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Phospholipase D in Stress Activated Lipid Signaling in Plants

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ABSTRACT

Phospholipid hydrolysing enzymes, phospholipase D are represented by multiple gene members encoding various isoforms in plants. Different PLD isoforms display a varying requirement for the Ca^{2+} and the substrate lipid molecules for their function. By hydrolysing the phosphodiester bond of phospholipids and generating phosphatidic acid (PA), and a soluble head group, phospholipase D regulates various cellular processes in plants such as abscisic acid (ABA) signaling, programmed cell death, defense response to wounding and pathogens, root growth, freezing tolerance and other physiological responses. Studies suggest association of phospholipase D members with various biotic and abiotic stresses and their possible role in stress mediated signaling in plants, as their transcript level and protein activity changes upon exposure to stress stimuli. The focus of this review is discussion of the expression pattern and the functional role of different phospholipase D isoforms under various abiotic and biotic stresses, and the modulation of the stress signaling events leading to stress adaptation and tolerance in plants.

Keywords: abiotic stress, biotic stress, gene expression, phosphatidic acid, phospholipase D, signal transduction
Abbreviations: **ABA**, abscisic acid; **ABI1**, abscisic acid insensitive 1; **JA**, jasmonic acid **MAPK**, mitogen activated protein kinase; **PA**, phosphatidic acid; **PC**, phosphatidylcholine; **PE**, phosphatidylethanolamine; **PG**, phosphatidylglycerol; **PIP_2**, phosphatidylinositol 4, 5-bisphosphate; **PS**, phosphatidylserine; **PLA**, phospholipase A; **PLC**, phospholipase C; **PLD**, phospholipase D; **PH**, pleckstrin homology; **ROS**, reactive oxygen species

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INTRODUCTION

In the environment, survival of plants is full of challenges as they have to cope up with diverse sets of adverse conditions. These adverse conditions are represented by biotic and abiotic stresses such as pathogen and herbivore attack, drought, high salinity, temperature fluctuation, nutrition deficiency, etc. One of the inherent property of plants is being sessile and unlike animals, they cannot move away from these adversities, hence they are destined to devise a way in order to adapt to these hostile growth conditions. Generally, plants respond to various environmental cues with specific changes in their metabolism, growth and development. These responses are governed by most important and prevalent adaptive mechanisms, including signal transduction events, triggering a number of signaling cascades involving Ca^{2+}, protein kinases, protein phosphatases, transcription factors and lipid molecules. In recent years, lipid signaling has been recognised as one of the important regulatory networks in response to abiotic and biotic stresses (Hong *et al*. 2008a, 2008b; Hong *et al*. 2009; Li *et al*. 2009; Munnik and Testerink 2009; Yamaguchi *et al*. 2009; Chen *et al*. 2011; Wang *et al*. 2012; Singh *et al*. 2012a, 2012b). Various environmental stresses have been known to trigger the hydrolysis of membrane phospholipids, which leads to generation of variety of lipid-derived secondary messengers (Bargmann and Munnik 2006; Boss *et al*. 2008; Tuteja and Sopory 2008). Phospholipases are the major enzymes, which catalyse the initial step of phospholipid hydrolysis, therefore are of utmost importance in lipid signaling. In plants, phospholipases are broadly classified into phospholipase A (PLA), phospholipase C (PLC) and phospholipase D (PLD) categories, based on their catalytic action at different sites on a glycerophospholipid molecule (Wang 2001). Phosphatidic acid (PA) is one of the major lipid-derived secondary messengers, which is normally present at low levels in the membrane lipids, its level has been found to dramatically escalate upon exposure to stress stimuli (Munnik 2001). Phospholipase D (PLD) is the key enzyme, which is responsible for this increased level of PA

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Invited Review

under stress conditions as it cleaves a phospholipid and produce PA and a head group such as choline (Meijer and Munnik 2003). In plants, PLD constitutes an important group of lipid hydrolysing enzymes represented by various isoforms, which are involved in a number of significant cellular processes both in monocots and dicot species. PLD gene family has been intensively studied under biotic as well as abiotic stresses in plants, which signify their possible involvement for enhancing stress tolerance and for developing high yielding crop varieties in future. The main focus of this report is on the expression and role of PLDs under biotic, abiotic stresses, and hormone signaling in plants. In addition, the structural diversity of PLD family in different plant species, the catalytic activity, and regulation of PLDs are also discussed.

PLD GENE FAMILY IN PLANTS

Whole genome sequencing of a number of plant species (especially of the model plants such as *Arabidopsis* and rice) has provided a thrust in the area of plant research and facilitated the researchers worldwide to adopt the genome-wide identification approach for a number of important gene families (Kerk *et al.* 2002; Arora *et al.* 2007; Liu *et al.* 2010; Singh *et al.* 2010, 2012a). Availability of full genome sequence, various cDNA and EST databases in recent time has also enabled the identification of PLD gene family in some important plant species.

PLD gene family exhibits great diversity as it is represented by multiple members and isoforms in various plant genomes, whereas only a single member in yeast and two members in mammals have been identified (Wang 2005). *Arabidopsis* PLD family has been studied most exhaustively among all plant species, and comprised of 12 members (Qin and Wang 2002). The genome of rice (*Oryza sativa*), the model monocot crop plant, encodes 17 PLD members (Li *et al*. 2007). Recently, the genome exploration of grape (*Vitis vinifera*) and woody plant poplar (*Populus trichocarpa*) lead to the discovery of 11 and 18 PLD members, respectively (Liu *et al*. 2010). Similar diversity of PLD family has been discovered in other higher plants (Elias *et al*. 2002). Apart from these genome-wide studies, PLD members have also been identified in plants such as castor bean (*Ricinus communis*) (Wang 2000), cabbage (*Brassica oleracea*) (Schaffner *et al*. 2002) poppy (*Papaver somniferum*) (Lerchner *et al*. 2005), sunflower (*Helianthus annuus*) (Moreno-Perez *et al*. 2010) and *Jatropha curcas* (Liu *et al*. 2010).

Similar to genomic diversity, PLDs exhibit great structural diversity and have been classified into subfamilies and isoforms. Domain structures (especially N-terminal lipid binding domain) in various PLD proteins demarcate two subfamilies in PLDs, designated as C2-PLD and PX/PH-PLD (Wang 2005). A unique PLD member called SP-PLD has been exclusively identified in the genome of rice, while it is absent in other plant genomes (Li *et al*. 2007). Plant C2-PLD members harbour a Ca^{2+}/phospholipid binding C2 domain, which is not found in mammalian PLD representatives (Burke and Dennis 2009). C2 domain in plant PLDs has been proposed to mediate the membrane localization of soluble proteins in calcium dependent manner (Kopka *et al*. 1998). PX/PH-PLD members are characterised by the presence of a phox (PX) and pleckstrin homology (PH) domain and they lack calcium binding (C2) domain (Qin and Wang 2002). PX and PH domains have been implicated in membrane targeting and polyphosphoinositide signaling of PLD (van Leeuwen *et al*. 2004). SP-PLD lacks C2, PX, and PH domains and instead, comprises a signal peptide at N-terminal, characteristic of some mammalian PLDs (Li *et al*. 2007). In the *Arabidopsis* genome, out of 12 members only two belong to PX/PH-PLDs while 10 are C2-PLD (Qin and Wang 2002). Similarly, the rice genome also encode for only two PX/PH-PLDs (Li *et al*. 2007). Based on the sequence homology at protein level and biochemical properties, PLD family has been grouped into six classes namely α, β, γ, δ, ε and ζ in plants (Qin and Wang 2002; Wang 2005). Li *et al*. (2007) identified two more classes of PLDs in rice genome, which have been designated as κ and φ. Yamaguchi *et al*. (2009), in their study have re-designated these classes as θ and *ι*, respectively. Additionally, *OsPLDα6* and *OsPLDα7* have been renamed to *OsPLDη1* and *OsPLDη2*, respectively; and *OsPLDα8*, based on its similarity with *AtPLD*ε, has been re-designated as *OsPLDε* (Yamaguchi *et al*. 2009). The ζ-PLD contains PX and PH domain and bear more resemblance with yeast and mammalian PLDs than any other class of plant PLDs (Bargmann and Munnik 2006). Entire PLD family members in eukaryotes, comprises of two highly conserved C-terminal HxKxxxxD (HKD) motifs and N-terminal lipid binding region (Bargmann and Munnik 2006). The HKD motifs have been identified as catalytic sites and are essential for the catalytic activity of these enzymes (Qin and Wang 2002).

ENZYMATIC ACTIVITY AND REGULATION OF PLDs

The HKD motif

The conserved HxKxxxxD motif in the all the eukaryotic PLDs is an important feature, essential for their catalytic activity. PLD members utilize the conserved His residue for the nucleophilic attack on the phosphorus of phospholipids. Basically, these enzymes act on the phospholipids and hydrolyse them in a two-step ping-pong reaction, where, in the first step the head-group is detached leading to the formation of a phosphatidyl-PLD intermediate between the phosphorus and histidine from one of the two HKD motifs. Subsequently, this intermediate is hydrolysed in second step resulting in the generation of PA (Stuckey and Dixon 1999). Plant PLDs mainly act on the lipid substrates such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) (Pappan *et al*. 1998).

Regulation by Ca^{2+} and lipids

Apart from the sequence and the structure, prerequisites including Ca^{2+} and phosphatidylinositol 4, 5-bisphosphate (PIP_2), substrate specificity and pH for *in-vitro* catalytic activity, also form a basis for the categorization of different PLDs (Wang 2001; Bargmann and Munnik 2006). PLDαs at neutral pH require millimolar concentrations of Ca^{2+} (Kuppe *et al*. 2008) while at the acidic pH, micromolars level of Ca^{2+} are adequate to support the PLDα activity (Li *et al*. 2009). PLDαs do not require PIP_2 for their activity, while catalysing a variety of phospholipid substrates such as PC, PE and PG in different plant species (Dippe and Ulbrich-Hofmann 2009; Li *et al*. 2009; Moreno-Perez *et al*. 2010). On the other hand, PLDβ and PLDγ, which are closely related isoforms of PLDs, have been found to be active at micromolar concentrations of Ca^{2+} at neutral pH and are PIP_2 dependent (Qin *et al*. 1997). This contrast between the PLD isoforms has been explained by the observation that PLDβ and PLDγ comprised of four acidic residues in their C2 domain while PLDα contains only two acidic residues (Zheng *et al*. 2000; Qin and Wang 2002). PLDβ and PLDγ have been known to be PIP_2 dependent (Pappan *et al*. 1997) and recombinant PLDβ and PLDγ from *Arabidopsis* could hydrolyse PE or PS in the presence of PIP_2 (Pappan *et al*. 1998). PLDδ, have been found to be active at millimolar range of Ca^{2+} at neutral pH. This PLD isoform is independent of PIP_2 and has been shown to be activated by oleic acid (Wang and Wang 2001). PLDζ isoform has a PH-PX domain combination and represented by two members both in *Arabidopsis* and rice. This isoform is calcium-independent similar to mammalian PLDs, and active at neutral pH and requires PIP_2 for its activity (Qin and Wang 2002). Another PLD isoform, PLDε, which is also known as PLDα4 is the most liberal of all the PLDs in terms of its substrate and cofactor requirements (Hong *et al*. 2009).

For the integrity and organization of cells and orga-

nelles, *in-vivo* activity of PLDs must be tightly regulated since uncontrolled hydrolysis of membrane could be detrimental to the cell. As discussed previously, *in-vitro* activity of plant PLDs is regulated by Ca^{2+}concentration, pH and PIP_2. In addition, some other elements have been identified, which participate in the regulation of PLD activity and includes, α-subunit of hetero-trimetric G protein (Lein and Saalbach 2001; Zhao and Wang 2004), actin (Kusner *et al*. 2003) and phosphorylation (Novotna *et al*. 2003). The regulation of PLD in plants by G protein is contrasting from mammals, where β/γ subunit of a trimeric G protein and monomeric G proteins are involved (Hiroyama and Exton 2005; Preininger *et al*. 2006). Recently, evidences for the regulation of PLD activity by actin have been provided in the study of tobacco PLDβ wherein, monomeric actin (G-actin) strongly hampered its activity and polymeric actin (F-actin) was stimulatory (Pleskot *et al*. 2010). They also showed that the effect of actin was more imposing on the activity of PLDβ than on the activity of PLDα and PLDδ. Study with cabbage (*Brassica oleracea*) PLDγ (*BoPLDγ*) showed that it could be regulated positively by phosphorylation (Novotna *et al*. 2003). Interestingly, one of the putative phosphorylation site (T640) of AtPLDγ1 corresponds to a tobacco PLDβ1 conserved threonine residue (T382), which was found to be critical for the actin binding as shown by Pleskot *et al*. (2010). This observation indicated a connection between the phosphorylation state and PLDβ1 interaction with actin.

PLDs IN ABIOTIC STRESS IN PLANTS

Various abiotic stresses such as cold, high salinity, dehydration, osmotic stress have been known to affect the longevity, life span and the productivity of the plant. In order to combat these adverse environmental conditions, plants have evolved an intricate adaptive mechanism, which involves the triggering of a number of signaling networks leading to adaptation and stress tolerance in plants. Recently, lipid signaling has been well studied and acknowledged in such adaptive signal transduction networks. PLD, one of the three major phospholipase classes (PLA, PLC being remaining two) have been well emphasized and found at the centre of these abiotic stress triggered signal transduction networks.

ABA, salt, drought and cold stresses

As discussed earlier, plant PLD family is comprised of multiple isoforms and the activity of many of these have been found to be altered in response to high salinity (Testerink and Munnik 2005; Zhang *et al*. 2008; Bargmann *et al.* 2009), dehydration (Katagiri *et al.* 2001; Sang *et al*. 2001; Mane *et al*. 2007), and cold (Welti *et al.* 2002; Li *et al.* 2004; Rajashekar *et al*. 2006) stresses, as well as phytohormone ABA (Zhang *et al.* 2004; Mishra *et al*. 2006). The significant role of PLDs in various abiotic stresses have been ascertained by their transcript expression profiles in various plant species such as *Arabidopsis*, rice and tomato (Wang *et al*. 2000; Li *et al*. 2007; Bargmann *et al.* 2009; Singh *et al*. 2012b), where different PLD isoforms displayed a significant differential expression. Among all the PLD isoforms, PLDα1 has been studied most extensively. In a knockout mutant based study in *Arabidopsis*, PLDα1 has been implicated in ABA mediated signaling and drought response (Zhang *et al.* 2004). It was demonstrated that the PLDα1 deficient *Arabidopsis* plants suffered from more transpirational water loss and reduced ABA induced stomatal closure than the wild type plants (Zhang *et al*. 2004). This reduction in the stomatal closure was negligible in PLDα1 deficient plants when compared with wild type after treatment with external PA (Zhang *et al*. 2004). In a prior study in tobacco (Sang *et al.* 2001), overexpression of PLDα1 made the plants more sensitive to ABA and lead to reduced transpirational water loss. They revealed that this effect on stomatal movement was mediated by the interaction of PLDα1 derived PA with ABI1 (protein phosphatase 2C), which has been known as a negative regulator of ABA signaling pathway (Merlot *et al*. 2001). This interaction of PA with ABI1 masked its phosphatase activity and therefore, refuted the negative regulation of ABA mediated response and leads to enhanced stomata closure (Zhang *et al*. 2004; Mishra *et al*. 2006). Furthermore, PLDα1 and its derivative PA were also acknowledged to prevent the closed stomata from opening through their interaction with a heterotrimeric G protein in an ABA dependent pathway (Zhang *et al*. 2004; Mishra *et al*. 2006). Moreover, in several studies, it has been established that plants, which overexpressed PLDα, exhibited hypersensitivity towards ABA, reduced level of water loss and infer drought and salinity tolerance (Zhang *et al*. 2008; Peng *et al*. 2010). However, in a study with tobacco, it was shown that PLDα1 exhibit a dual function under drought condition. In early stages of drought, the PLDα1 overexpressing plants showed reduced water loss due to efficient stomatal closure while at the later stages these plants exhibited more susceptibility to drought due to enhanced membrane degradation (Hong *et al*. 2008b). PLDα1 has also been implicated in salt stress response and signaling. The transcript level of tomato PLDα1 (*LePLDα1*) were escalated in cell suspension cultures after salt treatment (Bargmann *et al*. 2009). The study in *Arabidopsis* showed that *AtPLDα1* and *AtPLDδ* were activated in response to salt stress and the *pldα1* and *pldδ* single and double mutant plants exhibited sensitivity to high salinity stress. However, *pldα1/pldδ* double mutant plants were more susceptible to salinity than their respective single mutants (Bargmann *et al*. 2009). This observation indicated that *AtPLDα1* and *AtPLDδ* have their specific as well as overlapping function. Interestingly, both *AtPLDα1* and *AtPLDδ* mediate freezing response in *Arabidopsis*, but in an antagonistic manner (Welti *et al*. 2002; Li *et al*. 2004). PLDα1 deficient plants were more tolerant to freezing stress (Welti *et al*. 2002), whereas PLDδ knockout plants were susceptible to freezing stress (Li *et al*. 2004). Freezing tolerance of PLDα1 deficient plants was explained by the speculation that, deficiency of PLDα1 might have resulted in the reduced disturbance in the membrane composition and hence, lead to membrane stability under freezing conditions. Another member of plant PLDα group, PLDα3 have also been implicated in similar stress responses. In *Arabidopsis*, it has been shown that the varied expression level of PLDα3 resulted in the alteration of plant response to dehydration and salinity (Hong *et al*. 2008a). PLDα3 deficient (knock out mutant) plants were highly sensitive to salinity, and dehydration and ABA responsive genes were readily induced, while overexpressing plants showed reduced sensitivity (Hong *et al*. 2008a). Under the hyperosmotic conditions PLDα3 knockout mutant plants had lesser root growth whereas PLDα3 overexpressing plants had better root growth and lateral root hairs, some of the key features, which might help plants to adapt better under drought conditions (Hong *et al*. 2008a). Moreover, PLDα3 overexpressing plants bear flowers earlier than the wild type plants under mild drought condition while, PLDα3 mutant plants had delayed flowering (Hong *et al*. 2008a). These findings, suggested that PLDα3 regulate the hyperosmotic signaling and response and act positively to help plants adapt to adverse environmental conditions.

Arabidopsis PLDε was characterised and it was shown to mediate hyperosmotic responses since PLDε null mutants had reduced PA content under salinity and dehydration conditions (Hong *et al.* 2009). Also, PLDε mutant plants had lesser and shorter lateral roots, whereas the PLDε overexpressing plants had higher PA level and results in better growth of lateral root hairs, under salinity and dehydration conditions. The analysis with inhibitor such as 1-butanol confirmed that the responses were mediated by PLDε generated PA, as inhibition of the PA activity resulted in vanishing of the phenotypic difference between wild type and genetically altered plants (Hong *et al.* 2009).

Oxidative stress

Variety of abiotic stresses such as salinity, drought, cold and phytohormone ABA are known to induce the accumulation of various reactive oxygen species (ROS) like superoxide, hydrogen peroxide, and hydroxyl radicals (Guan *et al*. 2000; Hasegawa *et al*. 2000; Pei *et al*. 2000). These different ROS species may be either initiating a protective mechanism or may be damaging to the plant cell (Prasad *et al*. 1994). Plant PLDs are implicated to function in concert with ROS to mediate various hyperosmotic responses. *Arabidopsis* PLDs isoforms; *AtPLDα1* and *AtPLDδ* have been found to mediate both the production of ROS and their responses (Sang *et al*. 2001; Zhang *et al*. 2003, 2005). *AtPLDα1* and its reaction product PA have been shown to mediate the ROS production through regulation of NADPH oxidase activity in ABA mediated stomatal closure event (Zhang *et al.* 2009). In a recent study, deletion of AtPLDα1 lead to reduced ROS production in the mutant plants, while addition of PA resulted in the stimulated NADPH activity and enhanced ROS production, both in wild type and AtPLDα1 deficient plants (Zhang *et al.* 2009). These observations affirm the involvement of PLDα1 in ABA mediated ROS generation and stomatal closure and hence suggest that PLD generated PA is the central lipid molecule, which connect different cellular regulators in ABA and drought signaling networks. *Arabidopsis* PLDδ was found to be activated by hydrogen peroxide (H_2O_2) and the resulting PA could decrease the extent of H_2O_2 triggered programmed cell death (Zhang *et al.* 2003). The level of PA was reduced in PLDδ mutant plants and additionally the cells of these plants were having higher H_2O_2 induced death rate and hence high susceptibility to stress (Zhang *et al.* 2003). These findings established a connection between stress signaling and ROS production, which is mediated by PLDδ and suggested PLDδ regulate ROS mediated cell death positively and ultimately leads to cell protection.

PLDs IN BIOTIC STRESS

Like abiotic stresses, plants are constantly challenged by variety of biotic stresses including bacterial, viral, fungal, insects, and herbivores attacks during their life time. Similar to abiotic stresses, plants have also evolved a number of adaptive responses against pathogens or biotic stresses. These responses might be very specific, general or overlapping against various types of biotic challenges. Similar to abiotic stresses, PLDs have also been implicated in biotic stress responses. The transcript levels of PLD were found to be altered in rice leaves when challenged by bacterial blight pathogen *Xanthomonas oryzae* (Young *et al*. 1996). Moreover, expression analysis of *Arabidopsis* PLDα, PLDβ and PLDγ isoforms revealed a differential expression pattern upon treatment with virulent and avirulent strains of *Pseudomonas syringae* pv. *tomato* (de Torres Zabela *et al*. 2002). In another report, PLDγ1 and PLDβ were found to be induced in an early response to the pathogen attack and PLDγ1 was specifically up-regulated during gene-for-gene interaction, which resulted in the hypersensitive response (de Torres Zabela *et al*. 2002). Also, in suspension cultured rice cells after treatment with defense elicitor *N*-acetylchitooligosaccharide, PLD was activated and the level of PA was elevated (Yamaguchi *et al*. 2003). In tomato suspension cells and leaves, the PLDβ1 was activated and its transcripts were accumulated (more than five-fold relative to control) after treatment with the fungal elicitor xylanase (Laxalt *et al*. 2001). Moreover, the other elicitor chitotetraose could not trigger the same response as xylanase, indicating that it is not a general elicitor response. This observation was further supported by another study where RNAi based knockdown of *LePLDβ1* transcript in tomato suspension cells resulted in hampered xylanase induced PLD activity and confirmed that this isoform was responsible for the elicitor induced PLD activity (Bargmann *et al*. 2006). A very recent report by Wang and co-workers (2012), revealed that in tobacco suspension cell culture, expression of different PLD isoform varied differentially in response to riboflavin (a defense activator) treatment and by the use of PLD inhibitors and pharmacological agents, they confirmed involvement of PLDs in defense signaling. PA, the product of PLD activity was implicated in the ROS production in the rice suspension cells and also it induced the expression of elicitor responsive genes even in the absence of any elicitor (Yamaguchi *et al*. 2005). Moreover, the addition of PA resulted in the escalated phytoalexin production, which is a response to incompatible plant pathogen interaction. Recently, Yamaguchi *et al*. (2009) showed that *OsPLDβ1* knockdown rice plants exhibited an activated defense response and increased disease resistance in the absence of pathogen. RT-PCR and microarray analysis of *OsPLDβ1* knockdown plants revealed that hundreds of genes were differentially regulated and these included defense related genes such as PR-protein and ERF/WRKY transcription factor genes. Cell death and phytoalexin production were observed at an elevated level as a hypersensitive response in these plants. Also, these plants were assessed to be highly resistant to major rice pathogens such as rice blast (*Pyricularia grisea*) and rice bacterial blast (*Xanthomonas oryzae* pv. *oryzae*) (Yamaguchi *et al*. 2009). Therefore, it was concluded that *OsPLDβ1* act as a negative regulator of disease resistance and defense responses in rice. Such kind of regulation of defense responses is necessary for the normal plant growth as deregulated constitutive defense response might be damaging for the plants. Overall, these findings suggested active involvement and important role played by PLDs in plant defense mediated by pathogens.

PLDs IN WOUNDING

Wounding stress could be imparted to plants by both abiotic and biotic factors where plants respond by employing various defense strategies such as activation of signaling networks. In most of the cases, plant experience wounding stress by herbivores, pathogen attack, wind bruising or any other mechanical injury, where it leads to damage or disintegration of cell membrane. In order to survive the wounding or injury, as in case of abiotic or biotic stress, plants incorporate an array of complex signaling networks comprised of multiple signals and differential induction of gene expression at molecular level (Leon *et al*. 2001). Various molecules are produced in response to wounding, which includes hormones such as jasmonic acid, ethylene, oligopeptide like systemin (Farmer and Ryan 1991; Pearce *et al*. 1991; O'Donnell *et al*. 1996) and mediate the direct and indirect defense responses (Wasternack *et al.* 2006). Involvement of PLDs in such signaling networks and responses has been assessed in different plant species (Ryu and Wang 1996; Wang *et al*. 2000; Lee *et al*. 2001). Wounding triggered the activation of PLD in castor bean (*Ricinus communis* L.) leaves, which was assessed by the escalated PA and choline level at the site of wounding (Ryu and Wang 1996). Moreover, the wound activation of PLD was observed at unwounded cells and it was attributed to intracellular PLD translocation from cytosol to membranes, which was mediated by elevated cytosolic calcium upon wounding. In another study, Lee *et al*. (2001) established a connection between PLD activity and MAPK signaling in soybean (*Glycine max*). They showed that in response to wounding in soybean seedlings, a MAPK gets activated both in wounded and unwounded surrounding cells and this activation is contributed by the activity of PLD, as inhibition of PA production by addition of *n*-butanol suppressed the MAPK activity. Furthermore, addition of exogenous PA resulted in the activation of MAPK in suspension culture soybean cells (Lee *et al*. 2001). This finding recognized that PLD acts upstream to MAPK in a wound induced signaling network (Lee *et al*. 2001; Bargmann and Munnik 2006). In *Arabidopsis*, *AtPLDα1* activity was associated with the wounding response, as antisense silencing of this gene resulted in the reduced induction of PA and jasmonic acid (JA) and

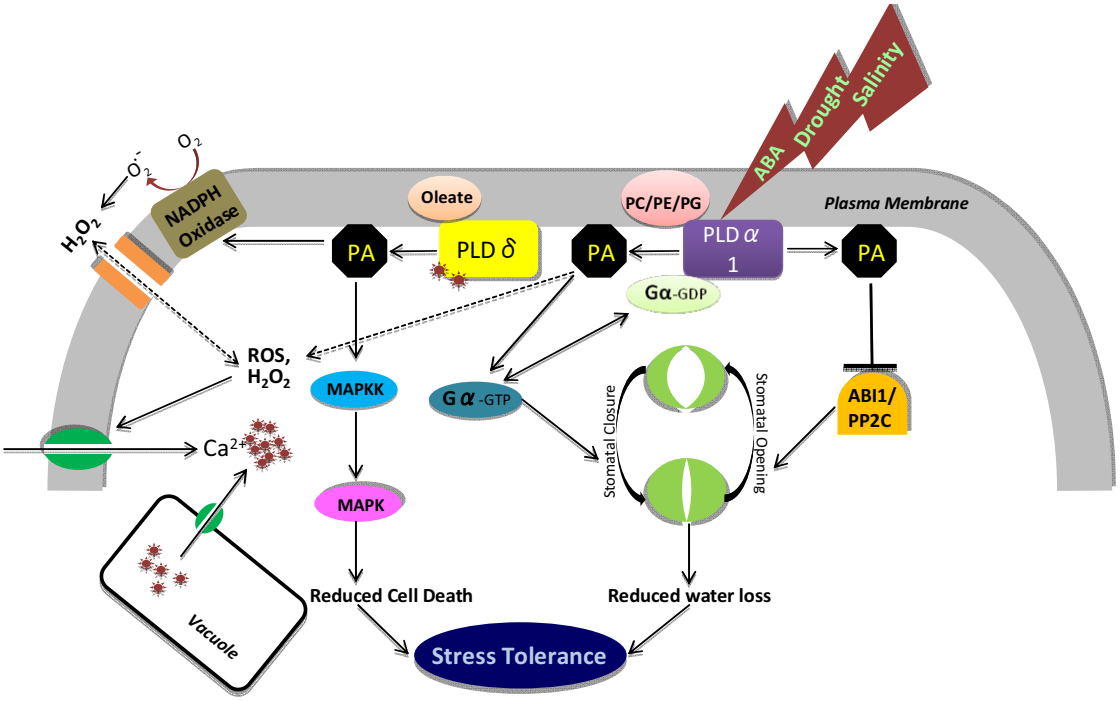

Fig. 1 A proposed model for the signaling networks activation by PLD in response to various abiotic stresses and ABA in plant cell. Various signals activate PLD, which hydrolyses the membrane lipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) to produce phosphatidic acid (PA). PLDα1 generated PA interact with a PP2C-ABI1 and removes its inhibition on the ABA induced stomatal closure. $G\alpha\text{-}_{GDP}$ binds to, and inactivates PLDα1. ABA signal activates Gα and causes the conversion of $G\alpha\text{-}_{GDP}$ to $G\alpha\text{-}_{GTP}$, which activates PLDα1 (by removal of $G\alpha\text{-}_{GDP}$ inhibition). PA acts upstream of $G\alpha\text{-}_{GTP}$ to promote stomatal closure, which ultimately results in the reduced transpirational water loss. Stress also causes the production of ROS and especially H_2O_2, which leads to enhanced cytosolic Ca^{2+} concentration. Ca^{2+} activates PLDδ in the presence of oleate and resulting PA further activates membrane localized NADPH oxidase to enhance the production of H_2O_2. PLDδ generated PA also activates the MAPK signaling, which culminates into reduced cell death and enhanced cell viability. Both the physiological responses; reduced water loss and enhanced cell viability leads to stress tolerance.

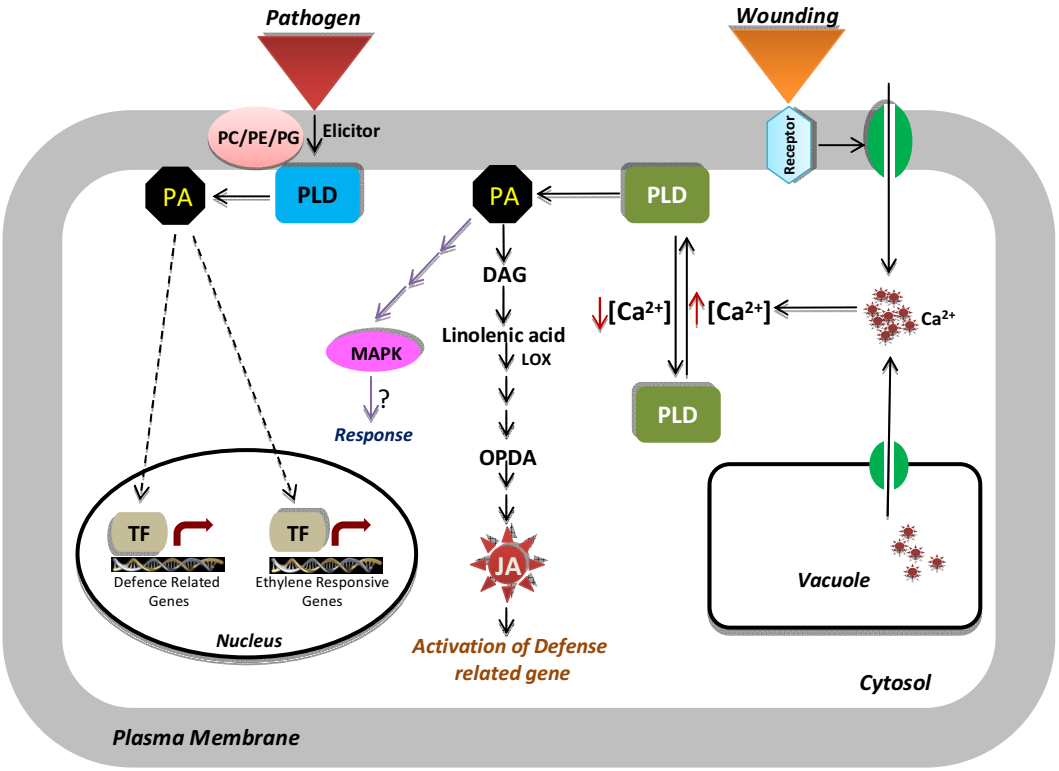

Fig. 2 A hypothetical model depicting the activation of PLD in response to biotic stress and wounding. Upon a pathogen attack, a signal is perceived at the plasma membrane, which leads to activation PLD, and production of PA, which in turn interact with various transcription factors (TF) such as ERF/WRKY family to enhance the transcription of defense related genes such as PR-protein and ethylene responsive genes. Wounding signal is perceived by the receptor at the plasma membrane, which results in the increased cytosolic Ca^{2+} concentration. Increased $[Ca^{2+}]_{cyto}$ promotes the translocation of PLD to the membrane, where it hydrolyses the lipids to produce PA, which initiate a series of reactions and leads to the production of jasmonic acid (JA), which activates the defense related genes. On the other hand, PA transduces the wound signal to a MAPK signaling cascade to regulate the physiological response. **DAG**, diacylglycerol; **LOX**, lipoxygenase; **OPDA**, 12-oxophytodienoic acid.

alteration in the wound induced gene expression (Wang *et al*. 2000). The reassessment of *Arabidopsis* PLD activity in wound response by Bargmann *et al*. (2009) revealed that although, the PLD activity was induced after wounding but it remained restricted to the ruptured cells. Furthermore, it was shown that *pldα1* knockout mutant plants had reduced level of PA and it was completely abolished in *pldα1/pldδ* double mutant plants. However, the wound induced protein kinase activation, defense gene expression and JA production remained unaffected in these PLD mutant plants (Bargmann *et al*. 2009), while the role of PLD in wounding response in *Arabidopsis* remains debatable. A recent study in rice has proven the role of two chloroplast localized phospholipase D; *OsPLDα4* and *OsPLDα5* in herbivore induced direct and indirect defense and mechanical wounding (Qi *et al*. 2011). The expression of these PLDs was found to be induced by the mechanical wounding, by rice striped stem borer (*Chilo suppressalis*) and by the treatment of JA. Antisense knockdown of *OsPLDα4* and *OsPLDα5* resulted in reduced levels of linolenic acid, JA, green leaf volatiles and ethylene; and also led to alteration in the expression of a *OsMPK3*, a lipoxygenase (*OsHI-LOX*), a hydroperoxide lyase (*OsHPL3*) and *OsACS2* genes (Qi *et al*. 2011). These observations suggested involvement of PLD and PA in plant responses to wounding, but detail mechanistic dissection of wound triggered signaling pathways involving PLDs need investigation.

CONCLUSION AND FUTURE PROSPECTS

The detail genomic surveys in various plant species have revealed that phospholipase D is a multi-gene family and represented by different isoforms. These isoforms could be grouped into various classes based on their sequences, domain structures and functional characteristics. The enzymatic activity of different PLD classes is dependent on several factors including Ca^{2+}, pH and the lipid substrates. Numerous studies have provided the evidences for the involvement and role of PLD enzymes in most of the stresses encountered by the plants. PLDα1, PLDα3, PLDδ and PLDε isoforms have been majorly found to be induced by various abiotic stresses and regulate the stress responses. The PLD isoforms catalyse membrane hydrolysis and lead to the production of PA, which has been shown to mediate most of the stress responses. In *Arabidopsis*, PLDα1 has been studied most extensively, and the knockout and overexpression plant studies have established a place for this isoform in the hyperosmotic and ABA mediated stress signaling and response. PLDs have also been found to regulate the abiotic stress responses through ROS generation (**Fig. 1**). Similarly, biotic stress such as pathogen attack and wounding events are also affected by various PLDs in plants. PLDβ1 has been found at the centre of biotic stress signaling, as its activity altered by pathogens and elicitors in important crop plant species such as rice and tomato. The PLDs have also been found to be involved in wound response pathway in plants such as soybean and *Arabidopsis*, arguably through regulation of MAPKs (**Fig. 2**). More recently, chloroplast localized PLDs (*OsPLDα4* and *OsPLDα5*) have been speculated to mediate the wound signaling in rice. Although, the knowledge has been expanded regarding the functions of PLDs in plants during stress responses, a lot is yet to be answered. Only a few PLD isoforms have been characterised functionally in plants, and many of these PLD isoforms opens future prospects for a detailed functional characterization. Studies can be done by generating multiple PLD knockout plants (such as double, triple and quadruple mutant) to understand the phenotypic differences, which are still not very decisive with specific PLD knockout mutants because of multiple gene redundancy. Most plant abiotic stress responses involving PLDs have been found to be mediated by ABA; therefore it would be interesting to find out role of PLDs in ABA independent abiotic stress responses. Study for the cross talk of signaling pathways under abiotic stresses will resolve the issue whether PLDs transduce signal in a linear signaling pathway or the responses are generated by the criss-cross connection of various factors. Also, the detail sub-cellular localization of various PLD isoforms will provide a clue about their site of action. Finally, it would be of significance to explore the components upstream or downstream of PLDs to reconstitute a stable and relevant signaling pathway in various plant stresses.

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