binding (Fujii *et al.* 2009, Singh *et al.*, 2003).

All plant PI-PLCs have a close resemblance to the PLC ζ isoform (Tuteja and Sopory, 2008). They are formed by a EF-hand, X/Y and C2 domains. Binding of Ca^{2+} occurs both at the C-2 domain and the X-Y linker region. Association with the G_{α} protein, membrane lipids has been demonstrated at the C2 domain in tobacco enzyme. In plant PLCs, the N-terminal undergoes a dimerization and association with the plasma membrane. In all known PI-PLC the catalytic X and Y domains are flanked by regulatory sequences.

The first structure of PLCδ1 comprising the EF-hands, catalytic domain and C2 domain, revealed that there are extensive inter-domain interactions; the central C2 domain interacts with both EF-hands and the catalytic TIM barrel based on the structural studies and domain organization of various PLCs, the EF-hands-C2 domain-TIM barrel unit probably will be structurally highly similar in all PLCs with the N-terminal PH domain attached loosely or making different interactions with other domains. The plant PLCs completely lack the PH domain (Rhee *et al.*, 1989, Rhee and Choi, 1992). Typically, a plant PI-PLC has a catalytic X (~170aa) and Y (~260aa) domains required for the phosphoesterase activity followed by a C2 domain at the C-terminal responsible for phospholipid binding and an EF-hand motif present at the N-terminal of the protein (Chen *et al.*, 2011).

The Catalytic X and Y Domain

In all mammalian PLC systems, highly-conserved domains are the X and Y boxes. The X and Y domains confer the catalytic activity of all PI-PLC as has been thought of till date. Experiments with amino acid substitution have shown that a single amino acid substitution in the X-domain abolished the catalytic capacity of the enzyme (Dowd *et al.*, 2006). The crystallographic structure of PLCδ revealed that this region of the amino acid consists of a distorted TIM barrel in the tertiary folding (Heinz *et al.*, 1995). All eukaryotic PI-PLCs have several highly conserved amino acid residues involved in the substrate binding and catalysis. In animal PLCδ1, Lys^{438}, Lys^{440}, Ser^{522} and Arg^{549} are found to interact with the 4 and 5 phosphate of the substrate head group and the positive charge of Arg^{549} has been attributed to the preferential hydrolysis of PtdIns-4,5-P_2 over PI (Ellis, 1998). Two conserved His residues in the X-domain have been found to take part in the mixed acid/base catalysed reaction of phosphoinositide hydrolysis (Essen *et al.*, 1996). These His residues have also been found to be conserved in plant PI-PLCs as in the His126 and His^{169} of the X-domain of mung bean enzyme. In Arabidopsis, AtPLC8 and AtPLC9 have been found to have the most divergent proteins compared to other AtPLCs. They have several long deletions in the Y region. It has also been found that AtPLC9 has a thermo-sensitive role as displayed in a PLC9 mutant (Ren *et al.*, 2016).

EF-hand Domain

Conventionally the EF domain consists of four helix-loop-helix folding motifs and it is often a characteristic of calcium binding proteins (Mikami *et al.*, 2004). The EF hand domain acts as an allosteric regulatory domain in animal PLCs that binds both calcium and lipids, stabilizes the enzyme and assist in active site formation (Kobayashi *et al.*, 2005). Though observations in several animal PLC isoforms suggest a major role of EF-hands in structural determinants, in plants no full length EF domain has been established. Most of them are truncated with only two helix-loop-helix motifs, and many plant PI-PLCs have no EF hand domain at all (Otterhag *et al.*, 2001, Hirayama *et al.*, 1997). In soybean PI-PLC1 the N-terminal EF domain is truncated but an additional putative EF-hand type calcium-binding motif is located between X and Y domains. It is interesting to note that plant PI-PLC with no N terminal EF hands can still show its catalytic activity. The role of EF hands in plant PI-PLC is yet to be understood (Otterhag *et al.*, 2001).

C2-domain

All identified plant PI-PLC contains a C2 domain. The C2 domain is responsible for the binding to phospholipids, wherein calcium may have a positive role in the entire process (Essen *et al.*, 1997). In potato as well as in rice PI-PLC specific hydrophobic residues and the polybasic stretch K-(K, R)-T-K in the C2 domain possibly brings the C2 domain binding to the anionic phospholipids. In many plant PI-PLC the C2 domain is sufficient to bind the enzyme to the membrane while in many others the C2 domain is involved only in membrane targeting where the EF hand is present (Hirayama *et al.*, 1997). Surface plasmon resonance spectroscopy and sedimentation

based phospholipid binding assay showed that C2 domain of plant PI-PLC alone is able to target membranes. Furthermore, alteration in surface hydrophobicity upon calcium stimulus is the major component in targeting plant PI-PLC to membranes from soluble fractions (Rupwate and Rajasekharan, 2012). This altered surface hydrophobicity property of PI-PLC plays a pivotal role in PLC regulation.

Other Structural Determinants

The linker region between the X and Y domain is a highly hydrophilic, divergent and believed to be essential and play different role in different PI-PLCs. In animals, this region has been found to have an auto-inhibitory function in most PI-PLC except PI-PLCæ (Hicks *et al.*, 2008). In comparison to this, plant PI-PLC contains a linker region containing a high percentage of acidic residues that are presumed to be exposed at the surface of the folded protein (Tasma *et al.*, 2008). The role of this linker region in plants is a subject of further research. The Cys7 residue is essential for disulphide bond formation resulting in homo-dimerization of *Chlamydomonas reinhardtii* PI-PLC and a lower lipid affinity. A cys residue is present in the first 10 amino acid of most plant PI-PLC but whether it is involved in homo-dimerization or not remains to be seen.

PI-PLC Biochemistry

Phosphoinositide specific phospholipase C can cleave phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P_2) to produce two downstream cellular regulators diacylglycerol (DAG) and inositol -1,4,5, trisphosphate (Krinke *et al.*, 2007). The activity of PI-PLC depends on Ca^{2+} that regulates not only the catalytic activity, but also the sub-cellular localization and substrate preference of the enzyme. PI-PLC demonstrated an intimate relationship between the functioning of enzyme with Ca^{2+} and the substrates phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate (PtdIns-4-P) and phosphatidylinositol -4,5-bisphosphate (PtdIns-4,5-P_2) (Krinke *et al.*, 2007, Chen *et al.*, 2011).

The substrate hydrolysis is accomplished by a two-step reaction. In the first step, a cleavage of phosphoinositide produces diacylglycerol and inositol 1,2 cyclic phosphates. In the second step, inositol 1,2 cyclic phosphate is converted into acyclic inositol phosphates. The mechanism proposed for these reactions involve a general base/acid catalysis (Katan 1996). Based on the structural studies, a number of amino acid residues (Lys438, Lys440, Ser522 and Arg549 in PLCä1) have been shown to interact with 4 or 5 phosphate of the substrate head group. Structural analysis of the mammalian PLCä1 has brought about valuable insights into the functioning of plant PI-PLC as both have a reasonably close resemblance. In PLCä1, the first step is the binding of PtdIns (4,5) P_2 through the PH domain and this binding helps the enzyme to associate with the membrane. In the next step, C2 domain along with the Ca^{2+} ions brings the catalytic domain in right conformation and the two conserved His residues participate to complete the catalysis (Wang, 2001). Studies on *Catharanthus roseus* PLC have shown that binding to lipid vesicles occurs through a single binding site, unlike their animal counterpart where binding occurs through multiple binding sites (Hernandez-Sotomayor *et al.*, 1999).

Biochemical analyses of plant PI-PLCs have shown that there are two kinds of PI-PLC; one localized in cytosol as the soluble form and the other is bound to membrane. Both the forms require availability of Ca^{2+} (Wang *et al.*, 2001, Dowd and Gilroy, 2010). Experimentally, it has been found that the concentration of Ca^{2+} determines substrate preference [PtdIns (4,5) P_2, PtdIns (4) P, or PtdIns)] of the enzyme. Soluble PI-PLC acts under the influence of millimolar levels of Ca^{2+}. On the other hand, membrane associated forms select PtdIns (4,5) P_2 and PtdIns (4) P under micromolar levels of Ca^{2+} (Chen *et al.*, 2011, Dowd and Gilroy, 2010). Different ions influence the activity of PI-PLCs in different ways. Millimolar levels of $Ca^{2+,}$ as well as Mn^{2+} and Co^{2+} inhibited wheat PI-PLC activity towards PtdIns (4,5) P_2, but promoted the activity towards PtdIns (4) P. The pH optimum for wheat PI-PLC is pH 6-7 for PtdIns (4) P and pH 6-6.5 for PtdIns (4,5) P_2 (Melin *et al.*, 1992). PI-PLC does not utilize phosphorylated D3 PtdIns derivatives, such as; PtdIns (3) P, PtdIns (3,4) P_2, PtdIns (3,5) P_2 and PtdIns (3,4,5) P_3. In potato plants, StPLC1, StPLC2 and StPLC3 were activated towards PtdIns (4, 5) P_2 at 10mM Ca^{2+} concentrations, but were inhibited by Al^{3+}. Application of calmodulin (CaM) enhances PI-PLC activity, whereas anti-CaM antibody suppressed this activity, such as observed in *Lilium daviddi* (Pan *et al.*, 2005). Plant PLCs unlike their animal counterparts lack the conserved

sequences involved in the interaction with G-proteins (Drobak, 1992). Nevertheless, they have been shown to be regulated by G-proteins. This has been seen in *Pisum sativum,* where a yeast two hybrid assay Gá1 is able to bind to the C2 domain (Misra *et al.*, 2007). Further, in wheat PI-PLC1 has been found to interact with GA3 (the central Gá subunit of the heterodimeric G-protein). Phosphorylation of tyrosine residues within the linker of animal PI-PLCã is responsible for the regulation of enzyme. In *Arabidopsis,* several sites of phosphorylation in the EF-domain have been identified by mass spectrophotometric peptide analysis (Nuhse *et al.*, 2004). Rupwate *et al.* (2012) provided evidence for the regulation of lipid biosynthesis by PI-specific PLC through upstream activating sequence inositol and the *trans*-acting elements. Gene expression analysis established that the over expression of rice PLC in yeast altered phospholipid biosynthesis at the transcript and enzyme levels (Rupwate *et al.*, 2012).

PI-PLC and Physiology of Plants

PI-PLC activities have been implicated in diverse development and physiological stages of the plant, such as; signal transduction in guard cells (Mills *et al.*, 2004), gravitropism (Perera et *al.*, 2001), Nod-factor signalling (Charron *et al.*, 2004) and carbon fixation in C4 plants (Coursol *et al.*, 2000). Regulation of stomata by abscisic acid (ABA) is one of the most well documented PI-PLC actions in plants. An early event in ABA signalling is the accumulation of cytosolic Ca^{2+} that stimulates stomatal closure (Hunts *et al.*, 2003; Mills *et al*., 2004). Conversely light is known to induce stomatal opening. In antisense transgenic lines producing lower levels of PI-PLC in guard cells, ABA-induced stomatal closure was low in relation to the wild type (Hunts *et al*., 2003). Both products of $PtdInsP_2$ turnover by PI-PLC have been shown to modulate guard cell activities. DAG has been reported to activate proton pump related to light induced guard cell opening, while increasing InsP3 levels are thought to induce cytoplasmic Ca^{2+}, leading to stomatal closure (Gilroy *et al.*, 1990; Peters *et al.*, 2014).

A second system in which PI-PLCs have been studied in some detail is in pollen and pollen tube growth. From several studies, it has been thought that PI-PLC is active in regulating the Ca^{2+} dynamics that are known to be required for pollen tube tip growth in a similar manner as to the $InsP_3$-based system (Holdaway-Clarke *et al.*, 2003). Pollen tubes elongated by localizing growth to the extreme tips and this very localization are determined by restriction of delivery of the new membrane material and the secretion of new wall components to the tip. In tobacco, germinating pollen tubes showed the accumulation of PI-PLC in the plasma membrane near the tip but not at the apex, whereas; PtdIns (4,5) P_2 exclusively accumulates at the apex of pollen tube (Dowd *et al.*, 2006). A tip-focused gradient in cytosolic Ca^{2+} levels coupled to the localized activity of a host of regulatory and structural proteins are required to maintain this spatial patterning of cell expansion. It is likely that in pollens, multiple isoforms of PI-PLC are expressed. This has been exhibited in the microarray and promoter expression analysis in Arabidopsis. Arabidopsis pollen shows at least three isoforms critical to pollen tube growth. InsP3 produced as a result of PI-PLC activities, is hypothesised to be important in plant gravitropic and phototropic responses (Perera *et al.*, 1999, 2001). Over-expression of inositol phosphate-5-phosphatase and suppression of PI-PLC showed a decreased plant gravitropic response (Melin *et al.*, 1992). The loss of PI-PLC1 resulted in insensitivity to cytokinin treatments and reduced gravitropic responses. Over-expression of BnPI-PLC2 in *Brassica napus*, resulted in multiple effects including enhanced photosynthesis accelerated flowering times, decreased maturation times, and changes in hormonal distribution in various tissues (Georges *et al.*, 2009).

Phosphoinositide Specific Phospholipase C in Relation to Plant Stress Response

PI-PLC has been found to have an active role in plant abiotic stress response. Significant participation of polyphosphoinositides produced by PI-PLC has been proposed in osmotic stress triggered signalling (Pokotylo *et al.*, 2014). Higher InsP3 levels due to the activity of PI-PLC during osmotic stress has been found to be accompanied by increase in PtdIns(4,5)P_2 levels, which indicates toward a concurrent activity of PI-PLC and phosphoinositide kinase (Pokotylo *et al.*, 2014). Increase of Ca^{2+} is central to plant cell stress perception. An increase in PI-PLC dependent Ca^{2+} in response to salt and other osmotic stresses, was seen in Arabidopsis root tip, seedling and tobacco cells (Perera *et al.*, 2008). Numerous studies have

indicated that PI-PLCs are responsible and influence numerous cellular processes under abiotic stresses. These include activation of mitogen activated protein kinases (MAPKs) and generation of reactive oxygen species (Im *et al.*, 2012), release of transcription factors (Tubby like) from the plasma membrane (Reitz *et al.*, 2012) and regulation of gene expression of PEP carboxylase kinase1 (Monreal *et al.*, 2013). Studies in Arabidopsis has further shown that PI-PLC, InsP3, and InsP3 gated Ca^{2+} increase influence several transcriptional and post-transcriptional events, that lead to the increased accumulation of osmolyte proline in response to ionic hyperosmotic stress (Parre *et al.*, 2007). Sub-cellular localization and genomic studies suggested that soybean PLCs have definitive role in environmental stress response and adaptation (Wang *et al.*, 2015).

Significant role of PLCs has been proposed under heat stress with evidence mounting from different plant species. In pea membranes, heat stress enhanced the membrane fluidity within 40 minutes of the induction of heat stress (Ruelland *et al.*, 2010). Under such conditions, PI-PLC proteins were found to increase appreciably (Liu *et al.*, 2006a). In Arabidopsis, a PLC gene AtPLC9 has been implicated in heat stress and the AtPLC9 mutants (*atplc9*) show high thermo-sensitive phenotype, following heat stress. A significantly lower accumulation of intracellular Ca^{2+} was detected in such *atplc9* mutants (Zheng *et al.*, 2012). AtPLC9 and AtPLC3 function co-ordinately in heat stress signalling as evidenced from the fact that atplc9/atplc3 double mutants showed high sensitivity, as compared to either of the single mutants.

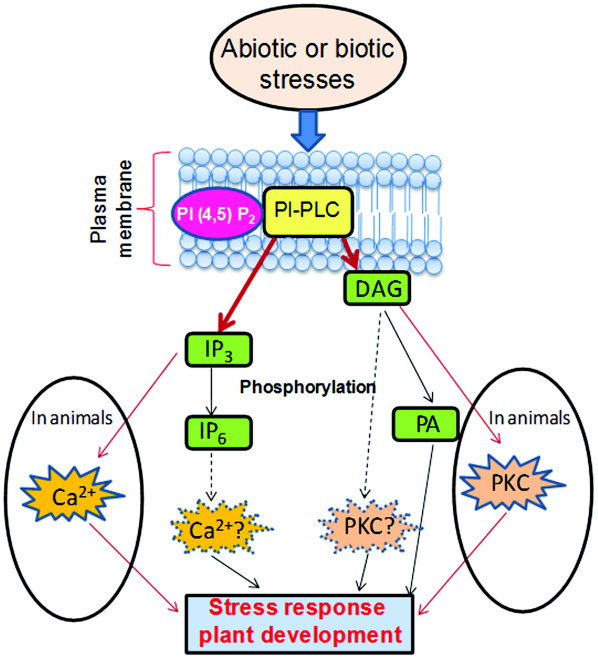
Being opposite to heat in physical sense, cold stress induces PI-PLC dependent signal transduction pathways. Increase in InsP3 accumulation with a simultaneous decrease in PI-4,5-P_2 levels was observed in winter grown wheat tissues, Arabidopsis suspension cultures and oilseed rape leaves (Bucolova *et al.*, 1994, Ruelland *et al.*, 2002, Smolenska-Sym *et al.*, 1994). Cold induced activation of PI-PLCs is dependent on calcium entry into the cells. The substrate for PI-PLC is supplied by type-III-phosphatidylinositol 4-kinases (Ruelland *et al.*, 2002, Delage *et al.*, 2013). Agents which altered the availability of Ca^{2+} inhibited the production of phosphatidic acid (PA), which implied that cold induced activation of phospholipase pathway is dependent on the entry of Ca^{2+} into the cell. Based on the studies of Arabidopsis desaturase mutant, a model was proposed where cold stress results in the membrane rigidity that leads to Ca^{2+} entry into the cell and consequent increase of PI-PLC activity (Vaultier *et al.*, 2006).

Toxic metal can inhibit plant cell metabolism by inhibiting key enzymatic reactions. Ni^{2+}, Zn^{2+} and Cu^{2+} decreased *in vitro* PI-PLC activity in both membrane and soluble fractions of *Catharanthus roseus* roots (Pina-Chable *et al.*, 1998). Role of PI-PLC based metal toxicity has been done extensively in aluminium stress. In *Coffea arabica* cells, aluminium treatment showed an increase of PI-PLC and rapid accumulation of InsP3. Prolonged exposure, however, reduced PI-PLC activity (Martinez-Estevez *et al.*, 2003, Yakimova *et al.*, 2007). The blockage of PI-PLC by aluminium may occur as a result of its binding to liposomal lipids substituting Ca^{2+} (Kopka *et al.*, 1998). However, the rapid activation of PI-PLC by Al^{2+} remains to be unravelled.

A model for PLC function under abiotic stress conditions is shown in Fig. 2.

Phosphoinositides in Relation to Plant Stress Response

Plants constantly observe their surroundings and interpret information which eventually reflects in their growth, reproduction and metabolism. The reactions obtained through both abiotic and biotic stresses are the best analysed through phosphoinositide signalling system described in plants. Abiotic stresses also have a considerable impact on the expression and regulation of genes linked with the signalling network. DNA chip analysis of different genes representing different isozymes involved in phosphoinositide signalling network and the expression pattern of different enzyme families were similar following treatments with auxin, abscisic acid and mannitol (Lin *et al.*, 2004). Furthermore, it has been established that many isoforms of phospholipase D (PLD), phospholipase C (PLC), phosphoinositide-phosphate 5-kinase (PIPK) and inositol polyphosphatase (IPPase) showed constitutive expression in unstimulated tissue and were differentially regulated by osmotic stress, salinity, drought and cold (Lin *et al.*, 2004). The most abundant IPP in plants is InsP6. Recently, Ins6 and its metabolites



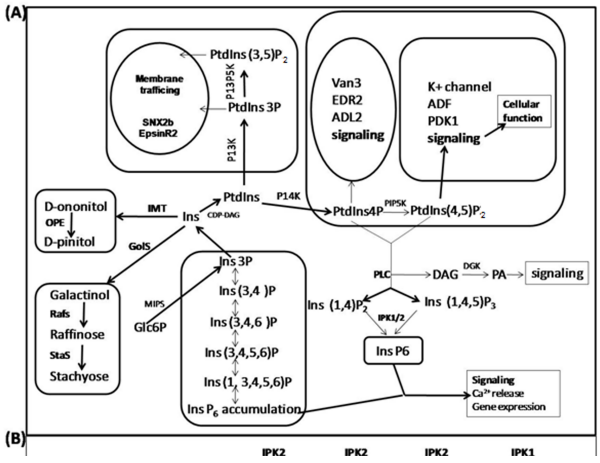


Fig. 2: An overview of PLC signalling pathway for stress response in plants. PI-PLC hydrolyzes PtdIns(4,5)P_2 or PI (4,5)P2 to Ins(1,4,5)P_3 or IP_3 and diacylglycerol (DAG). In plants Ins(1,4,5)P_3 is converted to InsP6 through inositol polyphosphate kinases, that attach to IP6 receptors and lead to the release of Ca^{2+} from reservoirs, hence IP6 act as secondary messenger in signalling pathways. DAG produced through PI-PLC can be converted to its phosphorylated product PA (phosphatidic acid) by DAG kinases. Ca^{2+} and PA work as signalling molecules in different signalling pathways such as abiotic stresses, biotic stresses, hormone signalling, phosphate starvation and plant development to produce favourable response to plants

have been implicated in cell signalling with crucial roles in chromatin re-modelling and gene expression (Shears 2009). Generally, two InsP6-generating pathways can be distinguished (1) lipid-dependent pathway through PLC (Fig. 3A) and (2) lipid - independent pathway through phosphorylation of MIPs- generated Ins3P (Fig. 3B). The 'lipid-dependent' signalling pathway hydrolyses PtdIns4P and/or PtdIns(4,5)P_2 through PLC, generating InsP_2 or InsP_3, which can be phosphorylated in a step-wise manner into InsP6 by the two inositol polyphosphate multikinases, IPK2 and IPK1, and DAG that is changed to PA as a signalling molecule. Under salinity conditions halophytes like common ice plant *Mesembryathemum* and *Porteresia* accumulate large amount of inositol derivatives such as D-ononitol and D-pinitol (Ishitani *et al.*, 1996; Sheveleva *et al.* 1997; Sengupta *et al.* 2008). Inositol is methylated to D-ononitol with the help of *myo*-inositol methyl-transferase (IMT) and subsequently epimerized to D-pinitol with the help of ononitol epimerase (OPE) (Rammesmayer *et al.*, 1995; Ishitani *et al.*, 1996). Other osmolytes such as raffinose and stachyose are also accumulated through the function of inositol. Galactinol synthase (GolS) catalyzes the first step in biosynthesis of galactinol and this galactinol is used as a substrate in raffinose and stachyose synthesis with the help of raffinose synthase (RafS) and stachyose synthase (StaS) respectively. This pathway