REVIEW

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PHOSPHATIDIC ACID FORMATION AND SIGNALING IN PLANT CELLS

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This review conteins updated information on the structure, localization and regulation of phosphatidic acid (PA)-producing enzymes phospholipase D, phosphoinositide-specific and non-specific phospholipases C and diacylglycerol kinases is analyzed. The specific role of PA and PA-producing enzymes in plant stress signaling is discussed.

Keywords: phosphatidic acid, plant cells, phospholipases D and C, diacylglycerol kinase, phosphatidic acid-binding proteins, plant stress signaling.

Membranes are composed of various types of phospholipids, and their role extends beyond structural to include informational functions. The latter involves membrane phospholipids serving as precursors to intracellular signaling molecules. One extensively studied informational function is their role as second messengers in intracellular signaling pathways. Phosphatidic acids (PA) are widely recognized as phospholipid second messengers that translate extracellular information, such as hormonal, stress, and developmental signals, into specific cellular responses. They play a crucial role in modulating cellular metabolism to maintain a balance in plant stress tolerance, growth, and development [1-3].

Phosphatidic acid is a minor membrane phospholipid containing phosphoryl glycerol with two fatty acid chains. Signaling phosphatidic acid is generated through the activation of phospholipases D, which cleave structural membrane phospholipids (e.g., phosphatidylcholine). This cleavage results in the production of phosphatidic acid and a free head group (e.g., choline) [2]. Additionally, PA can be produced through the phosphorylation of diacylglycerol (DAG), a process catalyzed by diacylglycerol kinase [3] (Fig. 1).

In response to stress and hormone action, PA level in cells undergoes rapid modification [4]. Once formed in response to extracellular stimuli within membranes, PA binds to specific proteins that regulate downstream responses crucial for its function in the regulation of growth, development, and stress responses. For example, PA inhibits autophagy by binding to GAPC or PGK3 proteins [5]. Additionally, PA binds to *Arabidopsis* arginase ARGAH2, stimulating its activity [6]. Given that PA is present at high levels in all cells under basal conditions, it was initially unclear how PA achieves signaling specificity in response to various extracellular actions. Possible determinants of PA signaling specificity in plants are listed below.

Recent developments on phospholipid signaling enzymes involved in PA production

Advances in defining the functions, regulation, and localization of phospholipase D isoforms

Structure and localization of plant PLDs. Phospholipase D (PLD) is the enzyme that hydrolyzes structural membrane phospholipids, directly producing phosphatidic acid. For instance, rice

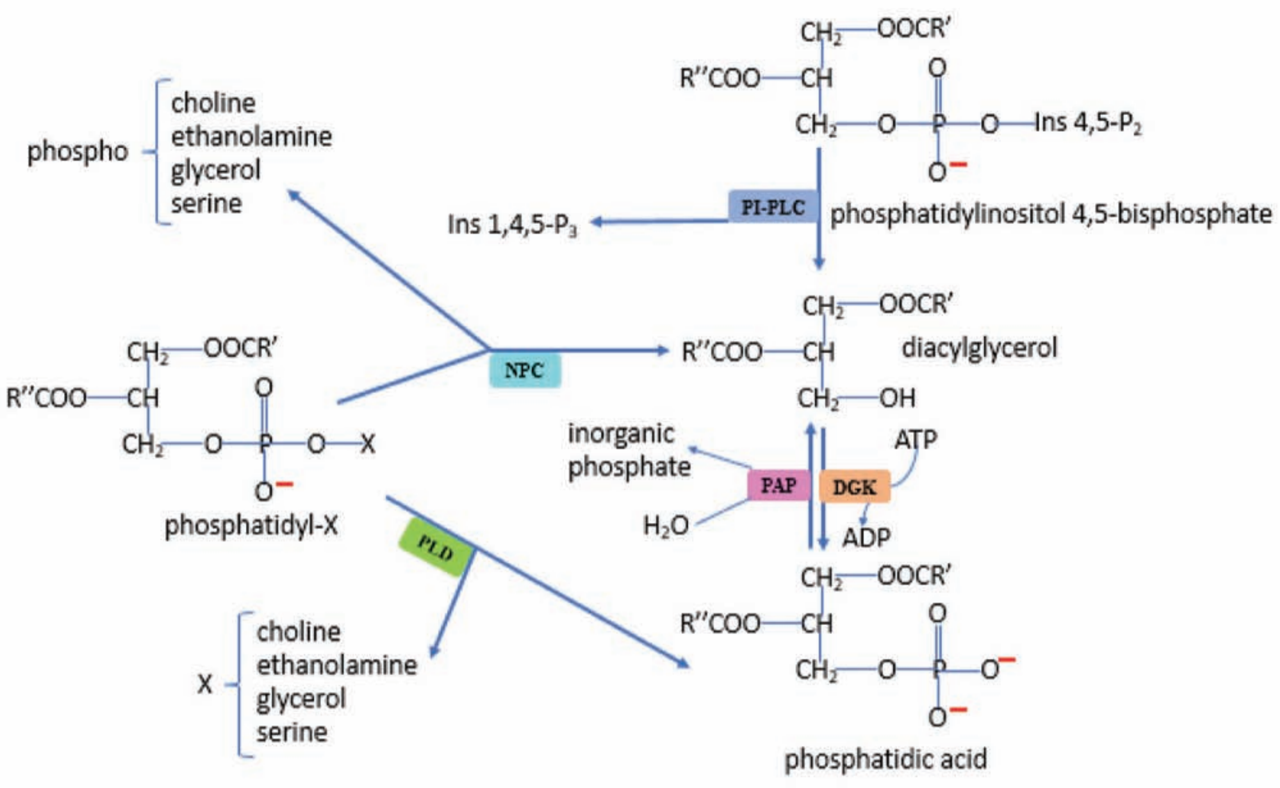

Fig. 1. Main pathways of phosphatidic acid metabolism in plants. DGK – diacylglycerol kinase, Ins – inositol, NPC – non-specific phospholipase C, PAP – phosphatidic acid phosphatase, PI-PLC – phosphoinositide-specific phospholipase C, PLD – phospholipase D

PLDα6 can hydrolyze various substrates, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylserine [7]. PLD activity, acting on glycosylinositol phosphoceramide, has also been detected in plants [8]. Additionally, the PLDγ isoform generates N-acylethanolamines together with PA in response to infection [9].

PLDs in plants are represented by various isoforms (α, β, γ, δ, ε, ζ, κ, and φ) [7, 10, 11] each possessing a typical domain structure (Fig. 2). In peanut, for example, isoforms α, β, γ, δ, and ε belong to the C2-PLDs, containing the calcium/phospholipid-binding C2 domain at the N-terminal. PLDζs containing PX and/or PH domains at the N-terminal are classified as PX/PH-PLDs. PLDφs contain a signal peptide at the N-terminal instead of the C2 or PX/PH domain, placing them in the SP-PLDs category [10]. Similarly, in rice, PLDαs, PLDβs, PLDδs, and PLDκ contain the C2 domain, while PLDζs have the PX and PH domains. PLDφ in rice has a signal peptide at the N-terminus. All rice C2-PLDs and PX/PH-PLDs possess two HKD (HxKxxxxD) catalytic motifs, except PLDα7, which has a mutation (RxKxxxxD) in the second HKD motif [7]. Notably, PLDγs isoforms were identified in *Arabidopsis* but not in rice, while PLDκ and PLDφ were found in rice but not in *Arabidopsis* [7]. Evolutionarily, PLDγ, PLDκ, and PLDφ were suggested to be duplicated later, as they are not found in lower plants and differ between monocot and dicot plants. In contrast, PLDαs and PLDδs are found in both lower and higher plant species, indicating their original and conserved nature among plant species [7]. All sorghum PLD family members harbor two conserved domains (HKD1 and 2) with catalytic activity, with most members containing a C2 domain. In the zeta subfamily, the C2 domain is replaced by the PX and PH domains [12]. Conservation of two HKD (HxKxxxxD) domains is found in all PLD genes of both jute species, except for *Co*PLDδ-2, which has only one HKD domain [13]. In alfalfa, two HKD structural domains are highly conserved, with some exceptions such as the mutation of D to K in the second HKD structural domain of *MsPLD03* and the deletion of D in the second HKD structural domain of *MsPLD56* [14].

Except for *Vvi*PLDφ, which is the only grapevine SP-PLD, all grapevine PLDs have a PIP2-binding motif [15]. In peanut PLD, this motif is represented by the sequence "xxGPRxPWHDxHxxxxGPAxxDVLTNFExRWRKxGx" [10]. Additionally, transmembrane helixes were predicted in some pineapple PLDs [16]. The tobacco PLDδ tertiary structure consists of a tightly packed globular catalytic domain with an attached C-terminal domain and a somewhat loosely connected N-terminal C2 domain [17]. Therefore, specific structure determinants within PLD could be involved in regulating the enzyme through interaction with modulators or in modifying its specific localization in cells, thereby affecting the number of PA molecules produced and the strength of the PA signal.

The cellular localization of PLD determines the site where phosphatidic acid is formed upon PLD activation, and this localization can vary within plant cells. For example, most of the alfalfa *MsPLDs* are predicted to be distributed in the cytoplasm, followed by the vacuole, endoplasmic reticulum, and chloroplast [14]. Similarly, the majority of *Sb*PLDs are predicted to be in the cytoplasm, with three *Sb*PLDs located in the endoplasmic reticulum and only *Sb*PLDβ1 in the chloroplast. *Sb*PLDα3 is experimentally supported to be located in the cytoplasm in *Arabidopsis* protoplasts [12]. *C. olitorius* and *C. capsularis* PLD proteins are predicted to be localized in the cytoplasm, followed by the endoplasmic reticulum, with *Cc*PLD-β1 and *Co*PLD-β1 uniquely found in the nucleus [13]. In peanuts, most PLD proteins are predicted in the cytoplasm, endoplasmic reticulum, and vacuole, with a few in the chloroplast, nucleus, and plasma membrane. *Ah*PLDφA/B are predicted to be localized at the plasma membrane [10]. Regarding the grapevine PLD, only two proteins had their cellular locations predicted: the *Vvi*PLDα4 was predicted to be in the mitochondria, and the *Vvi*PLDφ, possessing a signal peptide, was predicted to be secreted [15]. Experimental evidence indicates multiple subcellular locations of *Gm*PLDα1, including the cytoplasm, cytoskeleton-like structures, and, in part, chloroplasts [27]. Subcellular localization experiments indicated that apple *Md*PLD17 is a membrane protein mainly distributed in the cell membrane [11], while pineapple *Ac*PLD2 and *Ac*PLD9 [16] and cotton *Gh*PLD2 [28] were observed in the plasma membrane when expressed in tobacco leaf epidermal cells. In tobacco pollen tubes, *Nt*PLDδ1 and *Nt*PLDδ2 showed cytoplasmic localization, while all membrane-bound tobacco PLDδ(3-5) isoforms, with *Nt*PLDδ5 in particular, are attached to the plasma membrane. *Nt*PLDδ3 is only faintly detected at the plasma membrane, exclusively in the subapical zone, while plasma membrane localization of *Nt*PLDδ4-5 was more pronounced and extended further back to the pollen tube shank [17].

Recent studies indicate that the PLD that produces signaling PA seems to be localized at the plasma membrane. PA levels increased by PLDα1 and PLDδ in response to ammonium application were observed at the plasma membrane in *Arabidopsis* roots [29]. Rapid relocalization of *At*PLDδ to plasma membrane microdomains and its exocytosis in response to pathogen stimuli are involved in plant innate immunity responses [30].

In addition, specific structural determinants mediate PLD localization in cells. For example, PX and PH domains are responsible for membrane localization of *Arabidopsis* PLDζ1 and PLDζ2, mainly to the trans-Golgi network and post-Golgi compartments [31]. The N-terminus and central catalytic domain (VLREGTEI motif) of *Nt*PLDδ4 are both required for direct interaction with the plasma membrane. The catalytic domain is required, but not sufficient, for plasma membrane localization of *Nt*PLDδ4 [17]. *At*PLDγ1 at the plasma membrane associates with BIR2/3 proteins, the negative regulators of pattern-triggered immunity [32]. Therefore, the specific PLD localization within cells, coupled with its regulation at the structure level, plays a crucial role in determining the subcellular localization of PA signaling and its proximity to PA target proteins.

Regulation of plant PLDs. Results of the recent studies indicate that plant PLDs are directly regulated by a range of molecular mechanisms. For example, different PLD-binding proteins are known in plants, representing one level of PLD regulation. Regulator of flowering and stress *Bd*RFS protein binds to *Bd*PLDα1, affecting phospholipid metabolism [33]. Rice PLDα1 decorated microtubules and increased detyrosinated α-tubulin [34]. Low-affinity nitrate transporter NRT1.2 binds to *At*PLDα1 at the plasma membrane to positively affect ABA sensitivity during seed germination and seedling development [35]. Potato virus Y transmembrane protein 6K2 recruited *Nb*PLDα1 and PA to the membrane-bound viral replication complex, enhancing the production of *Nb*PLDα1-derived PA [36]. Various protein-protein interactions of peanut PLD were predicted to be with proteins involved in phospholipid transport, stress, defense, and plant development [10], supporting the important role of this regulatory mechanism in PLD modulations in plants. During allelochemical oridonin-induced stomatal closure in *Arabidopsis*, PLDα1 acted downstream of the heterotrimeric G-protein GPA1 [37], but the direct interaction of these proteins in these responses remains to be investigated.

PLD is actively modified at the protein level by post-translational modifications. For example, S-glutathionylation of apple tree PLD was found in the adult growth phase [38].

Another important mechanism of PLD regulation is protein phosphorylation. Phosphorylation of PLDδ was found in tomato plants resistant to biotic stress [39]. MPK3 and MPK6 interact with and phosphorylate PLDα1 and PLDδ, which may contribute to feedback inhibition of PA production under submergence [40]. Also, phosphorylation of PLDs from *Physcomitrella patens* (Phypa_117291, Phypa_163602, Phypa_213846) was found in response to ABA [41, 42]. Changes in phosphorylation level in response to cold were reported for tomato PLD (Solyc08g066800) [43]. MAPK cascade-dependent PLD phosphorylation in cotton was found in response to biotic stress [44]. Moreover, the prediction of protein phosphorylation (on serine, threonine, and tyrosine) of PLD in plants, including *Camelina sativa* and *Brassica napus* PLDs, suggested that PLD-alpha proteins are less influenced by this post-translational modification compared to other isoforms [45].

Lysine 2-hydroxyisobutyrylation was found in rice PLD in response to infection [46]. PLD S-nitrosylation was found for *Arachis hypogaea* PLD in response to aluminum stress [47]. Changes in lysine acetylation of poplar PLDs were observed during bud dormancy release [48]. ROS-induced cysteine oxidation in *Arabidopsis* PLDδ enhances its binding to calcium, which is involved in microtubule organization, stomatal movement, and thermotolerance [49].

Changes in PLD protein levels or stability represent another mechanism of PLD regulation in plants. PLD protein and PA accumulation in response to EPIP peptide were found in abscission zone cells of lupine flowers [50] suggesting a hormone-induced elevation of PA signaling machinery. Rice PLDα6 was found to be translocated from the cytosol to the nuclei in response to gibberellin treatment [7]. Accumulation of PLD proteins was observed in the roots and shoots of cowpea plants exposed to drought stress [51] and in groundnuts in response to *A. flavus* infection [52]. Exogenous hexaldehyde modulated PLD protein content in pineapple fruits [16]. MPK3 and MPK6 negatively regulate PLDα1 protein levels during submergence for feedback inhibition of PA production [40].

Key ions in cells are also well-known regulators of plant PLD enzymatic activity. Purified PLDα6 displayed Ca^{2+}-dependent hydrolysis of phospholipids with the highest activity at the mM levels of Ca^{2+} toward phosphatidylcholine [7]. *Nt*PLDδs possess a similar ability to bind multiple phospholipids *in vitro*, with a strong preference towards negatively charged phospholipids enriched in the plasma membrane. PA formed by *Nt*PLDδ3 positively affects *Nt*PLDδ3 plasma membrane binding via a positive feedback mechanism [17]. $PI(4,5)P_2$ is another PLD phospholipid effector required for substrate hydrolysis [53]. Taken together, PLD regulation by post-translational modifications, bound proteins, protein stability, and small molecules could modulate the PA signal strength and its velocity. This tight regulation is crucial for the precise interaction of PA with its target proteins.

Advances in defining the functions, regulation, and localization of phosphoinositide-specific phospholipase C

Structure and localization of plant PI-PLCs. PI-PLC hydrolyzes phosphoinositide phospholipids (phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate) generating diacylglycerol and inositol 1,4,5-trisphosphate. For example, rice *Os*PLC4 hydrolyzed PI, PI4P, and PIP_2 to produce DAG and exhibited a higher hydrolytic activity towards PIP_2 and PI4P than PI [54]. PI-PLC activity acting on glycosylinositol phosphoceramide was also detected in plants [8]. A typical PI-PLC structure is shown in Fig. 2. Tomato PI-PLCs, for example, have 4 domains, namely the EF-hand-like domain, the PLCXc catalytic domain, the PLCYc catalytic domain, and the Ca^{2+}/phospholipid-binding C2 domain [18]. Similarly, all the members of the orchid *Pe*PI-PLC, *Dc*PI-PLC, and *As*PI-PLC groups consist of X and Y catalytic domains and the calcium/phospholipid-binding domain [22]. In maize, *Zm*PI-PLCs contained the catalytic PI-PLC-X and PI-PLC-Y domains, the C2 domain, whereas an EF hand-like motif was found only

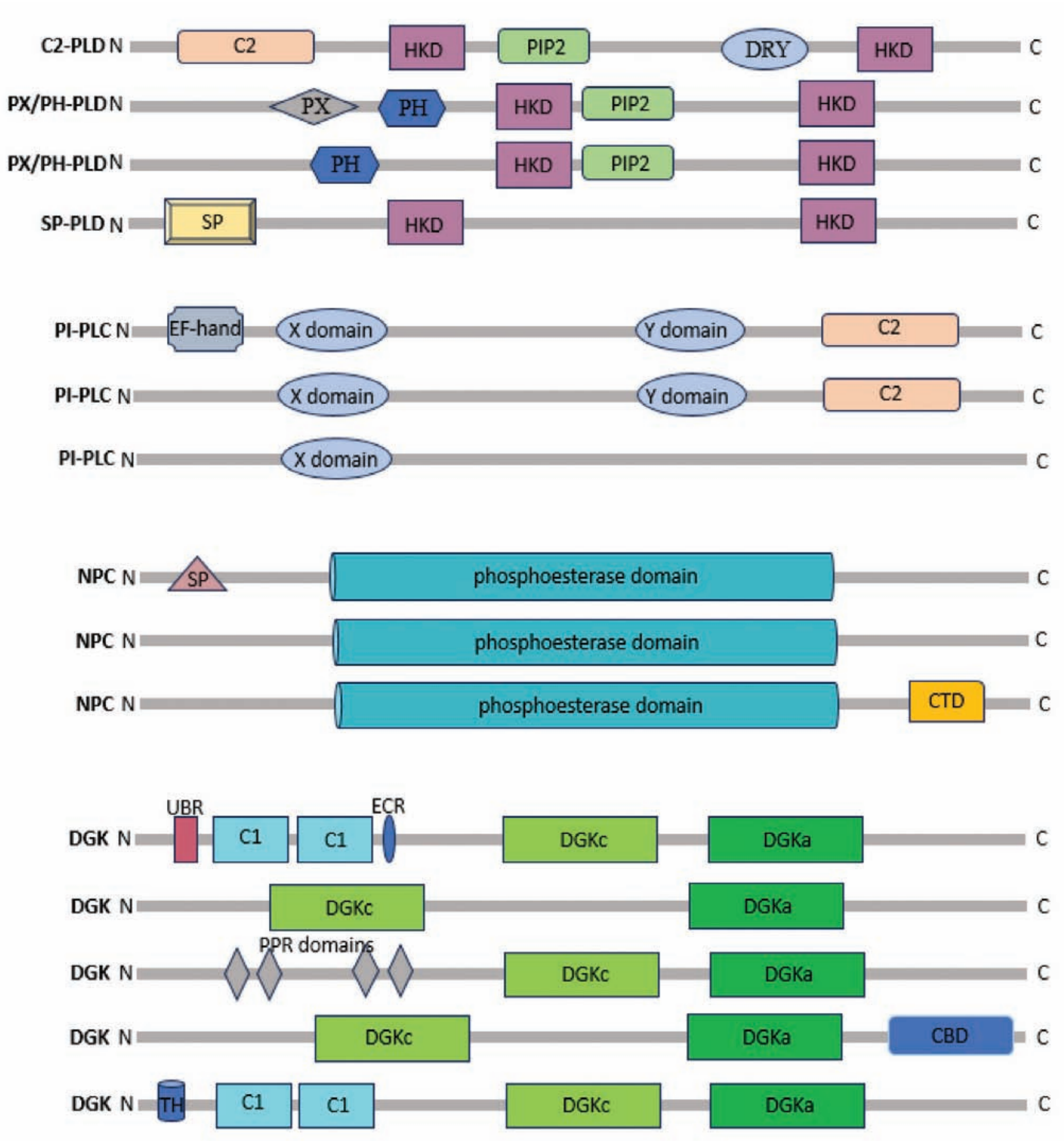

Fig. 2. The structure domains and main functional motifs of all possible types of the protein structures of plant PA-producing enzymes. Data on enzyme structures were taken from the literature [12, 18-26]. CBD – calmodulin-binding domain, CTD – C-terminal domain, C1 – DAG/PE binding motif and transmembrane domains, C2 – calcium/phospholipid-binding domain, DGKa – DGK accessory domain, DGKc – DGK catalytic domain, DRY – G-protein-binding motif, ECR – extended cysteine-rich (extCRD)-like domain, HKD – PLD catalytic domain, PIP2 – phosphoinositide binding region, PPR domain – pentatricopeptide repeat domain, SP – signal peptide, TH – transmembrane helix, UBR – upstream basic region, X and Y domains – PI-PLC catalytic domains

in *Zm*PI-PLC4 [19]. In contrast, grapevine PI-PLC presented four characteristic domains: EF-hand, PI-PLC X, PI-PLC Y and the C2 domain, except for *Vvi*PI-PLC4.2, which does not possess the EF-hand domain [15]. All cotton *GhP*IPLCs possessed four domains, with the exception of *Gh*PIPLC1A, *Gh*-PIPLC1D, and *Gh*PIPLC6D, which lacked the EF-hand-like domain [20]. Therefore, specific structure determinants within PI-PLC could be involved in the regulation of the enzyme catalytic activity or interaction with modulators/localization in cells, thus affecting the number of DAG molecules needed for PA production and thereby modulating the activity of the PA signal.

Results of recent investigations in different plants support that PI-PLCs are differentially localized within the cells. For example, *Zm*PI-PLCs were distributed throughout the nucleus and cytoplasm, but *Zm*PI-PLC2 was only located in the cytosol [19]. *Os*PLC3 was observed in the plasma membrane, cytoplasm, and nucleus of rice cells [55]. Grapevine PI-PLC proteins were predicted to be in the mitochondria (*Vvi*PI-PLC1, *Vvi*PI-PLC2, *Vvi*PI-PLC4, and *Vvi*PI-PLC6) and in the cytoplasmic membrane (*Vvi*PI-PLC3, *Vvi*PI-PLC4.2, *Vvi*PI-PLC5, and *Vvi*PI-PLC7) [15]. Chickpea *Ca*PLC2, *CaPLC3*, and *Ca*PLC6 were distributed throughout the cytoplasm, but *Ca*PLC1, *Ca*PLC4, *Ca*PLC5, and *Ca*NPC1-3 were confirmed to be localized at the plasma membrane [56]. *Os*PLC3 was observed in the plasma membrane, cytoplasm, and nucleus of rice protoplasts [55]. Salt-induced cytoplasm-to-plasma membrane translocation of *Os*PLC1 was shown in rice [57]. Banana *Ma*PLC1, *Ma*PLC2, *Ma*PLC4, *Mb*-PLC1, *Mb*PLC2, and *Mb*PLC3 were predicted to be localized to chloroplasts; *Ma*PLC5, *Ma*PLC6, and *Mb*PLC4 were localized in mitochondria, and only *Ma*PLC3 was localized in the cytoplasm. They are not transmembrane proteins [58]. Subcellular localization prediction showed that most of the orchid PI-PLC proteins were cytoplasmic and nuclear [22]. Therefore, specific PI-PLC localization in cells could determine the site of starting DAG production for PA signaling and its vicinity to target proteins.

Mechanisms of PI-PLC regulation. PI-PLC is differently regulated in plant cells. A protein-protein interaction was recently reported for PI-PLC in plants. The interaction between tomato *Sl*PLC1 and *Sl*PLC3 may result in the functioning of *Sl*PLC1 and *Sl*PLC3 as a dimer, and SlPLC3 can interact with *Sl*PLC4, *Sl*PLC6, and other proteins, forming a multimer, but *Sl*PLC7 does not interact with any *Sl*PLCs [18]. Although *Arabidopsis* PLC1 and G-protein GPA1 mediated the effect of allelochemical cycloastragenol on stomatal movements [59], the direct interaction of this PI-PLC with G-protein in regulating these responses was not investigated.

Phosphorylation is an important mechanism of PI-PLC regulation. Cold and cadmium-induced changes in phosphorylation of some PI-PLC were found in tomatos [43, 60]. Phosphorylation of PLC3 from *Nicotiana tabacum* was reported in response to the tobacco mosaic virus [61]. Also, MAPK cascade-dependent PLC phosphorylation in cotton was found in response to biotic stress [44]. PLC phosphorylation during fruit development was reported in pepper [62]. In addition, PLC phosphorylation was reported in soybean in response to aluminum stress [63]. *At*PI-PLC2 phosphopeptide abundance was found among significantly upregulated phosphopeptides in plants overexpressing C-TERMINALLY ENCODED PEPTIDE 5 (CEP5) [64]. Lysine crotonylation was found for some PLC proteins in *Camellia sinensis* in response to ammonium [65]. C-terminal proteolysis was found for PI-PLC (Solyc05g052760) from tomato [66].

An adaptor protein, *Os*GF14b, was reported to be an interaction partner of *Os*PI-PLC1 that promotes its activity and stability, thereby improving rice salt tolerance [67]. Heterotrimeric G-protein subunits β1 and α1 were reported to interact with PI-PLC in *M. truncatula*, but only the G-protein alpha 2 subunit could interact with *P. sativum Ps*PLC [68]. In addition, an ortholog protein of *Arabidopsis* PI-PLC2 from *C. roseus* (CRO_T004768) was *in silico* predicted by the “CroFGD” database to interact with the peptide receptors CLV1 ortholog (CRO_T007315), HAESA-like 1 ortholog (CRO_T002426), and BARELY ANY MERISTEM 2 ortholog (CRO_T011766) proteins [69].

Hydrolysis of PI by *Os*PI-PLC4 required Ca^{2+}, with the maximum activity being 50 mM Ca^{2+} [54] but *Os*PI-PLC1 maximal activity was observed at 100 μM Ca^{2+} [57]. Distinct requirements for Ca^{2+} ions in tomato *Sl*PLC2, *Sl*PLC4, and *Sl*PLC5 enzymes [70], and PI-PLC sensitivity to calcium entry into cells during cold stress action [71] support the well-known role of calcium in the modulation of PI-PLC. Additionally, PI-PLC regulation at the protein level was shown in response to melatonin in oat seeds [72] and by substrate supply produced by phosphoinositide kinases [73]. Taken together, PI-

PLC regulation by post-translational modifications, bound proteins, protein level, and small molecules could modulate the level of DAG production for subsequent modification of PA signal strength and its velocity. This is important for tight regulation of PA interaction with its targets.

Advances in defining the functions, regulation, and localization of non-specific phospholipase C isoforms

Non-specific phospholipase C (NPC or PC-PLC) usually hydrolyzes membrane structural phospholipids (i.e., phosphatidylcholine), producing diacylglycerol and phosphocholine [74]. However, it also catalyzes other types of reactions. Non-specific phospholipase C3 from *Raphanus sativus* produces phytoceramide 1-phosphate from glycosylinositol phosphoceramide [75]. *Arabidopsi*s NPC6 hydrolyzes not only phosphatidylcholine but also galactolipids [76]. Plant NPC is simply organized (Fig. 2). NPC4 from *Arabidopsis* is divided into a phosphoesterase domain (PD) and a C-terminal domain (CTD). The previously uncharacterized CTD is indispensable for the full activity of NPC4. Mechanistically, CTD contributes to NPC4 activity mainly via the CTDα1-PD interaction, which ultimately stabilizes the catalytic pocket in PD [21]. Ten orchid PC-PLC protein sequences (*Pe*PC-PLC1, *Pe*PC-PLC2A, *Dc*PC-PLC1A, *Dc*PC-PLC2, *Dc*PC-PLC1B, *Dc*PC-PLC5, *As*PC-PLC1, *As*PC-PLC2, *As*PC-PLC3, and *As*PC-PLC5) were reported to have signal peptides. *In silico* prediction indicated the presence of a transmembrane region in three proteins (*Pe*PC-PLC1, *Dc*PC-PLC1A, and *As*PC-PLC2) [22]. Six mazie *Zm*NPCs had only a phosphoesterase domain, which contains two highly conserved motifs, ENRSFDxxxG and TxPNR, and two invariable motifs, DExxGxxDHV and GxRVPxxxxxP [19]. The structures of cotton *Gh*NPCs were composed of the beta sheet and several alpha helices [77]. Members of orchid PC-PLC (characterized by the presence of a phosphodiesterase domain only) were observed with six beta-sheets in their tertiary structure, but *Dc*PC-PLC1A was predicted to have a large number of variations in their protein sequence at the alpha-helix region. In PC-PLC proteins, variations in the beta-sheets were observed to be greater in comparison to the alpha-helix, except in *Pe*PC-PLC1 and *As*PC-PLC1 [22]. Therefore, specific structure determinants within PC-PLC could be involved in the regulation of the enzyme by interaction with modulators or in modulating its specific localization in cells, thus affecting the number of DAG precursors of PA molecules produced and thereby affecting the level of PA signal.

Results of recent investigations in different plants indicate that NPCs are differentially localized within the cells. Although *Zm*NPCs were predicted to be multi-localized, *Zm*NPC3 was experimentally confirmed to be located in the cytosol [19]. *Glehnia littoralis Gl*NPC3 was predominantly localized at the plasma membrane, with some localization associated with the tonoplast [78]. In addition, rice NPCs were also localized at the cell periphery and plasma membrane of protoplasts [74]. Peach *Pp*NPC1 was experimentally located in the plasma membrane [79]. Also, subcellular localization predictive studies showed that most orchid NPC proteins were localized in the cytoplasm, nucleus, and mitochondria [22]. *At*NPC6 was found to be associated with chloroplast and microsomal fractions [76], but *At*NPC2 was present predominantly in Golgi apparatus, with a minor extent in other compartments of the secretory pathway, such as the endoplasmic reticulum or some post-Golgi compartments [80]. It can be proposed that specific PC-PLC localization in cells could determine the site of DAG production for subsequent PA generation and signaling and PAs vicinity to target proteins.

Some evidence has been reported regarding the post-translational regulation of NPC. The acylation of NPC4 was detected using NPC4 isolated from *Arabidopsis* and is important for membrane association and the hydrolysis of phosphosphingolipid glycosyl inositol phosphoryl ceramide during phosphate deficiency [81]. Changes in N-glycosylation were found for tomato NPC1 during ripening [35]. Taken together, PC-PLC regulation by post-translational modifications could potentially modulate the level of DAG production for subsequent modulation of PA signal strength and its velocity, which is important for tight regulation of PA interaction with its targets.

Advances in defining the functions, regulation, and localization of diacylglycerol kinases

DGK structure and localization. DGK carries out substrate diacylglycerol phosphorylation, producing phosphatidic acid as a product. DGKs in plants are represented by heterogenic enzyme families. These enzymes are characterized by a multidomain structure containing a range of functional motifs (Fig. 2). For example, DGK from common beans contains DAG/phorbol ester (PE)-binding domain 1, DAG/PE-binding domain 2, and the diacylglycerol kinase accessory (DGKa) domain [23]. *Ta*DGK harbored a diacylglycerol kinase catalytic domain (DGKc) and one accessory domain (DGKa) near the N-terminus, as well as an upstream basic region, an extCRD-like domain, and upstream basic regions near the C-terminus [82]. Almost all soybean DGKs contain an ATP-binding sequence (GXGXXG) in their catalytic domain (DGKc). C-terminal DAG/PE-binding domain C1 contains an additional 15-amino acid sequence (extCRD), whereas the sequence rich in basic amino acids is localized near the N-terminal C1 domain [26]. Rape DGKs also possess a conserved ATP-binding site, C1 domains, and an extCRD domain. However, *Bna*DGK2–1 lacks an N-terminal sequence rich in basic amino acids and an extended C1 sequence [24]. All DGKs in rice (*Os*DGK1-8) contain catalytic and accessory domains, but *Os*DGK4, *Os*DGK5, and *Os*DGK6 also contain two C1 domains. Among all known DGKs, only *Os*DGK6 contains a specific domain PPR instead of C1 domains, and this domain is known to play a role in macromolecular interactions [25]. Therefore, specific structure determinants within DGKs could be involved in the regulation of the enzyme by interaction with modulators or in modulating its specific localization in cells, thus affecting the number of PA molecules produced and the strength of the PA signal.

DGK localization in cells reflects the site of PA formation. Application of PA biosensors and pharmacological analysis suggest a role of DGK in the formation of 50-60% basal levels of PA localized in the plasma membrane and nucleus in the root epidermal cells of *Arabidopsis thaliana* [83]. Experimental evidence indicates that Arabidopsis DGK2 and DGK4 were localized to the endoplasmic reticulum and were involved in PA production for pollen tube growth [84]. Artificial expression of DGK2-GFP, DGK3-GFP, and DGK5-GFP in *Arabidopsis* indicated their localization in the cytosol [85]. In tobacco pollen tubes, DGK1-3 was observed to be localized within the endoplasmic reticulum, DGK4 – in the cytosol, DGK6 – in the cytoplasm, DGK5, DGK7, and DGK8 – on the plasma membrane. DGK5 was suggested to bind to the phospholipid bilayer by catalytic and accessory domains. Glycine-118 was proposed to be the key amino acid in DGK5 for binding to membranes, enzymatic activity, and regulation of pollen tube growth [86]. Other types of data on DGK localization are based on *in silico* predictions. Wheat DGKs were predicted to localize to the chloroplast, cytoplasm, and nucleus. *Ta*DGK2A and *Ta*DGK5B were expressed in the nucleus and cytomembrane, while *Ta*DGK3A and *Ta*DGK5A2 were mostly expressed in the cytomembrane based on confocal microscopy [82]. Also, *Gm*DGK2, *Gm*DGK11, and *Gm*DGK12 in soybean are predicted to be localized in the plasma membrane and endoplasmic reticulum, whereas *Gm*DGK1, *Gm*DGK3-4, *Gm*DGK8-9, *Gm*DGK5-7, and *Gm*DGK10 are localized in the cytoplasm and nucleus; *Gm*DGK10 is mostly localized in the nucleus [26]. Among different *Brassica* species (*Brassica napus* and *Brassica oleracea*), *Bna*DGK1–2, *Bna*DGK2–1, *Bol*DGK1–2, *Bol*DGK2–2 can be localized in nucleus, DGK3 and DGK7 – in peroxisome, DGK4 – in chloroplast, *Bna*DGK3–3 and *Bol*DGK3–2 – in mitochondria, whereas DGK5 and DGK6 – in peroxisomes and cytosol, respectively [24]. Prediction of apple DGK localization by *in silico* analysis suggests that *Md*DGK1, *Md*DGK3, and *Md*DGK7 can be localized in the nucleus and membranes of the endoplasmic reticulum [87]. Therefore, specific DGK localization in cells could determine the site of starting PA signaling and its vicinity to target proteins.

Regulatory mechanisms of plant DGKs. One mechanism of DGK regulation in plants is the modification of their protein structure. Among them, phosphorylation is one of the known mechanisms of DGK regulation. Cadmium-, cold-, and MAPK11-induced protein phosphorylation was observed for some tomato DGKs [43, 60, 88]. Exogenous peptide systemin [89] and TOR kinase [90] are other regulators that induce changes in DGK phosphorylation in plants. Bolting induced by high temperatures induces changes in lettuce DGK (A0A2J6JMK6) phosphorylation [91]. DGK phosphorylation during fruit development was reported in pepper [62]. Phosphorylation changes in *Arabidopsis* DGK5 and DGK7 were observed in systemic leaves in response to the bacteria *Pseudomonas syringae* pv. *maculicola* ES4326 [92]. C-terminal proteolysis was found for DGK (Solyc10g008640) from tomato [66]. Another mechanism of DGK regulation is a modulation of protein stability that was found for pear DGK4 [93]. Analysis of protein-protein interaction indicated that, for example, *Arabidopis At*DGK2 interacts physically with calmodulin CAM1 [94], whereas *At*DGK3 binds to MAP kinase MAPK6 [95], A-subunit of splicing factor U2af, and cytoskeletal protein myosin [96]. Results of pharmacological analysis suggest that calcium is involved in the activation of PA accumulation in response to the elicitors cryptogein [97] and flagellin [98], but whether calcium directly affects DGK in these responses needs further investigation. Summing up, DGK regulation by post-translational modifications, bound proteins, and protein stability could modulate the level of PA signal strength and its velocity, which is important for tight regulation of PA interaction with its targets.

Role of PA and PA-producing enzymes in plant stress signaling

Specific enzymes of PA production and downstream PA-binding proteins are involved in, for example, hormone [99] and stress signaling in plants (Fig. 3, Table). In Table, we briefly summarize recent data on the genetically supported functions of the individual isoforms of PA-producing enzymes in stress signaling and PA production and the specific PA targets involved. These data suggest that the induction of cellular responses by different stressors may be coded by each specific subset of the isoforms of PA signaling enzymes and PA targets. Despite the successes and analysis of PI-PLC isoforms in plant responses to a number of stresses, the question of the involvement of individual PI-PLC isoforms is being intensively analyzed [100]. However, redundancy among different isoforms of PA-producing enzymes is also sometimes possible during stress signaling. This was shown, for example, for PLD during effector-triggered immunity [101]. In addition, gene expression of PLD in response to elicitors and immunity inducers [102] and DGK in response to biotic and abiotic stressors [103] together with methylation of their genes [104] form the transcriptional control machinery of PA signaling specificity.

PA biosensor studies indicate that salt stress induces rapid PA accumulation with similar velocity but different degrees in plasma membranes of cells in different root zones [135]. Cold-induced PA also occurs at the plasma membrane [83], whereas heat stress induces rapid nuclear translocation of PA [4]. Fungal toxin botrydial [136] and elicitor chitosan [137] evoke rapid monophasic PA accumulation by PI-PLC/DGK and PLD pathways. Other elicitors induce PA accumulation mainly via the PI-PLC/DGK pathway [97]. Therefore, different dynamics of PA formation, in addition to specific sites of PA formation, is another level of PA signaling specificity.

Pathways downstream to PA-binding proteins may additionally specify PA signals into respective responses. For example, salicylic acid induces PLD-mediated translocation of the salicylic acid receptor, NPR1, to the nucleus [138]. PA accumulated in response to elicitors has been suggested to mediate elicitor-induced ROS and phytoalexin accumulation as well as elicitor-responsive defense gene expression [97]. Also, rapid elicitor-induced pH changes [70, 139] and endocytosis of the flagellin receptor [70] function downstream of PI-PLC. Flagellin-in-

T a b l e. Plant PA signaling in response to stress. Shown here are PLD, PI-PLC, NPC, and DGK isoforms, as well as PA-binding proteins involved in stress signaling pathways. Negative regulators of stress tolerance are shown in italics

Stress type	Enzyme isoform involved	Known PA-binding protein involved	References
Salt stress	*At*PLDα1/δ, *AtPLDζ1-2*, *At*NPC4, *Os*PI-PLC1/4, *Os*DGKs	*At*MAP65-1, *At*PINOID, *At*MKK7/9, GMK1, *At*SOS2, *At*CHC, *At*ANTH, *At*KAB1, ribosomal proteins (S3, L30), *At*GAPDH, *At*PI4Kγ	[4, 25, 54, 57, 105-116]
Cold	*AtDGK2-3*, *AtDGK5*, *Os*PLDα1	*Os*MPK6, OsSIZ1, *At*RbohD	[85, 117]
Heat stress	*At*PLDα1/δ, AtNPC1, *At*PLC5	*At*GAPDH	[4, 118-121]
Wounding	*Gh*PLDα/δ	*Zm*CPK11	[122, 123]
Hypoxia	*At*PLDα1/δ, *AtPLDζ1-2*	*At*CPK12, *At*MPK3/*At*MPK6	[40, 124-126]
Biotic stress	*Nb*PLDα1, *At*PLDα1, *AtPLDβ1-2*, *AtPLDγ1*, *At*PLDδ, *Sl*PLDα1/γ, *Nt*DGK5, *At*DGK5, *At*PI-PLC2, *NbPLC3*, *SlPLC2*, *At*NPC2	*At*PDK1, *At*WIPK, *At*CP	[9, 30, 32, 36, 80, 97, 98, 127-134]

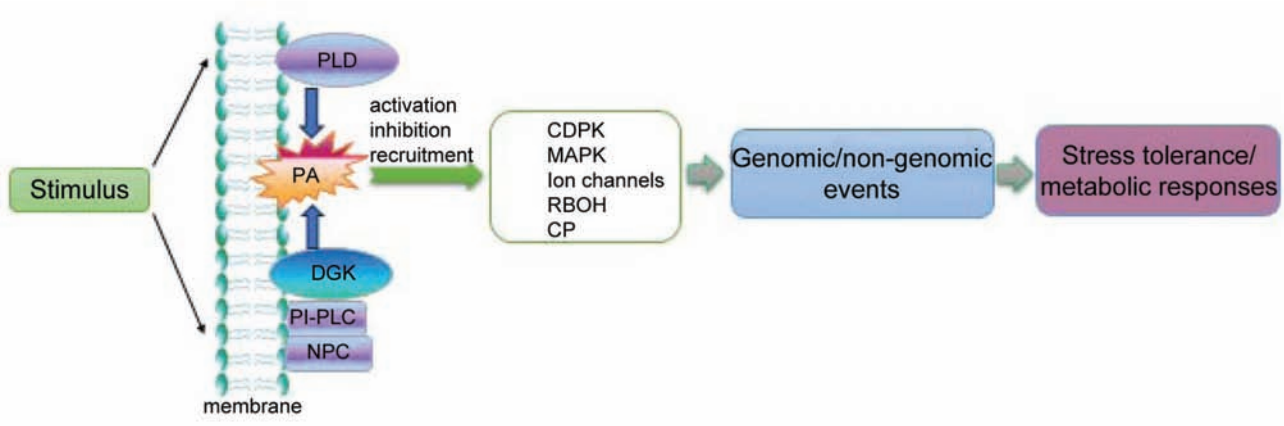

Fig. 3. A general model of plant PA signaling in plant cells. Specific extracellular stimuli induce the activation of a particular PA-producing enzyme isoform(s) by affecting localization, post-translational modification, protein-protein interaction, protein stability, or interaction with small regulatory molecules. Then, PA produced at a specific location binds to and modulates the localization and/or activity of target proteins. Subsequent genomic and/or non-genomic events coded by respective PA-target proteins further induce metabolic responses, leading to stress tolerance

duced changes in phosphorylation of some proteins are also found to be downstream of PLD in legume plants [140]. In addition, receptor-like kinase CRK2 [133], and auxin transporter AUX1 cellular relocalization [116] are also regulated downstream of salt stress-regulated PLD and PA formation. Finally, according to the analysis of gene expression, the PI-PLC and PLD pathways are upstream of different cold-induced signaling pathways leading to cold responses [141].

Conclusion. It is obvious that the specificity of phosphatidic acid formation and signaling in plants is multifaceted and complex. Despite numerous studies using modern methods of analyzing the factors that can activate (or potentially activate) phosphatidic acid formation and signaling in plant cells, new methods and approaches are needed to form a more holistic picture of this process. It can be suggested that specific catalytic activity modulated by enzyme structure, presence/absence of specific structural determinants (domains, functional motifs) of PA-producing enzymes, post-translational modification, their time and spatially regulated gene expression, and localization should be more deeply investigated. PA produced in response to the stimuli participates in genomic and non-genomic signaling events regulating gene expression, ion transport, cytoskeleton dynamics, and metabolic enzyme activity. To perform this, PA, as a phospholipid second messenger, directly modulates specific target proteins and enzymes, regulating their function, thus specifically directing and modulating downstream signaling events. Different plants possess their own isoform landscape of PA-producing enzymes and their intracellular localization, suggesting that signaling responses in cells to different factors action could be additionally regulated at the level of PA signaling enzymes.

The insufficient chromatographic resolution of anionic phospholipids, which include PA, significantly complicates the understanding of their role in the regulation of plant cell metabolism [142]. Important progress in understanding the localization and formation of phosphatidic acid in plant cells has certainly been achieved over the past year, thanks to new methodological approaches [100, 142]. These and other research findings will contribute to a more in-depth study of spatial and temporal changes in phosphatidic acid metabolism in plant cells during their growth, development, and formation of adaptive changes under the influence of numerous environmental stressors.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ФОРМУВАННЯ ФОСФАТИДНОЇ КИСЛОТИ ТА ПЕРЕДАЧА СИГНАЛІВ У КЛІТИНАХ РОСЛИН

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В огляді представлено оновлену інформацію про структуру, локалізацію та регуляцію ензимів формування фосфатидних кислот (ФК) фосфоліпази D, фосфоінозитид-специфічної та неспецифічної фосфоліпаз С та діацилгліцеролкіназ. Обговорюється специфічна роль ФК та ензимів, що продукують ФК, у процесах трансдукції сигналів у рослинах під час стресу.

Ключові слова: фосфатидна кислота, клітини рослин, фосфоліпази D і C, діацилгліцеролкіназа, зв'язувальні протеїни, сигналювання.

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