Biochimie 96 (2014) 144–157

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

Plant phosphoinositide-dependent phospholipases C: Variations around a canonical theme

Igor Pokotylo [a], Yaroslav Kolesnikov [a], Volodymyr Kravets [a], Alain Zachowski [b,c], Eric Ruelland [b,c,*]

[a] Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine, Kiev, Ukraine
[b] CNRS, EAC 7180, Physiologie Cellulaire et Moléculaire des Plantes, 75252 Paris cedex 05, France
[c] UPMC UnivParis06, UR5 7180, Physiologie Cellulaire et Moléculaire des Plantes, 75252 Paris cedex 05, France

ARTICLE INFO

Article history:
Received 28 March 2013
Accepted 4 July 2013
Available online 12 July 2013

Keywords:
Phospholipase C
Lipid signalling
Phosphoinositides
Inositol-phosphate
Phosphatidic acid

ABSTRACT

Phosphoinositide-specific phospholipase C (PI-PLC) cleaves, in a Ca^{2+}-dependent manner, phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2) into diacylglycerol (DAG) and inositol triphosphate (IP_3). PI-PLCs are multidomain proteins that are structurally related to the PI-PLCζs, the simplest animal PI-PLCs. Like these animal counterparts, they are only composed of EF-hand, X/Y and C2 domains. However, plant PI-PLCs do not have a conventional EF-hand domain since they are often truncated, while some PI-PLCs have no EF-hand domain at all. Despite this simple structure, plant PI-PLCs are involved in many essential plant processes, either associated with development or in response to environmental stresses. The action of PI-PLCs relies on the mediators they produce. In plants, IP_3 does not seem to be the sole active soluble molecule. Inositol pentakisphosphate (IP_5) and inositol hexakisphosphate (IP_6) also transmit signals, thus highlighting the importance of coupling PI-PLC action with inositol-phosphate kinases and phosphatases. PI-PLCs also produce a lipid molecule, but plant PI-PLC pathways show a peculiarity in that the active lipid does not appear to be DAG but its phosphorylated form, phosphatidic acid (PA). Besides, PI-PLCs can also act by altering their substrate levels. Taken together, plant PI-PLCs show functional differences when compared to their animal counterparts. However, they act on similar general signalling pathways including calcium homeostasis and cell phosphoproteome. Several important questions remain unanswered. The cross-talk between the soluble and lipid mediators generated by plant PI-PLCs is not understood and how the coupling between PI-PLCs and inositol-kinases or DAG-kinases is carried out remains to be established.

1. Introduction

Phosphoinositide-specific phospholipases C (PI-PLCs) are essential enzymes that cleave, in a Ca^{2+}-dependent manner, membrane phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2) to produce two second messengers: i) a lipid, diacylglycerol (DAG), and ii) a soluble molecule, inositol 1,4,5-triphosphate (IP_3). PI-PLCs have been identified in many eukaryotes from yeast to mammals [1,2] while simplified PI-PLCs are also present in bacteria [3], indicating the probable common evolutionary origin of all PI-PLCs.

In animals, the role and regulation of PI-PLC isozymes are well established [1]. The canonical model states that PI-PLC action is linked to the activation of protein kinase C (PKC) by DAG and to an intracellular Ca^{2+} release mediated by IP_3-sensitive channels (IP_3 receptors). The mode of action of plant PI-PLCs must be different from the animal model since the amount of PI-4,5-P_2 is lower in plant membranes [4], plants apparently lack conventional IP_3 receptors, and no plant PKC orthologs have been identified to date. However, clear roles (that we will detail) have been assigned to plant PI-PLCs in response to environmental stresses and during development. Despite the conclusive data concerning the role(s) of PI-PLCs in the plant cell, little is known about their *in planta*

Abbreviations: ABA, abscisic acid; DAG, diacylglycerol; DGK, DAG-kinase; IP_3, inositol triphosphate; IP_5, inositol pentakisphosphate; IP_6, inositol hexakisphosphate; PA, phosphatidic acid; PH, pleckstrin homology; PI, phosphatidylinositol; PI-4-P, phosphatidylinositol-4-phosphate; PI-4,5-P_2, phosphatidylinositol-4,5-bisphosphate; PI-PLC, phosphoinositide-dependent phospholipase C; PLD, phospholipase D; SA, salicylic acid; SUMO, small ubiquitin-like modifier.

* Corresponding author. Université Pierre et Marie Curie, 4 place Jussieu, case courrier 156, 75252 Paris cedex 05, France. Tel.: +33 1 44 27 96 47.
E-mail addresses: pokotylo@bpci.kiev.ua (I. Pokotylo), kolesnikov@bpci.kiev.ua (Y. Kolesnikov), kravets@bpci.kiev.ua (V. Kravets), alain.zachowski@upmc.fr (A. Zachowski), eric.ruelland@upmc.fr (E. Ruelland).

0300-9084/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2013.07.004

regulation. In this review we focus on the latest advances in plant PI-PLCs including their biochemical characteristics that precondition their role as regulators of many plant physiological reactions, their regulation and their modes of action. We will show that, as for animal systems, there is a tight connection between PI-PLC and Ca^{2+}-signalling while the important mediator for this action might not be IP_3, but inositol pentakisphosphate (IP_5) or inositol hexakisphosphate (IP_6). Finally, we will describe and discuss the active contribution of plant PI-PLCs to phosphatidic acid (PA) production in concert with diacylglycerol kinases (DGK). Indeed, PA is probably the lipid mediator produced by plant PI-PLC pathways, and protein targets of PA have been identified. Whether the lipid and soluble mediators act on separate signalling pathways or control the same pathways, synergistically or antagonistically, is an exciting ongoing field of investigation.

2. Structure of PI-PLCs

In every PI-PLC, the catalytic X and Y domains are flanked by regulatory sequences. In animals, PLCζ is the structurally simplest isoform, with the catalytic domains being flanked by an N-terminal EF-hand domain and a C-terminal C2 domain [1] (Fig. 1). The other mammalian PI-PLCs have additional domains. They all possess an N-terminal pleckstrin homology (PH) domain, involved in membrane targeting and protein binding [5,6] while additional domains depend on specific isoforms. For instance, a C-terminal PDZ-domain (Post synaptic density-95, Drosophila disc large tumour suppressor, and Zonula occludens-1 protein) with a protein binding motif required for supramolecular complex formation is present in the mammalian PLCβ [7]. All plant PI-PLCs are structurally related to the PLCζ isoform [8], as they are formed by the succession of EF-hand, X/Y and C2 domains.

2.1. X and Y domains

Catalytic activity of all PI-PLCs is believed to strictly rely on the X/Y domains. It was demonstrated that single aminoacid substitution in X-domain abolished PLC catalytic activity [9]. Many residues are highly conserved in these domains of all eukaryotic PI-PLCs and they are involved in substrate binding and catalysis. For example, in animal PLCδ1, Lys^{438}, Lys^{440}, Ser^{522} and Arg^{549} are involved in interacting with the 4- and 5-phosphates of the substrate headgroup [10] and the positive charge of Arg^{549} has been shown to be required for the preferential hydrolysis of PI-4,5-P_2 over PI [11]. Two conserved His residues in the X domain participate in the mixed acid/base-catalyzed reaction of phosphoinositide hydrolysis: one stabilizes the pentavalent phosphoryl transition state, and the other protonates and disjoints DAG and, being a nucleophile, attacks a 1,2-cyclic-inositolphosphate intermediate [12]. These His residues are conserved in plant PI-PLCs and correspond to His^{126} and His^{169} of the X domain of mung bean enzyme [13]. Substitution of a conserved Ser to Asn in the Y region of the active site of *Physcomitrella patens* PLC2 correlates with a reduced catalytic activity in comparison with the PLC1 isoform [14]. However, whether this Ser corresponds to Ser^{522} in animal PLCδ1 and therefore has a similar role in substrate interaction remains to be shown. Interestingly, *Arabidopsis thaliana* AtPLC8 and AtPLC9, that are the most divergent proteins compared to other AtPLCs, have long deletions in the Y region [15]. Consequently their catalytic activity is questionable. However, a distinct role of AtPLC9 in plant stress responses has been demonstrated using a plc9 mutant that displays a thermosensitive phenotype [16].

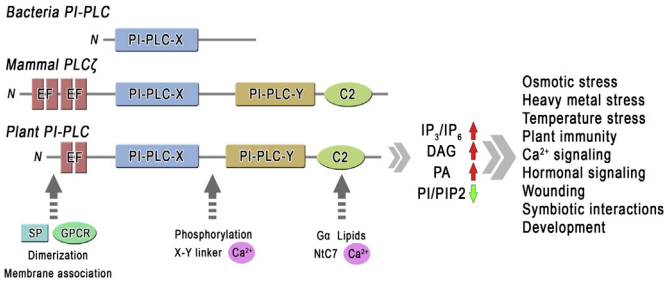

Fig. 1. Domain analysis and physiological role of plant PI-PLCs. Simplified representative polypeptide sequences of *Arabidopsis thaliana* PLC1, *Homo sapiens* PLCζ1 and *Bacillus cereus* PLCa with marked domains according to Pham (http://pfam.sanger.ac.uk). The spatial domain location in the sequence is relative. Inconstant sequence features of plant PI-PLC such as signal peptide sequence (SP) [144] and sequence with homology to G-protein coupled receptor (GPCR) [30] are indicated. Putative sites of plant PI-PLC regulation are marked: Ca^{2+} binds to plant PI-PLC both within the C2 domain and X–Y linker region [22,23,25,26]. Interactions with Gα protein [67], membrane lipids [26] and NtC7 in tobacco [68] have been observed at the C2 domain. At the N-terminus, plant PI-PLC may undergo dimerization [31] or associate with membranes [21]. Site of phosphorylation detected between X and Y domains of PI-PLC [193]. Also indicated are typical direct and indirect products of phosphoinositide hydrolysis by PI-PLC and plant physiological processes dependent on PI-PLC activity. IP_3, inositol trisphosphate; IP_6, inositol hexakisphosphate; DAG, diacylglycerol; PA, phosphatidic acid; PI, phosphatidylinositol, PIP_2, Phosphatidylinositol 4,5-bisphosphate.

2.2. EF-hand domain

The conventional EF-hand domain consists of four helix–loop–helix folding motifs and is often characteristic of calcium-binding proteins [17]. In mammalian PI-PLCs, the EF-hand acts as an allosteric regulatory domain that binds calcium and lipids, stabilizes PI-PLC structure and assists in active site formation [18]. Moreover, in PLCβ3, the EF-hand participates in the formation of an active complex with Gα protein [19], while in PLCγ, it facilitates binding to tyrosine kinases [20]. These observations plead for a major role of the EF-hand as a structural determinant of PI-PLCs. However, plant PI-PLCs have no full length EF-hand domain since most of them have a truncated EF-hand consisting of only two helix–loop–helix motifs [12,21] while several plant PI-PLCs have no N-terminal EF-hand, such as AtPLC2 [22]. Soybean PI-PLC1 contains an additional putative EF-hand type calcium-binding motif located in between the X and Y domains, while the N-terminal EF-hand is truncated [23]. Because the EF-hand mediates the calcium-dependent activation of PLCζ [24], it is tempting to postulate a similar role in plant PI-PLCs. Whether a truncated EF-hand can still carry out this function is unknown. Interestingly, PI-PLCs with no N-terminal EF-hand still show a catalytic activity [25]. Clearly the importance of the EF-hand domain is, as yet, not understood for plant PI-PLCs.

2.3. C2 domain

All identified plant PI-PLCs contain a C2 domain. This domain is described to bind phospholipid, and calcium may positively participate in this process [24]. In potato and rice PI-PLCs, specific hydrophobic residues [26] and the polybasic region K-(K,R)-T-K [25] in the C2 domain possibly mediate C2 domain binding to anionic phospholipids. In some plants, the C2 domain is sufficient to address PI-PLCs to the membranes [9,13,26] while in other cases, the C2 domain is involved in membrane targeting only when the EF-hand is present [27].

2.4. Other structural determinants

A highly hydrophilic and extremely divergent linker region between the X and Y domains is also believed to be essential and to play different roles in different PI-PLCs. In animals, the X–Y linker

has an autoinhibitory function in all PI-PLCs, except PLCζ [28]. In PLCζ, the linker is rather positively charged and it is required for PI-4,5-P_2 binding [29]. In contrast, plant PI-PLCs contain a linker region containing a high percentage of acidic residues that are presumed to be exposed at the surface of the folded protein [22]. To date, the role of the linker in plant PI-PLC activity remains to be identified.

A sequence with high homology to the G protein coupled-receptor motif has been found in the N-terminus region of *Brassica napus* PI-PLC2 [30] as well as in AtPLC2 [22]. Its function is unknown.

The Cys^7 residue is essential for disulphide bond formation resulting in homodimerization of *Chlamydomonas reinhardtii* PI-PLC and a lower lipid affinity [31]. A Cys residue is present in the first 10 residues of most plant PI-PLCs but whether it is involved in homodimerization remains to be shown. Homodimerization via uncharacterized mechanisms have been demonstrated previously for putative animal and bacterial PLCs [32,33].

2.5. PI-PLC-like proteins

Several PI-PLC-like proteins lacking one or several canonical PI-PLC domains are found in plants. A *Medicago truncatula* DNF2 protein containing only the PI-PLC X domain, and thus closer to bacterial PI-PLCs than to eukaryotic PI-PLCs, has been shown to be involved in the control of symbiotic relations with *Sinorhizobium meliloti* [34]. Whether this protein possesses a PI-PLC catalytic activity or only interacts with PLC substrates remains to be shown. Putative PI-PLC proteins with a similar structure are also found in Arabidopsis, maize and rice (Supplemental Table 1).

Taken together, these data indicate that plant PI-PLCs possess a relatively simple structure when compared to animal PI-PLCs, they contain several essential domains – namely the X/Y catalytic domains, a C2 domain and truncated EF-hand domains. Regardless of these limited structural features, plant PI-PLC are regulatory targets that have important functions within plant cells.

3. Phylogenetic considerations

Due to the high level of structural identity, all PI-PLCs might share a common ancestor. Throughout evolution, PI-PLCs have evolved into distinct groups that are now represented as isozymes within multigene families. We have conducted a phylogenetic analysis of a number of plant PI-PLC enzymes (Fig. 2). They are usually grouped according to taxonomical traits. Algae PI-PLC sequences are more divergent from those of other plant species and more related to human and yeast PI-PLCs. Higher plant PI-PLCs are separated from human and yeast PI-PLC proteins which group together. Grouping of PI-PLCs from non-vascular plants – lycophytes and moss – was also observed, suggesting that PI-PLC sequences further independently multiplied and evolved after the separation of mosses, lycophytes, and vascular plants. All but one of monocot PI-PLCs were found to be grouped together. Multiple independent intraspecies duplications must have occurred, leading to PI-PLC family expansion in each species. For example, in Arabidopsis, genes *AtPLC8* and *AtPLC9* occur in a tandem array on chromosome 3 and may represent a relatively recent local duplication. Progenitors of *AtPLC1*/*AtPLC3* possibly arose from a single gene by a duplication event on chromosome 5, with a subsequent duplication and relocation of *AtPLC3* to chromosome 4 [15,35]. Highly similar *AtPLC1*, *AtPLC4* and *AtPLC5* genes are organized in tandem in a small DNA region of chromosome 5 possibly due to recent gene duplication events [35].

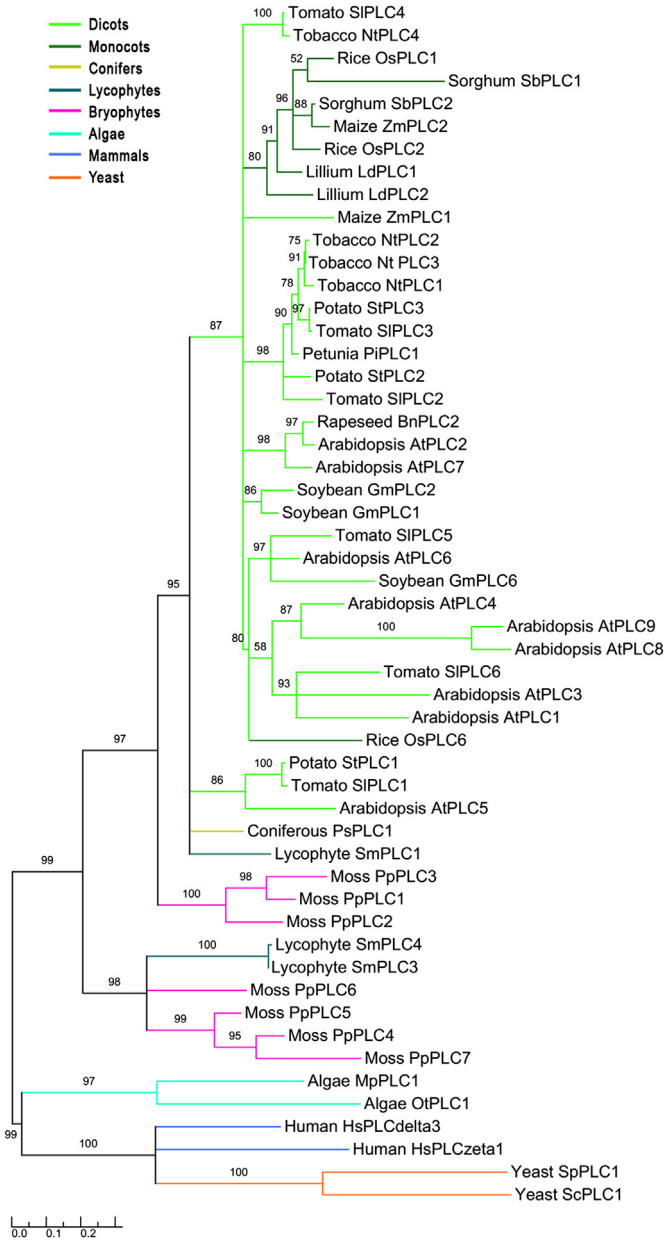

Fig. 2. Phylogenetic analysis of selected plant, mammal and yeast PI-PLCs. Sequences of PI-PLCs were obtained from NCBI and UniProtKB and aligned using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/). Conserved blocks in PI-PLC-X, PI-PLC-Y and C2 domains from 53 PI-PLC sequences were subjected to phylogenetic analysis. An unrooted phylogenetic tree was created using the maximum likelihood method in PhyMl (WAG substitution model). Numbers at nodes represent values of neighbour-joining bootstrap support obtained using the aLRT (approximate likelihood-ratio test) method. Branches below 50% were collapsed. The scale bar represents the number of substitutions per site. Unclassified proteins were named according to maximum similarity with the respective PI-PLC isozyme of Arabidopsis. Species abbreviations: At, *Arabidopsis thaliana*; Gm, *Glycine max*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*; Ps, *Picea sitchensis*; Nt, *Nicotiana tabacum*; Sl, *Solanum licopersicum*; Ld, *Lilium davidii*; Sb, *Sorghum bicolor*; Pi, *Petunia integrifolia*; St. *Solanum tuberosum*; Bn, *Brassica napus*; Sm, *Selaginella moellendorffii*; Mp, *Micromonas pusilla*; Ot, *Ostreococcus tauri*; Hs, *Homo sapiens*; Sp, *Schizosaccharomyces pombe*; Sc, *Saccharomyces cerevisiae*; Zm, *Zea mays*.

4. Regulation of plant PI-PLCs

Due to their intrinsic role as essential regulators of metabolism, PI-PLCs constantly undergo a fine tuning of activity mediated by effectors, regulatory domains and intracellular localization.

4.1. Localization

PI-PLCs have no predicted transmembrane domains (TMHMM Server v. 2.0) and there are no current experimental data suggesting palmitoylation of plant PI-PLCs. However, PI-PLC activity strictly requires either transient or permanent association with cell membranes where phosphoinositides are located.

In plants, PI-PLC activities were traditionally studied as either cytosolic or membrane-associated, with differences in preferred substrates and Ca^{2+} requirements [23]. Yet these two PI-PLC fractions may represent pools of the same proteins since a specific antibody raised against the N-terminal domain of AtPLC4 reacted with a 68 kDa protein both in the plasma membrane and cytosolic fractions [36]. Whether a mechanism of stress-induced translocation towards membranes, as shown for phospholipase D, is applicable to PI-PLC is currently unclear.

PI-PLC proteins apparently employ several means for being targeted to membranes. Critical roles of EF-hand [27] and C2 domains [13,26] in Ca^{2+}-regulated membrane-targeting has been demonstrated in plants. PI-4,5-P_2 also appears to be important for PI-PLC membrane targeting. In mammals, the PH domain directs PLC activation and high affinity association with PI-4,5-P_2 in membranes [37]. A role of the X–Y linker region in the binding of PLCζ, that lacks the PH domain, to PI-4,5-P_2, has been suggested [29]. Plant PI-PLCs also lack the PH domain, and their X–Y linker is negatively charged, contrary to that of PLCζ, and plant cell membranes are poor in PI-4,5-P_2 when compared to animal membranes [38]. Therefore, a PI-4,5-P_2-dependent mechanism of plant PLC membrane targeting still requires clarification.

So, to which membranes are PLCs associated? Most data point to a plasma membrane localization, based on studies with fluorescent fusion proteins [9,27,39] or tandem mass spectrometry studies [40–43]. However, PI-PLC has been observed with the endoplasmic reticulum of wheat [44] and barley [45]. Interestingly, PI-4,5-P_2 is found only in plasma membranes [4], thus raising the question as to the PI-PLC substrate in other membranes.

Interestingly, several studies indicate the presence of cytosol-located PI-PLCs. NtPLCδ1 was shown to be cytoplasmic in quiescent tobacco BY2 cells [46] and a strong association to the actin cytoskeleton of a soluble protein that reacted with anti-bovine PLCβ1 polyclonal antibodies was demonstrated in oat roots [47].

Less data are available concerning the tissue localization of PI-PLCs. Genevestigator data [48] points out that the root and pollen are organs with high PLC transcript levels (Fig. 3A, B). GUS expression under the control of the *AtPLC1* promoter was detected in petioles and vascular tissues. *AtPLC5*::*GUS* was detected in guard cells, roots and vascular cells. *AtPLC4*::*GUS* was detected in pollen and certain floral organs (Fig. 3C) [35].

4.2. Ca^{2+} dependency

All Arabidopsis PI-PLCs, with the possible exception of AtPLC4 [35], strictly require Ca^{2+} for their activity. Our current knowledge provides clues for both Ca^{2+}-driven activation and membrane targeting of plant PI-PLC [26]. Ca^{2+} requirement is often linked to substrate preference. PI-PLCs from *Cupressus lusitanica* [49] and *P. patens* [14] can use non-phosphorylated PI as a substrate at high Ca^{2+} concentrations. Not only do PI-PLCs depend on Ca^{2+}, but in mammals they also actively contribute to its liberation from intracellular stores (via IP_3 receptors), thus making them essential enzymes of Ca^{2+} turnover. Although no IP_3 receptor has been genetically characterized in plants, there is evidence for IP_3-induced Ca^{2+} release, suggesting the presence of a conserved mechanism of Ca^{2+} regulation (see 9.3).

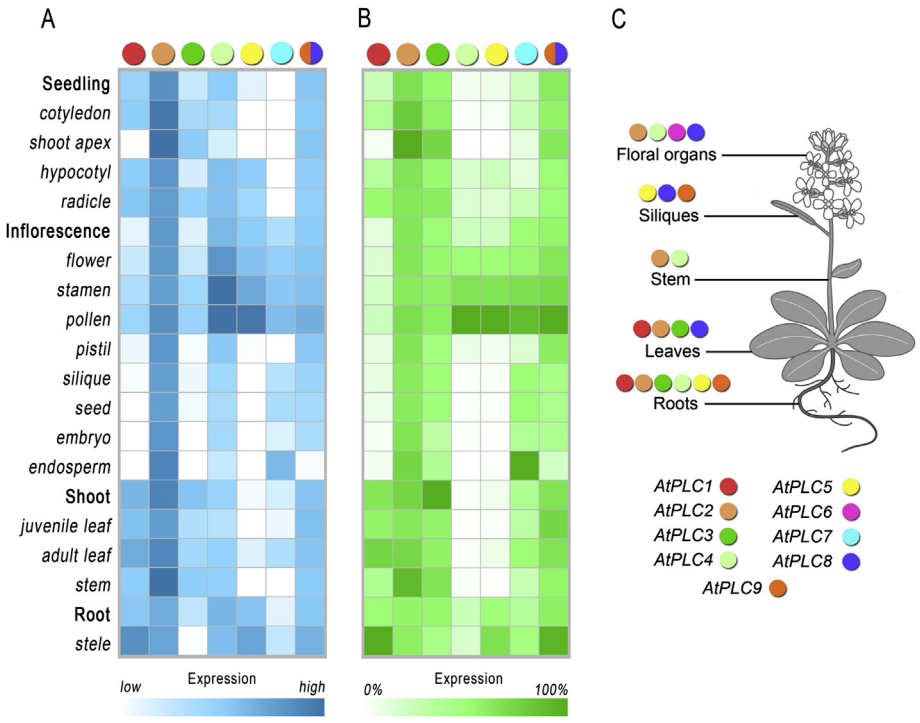

Fig. 3. Organ-specific expression of Arabidopsis PI-PLCs. Each PI-PLC isoform is colour-coded. (A, B) Microarray data were collected using Genevestigator interface [48]. In (A) colour saturation represents the absolute level of transcript abundance. In (B) the relative transcript prevalence is given as percentage of the level in the organ where the isoform is expressed at maximum level. (C) Organs and tissues where Arabidopsis PI-PLC isoforms are mainly expressed as shown by real-time PCR or promoter activity studies [15,35]. In (C), the expression of *PI-PLC6*, *PI-PLC8* and *PI-PLC9* of Arabidopsis can be distinguished.

4.3. Post translational modifications

4.3.1. Phosphorylation

In animals, regulation of PLCγ activity by phosphorylation of tyrosine residues of X/Y linkers within the catalytic domain has been reported [50]. In plants, phosphorylation of PI-PLCs has also been shown. Several phosphorylation sites were identified in AtPLCs by mass spectrometry peptide analyses [51] (Table 1), some of them located within functional domains, such as the phosphorylation of Thr^{29} within an EF-hand like motif of AtPLC2, and phosphorylation of either Ser^{346} or Ser^{348} in the Y domain [52–54] while AtPLC7 contains adjacent phosphorylation sites at Thr^{169} and Ser^{170} in the X catalytic domain [53].

4.3.2. Palmitoylation

A covalent attachment of a fatty acid residue (S-acylation) is a common post-translational control of various protein properties [55]. Protein lipid modifications facilitate membrane targeting and interaction with negatively charged head groups of lipids such as PI-4,5-P_2. Currently, there are no experimental data concerning palmitoylation of plant PI-PLCs. However, AtPLC1 and AtPLC3 have several high-probability putative palmitoylation sites at cysteine residues (Table 2) [56].

4.3.3. Ubiquitination

To our knowledge, no experimental data are available regarding ubiquitination of plant PI-PLCs. However, computational predictions suggest the presence of putative ubiquitination sites in AtPLC1, AtPLC2, AtPLC5, AtPLC6, AtPLC7 and AtPLC8 (BDM-PUB) (Table 2).

4.3.4. SUMOylation

Attachment of small ubiquitin-like modifiers (SUMOylation) to specific lysine residues in protein targets is another conserved mechanism of protein post-translational regulation [57]. Unlike the related ubiquitination, SUMOylated proteins are not directed for degradation and instead SUMOylation affects protein stability, activity and localization. Limited data are available regarding *bona fide* SUMOylation of plant PI-PLCs. SUMOylation of PLC8 was detected in heat-treated transgenic Arabidopsis plants overexpressing AtSUMO1 [58]. In addition, numerous putative SUMOylation sites were detected in PI-PLCs of Arabidopsis, and AtPLC2, AtPLC5, AtPLC6 and AtPLC8 have conventional tetrapeptide Ψ-K-x-E motifs while AtPLC7 has a good probability for the presence of a non-consensual SUMOylation site [59]. These predictions (Table 2) now require experimental proof.

4.3.5. Other modifications

In plants, treatment with the NO-donor S-nitroso-N-acetylpenicilamine induced PA formation both by PI-PLC and phospholipase D (PLD) [60] suggesting a possible mechanism of PI-PLC activity regulation via nitrosylation or nitration of cysteine/tyrosine residues. Whether PI-PLC is the direct target of nitrosylation is not known.

4.4. Protein–protein interactions

In mammalian cells, PI-PLCs interact with a range of regulatory elements including G-proteins [61], tyrosine kinases [62] and others [63]. Such interactions are thought to provide basic mechanisms of PI-PLC activity regulation and coordination with other cell effectors. Less is known about PI-PLC protein partners in plant cells. Current data point out a possible interaction with a G-protein coupled receptor system. PI-PLC activity was increased by cholera toxin (a G-protein agonist) and inhibited by pertussis toxin (a G-protein antagonist) in *Lilium daviddi* pollen protoplasts [64]. PI-PLC activity from the cytosolic fraction of *Phaseolus vulgaris* root nodules was stimulated *in vitro* by mastoparan, a G-protein agonist [65]. Yeast two-hybrid experiments provided evidence for an interaction between PI-PLC and some G-protein subunits in *L. daviddi* [66] and *Pisum sativum* [67]. Interestingly, Gα1, but not Gβ, is able to bind to the C2 domain of PLCδ from *P. sativum* [67]. PI-PLC1 from wheat (*Triticum aestivum*) was reported to interact with the Ga3 Gα subunit when expressed in tobacco epidermal leaf tissues [44].

Other proteins were also found to interact with PLCs. An example is *Nicotiana tabacum* NtC7 receptor-like protein implicated in plant reaction to wounding and osmotic stress resistance, which interacts with the C2 domain of NtPI-PLC in yeast two-hybrid screening and *in planta* when assayed by bimolecular fluorescence complementation [68]. Both proteins were detected in the plasmalemma when expressed in onion epidermal cell layers. Authors postulated that NtC7, that contains a C-terminal transmembrane domain, may drive PI-PLC plasma membrane targeting [68]. No protein encoded by the Arabidopsis genome shows a significant homology to NtC7.

Table 1
Biochemical properties and localization of Arabidopsis PI-PLCs. PM, plasma membrane.

Name	AGI	Ca^{2+} dependency[a]	Cell Localization[b]	Protein–protein interaction[a]	Co-expression[c]
AtPLC1	At5g58670	+	PM	At4g01010	At5g42650, At3g51450
AtPLC2	At3g08510	+	PM	At4g00430, At5g02620, At4g31340, At5g27370, At5g64870	At1g06460, At3g45780, At4g04340
AtPLC3	At4g38530	+	PM		At5g02600, At1g61660, At5g64240
AtPLC4	At5g58700	+/−	PM		At5g63990, At5g58690
AtPLC5	At5g58690	+	?		At5g58700
AtPLC6	At2g40116	?	?		
AtPLC7	At3g55940	?	PM	At5g63770	At4g17550
AtPLC8	At3g47290	?	?		At4g16950, At4g19530
AtPLC9	At3g47220	?	?	At1g12840, At2g22425, At3g08040, At3g12180, At3g48890, At4g30850, At5g47180, At1g21240, At4g20790, At5g59650, At1g31812, At2g26180, At2g41400, At3g25805, At5g37050	

AtPLC8 and *AtPLC9* cannot be distinguished in microarrays experiments used for co-expression analysis.
[a] Identified experimentally [36,69].
[b] MS/MS [40–43].
[c] *In silico* analysis [48].

Table 2
Post-translational modifications of Arabidopsis PI-PLCs identified in Arabidopsis. Phosphorylation sites are experimentally obtained [51] while the other modifications are putative, as predicted using SUMOsp 2.0, CSS-Palm 3.0 and BDM-PUB software [56,59].

Name	AGI	Phosphorylation sites	SUMOylation	Palmitoylation	Ubiquitination
AtPLC1	At5g58670	S^{260}		Cys^{8}, Cys^{10}, Cys^{11}, Cys^{226}	Lys^{2}
AtPLC2	At3g08510	S^{280}, S^{286}, T^{29}, S^{346}/S^{348}	Lys^{577}	Cys^{9}, Cys^{11}	Lys^{3}, Lys^{358}
AtPLC3	At4g38530			Cys^{8}, Cys^{10}, Cys^{11}, Cys^{313}	
AtPLC4	At5g58700	S^{280}		Cys^{14}, Cys^{439}	
AtPLC5	At5g58690		Lys^{2}, Lys^{295}, Lys^{497}	Cys^{13}, Cys^{14}	Lys^{264}, Lys^{266}, Lys^{275}, Lys^{448}
AtPLC6	At2g40116		Lys^{6} Lys^{364}		Lys^{3}
AtPLC7	At3g55940	T^{169}, S^{170}, T^{19}/S^{21}, S^{287}/S^{293}	Lys^{60}	Cys^{9}, Cys^{11}	Lys^{3}, Lys^{364}
AtPLC8	At3g47290		Lys^{202}	Cys^{519}	Lys^{60}
AtPLC9	At3g47220			Cys^{519}	

High throughput screening for protein–protein interactions using the split-ubiquitin method yielded additional putative PI-PLC protein partners [69]. The roles of AtPLC protein partners (Table 1) could give indications concerning the role(s) of PI-PLCs. As an example, AtPLC1 interacted with cyclic nucleotide-gated channel 13 protein, a putative cyclic nucleotide and calmodulin-regulated ion channel that affects Ca^{2+} cell signalling [70]. AtPLC7 cross-reacted with diacylglycerol kinase 2 (DGK2), thus showing that they interact not only functionally but also physically in DAG- and PA-dependent lipid signalling [71]. Interactions between PI-PLC and protein kinases have also been reported (Table 1). Such interactions may result in the phosphorylation of PI-PLC or in regulating the phosphorylation of other protein partners. However, the physiological significance of most of the observed protein–protein interactions remains to be elucidated.

5. Plant PI-PLC expression and co expression web

The role of plant PI-PLCs can be assessed using transcriptome data. Expression studies of individual PI-PLC genes from different species have been performed but for this review we focus on transcriptome-wide analyses, in response to hormone treatment or environmental stresses (Fig. 4). For species where expression data of different isoforms are available, the expression pattern is usually characterized by a multidirectional regulation of the different isoforms. This is the case during elicitor and heat treatments for the PI-PLC genes of wheat and Arabidopsis. This kind of data can be interpreted as an evidence that PI-PLC genes in families are not necessarily redundant, and that some have specific and distinct roles, therefore have distinct expression regulation. Yet for some elicitations, all PI-PLC genes of a same species are regulated in the same way, as seen for cold treatment, abscisic acid (ABA shoots), drought, salt stress and wounding in Arabidopsis. These treatments, in the organ(s) considered, induce the expression of some PLC genes, while others are not affected. This is in agreement with a PI-PLC role in response to cold and osmotic stresses (see below).

It is conceivable that co-expression of genes may signify their functional association. A co-expression study was performed using the Genevestigator interface [48] (Table 1). Several AtPLCs co-express with genes implicated in plant stress and hormonal responses. AtPLC1 was found to be co-expressed with an allene oxide synthase (*At5g42650*) involved in the jasmonic acid (JA) biosynthetic pathway [72] and with a member of the calcium-dependent phosphotriesterase superfamily (*At3g51450*) implicated in plant responses to salicylic acid (SA), methyl jasmonate, wounding or pathogen inoculation [73]. AtPLC4 co-expressed with *At5g63990* encoding a 3′(2′),5′-bisphosphate nucleotidase/inositol polyphosphate 1-phosphatase, with a possible role during cold stress [74] and functionally related to the stress-responsive FRY1 gene implicated in the attenuation of the IP_3 signal [75]. Due to their high similarity, *AtPLC8* and *AtPLC9* expression cannot be distinguished in Arabidopsis ATH1 22k arrays. *AtPLC8/9* co-expressed with *At4g16950* and *At4g19530*, both genes involved in plant immunity encoding NB-LRR receptor-like proteins [76,77]. *AtPLC5* co-expressed with another biotic-stress protein, an SOS2-like Protein Kinase 5 (*At2g30360*) that is involved in controlling several plant activities and shown to directly phosphorylate NPR1 – a critical component of plant immunity [78]. *AtPLC3* appears to co-express with type I metacaspase (*At5g64240*) that is known to be involved in the control of cell death [79] and *AtPLC7* co-expresses with a member of the phosphate starvation-induced glycerol-3-phosphate permease gene family (*At4g17550*) possibly implicated

Fig. 4. Changes in plant PI-PLC gene expression in response to hormone treatments and during stresses. Transcriptional analysis of plant PI-PLC gene expression was performed *in silico* using Genevestigator (https://www.genevestigator.com/). Shown here are cumulative representative data concerning changes in PI-PLC gene expression in different growth conditions. When several expression data points with different time or different dose of treatment were available, the one with the most apparent and consistent changes in PI-PLC expression was chosen. ABA, abscisic acid; CK, cytokinins; Bl, brassinolide; GA, gibberelic acid; IAA, indolacetic acid; ET, ethylene; MeJa, methyl jasmonate; SA, salycilic acid. Species abbreviations: At, *Arabidopsis thaliana*; Si, *Solanum licopersicum*; Os, *Oryza sativa*; Zm, *Zea mays*; Hv, *Hordeum vulgare*; Ta, *Triticum aestivum*; Gm, *Glycine max*.

in phosphorus transport during phosphate starvation [80]. Interestingly, *AtPLC4* significantly co-expressed with *AtPLC5*, indicating the possible redundancy of these 2 isoforms.

6. PI-PLCs in plant development

PI-PLCs are often considered to be stress-activated enzymes. However, they also play a role in regulating growth and development-related processes both in animals [81] and plants [82]. The role of PI-PLC in plant development seems to be multifaceted. For instance, over-expression of *BnPI-PLC2* caused both an early shift from vegetative to reproductive phases, and shorter maturation periods, together with alterations in hormonal distribution patterns in plant tissues [83]. IP_3 is involved in the differentiation of xylem vessels [84] and stolon-to-tuber transition [85]. An influence of PI-PLC was also noted during asymmetric cell divisions that produce stomatal complexes in *Zea mays* [86]. PI-PLCs also seem to participate in cell cycle progression in tobacco, through DNA synthesis control [87].

Involvement of PI-PLCs in plant development has been well studied during polarized pollen growth. Such an asymmetric cell expansion is known to rely on several events including calcium signalling, vesicular trafficking and cytoskeleton rearrangements [88].

In the elongating pollen tube, PI-PLC accumulates in the plasma membrane specifically at the flanks of the tip, but not at the very apex. On the contrary, PI-4,5-P_2 exclusively accumulates at the apex of the pollen tube [9,27]. It was shown that the PI-PLC inhibitor U73122 strongly inhibited pollen tube growth and led to swollen tips, thus indicating that expansion is no longer polarized [27]. The same effect was produced when a PI-PLC inactive form, that competes with the native protein for the localization in the membrane, is expressed in the pollen tube [9]. These effects (reduction of growth and swelling) correlate with the spreading of PI-4,5-P_2 to the flanks of the tip. Therefore PI-PLC has an active role to create and maintain a PI-4,5-P_2 gradient in the pollen tip, between the apical and lateral membranes, and this gradient is necessary for polarized growth [9,27,89]. The role of PI-4,5-P_2 might be related to the control of actin cytoskeleton dynamics, of membrane trafficking including clathrin-dependent endocytosis, and to the control of apical pectin deposition [90–92].

In root hair tips, PI-4,5-P_2 is also important for efficient growth. Arabidopsis mutants deficient in phosphatidylinositol-4-phosphate 5-kinase gene – enzyme producing PI-4,5-P_2 – were significantly impaired in root hair development [93,94].

7. PI-PLCs in abiotic stress

Plants are sessile organisms that require specific mechanisms in order to resist or adapt to undesirable environmental conditions, such as increased soil salt concentration, high or low temperature, and water shortage.

7.1. Osmotic stress

The role of PI-PLC in the production of polyphosphoinositides and their role during osmotic-stress induced cell signalling have been discussed in detail by Munnik et al. [95]. Different salts (NaCl, KCl) and osmotic stress inducers (mannitol, sorbitol and mannose) [96–99] evoke a rapid (often within seconds) increase in IP_3 levels. This increase has a positive role regarding osmotic stress resistance as supported by genetic studies of inositol polyphosphate kinases [100,101] and phosphatases [75,102]. Apparently not all PI-PLCs participate in stress signalling as PA, PI-4-P or PI-4,5-P_2 levels were found to be identical in wild type and in a *AtPLC3*/*AtPLC6*/*AtPLC9* triple mutant in response to salts [103].

The increase in IP_3 production during osmotic-stress is accompanied by a rise of PI-4,5-P_2 levels [96,104]. This may suggest that PI-PLC functions simultaneously with phosphoinositide kinases in stress signalling. Whether this PI-4,5-P_2 is produced to serve as a PI-PLC substrate [105] or as a specific signalling molecules *per se* [106] is not known.

PI-PLC activation in response to osmotic stress may be dependent on activation of a specific receptor or an unspecific destabilization of cellular compartments resulting in a calcium increase [107]. Interestingly, the rapid PLC activation in response to hyperosmotic conditions caused by mannitol in wheat roots was shown to be the result of microtubule destabilization [108].

Rapid calcium release into the cytosol in response to salt and osmotic stress observed in *Arabidopsis* root tips [96], seedlings [102] and tobacco cells [109] was shown to be dependent on PI-PLC. PI-PLCs affect several cellular processes in hyperosmotic stress conditions including microtubule polymer recovery in plasmolyzed wheat root cells [108], rapid MAPK activation and ROS generation in soybean [110], release of Tubby transcriptional factors from the plasma membrane [111] and the control of phosphoenolpyruvate carboxylase kinase 1 gene expression in sorghum [112].

Abscisic acid (ABA) is one of the key plant stress hormones that accumulate upon stress exposure and it controls many plant defence reactions [113]. In plants, PI-PLC seems to be actively involved in ABA-dependent signalling. As an example, exogenous ABA treatment evoked IP_3 accumulation in Arabidopsis seedlings [75]. More importantly, in *Commelina communis* ABA-sensitivity of guard-cells that control leaf transpiration was impaired by the addition of heparin – an IP_3 antagonist [114]. Previously, inhibitor studies demonstrated that the regulatory role of PI-PLC on guard-cell movements is likely to be implemented via calcium oscillations [115].

Proline is an osmolyte that accumulates in plants in order to alleviate osmotic stress action. In *Arabidopsis*, PI-PLC, IP_3 and IP_3-gated calcium release regulate transcriptional and post-transcriptional events which subsequently lead to proline accumulation in response to ionic, but not to non-ionic hyperosmotic stress [107]. Gene expression changes are also known to be mediated by PI-PLC in response to dehydration [102,116]. However, an artificial decrease in IP_3 levels by expressing inositol polyphosphate phosphatase increased drought tolerance [102]. This discrepancy may be in part explained by pharmacological studies conducted with the halophyte plant *Thellungiella halophita*. In this species, in the absence of stress and in response to moderate salt stress (200 mM), PI-PLC negatively regulated proline accumulation and expression of genes coding proline metabolism enzymes. However, during severe salt and osmotic stress conditions, PI-PLC positively affected the osmolyte level [117]. This supports a notion about divergence in metabolism responses to osmotic stress evoked by PI-PLC depending on stress severity or plant robustness.

7.2. Heat stress

Heat exposure increased PI-PLC activity in pea membranes with a maximum at 40 min [118]. This observation was consistent with the observed PI-PLC protein accumulation in heat-treated plants [39]. In *Arabidopsis*, rapid (within minutes) and substantial IP_3 accumulation in response to heat stress was reported [119]. The heat-activated AtPLC9 activity was required for intracellular calcium accumulation, *AtHsp* promoter activity, stress-dependent gene expression and heat acclimation [16,119].

7.3. Cold stress

Despite its opposite physical nature, cold stress induces signal transduction in cells that also involves PI-PLC pathway(s). Rapid and transient IP_3 accumulation with a simultaneous decrease in PI-4-P and PI-4,5-P_2 levels was observed in winter wheat tissues [120], *Arabidopsis* suspension cells [121] and oilseed rape leaves [122] subjected to cold stress. Later, mutant analyses of inositol polyphosphate kinases [99] and phosphatases [75,123] validated the role of IP_3 in cold tolerance. Cold-induced activation of PI-PLCs is dependent on calcium entry into the cells [121] while substrates for PI-PLCs are supplied by type III-phosphatidylinositol 4-kinases [106]. Analysis of *Arabidopsis* mutants altered in fatty acid desaturase activity suggests that cold-induced membrane rigidification is upstream of PI-PLC activation [124], leading to the model where cold would rigidify membranes, leading to calcium entry and then to PI-PLC activity. To date, main downstream components of cold-induced PI-PLC activation are poorly studied. A plant expressing the mammalian type I inositol polyphosphate 5-phosphatase (that cleaves IP_3) accumulates 30% less calcium in response to cold [102]. In addition, pharmacological studies have suggested that phosphoinositide metabolism may be upstream of microtubule depolymerization in response to cold stress [125] while expression of a subset of genes regulated by low temperature was dependent on PI-PLC activity [126]. It should be noted that cold stress also induces the expression of PI-PLC genes in *B. napus* [30], *Z. mays* [127,128], winter wheat [129] and *Arabidopsis* [15], thus supporting a role of PI-PLC in cold acclimation.

7.4. Heavy metal stress

Toxic metals can severely inhibit plant cell metabolism by affecting many main enzymatic reactions. Ni^{2+}, Zn^{2+} and especially Cu^{2+} decreased *in vitro* PI-PLC activity in both membrane and soluble fractions of *Catharanthus roseus* roots [130]. However, in another study, copper excess rapidly increased *in vivo* DAG accumulation in roots of *B. napus* that could reflect PI-PLC activation [131]. Moreover, copper-induced release of intracellular calcium in marine alga *Ulva compressa* seems to be dependent on PI-PLC activity [132].

Aluminium is currently the best studied toxic metal in terms of affecting plant PI-PLC activity. In *Coffea arabica* suspension cells, aluminium treatment induced a rapid PI-PLC activation and IP_3 accumulation [133]. However, a prolonged exposure led to inhibition of PI-PLC [134,135]. Aluminium may block PI-4,5-P_2 hydrolysis by binding to it or substituting calcium bound to liposomal lipids [25]. The mechanisms involved in the rapid activation of PI-PLC by aluminium remain to be revealed.

7.5. Other stresses

Several other stress signals appear to be transmitted in plant cells by a PI-PLC pathway. Hypoxia induces a rapid G-protein-dependent IP_3 accumulation in rice roots. This PLC activation is further transmitted by IP_3-sensitive calcium channels, calcium and calmodulin that are required for gamma-aminobutyric acid accumulation and cellular potassium loss during anaerobic stress [136]. Interestingly, in *Peganum harmala* calli, extremely low frequency electromagnetic fields rapidly reduced PI-4,5-P_2 levels [137]. This effect was blocked by PI-PLC inhibitors, indicating that membranes may be the primary site of electromagnetic stimulus perception that induces PI-PLC activation.

8. PI-PLCs in biotic stress

Plants possess unique defence mechanisms that enable a single cell to fulfil the role of an integrated immune system. Two defence strategies are usually employed during plant–pathogen interactions [138]. Upon contact with a pathogen, plants are able to rapidly develop a hypersensitive response (HR) leading to cell death at the point of infection and thus restricting pathogen propagation. Alternatively plants can induce systemic acquired resistance (SAR) or induced systemic resistance (ISR) by activation of defence genes and production of antimicrobial metabolites that limit pathogen growth at the whole plant level. Phospholipases and phospholipid-derived molecules are recognized to be intrinsic components of both defence strategies [139].

PI-PLC-derived molecules seem to be involved in plant defence reactions. DAG rapidly accumulated in rice cells following exposure to the elicitor *N*-acetylchitooligosaccharide [140] while rapid and transient accumulation of IP_3 was observed in elicited *C. lusitanica* cells [49]. PI-PLC inhibitor U73122 reduced accumulation of phytoalexin and ROS production in tobacco cells elicited with riboflavin [141] and in transgenic tobacco cells elicited during a simulated Cf-4/Avr4 interaction [142]. U73122 also reduced ROS production in tomato cells elicited with chitosan [143].

Transcriptional activation of PI-PLC genes is common during biotic stress conditions. In tomato, several PI-PLC gene family members were identified as crucial components of plant defence systems [144]. Prominent roles were ascribed to tomato *SlPLC4* and *SlPLC6* genes that are induced under biotic stress and differentially control the onset of HR and plant resistance against *Cladosporium fulvum*, *Verticillium dahlia* and *Pseudomonas syringae* pathogens.

Despite numerous biochemical evidences, the roles of the molecules produced by PI-PLC in mediation of defence reactions are not entirely clear. The addition of synthetic DAG induced quick ROS accumulation in rice cells [140] and activated expression of defence-related genes [145]. It was also shown that phorbol myristate acetate, an analogue of DAG that cannot be converted to PA, mimicked the effects of chitosan elicitor and induced anthraquinone accumulation in cultured *Rubia tinctorum* cells [146], suggesting that DAG can act not only as a PA precursor, but can also have a role *per se* during plant immune signalling.

Arabidopsis mutants expressing a mammalian type I inositol polyphosphate 5-phosphatase, thus characterized by low levels of IP_3 and IP_6, had a reduced cytosolic Ca^{2+} increase in response to flagellin [147]. Therefore, it is possible that PI-PLC can regulate plant defence reactions by bringing about changes to cytosolic Ca^{2+} that are perceived by Ca^{2+} dependent protein kinases [148].

Symbiotic relations are special cases of plant–bacteria interactions mediated by a special class of molecules named nodulation factors (Nod) recognized by special plant receptor kinases. An essential role of PI-PLC, PLD and their corresponding lipid products, DAG and PA, in controlling downstream responses of *M. truncatula* root hair cells during Nod factor-induced signalling has been convincingly revealed [149]. Interestingly, the symbiotic relationship between *M. truncatula* and *S. meliloti* was shown to be controlled by DNF2 protein that contains the X domain of PI-PLC [34]. The other conventional domains of PI-PLC are absent in DNF2. The authors speculated that DNF2 may bind, but not cleave, phosphoinositides, thus preventing their hydrolysis by PI-PLC and the onset of defence reactions that may result in bacteroid degradation.

Intriguingly, plant pathogens also frequently make use of PI-PLC signalling. Despite having biochemical properties similar to those of plant PI-PLCs, bacterial PI-PLC activity is often a prerequisite for pathogenicity [150]. It is thought that they act to either suppress or overwhelm plant innate defence mechanisms or to lyse cells. This

indicates the requirement for a precise balancing of defence mechanisms *in vivo*, granting either plant resistance or disease. Such knowledge is important for the future biotechnological development of disease-resistant plants.

In summary, plant PI-PLC plays an important role in signal transduction in response to different stresses, representing a key hub within the complex network of cellular regulatory systems. However, the regulation of PI-PLCs, the involvement of specific PI-PLC genes, the mechanistic action of generated second messengers in abiotic and biotic stress signalling and responses require further investigation.

9. PI-PLC modes of action in plant cells

Taking into account the multitude of signalling events involving PI-PLCs, it is of great interest to know which downstream events convey PI-PLC signals. PI-PLC signalling in plants can be associated with changes in the cellular concentration of several molecules; either direct (DAG, IP_3) or indirect (PA) products [151], and phosphoinositide substrates [152].

9.1. Diacylglycerol

In animals, DAG serves as a classical second messenger that activates protein kinase C (PKC) by binding to its C1 domain [153]. However, no homologous PKC genes have been identified in plants. In addition, DAG has not been shown to activate any purified plant protein kinase. Although different effects of the DAG analogue phorbol myristate acetate have been described [154,155], their relation to PI-PLC activity and DAG action remains to be established. Thus, the canonical role of DAG as a protein kinase activator in plants is controversial. On the other hand, many different C1 domain-containing proteins are encoded by *Arabidopsis* [156], rice and other plant genomes. C1 domain containing-proteins are not limited to predicted protein kinase activities, one such protein was shown to possess a transactivation activity in wheat [157], but the role of DAG has yet to be investigated.

Taking into account that DAG is also a precursor of galactolipids, structural phospholipids and storage lipids, only specifically localized, tightly controlled and transient DAG accumulation may fulfil signalling functions in plants. Determination of such DAG signalling levels in plants is still a challenge and complicated by the observation that basal DAG levels differ between plant organs [158].

9.2. Phosphatidic acid

DAG, a direct product of PI-PLC [159], can be phosphorylated to PA by diacylglycerol kinases (DGK) [160,161]. PA can also be produced by the action of phospholipases D on structural phospholipids. The coupling of DGK with PI-PLC, leading to PA synthesis, has been established in response to salt in rice [104], to cold in *Arabidopsis* cells [121,126], seedlings [106] and adult leaves [161], and to shear stress in *Taxus cuspidata* cells [162].

In plants, as in animals, PA is a well-known second messenger [163]. In Arabidopsis, PA binds and mediates membrane recruitment of glyceraldehyde 3-phosphate dehydrogenase, clathrin heavy chain proteins and sucrose non-fermenting-1-related protein kinase 2 during salt stress [164,165]. PA activates calcium-dependent protein kinase [166], MAP kinase GMK1 [110] and monogalactosyldiacylglycerol synthase [167]. In contrast, inhibition of actin-capping proteins [168] and protein phosphatase 1 [169] by PA has been reported. PA has also been suggested to play a role in ATPase interactions with 14-3-3 proteins, causing inhibition of ATPase activity [170], and induced proteolytic cleavage of bound glyceraldehyde 3-phosphate dehydrogenase [171].

The fact that PA is an active mediator in the PLC/DGK pathway is reinforced considering the effect of DGK inhibition or overexpression. Overexpression of rice *OsBIDK1* (a DGK) in tobacco conferred amplified resistance against tobacco mosaic virus and *Phytophthora parasitica* var. *nicotianae* pathogen [172]. Application of R59022, a DGK inhibitor, hampered defensive ROS generation in xylanase-induced ROS production in tomato cells [173]. In another study, PI-PLC inhibitor U73122 reduced ROS production in tomato cells elicited with chitosan [143] and the authors assumed that this was dependent on NO-induced PLC/DGK generation of PA. DGK inhibitor R59022 reduced primary root elongation and plant growth in Arabidopsis [174] and inhibited tobacco pollen tube growth [175].

9.3. Soluble inositol-phosphates

PI-PLC action in plants is thought to be tightly related to polyphosphoinositide signalling [176]. Previously, many effects of soluble phosphorylated inositols produced by PI-PLC have been studied in relation to calcium signalling [177]. In animals, a role of IP_3 in the regulation of Ca^{2+} fluxes by binding to IP_3 receptors (Ca^{2+} channel) located mainly on the ER is dogmatic [178,179]. The physiological role of IP_3 is also obvious in plants however it is most likely mediated by different mechanisms. Early findings concerning IP_3 influence on cell calcium oscillations are thoroughly summarized in the review by Krinke et al. [180]. More recent studies revealed that IP_3 is also implicated in regulation of diurnal cytosolic Ca^{2+} oscillations induced by exogenous calcium [181] and Ca^{2+}-dependent proline accumulation in salt-stressed Arabidopsis [107]. Apart from PI-PLC, several other enzymes are involved in the control of IP_3 abundance and induced calcium signalling. Studies of the *supo1* mutant of Arabidopsis defective in inositol polyphosphate 1-phosphatase have shown that regulation of auxin distribution through PIN transporters is mediated by cytosolic calcium release controlled by IP_3 [182]. It was also shown that elevated IP_3 levels in Arabidopsis inositol polyphosphate 5-phosphatase mutants resulted in elevated concentrations and altered distributions of cytosolic Ca^{2+} in developing pollen [183]. However, there is no direct genetic or molecular evidence for the existence of plant IP_3 receptors. If such a receptor exists, it apparently does not share sequence homology with the analogous animal proteins, and most likely represents a multidomain heteromeric complex [180]. Nevertheless, several studies have provided indirect evidence of a receptor-like IP_3-binding protein or complex in plant cells. Indeed, IP_3 had a high affinity towards reticulum-enriched membrane fractions prepared from *Chenopodium rubrum* leaves [184]. While, 2-aminoethoxydiphenylborate, an inhibitor of IP_3-mediated calcium release described in animals, was able to reduce cytosolic Ca^{2+} spiking in *M. truncatula* root hairs induced by Nod factors [185].

It is also apparent that the regulatory role of soluble PI-PLC products in plants is not limited to IP_3 production. Products of further IP_3 phosphorylation, inositol tetrakisphosphate (IP_4), inositol pentakisphosphate (IP_5) and inositol hexakisphosphate (IP_6), seem to be equally important. According to several studies, phosphorylated products of IP_3 produced via inositol-phosphate kinase activity fulfil their own signalling role in plants. IP_6 (also named phytate) is relatively abundant in plant cells and sometimes regarded as a phosphorus storage molecule. However its signalling role has been demonstrated since it triggered intracellular calcium release after ABA addition in patch-clamped guard cell protoplasts of *Vicia faba* [186] and regulated potassium-inward rectifying channel conductance [187]. The role of IP_6 in plant resistance was also demonstrated [188] with authors suggesting that low levels of IP_6 in mutant plants lacking *myo*-inositol phosphate synthase or IP_5-2-kinase genes corresponded to a higher sensitivity to infection

by different groups of pathogens attributed to impaired SA accumulation. Other genetic studies indicate that components of a phosphoinositide signalling pathway are involved in the induction of wounding-inducible defence gene expression and resistance to herbivores [98]. In turn, tolerance to oxidative stress may be mediated in part by products of IP_3 phosphorylation [100,101].

IP_4 and IP_5, but not IP_3, have also been reported to be potent activators of the COI–JAZ co-receptor interaction with coronatine, an analogue of JA [189]. Beyond JA, the metabolism of soluble inositol polyphosphates might also affect auxin signalling through modulation of the level of the IP_6 ligand of auxin receptor TIR1 [190] but how this affects auxin response remains unclear. Moreover, whether other hormone receptors are affected by these metabolites has not yet been described.

9.4. Phosphoinositide level

PI-PLC activity will not only result in the production of molecules. It will also lead to a decrease in its substrates, the PI-4,5-P_2. Because PI-4,5-P_2 and its precursor PI-4-P, can bind to proteins, thus modifying their localization and/or activity [4], theoretically PI-PLC action could be transduced through the modification of the level of these molecules, the phosphoinositides. Does it really occur in plant cells? It seems that it is indeed the role of PI-PLCs in the pollen tube. In this organ, inhibiting PI-PLC [9,27], or overexpressing PI-4-P-5-kinases [90,191], that produce PI-4,5-P_2, result in the same phenotype, the swelling of the pollen tip (which corresponds to a loss of polarized growth). The common action of inhibiting PI-PLC and of overexpressing PI-4-P-5-kinases is to lead to an increase in PI-4,5-P_2. As already explained, the strict control of the level of PI-4,5-P_2, in a particular pollen tube zone, is necessary for pollen polarized growth. PI-PLC has a major role in this process.

10. Conclusion and open questions

In plants, PI-PLCs have major roles in responses to environmental stresses and in plant development. Despite the importance of these roles, the functioning of the PI-PLC pathway is far from being fully understood.

To function, PI-PLCs must be coupled to the enzymes that produce the substrates, i.e. the phosphoinositides. It is important to understand if stimulated PI-PLCs use the phosphoinositide pool already present in plant membranes before the activating stress, or if a *de novo* phosphoinositide synthesis, occurring concomitantly with PI-PLC activity, participates in providing the substrate used by PI-PLC. Our data show that during a cold stress, *de novo* synthesis by PI-4 kinases participates in substrate providing [106] but whether this can be generalised to other activation situations is not known. Besides, the phosphorylation of PI into PI-4-P by PI-4-kinases takes place most probably in endomembranes. How the *de novo* synthesis of PI-4-P is compatible with PI-4,5-P_2 production and subsequent hydrolysis by PI-PLC in the plasma membrane is another intriguing question [105].

We have also seen that most PI-PLC actions do not rely on the direct enzyme products, but on their phosphorylation products, such as IP_5/IP_6 or PA. Therefore a functional coupling between PI-PLC and DGK and inositol-phosphate-kinase exists. Does this coupling require a physical interaction between these enzymes? And if yes, which isoforms are involved? The data discussed above suggesting an interaction between Arabidopsis PLC7 and DGK2 support the existence of physical coupling but it needs more characterization, including structural characterization. Does this mean that PI-PLC is part of a supramolecular complex, a so-called *signalosome* or *signalling platform* that comprise PI-PLC, the kinases necessary for the phosphorylation of the products, together with the proteins that are the targets of these phosphorylated products? The existence of such a platform is an exciting hypothesis to investigate.

The roles of IP_5/IP_6 or PA in the PI-PLC transducing pathway seem clearly established. Does this mean that PI-PLC action is not mediated also by other molecules? First, the potential of DAG as an active molecule cannot be ruled out. At least the capacity of plant C1 domain-bearing proteins to actually bind DAG should be considered. Moreover, proteins can certainly bind DAG through non-conserved motifs. Besides, PI-4-P and PI-4,5-P_2 are active molecules *per se* [4] and therefore PI-PLC could also affect cells by diminishing phosphoinositide levels. Such a regulatory role occurs during polarized growth. Does it also intervene in response to environmental stresses and/or hormones.

Calcium metabolism clearly appears to be the main cellular process controlled by PI-PLC via soluble inositol-phosphates and their action on calcium channels. But it is also possible that PI-PLCs influence Ca^{2+} signalling indirectly by affecting Ca^{2+}-sensitive cell targets. Indeed, recently it has been shown that the activity of stress-induced calcium-dependent protein kinase CaCDPK1 from chickpea, implicated in salt-stress responses, is regulated both by PA and, less efficiently, by DAG [166]. Furthermore, PCaP2 protein located predominantly in the plasma membrane of Arabidopsis root hair cells was shown to interact with PI-4,5-P_2 [192]. This raises the question of how the different active molecules generated or controlled directly by PI-PLC (DAG/PA, inositol-phosphates and phosphoinositides) act together. Is there necessarily a synergetic action between lipid and soluble messengers, or can antagonistic actions be envisaged?

Finally, more studies are necessary to understand PI-PLC signalling since the actual *in planta* substrate is still undetermined. PI-4-P is much more abundant in plant cell membranes than PI-4,5-P_2, and the PI-4,5-P_2 vs. PI-4-P ratio is 10-fold higher in animals that in plants. Taking into account that PI-PLC can use PI-4-P as a substrate *in vitro* raises the possibility of this monophosphorylated PI to be an *in vivo* PI-PLC substrate. When U73122, a PI-PLC inhibitor, is added to plant cells, PIP_2 level increases [38] thus indicating that PI-4,5-P_2 is an *in vivo* PI-PLC substrate, but this does not rule out that PI-4-P also has this role. Besides, it was shown that, *in vitro*, tomato *SlPLC4* and *SlPLC6* do not hydrolyse PI-4,5-P_2 but PI [144].

In conclusion, plant PI-PLCs are amongst the structurally simplest eukaryotic PI-PLCs. Like the mammalian PLCζs, they are only composed of EF-hand, X/Y and C2 domains and they do not possess a PH domain. Nevertheless, they are targets of many regulatory processes that require further characterization, including the role of protein–protein interactions in the control of plant PI-PLC activity. Even though significant differences with the mammalian canonical PI-PLC transduction module exist, including the importance of phosphorylated forms of the direct PI-PLC products as mediators, the cellular effects controlled by PI-PLCs are similar and include calcium signatures and cell phosphoproteome.

Acknowledgements

This work was supported by grants from NAS of Ukraine (9.1-06-13 and 8-13) and from SFFR of Ukraine (F41.4/042-2012). The authors acknowledge funding from the French Agence Nationale de la Recherche Programme Blanc PANACEA NT09_517917 (contract to ER). We would like to thank Michael Hodges (Institut de Biologie des Plantes, CNRS UMR8618, Saclay Plant Sciences, Université Paris Sud, France) for carefully reading the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2013.07.004.

References

[1] G. Kadamur, E.M. Ross, Mammalian phospholipase C, Annu. Rev. Physiol. 75 (2013) 127–154.
[2] T. Yoko-o, Y. Matsui, H. Yagisawa, H. Nojima, I. Uno, A. Toh-e, The putative phosphoinositide-specific phospholipase C gene, PLC1, of the yeast *Saccharomyces cerevisiae* is important for cell growth, PNAS 90 (1993) 1804–1808.
[3] Z. Wei, L.A. Zenewicz, H. Goldfine, *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C has evolved for virulence by greatly reduced activity on GPI anchors, PNAS 102 (2005) 12927–12931.
[4] E. Delage, J. Puyaubert, A. Zachowski, E. Ruelland, Signal transduction pathways involving phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate: convergences and divergences among eukaryotic kingdoms, Prog. Lipid Res. 52 (2013) 1–14.
[5] S.M. Singh, D. Murray, Molecular modeling of the membrane targeting of phospholipase C pleckstrin homology domains, Protein Sci. 12 (2003) 1934–1953.
[6] M. Fujii, K.S. Yi, M.J. Kim, S.H. Ha, S.H. Ryu, P.-G. Suh, H. Yagisawa, Phosphorylation of phospholipase C-δ1 regulates its enzymatic activity, J. Cell. Biochem. 108 (2009) 638–650.
[7] J.K. Kim, O. Kwon, J. Kim, E.-K. Kim, H.K. Park, J.E. Lee, K.L. Kim, J.W. Choi, S. Lim, H. Seok, W. Lee-Kwon, J.H. Choi, B.H. Kang, S. Kim, S.H. Ryu, P.-G. Suh, PDZ domain-containing 1 (PDZK1) protein regulates phospholipase C-β3 (PLC-β3)-specific activation of somatostatin by forming a ternary complex with PLC-β3 and somatostatin receptors, J. Biol. Chem. 287 (2012) 21012–21024.
[8] X. Wang, Lipid signaling, Curr. Opin. Plant Biol. 7 (2004) 329–336.
[9] P.E. Dowd, S. Coursol, A.L. Skirpan, T.-H. Kao, S. Gilroy, Petunia phospholipase C1 is involved in pollen tube growth, Plant Cell 18 (2006) 1438–1453.
[10] M.V. Ellis, S.R. James, O. Perisic, C.P. Downes, R.L. Williams, M. Katan, Catalytic domain of phosphoinositide-specific phospholipase C (PLC): mutational analysis of residues within the active site and hydrophobic ridge of PLCδ1, J. Biol. Chem. 273 (1998) 11650–11659.
[11] L.-P. Wang, C. Lim, Y.-S. Kuan, C.-L. Chen, H.-F. Chen, K. King, Positive charge at position 549 is essential for phosphatidylinositol 4,5-bisphosphate-hydrolyzing but not phosphatidylinositol-hydrolyzing activities of human phospholipase C δ1, J. Biol. Chem. 271 (1996) 24505–24516.
[12] L.-O. Essen, O. Perisic, R. Cheung, M. Katan, R.L. Williams, Crystal structure of a mammalian phosphoinositide-specific phospholipase C delta, Nature 380 (1996) 595–602.
[13] Y.J. Kim, J.E. Kim, J.-H. Lee, M.H. Lee, H.W. Jung, Y.Y. Bahk, B.K. Hwang, I. Hwang, W.T. Kim, The Vr-PLC3 gene encodes a putative plasma membrane-localized phosphoinositide-specific phospholipase C whose expression is induced by abiotic stress in mung bean (*Vigna radiata* L.), FEBS Lett. 556 (2004) 127–136.
[14] K. Mikami, A. Repp, E. Graebe-Abts, E. Hartmann, Isolation of cDNAs encoding typical and novel types of phosphoinositide-specific phospholipase C from the moss *Physcomitrella patens*, J. Exp. Bot. 55 (2004) 1437–1439.
[15] I.M. Tasma, V. Brendel, S.A. Whitham, M.K. Bhattacharyya, Expression and evolution of the phosphoinositide-specific phospholipase C gene family in *Arabidopsis thaliana*, Plant Physiol. Biochem. 46 (2008) 627–637.
[16] S.-Z. Zheng, Y.-L. Liu, B. Li, Z.-l. Shang, R.-G. Zhou, D.-Y. Sun, Phosphoinositide-specific phospholipase C9 is involved in the thermotolerance of Arabidopsis, Plant J. 69 (2012) 689–700.
[17] M. Kumar, S. Ahmad, E. Ahmad, M.A. Saifi, R. Khan, *In silico* prediction and analysis of *Caenorhabditis* EF-hand containing proteins, PLoS One 7 (2012) e36770.
[18] M. Kobayashi, Z. Gryczynski, J. Lukomska, J. Feng, M.F. Roberts, J.R. Lakowicz, J.W. Lomasney, Spectroscopic characterization of the EF-hand domain of phospholipase C δ1: identification of a lipid interacting domain, Arch. Biochem. Biophys. 440 (2005) 191–203.
[19] G. Waldo, T. Ricks, S. Hicks, M. Cheever, T. Kawano, K. Tsuboi, X. Wang, C. Montell, T. Kozasa, J. Sondek, T. Harden, Kinetic scaffolding mediated by a phospholipase C-β and Gq signaling complex, Science 330 (2010) 974–980.
[20] L.-O. Essen, O. Perisic, D.E. Lynch, M. Katan, R.L. Williams, A ternary metal binding site in the C2 domain of phosphoinositide-specific phospholipase C-δ1, Biochemistry 36 (1997) 2753–2762.
[21] L. Otterhag, M. Sommarin, C. Pical, N-terminal EF-hand-like domain is required for phosphoinositide-specific phospholipase C activity in *Arabidopsis thaliana*, FEBS Lett. 497 (2001) 165–170.
[22] T. Hirayama, N. Mitsukawa, D. Shibata, K. Shinozaki, AtPLC2, a gene encoding phosphoinositide-specific phospholipase C, is constitutively expressed in vegetative and floral tissues in *Arabidopsis thaliana*, Plant Mol. Biol. 34 (1997) 175–180.
[23] J. Shi, R.A. Gonzales, M.K. Bhattacharyya, Characterization of a plasma membrane-associated phosphoinositide-specific phospholipase C from soybean, Plant J. 8 (1995) 381–390.
[24] Z. Kouchi, T. Shikano, Y. Nakamura, H. Shirakawa, K. Fukami, S. Miyazaki, The role of EF-hand domains and C2 domain in regulation of enzymatic activity of phospholipase Cζ, J. Biol. Chem. 280 (2005) 21015–21021.
[25] J. Kopka, C. Pical, J.E. Gray, B. Müller-Röber, Molecular and enzymatic characterization of three phosphoinositide-specific phospholipase C isoforms from potato, Plant Physiol. 116 (1998) 239–250.
[26] S. Rupwate, R. Rajasekharan, C2 domain is responsible for targeting rice phosphoinositide specific phospholipase C, Plant Mol. Biol. 78 (2012) 247–258.
[27] D. Helling, A. Possart, S. Cottier, U. Klahre, B. Kost, Pollen tube tip growth depends on plasma membrane polarization mediated by tobacco PLC3 activity and endocytic membrane recycling, Plant Cell 18 (2006) 3519–3534.
[28] S.N. Hicks, M.R. Jezyk, S. Gershburg, J.P. Seifert, T.K. Harden, J. Sondek, General and versatile autoinhibition of PLC isozymes, Mol. Cell 31 (2008) 383–394.
[29] M. Nomikos, K. Elgmati, M. Theodoridou, B.L. Calver, G. Nounesis, K. Swann, F.A. Lai, Phospholipase Cζ binding to PtdIns(4,5)P_2 requires the XY-linker region, J. Cell Sci. 124 (2011) 2582–2590.
[30] S. Das, A. Hussain, C. Bock, W. Keller, F. Georges, Cloning of *Brassica napus* phospholipase C2 (BnPLC2), phosphatidylinositol 3-kinase (BnVPS34) and phosphatidylinositol synthase1 (BnPtdIns S1)—comparative analysis of the effect of abiotic stresses on the expression of phosphatidylinositol signal transduction-related genes in *B. napus*, Planta 220 (2005) 777–784.
[31] M. Awasthi, J. Batra, S. Kateriya, Disulphide bridges of phospholipase C of *Chlamydomonas reinhardtii* modulates lipid interaction and dimer stability, PLoS One 7 (2012) e39258.
[32] C. Shao, X. Shi, H. Wehbi, C. Zambonelli, J.F. Head, B.A. Seaton, M.F. Roberts, Dimer structure of an interfacially impaired phosphatidylinositol-specific phospholipase C, J. Biol. Chem. 282 (2007) 9228–9235.
[33] A.U. Singer, G.L. Waldo, K.T. Harden, J. Sondek, A unique fold of phospholipase C-beta mediates dimerization and interaction with Galphaq, Nat. Struct. Biol. 9 (2001) 32–36.
[34] M. Bourcy, L. Brocard, C.I. Pislariu, V. Cosson, P. Mergaert, M. Tadege, K.S. Mysore, M.K. Udvardi, B. Gourion, P. Ratet, *Medicago truncatula* DNF2 is a PI-PLC-XD-containing protein required for bacteroid persistence and prevention of nodule early senescence and defense-like reactions, New Phytol. 197 (2013) 1250–1261.
[35] L. Hunt, L. Otterhag, J.C. Lee, T. Lasheen, J. Hunt, M. Seki, K. Shinozaki, M. Sommarin, D.J. Gilmour, C. Pical, J.E. Gray, Gene-specific expression and calcium activation of *Arabidopsis thaliana* phospholipase C isoforms, New Phytol. 162 (2004) 643–654.
[36] Z. Cao, J. Zhang, Y. Li, X. Xu, G. Liu, M.K. Bhattacharrya, H. Yang, D. Ren, Preparation of polyclonal antibody specific for AtPLC4, an Arabidopsis phosphatidylinositol-specific phospholipase C in rabbits, Protein Expr. Purif. 52 (2007) 306–312.
[37] J.W. Lomasney, H.-F. Cheng, L.-P. Wang, Y.-S. Kuan, S.-M. Liu, S.W. Fesik, K. King, Phosphatidylinositol 4,5-bisphosphate binding to the pleckstrin homology domain of phospholipase C-δ1 enhances enzyme activity, J. Biol. Chem. 271 (1996) 25316–25326.
[38] W. Van Leeuwen, J.E.M. Vermeer, T.W.J. Gadella, T. Munnik, Visualization of phosphatidylinositol 4,5-bisphosphate in the plasma membrane of suspension-cultured tobacco BY-2 cells and whole Arabidopsis seedlings, Plant J. 52 (2007) 1014–1026.
[39] H.-T. Liu, W.-D. Huang, Q.-H. Pan, F.-H. Weng, J.-C. Zhan, Y. Liu, S.-B. Wan, Y.-Y. Liu, Contributions of PIP2-specific-phospholipase C and free salicylic acid to heat acclimation-induced thermotolerance in pea leaves, J. Plant Physiol. 163 (2006) 405–416.
[40] J.J. Benschop, S. Mohammed, M. O'Flaherty, A.J.R. Heck, M. Slijper, F.L.H. Menke, Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis, Mol. Cell. Proteomics 6 (2007) 1198–1214.
[41] J.M. Elmore, J. Liu, B. Smith, B. Phinney, G. Coaker, Quantitative proteomics reveals dynamic changes in the plasma membrane during Arabidopsis immune signaling, Mol. Cell. Proteomics 11 (2012). M111.014555.
[42] B. Li, D. Takahashi, Y. Kawamura, M. Uemura, Comparison of plasma membrane proteomic changes of Arabidopsis suspension-cultured cells (T87 line) after cold and ABA treatment in association with freezing tolerance development, Plant Cell Physiol. 53 (2012) 543–554.
[43] S.K. Mitra, B.T. Walters, S.D. Clouse, M.B. Goshe, An efficient organic solvent based extraction method for the proteomic analysis of Arabidopsis plasma membranes, J. Proteome Res. 8 (2009) 2752–2767.
[44] H. Khalil, Z. Wang, J. Wright, A. Ralevski, A. Donayo, P. Gulick, Heterotrimeric Gα subunit from wheat (*Triticum aestivum*), GA3, interacts with the calcium-binding protein, Clo3, and the phosphoinositide-specific phospholipase C, PI-PLC1, Plant Mol. Biol. 77 (2011) 145–158.
[45] M.K. Johnston, N.P. Jacob, M.R. Brodl, Heat shock-induced changes in lipid and protein metabolism in the endoplasmic reticulum of barley aleurone layers, Plant Cell Physiol. 48 (2007) 31–41.
[46] M. Tripathy, W. Tyagi, M. Goswami, T. Kaul, S. Singla-Pareek, R. Deswal, M. Reddy, S. Sopory, Characterization and functional validation of tobacco PLC delta for abiotic stress tolerance, Plant Mol. Biol. Rep. 30 (2012) 488–497.
[47] C.-H. Huang, R. Crain, Phosphoinositide-specific phospholipase C in oat roots: association with the actin cytoskeleton, Planta 230 (2009) 925–933.
[48] T. Hruz, O. Laule, G. Szabo, F. Wessendorp, S. Bleuler, L. Oertle, P. Widmayer, W. Gruissem, P. Zimmermann, Genevestigator V3: a reference expression database for the meta-analysis of transcriptomes, Adv. Bioinf. 2008 (2008) 420747.
[49] J. Zhao, Y. Guo, A. Kosaihira, K. Sakai, Rapid accumulation and metabolism of polyphosphoinositol and its possible role in phytoalexin biosynthesis in yeast elicitor-treated *Cupressus lusitanica* cell cultures, Planta 219 (2004) 121–131.
[50] A. Gresset, S.N. Hicks, T.K. Harden, J. Sondek, Mechanism of phosphorylation-induced activation of phospholipase C-γ isozymes, J. Biol. Chem. 285 (2010) 35836–35847.

[51] P. Durek, R. Schmidt, J.L. Heazlewood, A. Jones, D. MacLean, A. Nagel, B. Kersten, W.X. Schulze, PhosPhAt: the *Arabidopsis thaliana* phosphorylation site database. An update, Nucleic Acids Res. 38 (2010) D828–D834.
[52] S.-A. Whiteman, L. Serazetdinova, A.M.E. Jones, D. Sanders, J. Rathjen, S.C. Peck, F.J.M. Maathuis, Identification of novel proteins and phosphorylation sites in a tonoplast enriched membrane fraction of *Arabidopsis thaliana*, Proteomics 8 (2008) 3536–3547.
[53] T.S. Nühse, A. Stensballe, O.N. Jensen, S.C. Peck, Phosphoproteomics of the Arabidopsis plasma membrane and a new phosphorylation site database, Plant Cell 16 (2004) 2394–2405.
[54] Y. Chen, W. Hoehenwarter, W. Weckwerth, Comparative analysis of phytohormone-responsive phosphoproteins in *Arabidopsis thaliana* using TiO_2-phosphopeptide enrichment and mass accuracy precursor alignment, Plant J. 63 (2010) 1–17.
[55] P.A. Hemsley, C.S. Grierson, Multiple roles for protein palmitoylation in plants, Trends Plant Sci. 13 (2008) 295–302.
[56] J. Ren, L. Wen, X. Gao, C. Jin, Y. Xue, X. Yao, CSS-Palm 2.0: an updated software for palmitoylation sites prediction, Protein Eng. Des. Sel. 21 (2008) 639–644.
[57] M. Mazur, H.A. Van Den Burg, Global SUMO proteome responses guide gene regulation, mRNA biogenesis, and plant stress responses, Front. Plant Sci. 3 (2012).
[58] H. Park, W. Choi, H. Park, M. Cheong, Y. Koo, G. Shin, W. Chung, W.-Y. Kim, M. Kim, R. Bressan, H. Bohnert, S. Lee, D.-J. Yun, Identification and molecular properties of SUMO-binding proteins in Arabidopsis, Mol. Cells 32 (2011) 143–151.
[59] J. Ren, X. Gao, C. Jin, M. Zhu, X. Wang, A. Shaw, L. Wen, X. Yao, Y. Xue, Systematic study of protein sumoylation: development of a site-specific predictor of SUMOsp 2.0, Proteomics 9 (2009) 3409–3412.
[60] A.M. Distéfano, C. García-Mata, L. Lamattina, A.M. Laxalt, Nitric oxide-induced phosphatidic acid accumulation: a role for phospholipases C and D in stomatal closure, Plant Cell Environ. 31 (2008) 187–194.
[61] O. Gutman, C. Walliser, T. Piechulek, P. Gierschik, Y.I. Henis, Differential regulation of phospholipase C-β2 activity and membrane interaction by Gαq, Gβ1γ2, and Rac2, J. Biol. Chem. 285 (2010) 3905–3915.
[62] X. Zhang, A. Chattopadhyay, Q.-s. Ji, J.D. Owen, P.J. Ruest, G. Carpenter, S.K. Hanks, Focal adhesion kinase promotes phospholipase C-γ1 activity, PNAS 96 (1999) 9021–9026.
[63] O.R. Aisiku, L.W. Runnels, S. Scarlata, Identification of a novel binding partner of phospholipase Cβ1: translin-associated factor X, PLoS One 5 (2010) e15001.
[64] Y.-Y. Pan, X. Wang, L.-G. Ma, D.-Y. Sun, Characterization of phosphatidylinositol-specific phospholipase C (PI-PLC) from *Lilium daviddi* pollen, Plant Cell Physiol. 46 (2005) 1657–1665.
[65] C. De Los Santos-Briones, L. Cárdenas, G. Estrada-Navarrete, O. Santana, Y. Minero-García, C. Quinto, F. Sánchez, GTPγS antagonizes the mastoparan-induced *in vitro* activity of PIP2-phospholipase C from symbiotic root nodules of *Phaseolus vulgaris*, Physiol. Plant. 135 (2009) 237–245.
[66] J. Sun, X. Liu, Y. Pan, The physical interaction between LdPLCs and Arabidopsis G beta in a yeast two-hybrid system, Front. Agric. China 5 (2011) 64–71.
[67] S. Misra, Y. Wu, G. Venkataraman, S.K. Sopory, N. Tuteja, Heterotrimeric G-protein complex and G-protein-coupled receptor from a legume (*Pisum sativum*): role in salinity and heat stress and cross-talk with phospholipase C, Plant J. 51 (2007) 656–669.
[68] K. Nakamura, H. Sano, A plasma-membrane linker for the phosphoinositide-specific phospholipase C in tobacco plants, Plant Signaling Behav. 4 (2009) 26–29.
[69] S. Lalonde, D.W. Ehrhardt, D. Loqué, J. Chen, S.Y. Rhee, W.B. Frommer, Molecular and cellular approaches for the detection of protein–protein interactions: latest techniques and current limitations, Plant J. 53 (2008) 610–635.
[70] W. Ma, G.A. Berkowitz, Ca^{2+} conduction by plant cyclic nucleotide gated channels and associated signaling components in pathogen defense signal transduction cascades, New Phytol. 190 (2011) 566–572.
[71] S.A. Arisz, C. Testerink, T. Munnik, Plant PA signaling via diacylglycerol kinase, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1791 (2009) 869–875.
[72] S.K. Park, J.R. Lee, S.S. Lee, H.J. Son, J.Y. Yoo, J.C. Moon, H.Y. Kwon, C.O. Lim, J.D. Bahk, M.J. Cho, S.Y. Lee, Partial purification and properties of a phosphatidylinositol 4,5-bisphosphate hydrolyzing phospholipase C from the soluble fraction of soybean sprouts, Mol. Cells 13 (2002) 377–384.
[73] M.M. Sohani, P.M. Schenk, C.J. Schultz, O. Schmidt, Phylogenetic and transcriptional analysis of a strictosidine synthase-like gene family in *Arabidopsis thaliana* reveals involvement in plant defence responses, Plant Biol. 11 (2009) 105–117.
[74] B.-H. Lee, D.A. Henderson, J.-K. Zhu, The Arabidopsis cold-responsive transcriptome and its regulation by ICE1, Plant Cell 17 (2005) 3155–3175.
[75] L. Xiong, B.-H. Lee, M. Ishitani, H. Lee, C. Zhang, J.-K. Zhu, FIERY1 encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in Arabidopsis, Genes Dev. 15 (2001) 1971–1984.
[76] K. Bailey, V. Çevik, N. Holton, J. Byrne-Richardson, K.H. Sohn, M. Coates, A. Woods-Tör, H.M. Aksoy, L. Hughes, L. Baxter, J.D.G. Jones, J. Beynon, E.B. Holub, M. Tör, Molecular cloning of ATR5Emoy2 from *Hyaloperonospora arabidopsidis*, an avirulence determinant that triggers RPP5-mediated defense in Arabidopsis, Mol. Plant-Microbe Interact. 24 (2011) 827–838.
[77] M.R. Swiderski, D. Birker, J.D.G. Jones, The TIR domain of TIR-NB-LRR resistance proteins is a signaling domain involved in cell death induction, Mol. Plant-Microbe Interact. 22 (2009) 157–165.
[78] C. Xie, X. Zhou, X. Deng, Y. Guo, PKS5, a SNF1-related kinase, interacts with and phosphorylates NPR1, and modulates expression of WRKY38 and WRKY62, J. Genet. Genomics 37 (2010) 359–369.
[79] N.S. Coll, D. Vercammen, A. Smidler, C. Clover, F. Van Breusegem, J.L. Dangl, P. Epple, Arabidopsis type I metacaspases control cell death, Science 330 (2010) 1393–1397.
[80] M. Ramaiah, A. Jain, J.C. Baldwin, A.S. Karthikeyan, K.G. Raghothama, Characterization of the phosphate starvation-induced gycerol-3-phosphate permease gene family in *Arabidopsis*, Plant Physiol. 157 (2011) 279–291.
[81] C.-B. Jing, Y. Chen, M. Dong, X.-L. Peng, X.-E. Jia, L. Gao, K. Ma, M. Deng, T.-X. Liu, L.I. Zon, J. Zhu, Y. Zhou, Y. Zhou, Phospholipase C gamma-1 is required for granulocyte maturation in zebrafish, Dev. Biol. 374 (2013) 24–31.
[82] M. Khodakovskaya, C. Sword, Q. Wu, I.Y. Perera, W.F. Boss, C.S. Brown, H. Winter Sederoff, Increasing inositol (1,4,5)-trisphosphate metabolism affects drought tolerance, carbohydrate metabolism and phosphate-sensitive biomass increases in tomato, Plant Biotechnol. J. 8 (2010) 170–183.
[83] F. Georges, S. Das, H. Ray, C. Bock, K. Nokhrina, V.A. Kolla, W. Keller, Overexpression of *Brassica napus* phosphatidylinositol-phospholipase C2 in canola induces significant changes in gene expression and phytohormone distribution patterns, enhances drought tolerance and promotes early flowering and maturation, Plant Cell Environ. 32 (2009) 1664–1681.
[84] X. Zhang, G. Coté, R. Crain, Involvement of phosphoinositide turnover in tracheary element differentiation in *Zinnia elegans* L. cells, Planta 215 (2002) 312–318.
[85] A. Cenzano, R. Cantoro, G. Racagni, C. Los Santos-Briones, T. Hernández-Sotomayor, G. Abdala, Phospholipid and phospholipase changes by jasmonic acid during stolon to tuber transition of potato, Plant Growth Regul. 56 (2008) 307–316.
[86] P. Apostolakos, E. Panteris, B. Galatis, The involvement of phospholipases C and D in the asymmetric division of subsidiary cell mother cells of *Zea mays*, Cell Motil. Cytoskeleton 65 (2008) 863–875.
[87] F. Apone, N. Alyeshmerni, K. Wiens, D. Chalmers, M.J. Chrispeels, G. Colucci, The G-protein-coupled receptor GCR1 regulates DNA synthesis through activation of phosphatidylinositol-specific phospholipase C, Plant Physiol. 133 (2003) 571–579.
[88] R.A. Cole, J.E. Fowler, Polarized growth: maintaining focus on the tip, Curr. Opin. Plant Biol. 9 (2006) 579–588.
[89] E. Sousa, B. Kost, R. Malho, Arabidopsis phosphatidylinositol-4-monophosphate 5-kinase 4 regulates pollen tube growth and polarity by modulating membrane recycling, Plant Cell 20 (2008) 3050–3064.
[90] T. Ischebeck, I. Stenzel, F. Hempel, X. Jin, A. Mosblech, I. Heilmann, Phosphatidylinositol-4,5-bisphosphate influences Nt-Rac5-mediated cell expansion in pollen tubes of *Nicotiana tabacum*, Plant J. 65 (2011) 453–468.
[91] Y. Zhao, A. Yan, J.A. Feijó, M. Furutani, T. Takenawa, I. Hwang, Y. Fu, Z. Yang, Phosphoinositides regulate clathrin-dependent endocytosis at the tip of pollen tubes in Arabidopsis and tobacco, Plant Cell 22 (2010) 4031–4044.
[92] T. Ischebeck, I. Stenzel, I. Heilmann, Type B phosphatidylinositol-4-phosphate 5-kinases mediate Arabidopsis and *Nicotiana tabacum* pollen tube growth by regulating apical pectin secretion, Plant Cell 20 (2008) 3312–3330.
[93] H. Kusano, C. Testerink, J.E.M. Vermeer, T. Tsuge, H. Shimada, A. Oka, T. Munnik, T. Aoyama, The Arabidopsis phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth, Plant Cell 20 (2008) 367–380.
[94] I. Stenzel, T. Ischebeck, S. König, A. Hołubowska, M. Sporysz, B. Hause, I. Heilmann, The type B phosphatidylinositol-4-phosphate 5-kinase 3 is essential for root hair formation in *Arabidopsis thaliana*, Plant Cell 20 (2008) 124–141.
[95] T. Munnik, J.E.M. Vermeer, Osmotic stress-induced phosphoinositide and inositol phosphate signalling in plants, Plant Cell Environ. 33 (2010) 655–669.
[96] D.B. DeWald, J. Torabinejad, C.A. Jones, J.C. Shope, A.R. Cangelosi, J.E. Thompson, G.D. Prestwich, H. Hama, Rapid accumulation of phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate correlates with calcium mobilization in salt-stressed Arabidopsis, Plant Physiol. 126 (2001) 759–769.
[97] Y.J. Im, I.Y. Perera, I. Brglez, A.J. Davis, J. Stevenson-Paulik, B.Q. Phillippy, E. Johannes, N.S. Allen, W.F. Boss, Increasing plasma membrane phosphatidylinositol(4,5)bisphosphate biosynthesis increases phosphoinositide metabolism in *Nicotiana tabacum*, Plant Cell 19 (2007) 1603–1616.
[98] A. Mosblech, S. König, I. Stenzel, P. Grzeganek, I. Feussner, I. Heilmann, Phosphoinositide and inositolpolyphosphate signalling in defense responses of *Arabidopsis thaliana* challenged by mechanical wounding, Mol. Plant 1 (2008) 249–261.
[99] I. Heilmann, I.Y. Perera, W. Gross, W.F. Boss, Changes in phosphoinositide metabolism with days in culture affect signal transduction pathways in *Galdieria sulphuraria*, Plant Physiol. 119 (1999) 1331–1340.
[100] L. Yang, R. Tang, J. Zhu, H. Liu, B. Mueller-Roeber, H. Xia, H. Zhang, Enhancement of stress tolerance in transgenic tobacco plants constitutively expressing AtIpk2β, an inositol polyphosphate 6-/3-kinase from *Arabidopsis thaliana*, Plant Mol. Biol. 66 (2008) 329–343.

[101] J.-Q. Zhu, J.-T. Zhang, R.-J. Tang, Q.-D. Lv, Q.-Q. Wang, L. Yang, H.-X. Zhang, Molecular characterization of *ThIPK2*, an inositol polyphosphate kinase gene homolog from *Thellungiella halophila*, and its heterologous expression to improve abiotic stress tolerance in *Brassica napus*, Physiol. Plant. 136 (2009) 407–425.
[102] I.Y. Perera, C.-Y. Hung, C.D. Moore, J. Stevenson-Paulik, W.F. Boss, Transgenic Arabidopsis plants expressing the type 1 inositol 5-phosphatase exhibit increased drought tolerance and altered abscisic acid signaling, Plant Cell 20 (2008) 2876–2893.
[103] B. van Schooten, Dissecting Arabidopsis Phospholipid Signaling Using Reverse Genetics, Faculty of Science, FNWI: Swammerdam Institute for Life Sciences, Amsterdam, 2008, pp. 103–124.
[104] E. Darwish, C. Testerink, M. Khalil, O. El-Shihy, T. Munnik, Phospholipid signaling responses in salt-stressed rice leaves, Plant Cell Physiol. 50 (2009) 986–997.
[105] E. Delage, E. Ruelland, A. Zachowski, J. Puyaubert, Eat in or take away? How phosphatidylinositol 4-kinases feed the phospholipase C pathway with substrate, Plant Signaling Behav. 7 (2012) 1197–1199.
[106] E. Delage, E. Ruelland, I. Guillas, A. Zachowski, J. Puyaubert, Arabidopsis type-III phosphatidylinositol 4-kinases β1 and β2 are upstream of the phospholipase C pathway triggered by cold exposure, Plant Cell Physiol. 53 (2012) 565–576.
[107] E. Parre, M.A. Ghars, A.-S. Leprince, L. Thiery, D. Lefebvre, M. Bordenave, L. Richard, C. Mazars, C. Abdelly, A. Savouré, Calcium signaling via phospholipase C is essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in Arabidopsis, Plant Physiol. 144 (2007) 503–512.
[108] G. Komis, B. Galatis, H. Quader, D. Galanopoulou, P. Apostolakos, Phospholipase C signaling involvement in macrotubule assembly and activation of the mechanism regulating protoplast volume in plasmolyzed root cells of *Triticum turgidum*, New Phytol. 178 (2008) 267–282.
[109] S.G. Cessna, T.K. Matsumoto, G.N. Lamb, S.J. Rice, W.W. Hochstedler, The externally derived portion of the hyperosmotic shock-activated cytosolic calcium pulse mediates adaptation to ionic stress in suspension-cultured tobacco cells, J. Plant Physiol. 164 (2007) 815–823.
[110] J. Im, H. Lee, J. Kim, H. Kim, C. An, Soybean MAPK, GMK1 is dually regulated by phosphatidic acid and hydrogen peroxide and translocated to nucleus during salt stress, Mol. Cells 34 (2012) 271–278.
[111] M.U. Reitz, J.K. Bissue, K. Zocher, A. Attard, R. Hückelhoven, K. Becker, J. Imani, R. Eichmann, P. Schäfer, The subcellular localization of Tubby-like proteins and participation in stress signaling and root colonization by the mutualist *Piriformospora indica*, Plant Physiol. 160 (2012) 349–364.
[112] J. Monreal, C. Arias-Baldrich, F. Pérez-Montaño, J. Gandullo, C. Echevarría, S. García-Mauriño, Factors involved in the rise of phosphoenolpyruvate carboxylase-kinase activity caused by salinity in sorghum leaves, Planta (2013) 1–13.
[113] Y. Fujita, M. Fujita, K. Shinozaki, K. Yamaguchi-Shinozaki, ABA-mediated transcriptional regulation in response to osmotic stress in plants, J. Plant Res. 124 (2011) 509–525.
[114] L.N. Mills, L. Hunt, C.P. Leckie, F.L. Aitken, M. Wentworth, M.R. McAinsh, J.E. Gray, A.M. Hetherington, The effects of manipulating phospholipase C on guard cell ABA-signalling, J. Exp. Bot. 55 (2004) 199–204.
[115] I. Staxén, C. Pical, L.T. Montgomery, J.E. Gray, A.M. Hetherington, M.R. McAinsh, Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C, PNAS 96 (1999) 1779–1784.
[116] S. Takahashi, T. Katagiri, T. Hirayama, K. Yamaguchi-Shinozaki, K. Shinozaki, Hyperosmotic stress induces a rapid and transient increase in inositol 1,4,5-trisphosphate independent of abscisic acid in Arabidopsis cell culture, Plant Cell Physiol. 42 (2001) 214–222.
[117] M.A. Ghars, L. Richard, D. Lefebvre-De Vos, A.-S. Leprince, E. Parre, M. Bordenave, C. Abdelly, A. Savouré, Phospholipases C and D modulate proline accumulation in *Thellungiella halophila*/*salsuginea* differently according to the severity of salt or hyperosmotic stress, Plant Cell Physiol. 53 (2012) 183–192.
[118] E. Ruelland, A. Zachowski, How plants sense temperature, Environ. Exp. Bot. 69 (2010) 225–232.
[119] H.T. Liu, F. Gao, S.J. Cui, J.L. Han, D.Y. Sun, R.G. Zhou, Primary evidence for involvement of IP_3 in heat-shock signal transduction in Arabidopsis, Cell Res. 16 (2006) 394–400.
[120] T.P. Bucolova, N.V. Volovik, V.S. Kravets, A possible role of phosphatidylinositols in transduction of temperature signal in winter wheat, Biol. Plant. 36 (1994) 45.
[121] E. Ruelland, C. Cantrel, M. Gawer, J.-C. Kader, A. Zachowski, Activation of phospholipases C and D is an early response to a cold exposure in Arabidopsis suspension cells, Plant Physiol. 130 (2002) 999–1007.
[122] G. Smoleńska-Sym, A. Kacperska, Phosphatidylinositol metabolism in low temperature-affected winter oilseed rape leaves, Physiol. Plant. 91 (1994) 1–8.
[123] M.E. Williams, J. Torabinejad, E. Cohick, K. Parker, E.J. Drake, J.E. Thompson, M. Hortter, D.B. DeWald, Mutations in the Arabidopsis phosphoinositide phosphatase gene *SAC9* lead to overaccumulation of $PtdIns(4,5)P_2$ and constitutive expression of the stress-response pathway, Plant Physiol. 138 (2005) 686–700.
[124] M.-N. Vaultier, C. Cantrel, C. Vergnolle, A.-M. Justin, C. Demandre, G. Benhassaine-Kesri, D. Çiçek, A. Zachowski, E. Ruelland, Desaturase mutants reveal that membrane rigidification acts as a cold perception mechanism upstream of the diacylglycerol kinase pathway in *Arabidopsis* cells, FEBS Lett. 580 (2006) 4218–4223.
[125] M.E. Bartolo, J.V. Carter, Lithium decreases cold-induced microtubule depolymerization in mesophyll cells of spinach, Plant Physiol. 99 (1992) 1716–1718.
[126] C. Vergnolle, M.-N. Vaultier, L. Taconnat, J.-P. Renou, J.-C. Kader, A. Zachowski, E. Ruelland, The cold-induced early activation of phospholipase C and D pathways determines the response of two distinct clusters of genes in *Arabidopsis* cell suspensions, Plant Physiol. 139 (2005) 1217–1233.
[127] S. Zhai, Z. Sui, A. Yang, J. Zhang, Characterization of a novel phosphoinositide-specific phospholipase C from *Zea mays* and its expression in *Escherichia coli*, Biotechnol. Lett. 27 (2005) 799–804.
[128] Z. Sui, L. Niu, G. Yue, A. Yang, J. Zhang, Cloning and expression analysis of some genes involved in the phosphoinositide and phospholipid signaling pathways from maize (*Zea mays* L.), Gene 426 (2008) 47–56.
[129] D.Z. Skinner, B.S. Bellinger, S. Halls, K.-H. Baek, K. Garland-Campbell, W.F. Siems, Phospholipid acyl chain and phospholipase dynamics during cold acclimation of winter wheat, Crop Sci. 45 (2005) 1858–1867.
[130] M.L. Piña-Chable, C. de los Santos-Briones, J. Muñoz-Sánchez, I. Echevarría Machado, S.M. Hernández-Sotomayor, Effect of different inhibitors on phospholipase C activity in *Catharanthus roseus* transformed roots, Prostaglandins Other Lipid Mediators 56 (1998) 19–31.
[131] M. Russo, C. Sgherri, R. Izzo, F. Navari-Izzo, *Brassica napus* subjected to copper excess: phospholipases C and D and glutathione system in signalling, Environ. Exp. Bot. 62 (2008) 238–246.
[132] A. González, M.D.L.Á. Cabrera, M. Mellado, S. Cabello, S. Márquez, B. Morales, A. Moenne, Copper-induced intracellular calcium release requires extracellular calcium entry and activation of L-type voltage-dependent calcium channels in *Ulva compressa*, Plant Signaling Behav. 7 (2012) 728–732.
[133] M. Martínez-Estévez, G.R.-D. Palma, J.A. Muñoz-Sánchez, L. Brito-Argáez, V.M. Loyola-Vargas, S.M. Teresa Hernández-Sotomayor, Aluminium differentially modifies lipid metabolism from the phosphoinositide pathway in *Coffea arabica* cells, J. Plant Physiol. 160 (2003) 1297–1303.
[134] E.T. Yakimova, V.M. Kapchina-Toteva, E.J. Woltering, Signal transduction events in aluminum-induced cell death in tomato suspension cells, J. Plant Physiol. 164 (2007) 702–708.
[135] A. Ramos-Díaz, L. Brito-Argáez, T. Munnik, S.M.T. Hernández-Sotomayor, Aluminum inhibits phosphatidic acid formation by blocking the phospholipase C pathway, Planta 225 (2007) 393–401.
[136] R. Reggiani, P. Laoreti, Evidence for the involvement of phospholipase C in the anaerobic signal transduction, Plant Cell Physiol. 41 (2000) 1392–1396.
[137] M.P. Piacentini, E. Piatti, D. Fraternale, D. Ricci, M.C. Albertini, A. Accorsi, Phospholipase C-dependent phosphoinositide breakdown induced by ELF-EMF in *Peganum harmala* calli, Biochimie 86 (2004) 343–349.
[138] S.H. Spoel, X. Dong, How do plants achieve immunity? Defence without specialized immune cells, Nat. Rev. Immunol. 12 (2012) 89–100.
[139] J. Canonne, S. Froidure-Nicolas, S. Rivas, Phospholipases in action during plant defense signaling, Plant Signaling Behav. 6 (2011) 13–18.
[140] T. Yamaguchi, E. Minami, N. Shibuya, Activation of phospholipases by *N*-acetylchitooligosaccharide elicitor in suspension-cultured rice cells mediates reactive oxygen generation, Physiol. Plant. 118 (2003) 361–370.
[141] L. Wang, X. Zhu, J. Liu, X. Chu, J. Jiao, Y. Liang, Involvement of phospholipases C and D in the defence responses of riboflavin-treated tobacco cells, Protoplasma (2012) 1–9.
[142] C.F. De Jong, A.M. Laxalt, B.O.R. Bargmann, P.J.G.M. De Wit, M.H.A.J. Joosten, T. Munnik, Phosphatidic acid accumulation is an early response in the Cf-4/Avr4 interaction, Plant J. 39 (2004) 1–12.
[143] N. Raho, L. Ramirez, M.L. Lanteri, G. Gonorazky, L. Lamattina, A. Ten Have, A.M. Laxalt, Phosphatidic acid production in chitosan-elicited tomato cells, via both phospholipase D and phospholipase C/diacylglycerol kinase, requires nitric oxide, J. Plant Physiol. 168 (2011) 534–539.
[144] J.H. Vossen, A. Abd-El-Haliem, E.F. Fradin, G.C.M. Van Den Berg, S.K. Ekengren, H.J.G. Meijer, A. Seifi, Y. Bai, A. Ten Have, T. Munnik, B.P.H.J. Thomma, M.H.A.J. Joosten, Identification of tomato phosphatidylinositol-specific phospholipase-C (PI-PLC) family members and the role of PLC4 and PLC6 in HR and disease resistance, Plant J. 62 (2010) 224–239.
[145] T. Yamaguchi, E. Minami, J. Ueki, N. Shibuya, Elicitor-induced activation of phospholipases plays an important role for the induction of defense responses in suspension-cultured rice cells, Plant Cell Physiol. 46 (2005) 579–587.
[146] A. Vasconsuelo, A. Mara Giuletti, G. Picotto, J. Rodriguez-Talou, R. Boland, Involvement of the PLC/PKC pathway in chitosan-induced anthraquinone production by *Rubia tinctorum* L. cell cultures, Plant Sci. 165 (2003) 429–436.
[147] Y. Ma, R.K. Walker, Y. Zhao, G.A. Berkowitz, Linking ligand perception by PEPR pattern recognition receptors to cytosolic Ca^{2+} elevation and downstream immune signaling in plants, PNAS 109 (2012) 19852–19857.
[148] X. Gao, X. Chen, W. Lin, S. Chen, D. Lu, Y. Niu, L. Li, C. Cheng, M. McCormack, J. Sheen, L. Shan, P. He, Bifurcation of Arabidopsis NLR immune signaling via Ca^{2+}-dependent protein kinases, PLoS Pathog. 9 (2013) e1003127.
[149] D. Charron, J.-L. Pingret, M. Chabaud, E.-P. Journet, D.G. Barker, Pharmacological evidence that multiple phospholipid signaling pathways link rhizobium nodulation factor perception in *Medicago truncatula* root hairs to intracellular responses, including Ca^{2+} spiking and specific *ENOD* gene expression, Plant Physiol. 136 (2004) 3582–3593.

[150] M.A. Poussin, M. Leitges, H. Goldfine, The ability of *Listeria monocytogenes* PI-PLC to facilitate escape from the macrophage phagosome is dependent on host PKCβ, Microb. Pathog. 46 (2009) 1–5.
[151] G. Wang, S. Ryu, X. Wang, Plant phospholipases: an overview, in: G. Sandoval (Ed.), Lipases and Phospholipases, Humana Press, 2012, pp. 123–137.
[152] W.F. Boss, Y.J. Im, Phosphoinositide signaling, Annu. Rev. Plant Biol. 63 (2012) 409–429.
[153] G.M. Rahman, S. Shanker, N.E. Lewin, N. Kedei, C.S. Hill, B.V.V. Prasad, P.M. Blumberg, J. Das, Identification of the activator-binding residues in the second cysteine-rich regulatory domain of protein kinase Cθ (PKCθ), Biochem. J. 451 (2013) 33–44.
[154] M.R. Chandok, S.K. Sopory, Phorbol myristate acetate replaces phytochrome-mediated stimulation of nitrate reductase in maize, Phytochemistry 31 (1992) 2255–2258.
[155] N. Ishioka, S. Tanimoto, Adventitious bud induction by protein kinase C activators in Torenia stem segments, Plant Tissue Cult. Lett. 9 (1992) 86–89.
[156] M. Janda, S. Planchais, N. Djafi, J. Martinec, L. Burketova, O. Valentova, A. Zachowski, E. Ruelland, Phosphoglycerolipids are master players in plant hormone signal transduction, Plant Cell Rep. 32 (2013) 839–851.
[157] X. Zhao, M. Wang, T. Quan, G. Xia, The role of *TaCHP* in salt stress responsive pathways, Plant Signaling Behav. 7 (2012) 71–74.
[158] Y. Nakamura, H. Ohta, The diacylglycerol forming pathways differ among floral organs of *Petunia hybrida*, FEBS Lett. 581 (2007) 5475–5479.
[159] I. Pokotylo, P. Pejchar, M. Potocký, D. Kocourková, Z. Krčková, E. Ruelland, V. Kravets, J. Martinec, The plant non-specific phospholipase C gene family. Novel competitors in lipid signalling, Prog. Lipid Res. 52 (2013) 62–79.
[160] D.J. Sueldo, N.P. Foresi, C.A. Casalongué, L. Lamattina, A.M. Laxalt, Phosphatidic acid formation is required for extracellular ATP-mediated nitric oxide production in suspension-cultured tomato cells, New Phytol. 185 (2010) 909–916.
[161] S.A. Arisz, R. van Wijk, W. Roels, J.-K. Zhu, M.A. Haring, T. Munnik, Rapid phosphatidic acid accumulation in response to low temperature stress in Arabidopsis is generated through diacylglycerol kinase, Front. Plant Sci. 4 (2013).
[162] P.-p. Han, Y.-j. Yuan, Lipidomic analysis reveals activation of phospholipid signaling in mechanotransduction of *Taxus cuspidata* cells in response to shear stress, FASEB J. 23 (2009) 623–630.
[163] C. Testerink, T. Munnik, Molecular, cellular, and physiological responses to phosphatidic acid formation in plants, J. Exp. Bot. 62 (2011) 2349–2361.
[164] F. McLoughlin, C.S. Galvan-Ampudia, M.M. Julkowska, L. Caarls, D. van der Does, C. Laurière, T. Munnik, M.A. Haring, C. Testerink, The Snf1-related protein kinases SnRK2.4 and SnRK2.10 are involved in maintenance of root system architecture during salt stress, Plant J. 72 (2012) 436–449.
[165] F. McLoughlin, S.A. Arisz, H.L. Dekker, G. Kramer, C.G. de Koster, M.A. Haring, T. Munnik, C. Testerink, Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of *Arabidopsis thaliana* roots, Biochem. J. 450 (2013) 573–581.
[166] A.K. Dixit, C. Jayabaskaran, Phospholipid mediated activation of calcium dependent protein kinase 1 (*Ca*CDPK1) from chickpea: a new paradigm of regulation, PLoS One 7 (2012) e51591.
[167] J. Rocha, M. Audry, G. Pesce, V. Chazalet, M.A. Block, E. Maréchal, C. Breton, Revisiting the expression and purification of MGD1, the major galactolipid synthase in Arabidopsis to establish a novel standard for biochemical and structural studies, Biochimie 95 (2013) 700–708.
[168] R. Pleskot, P. Pejchar, V. Žárský, C.J. Staiger, M. Potocký, Structural insights into the inhibition of actin-capping protein by interactions with phosphatidic acid and phosphatidylinositol (4,5)-bisphosphate, PLoS Comput. Biol. 8 (2012) e1002765.
[169] A. Takemiya, K.-i. Shimazaki, Phosphatidic acid inhibits blue light-induced stomatal opening via inhibition of protein phosphatase 1, Plant Physiol. 153 (2010) 1555–1562.
[170] L. Camoni, C. Di Lucente, R. Pallucca, S. Visconti, P. Aducci, Binding of phosphatidic acid to 14-3-3 proteins hampers their ability to activate the plant plasma membrane H^+-ATPase, IUBMB Life 64 (2012) 710–716.
[171] S.-C. Kim, L. Guo, X. Wang, Phosphatidic acid binds to cytosolic glyceraldehyde-3-phosphase dehydrogenase and promotes its cleavage in Arabidopsis, J. Biol. Chem. 288 (2013) 11834–11844.
[172] W. Zhang, J. Chen, H. Zhang, F. Song, Overexpression of a rice diacylglycerol kinase gene OsBIDK1 enhances disease resistance in transgenic tobacco, Mol. Cells 26 (2008) 258–264.
[173] A.M. Laxalt, N. Raho, A.t. Have, L. Lamattina, Nitric oxide is critical for inducing phosphatidic acid accumulation in xylanase-elicited tomato cells, J. Biol. Chem. 282 (2007) 21160–21168.
[174] F.C. Gómez-Merino, F.A. Arana-Ceballos, L.I. Trejo-Téllez, A. Skirycz, C.A. Brearley, P. Dörmann, B. Mueller-Roeber, Arabidopsis AtDGK7, the smallest member of plant diacylglycerol kinases (DGKs), displays unique biochemical features and saturates at low substrate concentration: the DGK inhibitor R59022 differentially affects AtDGK2 and AtDGK7 activity *in vitro* and alters plant growth and development, J. Biol. Chem. 280 (2005) 34888–34899.
[175] R. Pleskot, P. Pejchar, R. Bezvoda, I.K. Lichtscheidl, M. Wolters-Arts, J. Marc, V. Žárský, M. Potocký, Turnover of phosphatidic acid through distinct signalling pathways affects multiple aspects of tobacco pollen tube tip growth, Front. Plant Sci. 3 (2012).
[176] T. Munnik, E. Nielsen, Green light for polyphosphoinositide signals in plants, Curr. Opin. Plant Biol. 14 (2011) 489–497.
[177] O. Batistič, J. Kudla, Analysis of calcium signaling pathways in plants, Biochim. Biophys. Acta Gen. Subj. 1820 (2012) 1283–1293.
[178] A.M. Rossi, S.C. Tovey, T. Rahman, D.L. Prole, C.W. Taylor, Analysis of IP_3 receptors in and out of cells, Biochim. Biophys. Acta Gen. Subj. 1820 (2012) 1214–1227.
[179] S. Zhang, N. Fritz, C. Ibarra, P. Uhlén, Inositol 1,4,5-trisphosphate receptor subtype-specific regulation of calcium oscillations, Neurochem. Res. 36 (2011) 1175–1185.
[180] O. Krinke, Z. Novotná, O. Valentová, J. Martinec, Inositol trisphosphate receptor in higher plants: is it real? J. Exp. Bot. 58 (2007) 361–376.
[181] R.-H. Tang, S. Han, H. Zheng, C.W. Cook, C.S. Choi, T.E. Woerner, R.B. Jackson, Z.-M. Pei, Coupling diurnal cytosolic Ca^{2+} oscillations to the CAS-IP_3 pathway in Arabidopsis, Science 315 (2007) 1423–1426.
[182] J. Zhang, S. Vanneste, Philip B. Brewer, M. Michniewicz, P. Grones, J. Kleine-Vehn, C. Löfke, T. Teichmann, A. Bielach, B. Cannoot, K. Hoyerová, X. Chen, H.-W. Xue, E. Benková, E. Zažímalová, J. Friml, Inositol trisphosphate-induced Ca^{2+} signaling modulates auxin transport and PIN polarity, Dev. Cell 20 (2011) 855–866.
[183] Y. Wang, Y.-J. Chu, H.-W. Xue, Inositol polyphosphate 5-phosphatase-controlled Ins(1,4,5)P_3/Ca^{2+} is crucial for maintaining pollen dormancy and regulating early germination of pollen, Development 139 (2012) 2221–2233.
[184] J. Martinec, T. Feltl, C.H. Scanlon, P.J. Lumsden, I. Macháčková, Subcellular localization of a high affinity binding site for d-myo-inositol 1,4,5-trisphosphate from *Chenopodium rubrum*, Plant Physiol. 124 (2000) 475–483.
[185] E.M. Engstrom, D.W. Ehrhardt, R.M. Mitra, S.R. Long, Pharmacological analysis of Nod factor-induced calcium spiking in *Medicago truncatula*. Evidence for the requirement of type IIA calcium pumps and phosphoinositide signaling, Plant Physiol. 128 (2002) 1390–1401.
[186] F. Lemtiri-Chlieh, E.A.C. MacRobbie, A.A.R. Webb, N.F. Manison, C. Brownlee, J.N. Skepper, J. Chen, G.D. Prestwich, C.A. Brearley, Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells, PNAS 100 (2003) 10091–10095.
[187] F. Lemtiri-Chlieh, E.A.C. MacRobbie, C.A. Brearley, Inositol hexakisphosphate is a physiological signal regulating the K^+-inward rectifying conductance in guard cells, PNAS 97 (2000) 8687–8692.
[188] A.M. Murphy, B. Otto, C.A. Brearley, J.P. Carr, D.E. Hanke, A role for inositol hexakisphosphate in the maintenance of basal resistance to plant pathogens, Plant J. 56 (2008) 638–652.
[189] L.B. Sheard, X. Tan, H. Mao, J. Withers, G. Ben-Nissan, T.R. Hinds, Y. Kobayashi, F.-F. Hsu, M. Sharon, J. Browse, S.Y. He, J. Rizo, G.A. Howe, N. Zheng, Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor, Nature 468 (2010) 400–405.
[190] X. Tan, L.I.A. Calderon-Villalobos, M. Sharon, C. Zheng, C.V. Robinson, M. Estelle, N. Zheng, Mechanism of auxin perception by the TIR1 ubiquitin ligase, Nature 446 (2007) 640–645.
[191] I. Stenzel, T. Ischebeck, M. Quint, I. Heilmann, Variable regions of PI4P 5-kinases direct PtdIns(4,5)P(2) toward alternative regulatory functions in tobacco pollen tubes, Front. Plant Sci. 2 (2011) 114.
[192] M. Kato, N. Nagasaki-Takeuchi, Y. Ide, M. Maeshima, An Arabidopsis hydrophilic Ca^{2+}-binding protein with a PEVK-rich domain, PCaP2, is associated with the plasma membrane and interacts with calmodulin and phosphatidylinositol phosphates, Plant Cell Physiol. 51 (2010) 366–379.
[193] T.S. Nühse, A.R. Bottrill, A.M.E. Jones, S.C. Peck, Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses, Plant J. 51 (2007) 931–940.