Environmental and Experimental Botany xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Role of phospholipid signalling in plant environmental responses

E. Ruelland [a], V. Kravets [b], M. Derevyanchuk [b], J. Martinec [c], A. Zachowski [a], I. Pokotylo [b,*]

[a] Institut d'écologie et des sciences de l' environnement de Paris, CNRS UMR 7618, Université Paris-Est Créteil, Creteil, France
[b] Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, Murmanska 1, 02660 Kyiv-94, Ukraine
[c] Department of Biology, Faculty of Sciences, Jan Evangelista Purkyně University, Ústí nad Labem, Czech Republic

ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form 15 July 2014
Accepted 25 August 2014
Available online xxx

Keywords:
Lipid signalling
Phospholipases
Lipid-kinases
Lipid-phosphatases
Environmental responses
Membranes

ABSTRACT

Despite the fact that all plants follow strict developmental programmes, they also have intrinsic mechanisms to monitor the environment and activate appropriate responses at the (sub-)cellular level, facilitating adaptation to abiotic and biotic fluctuations. The functionality of plant adaptive systems always relies on the sum of signalling machineries that control their transition from the resting state. Phosphoglycerolipids play a role in such signalling mechanisms. These structural components of cell membranes can be converted into multiple bioactive lipids, but also into soluble molecules. Together they shape cell metabolism via binding to downstream protein targets, thus affecting enzymatic activities, vesicle trafficking and ion fluxes. The conversion of lipids is catalysed by the hydrolytic activity of phospholipases and by the action of lipid-kinases and lipid-phosphatases. These activities are strictly regulated in plant cells and are highly reactive to various environmental signals. While phospholipases have been shown to be essential for plant growth and adaptability, many aspects of phosphoglycerolipid signalling at the molecular level remain unknown. Here, we summarise the latest concepts and challenges associated with phosphoglycerolipid signalling in relation to environmental responses in plants.

1. Introduction

Production of biologically active lipids, such as phosphatidic acid (PA), results from the action of phospholipases and/or lipid-kinases (Fig. 1). Phospholipases are found in all living organisms and typically constitute single-polypeptide multi-domain hydrolytic enzymes acting on (phospho)ester bonds of phospholipids. They are classified according to the site of phospholipid cleavage, and consistently, to the nature of their products, into phospholipases A_1, A_2, C and D (PLA_1, PLA_2, PLC and PLD; Fig. 2) (Wang et al., 2012). Some phospholipases are characterised by their strict substrate preference. For instance, PLCs are either phosphoinositide-specific PLCs (PI-PLCs) or non-specific PLCs (NPCs). The latter hydrolyse membrane structural phospholipids such as phosphatidylcholine (PC) or phosphatidylethanolamine (PE). Several types of lipid-kinases are found in plants. One can distinguish kinases acting on diacylglycerol (DAG) – the DAG-Kinases (DGK) – from those acting on phosphatidylinositol (PI), – the PI-kinases (*e.g.* phosphatidylinositol 4-kinase, PI4K) – and those acting on phosphorylated PI (PIP) – the PIP-kinases (*e.g.* phosphatidylinositol-4-phosphate 5-kinase, PI4P5K). In plant genomes, many lipid-processing enzymes are encoded as multigenic families. For instance, the genome of *Arabidopsis thaliana* encodes 12 PLDs, 9 PI-PLCs, 6 NPCs and 7 DGKs and 3 PI4Ks. It is not uncommon that each isogene codes for an enzyme with unique regulatory properties, organ localisation or stress-induced expression patterns (Pinosa et al., 2013; Zheng et al., 2012). Moreover, the activity of phospholipases can be rapidly altered by post-translational regulation (*e.g.* G-protein-mediated activation, protein phosphorylation), granting them a role in rapid signalling events. Phospholipases and lipid-kinases are also functionally connected to other plant signalling systems including nitric oxide (NO; Lanteri et al., 2008), reactive oxygen species (ROS; Zhang et al., 2009) and Ca^{2+} (Parre et al., 2007).

2. Plant phospholipases and lipid processing enzymes

2.1. Phospholipase D

PLD (EC 3.1.4.4) hydrolyses structural membrane phospholipids (PC, PE and phosphatidylglycerol, PG) into phosphatidic acid (PA) and the corresponding soluble headgroup. Only two PLD genes are known in mammals (Exton, 2002). In contrast, plant PLDs are encoded by large multigenic families. Twelve PLD genes were identified in *A. thaliana* (Qin and Wang, 2002), 17 in rice (Li et al.,

* Corresponding author. Tel.: +380 445732705; fax: +380 445732552.
E-mail address: pokotylo@bpci.kiev.ua (I. Pokotylo).

http://dx.doi.org/10.1016/j.envexpbot.2014.08.009
0098-8472/

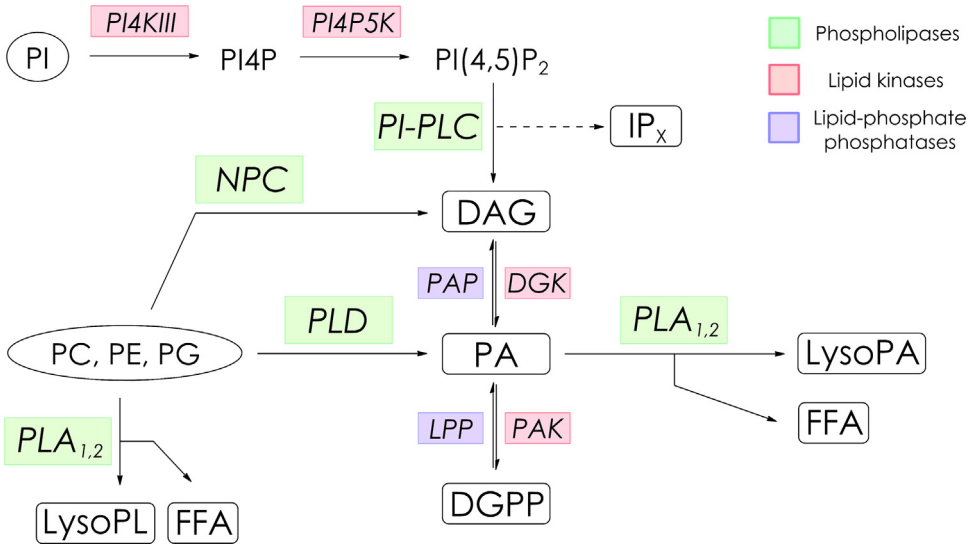

Fig. 1. Phospholipid signalling pathways in plants. Lipid processing enzymes are shown in red. DAG, diacylglycerol; DGK, diacylglycerol kinase; DGPP, diacylglycerol pyrophosphate; FFA, free fatty acids; IP_x, inositol polyphosphate; LysoPA, lysophosphatidic acid; LysoPL, lysophospholipids; LPP, lipid phosphate phosphatase; NPC, non-specific phospholipase C; PA, phosphatidic acid; PAK, phosphatidic acid kinase; PAP, phosphatidic acid phosphatase; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PI4K, phosphatidylinositol 4-kinase; PI4P5K, phosphatidylinositol-4-phosphate 5-kinase; PI4P, phosphatidylinositol 4-phosphate; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PI-PLC, phosphatidylinositol-specific phospholipase C; PLA, phospholipase A; PLD, phospholipase D.

2007), 18 in soybean (Zhao et al., 2012), 17 in poplar and 11 in grape (Liu et al., 2010). Plant PLDs are characterised by a complex domain structure. They facultatively contain C2, PH, PX and DRY regulatory domains (Fig. 3) (Kolesnikov et al., 2012). The differences in primary structure, together with differences in enzymatic properties, distribute plant PLDs into α, β, γ, δ, ε and ζ sub classes. In particular plant species, PLD isoforms named κ and φ (the latter bearing a signal peptide sequence) are also present (Li et al., 2007). The majority of PLDs are thought to be membrane-associated and require Ca^{2+} for their activity (Pappan et al., 1997). The stress-induced, calcium-dependent PLD allocation to membranes is considered as a key mechanism of PLD activation (Ryu and Wang, 1996). Among other post-translational regulators of PLD activity in plants are G-proteins (Munnik et al., 1995), lipids (such as phosphatidylinositol-4,5-bisphosphate, $PI(4,5)P_2$) and protein-kinases (Novotná et al., 2003). The different classes of plant PLDs have distinct modes of regulation. For instance, PLDα activity is $PI(4,5)P_2$-independent and calcium-dependent, and that of PLDζ is calcium-independent and $PI(4,5)P_2$-dependent. PLDs can participate in a characteristic transphosphatidylation reaction with primary alcohols leading to a production of non-metabolisable phosphatidylalcohol (Rainteau et al., 2012). This reaction is used both to inhibit PA production and monitor PLD activity *in vivo* (Munnik and Laxalt, 2013). The primary alcohol most commonly used for these experiments is *n*-butanol. Tertiary-butanol, which is not a substrate of PLD, is used as a control. PLDs have long been considered as the main contributors to PA signalling. However their hegemony is now being challenged by the PLC/DGK pathway.

2.2. Phosphoinositide-specific phospholipase C

Phosphoinositides, such as phosphatidylinositol-4-phosphate (PI4P) or $PI(4,5)P_2$, are minor constituents of plant membranes. PI-PLC (EC 3.1.4.11) selectively hydrolyses phosphoinositides into diacylglycerol (DAG) and phosphorylated-*myo*-inositol. When compared to animal PI-PLCs, the plant PI-PLC structure (close to that of animal ζ isoform) is relatively simpler, with X and Y catalytic domains, C2 domain and truncated EF hand regulatory domains (Fig. 3) (Pokotylo et al., 2014a). Nine PI-PLC genes were identified in *A. thaliana* (Hunt et al., 2004) and rice (Singh et al., 2013) and six in tomato (Vossen et al., 2010). In animals, it is well established that DAG activates proteins, such as protein kinases C (PKC), while inositol trisphosphate (IP_3) binds calcium channels and thus triggers the release of the cation from internal reservoirs. However in plants, the mode of action of PI-PLCs is different. Identification of gene(s) encoding a receptor protein of IP_3 in plant cells has not been successful (Krinke et al., 2007a) and no plant proteins orthologous to mammalian PKCs have been found. In plants, IP_3 might be phosphorylated into inositol hexakisphosphate (IP_6) – a putative signalling molecule (Lemtiri-Chlieh et al., 2003). DAG on the other hand is phosphorylated into PA and thus contributes to PA-dependent plant stress responses (Arisz et al., 2013).

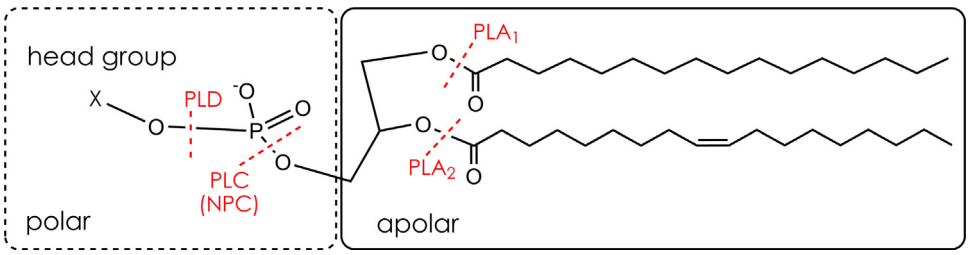

Fig. 2. Diversity of phospholipases. A generalised representastion of the structure of phospholipid molecule with an arbitrary fatty acid composition is shown with the sites of phospholipid cleavage indicated. The polar and apolar regions of the phospholipid and corresponding lipid products are marked. PLA_1, phospholipase A1; PLA_2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D.

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

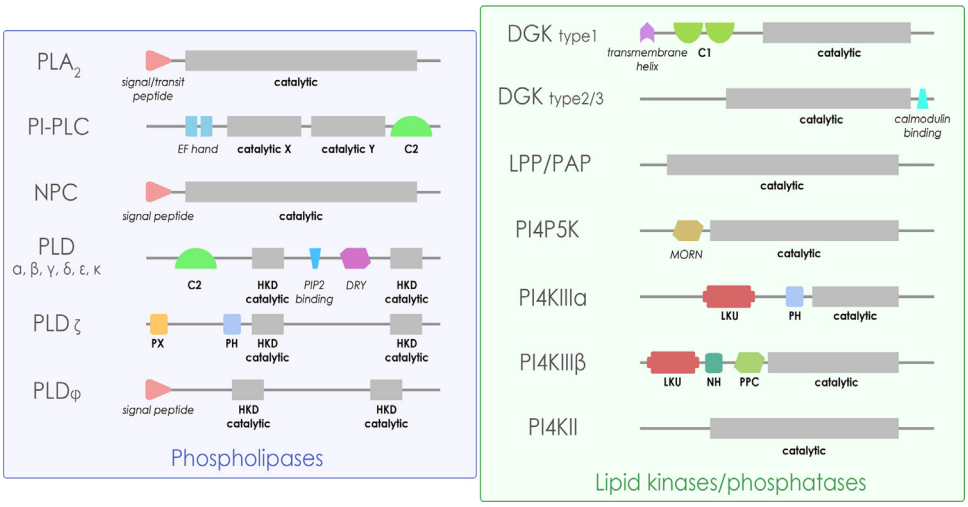

Fig. 3. Domain structure of lipid-processing enzymes in plants. Domains that are mandatory in the particular enzyme subtypes are shown in bold whereas facultative domains and motives are given in italics. LKU, lipid kinase unique domain; MORN, membrane occupation and recognition nexus domain; NH, novel homology domain; PH, pleckstrin homology domain; PPC, plant PI4K charged domain; PX, phox domain.

Furthermore, the changes in phosphoinositide levels resulting from PI-PLC activity may also have a regulatory role per se. Phosphoinositides are indeed active molecules, that bind and regulate the activity and/or localisation of plant proteins (Delage et al., 2013; Oxley et al., 2013). PI-PLC activity regulation involves Ca^{2+} (Hunt et al., 2004), C2 domain-mediated membrane targeting (Rupwate and Rajasekharan, 2012), protein–protein interactions and putative post-translational modifications (Pokotylo et al., 2014a).

2.3. Non-specific phospholipase C

NPCs (EC 3.1.4.3) are phospholipases that predominantly hydrolyse PC, an abundant structural phospholipid, as well as PE and PG. They produce DAG and the corresponding phosphorylalcohol. Plant NPCs are characterised by a plain domain structure, with little or no regulatory sequences identified (Fig. 3) (Pokotylo et al., 2013). They share no sequence similarities with, and are structurally unrelated to, PI-PLC or other plant phospholipases. Instead, a similarity to bacterial non-toxic PC-PLC has been reported (Nakamura et al., 2005). Rapid post-translational regulation of NPC activity was reported in response to stress and hormonal stimuli (Kocourková et al., 2011; Pejchar et al., 2010; Wimalasekera et al., 2010). The molecular mechanisms behind these regulatory events remain unclear. Unlike PI-PLC, the soluble product of NPC activity, a phosphorylalcohol, is thought to be irrelevant to signalling events in plants.

2.4. Phospholipase A

PLAs (EC 3.1.1.4) are hydrolases cleaving many membrane phospholipids, thus releasing a free fatty acid (FA) and a lysophospholipid. Both these molecules can be biologically active (Scherer, 2010). PLAs are classified into PLA_1 and PLA_2 depending on whether the *sn-1* or *sn-2* acyl chain is released from the phospholipid molecule. In plants, the PLA_2 group is further diversified into small secreted PLA_2 ($sPLA_2$), patatin-like PLA_2 ($pPLA_2$) and calcium-activated PLA_2 (Scherer, 2010). This classification is essentially based on the enzyme biochemical properties and not so much on the presence of structural motifs (Fig. 3) (Chen et al., 2013). Patatin-like phospholipases frequently combine the activities of both PLA_2 and PLA_1. PLA_2-like activity has been also detected in structurally divergent plant proteins (Chen et al., 2012). The implication of PLA_2 in lipid signalling has now been demonstrated in relation to plant defence responses (Viehweger et al., 2002) and reaction to auxin that rapidly activates patatin-like phospholipases A (Effendi et al., 2014; Labusch et al., 2013). The putative role of lyso-PA (a product of PLA activity) in salt responses of *Chlamydomonas* has also been reported (Arisz and Munnik, 2011). Amongst the mechanisms involved in the regulation of PLA_2 activities in plants, a critical role has been given to G-proteins (Heinze et al., 2013). Less is known about the signalling role and mode of regulation of plant PLA_1.

2.5. Diacylglycerol kinase

DGKs (EC 2.7.1.107) catalyse the ATP-dependent DAG phosphorylation resulting in PA synthesis. Seven DGK genes were identified in *A. thaliana* genome (Gómez-Merino et al., 2004) and eight in rice genome (Ge et al., 2012). The structure of plant DGK is simpler than that of animals (Fig. 3) (Liu et al., 2013b). For instance, five out of seven DGKs from *A. thaliana* lack the C1 domain characteristic of animal enzymes. There is increasing evidence suggesting an essential role of DGKs in regulation of plant cell activities (for review see Arisz et al., 2009). For example, DGK has been demonstrated to play a role in the signal-specific PA production in cold-stressed *A. thaliana* (Delage et al., 2013; Ruelland et al., 2002; Vaultier et al., 2006). Inhibition of DGK activity results in the retardation of plant growth (Gómez-Merino et al., 2005) and defence responses (Ge et al., 2012). The DAG substrate required for DKG activity can be produced by both NPC and PI-PLC. However, FA composition is usually different in phosphoinositides and structural lipids such as PC or PE (Rainteau et al., 2012), thus the PA produced by the two pathways will be composed of essentially distinct molecular species. In fact, based on FA composition, the PA produced from NPC-derived DAG (via DGK activity) will be much closer to the PLD-generated PA, than to a PA that originated from PI-PLC.

2.6. PA kinase

In some cases, PA is phosphorylated into DGPP by a PA kinase (EC 2.7.4.). This enzyme has not yet been cloned in plants. DGPP acts as a stress-activated signalling molecule (Munnik and Testerink, 2009; van Schooten et al., 2006), namely in the ABA-mediated responses in *A. thaliana* cells (Zalejski et al., 2005).

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

2.7. PI- and PIP-kinases

PI4P and PI(4,5)P_2 are not solely substrates of PI-PLC, they can participate in signalling cascades by recruiting/sequestering proteins through PH or PX domains (D'Angelo et al., 2008; Delage et al., 2013). The synthesis of PI4P and PI(4,5)P_2 in plant cell relies on the activity of PI4 kinase (EC 2.7.1.67) and PI4P5 kinase (EC 2.7.1.149) respectively. Plant PI4 kinases are classified as type IIIα, type IIIβ and type II enzymes that differ in their molecular weight and domain composition (Fig. 3) (Drøbak et al., 1999). A unique gene coding for PI3 kinase that facilitates production of phosphatidylinositol 3-phosphate has been found in *A. thaliana* (Lee et al., 2008). The structure of PI4P5 kinases (10 isoforms in *A. thaliana*) is more homogenous with a N-terminal Membrane Occupation and Recognition Nexus (MORN) regulatory domain (Saavedra et al., 2012). The PI- and PIP-kinases have distinct biological roles in plants. For example, the contribution of type III PI4 kinases has been shown to be indispensable for phosphoinositide-mediated stress responses in *A. thaliana* (Delage et al., 2012).

2.8. Lipid phosphatases

Phosphoinositide phosphatases (EC 3.1.3.) act together with kinases to maintain equilibrium between the pools of phosphorylated and dephosphorylated phosphoinositides. Recently, Suppressor of Actin (SAC) phosphoinositide phosphatases have been demonstrated to play a role in vacuolar development and trafficking in *A. thaliana* (Nováková et al., 2014).

Lipid phosphate phosphatase (EC 3.1.3.4) dephosphorylates PA (and DGPP) thus quenching the signal generated by those lipids. This step is essential in a signalling pathway and such a role has been assigned to lipid phosphate phosphatases in plant responses to drought, ABA and the elicitor harpin (França et al., 2008; Paradis et al., 2011; Pierrugues et al., 2001).

3. Implication of lipid signalling in plant stress and hormonal responses

Plants are constantly challenged by their environment but they cope with the majority of stresses by adjusting their metabolism and physiological responses. Lipid signalling offers the perfect mechanism for the information transmission between a plasma membrane, the cytosol and other organelles, particularly the nucleus. Membranes are the sites where many signals are perceived by the cell and offer a unique environment for inducible enzyme activity (Horváth et al., 2012; Sun et al., 2013). The lipid-processing enzymes described above are responsible for the production or the catabolism of lipid mediators involved in plant responses to stresses. The majority of them stay, at least transiently, in membranes. However, the binding of soluble proteins will allow the transduction of the signal into the soluble compartments of the cell. PI-PLC-class phospholipases furthermore produce a biologically active soluble molecule that is directly released to the cytosol.

Moreover, an environmental stress, considered as a primary signal, will trigger the synthesis of hormones, *e.g.* ABA or SA, that will act as a secondary signal and activate plant adaptive responses.

3.1. Stress responses

3.1.1. Biotic stress

Pathogens, such as bacteria or fungi, are a major threat to plants. Throughout evolution, sophisticated defence mechanisms aimed at counteracting pests have been selected. This has resulted in a highly structured plant immune system (Wirthmueller et al., 2013). The first line of plant defence comprises mechanical barriers (*e.g.* a cuticle, wax deposits) and constitutive synthesis of antimicrobial secondary metabolites such as phytoalexins. Then, two components of plant immunity can be induced: a pathogen-associated molecular pattern triggered immunity (PTI) and an effector triggered immunity (ETI). A PTI is activated upon recognition of unspecific pathogen-derived molecules (*e.g.* a flagellin) by pattern recognition receptor (PRR), whereas a more specialised ETI is responsible for recognition of race-specific pathogen effectors by nucleotide-binding leucine-rich repeat (NB-LRR) proteins (Senthil-Kumar and Mysore, 2013). Both systems often act simultaneously, leading to an expression of defence genes and, facultatively, to a hypersensitive cell death response (HR) that restricts pathogen propagation at the infection site.

Plant phospholipases are essential components of these systems (Canonne et al., 2011), in particular for PTI-responses. A significant increase of PA production in tomato cells implicating both PLD and PLC/DGK pathways was observed as soon as after 30 min of treatment with chitosan, a polysaccharide elicitor (Raho et al., 2011). A rapid accumulation of lysophosphatidylcholine, resulting from PLA_2 activation, was reported in cultured cells of California poppy elicited with a yeast glycoprotein (Viehweger et al., 2002). In contrast, two oomycete-derived elicitors inhibited NPC activity in parsley and tobacco cell suspensions (Scherer et al., 2002). This suggests that each phospholipase has its specific role in plant immunity. The differently directed changes of expression of numerous phospholipase-coding genes following pathogen encounter or elicitor treatment are evident (Fig. 4).

An implication of lipid signalling to ETI response is also evident. Indeed, a biphasic PA accumulation (from PLD and PLC/DGK pathways) was observed in *A. thaliana* after recognition of AvrRpm1 and AvrRpt2 by membrane receptors RPM1 and RPS2, respectively (Andersson et al., 2006). Moreover, silencing of PLC4 in tomato has resulted in weakened HR following Avr4 recognition in plants expressing the Cf-4 R-gene. In contrast, the silencing of PLC6 did not affect plant responses in similar conditions, but PLC6 was required for efficient responses mediated by Ve1 and Pto/Prf receptor proteins (Vossen et al., 2010).

Phospholipases are likely to participate in the immune responses at the plasma membrane level, where PRRs, as well as some of the NB-LRR, are located (Monaghan and Zipfel, 2012; Mackey et al., 2002). Quantitative proteomics of plasma membrane-enriched fractions from dexamethasone-inducible GVG-AvrRpt2 *A. thaliana* plants, in which an ETI response mediated by RPS2 resistance gene is evoked, have revealed that among proteins whose abundance was increased upon onset of the immune reactions are some members of phosphoglycerolipid signalling system: DGK 5, PLDα1 and PLDγ1, and several members of PLA_2 family (PLP2a/pPLA-IIα and SOBER1) (Elmore et al., 2012).

The PLD-mediated accumulation of PA has also been observed in *A. thaliana* in response to wounding and thus may play a role in biotic stress signalling necessary for resistance to herbivore attack (Bargmann et al., 2009).

For this reason, PA appears to act as an active molecule in the immune response. The application of exogenous PA (or DAG) could mimic the effects of the *N*-acetylchitooligosaccharide elicitor in rice cells, as suggested by the triggering of ROS generation, or expression of defence-related genes (Yamaguchi et al., 2005). PA application was also sufficient to induce the accumulation of phytoalexin in tobacco cells (Wang et al., 2013a).

The participation of several types of phospholipases in plant immunity has been demonstrated at the molecular level. A role in *A. thaliana* resistance to powdery mildew fungus (*Blumeria graminis* f. sp. *Hordei*) has been specifically ascribed to PLDδ that accumulated in the plasma membrane near the site of fungal attack (Pinosa et al., 2013). Analysis of PLDδ-deficient plants demonstrated a declined resistance to fungal spore penetration. The chitin-induced expression of *NHL10*, *FRK1*, and *PHI-1* defence genes was also retarded

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

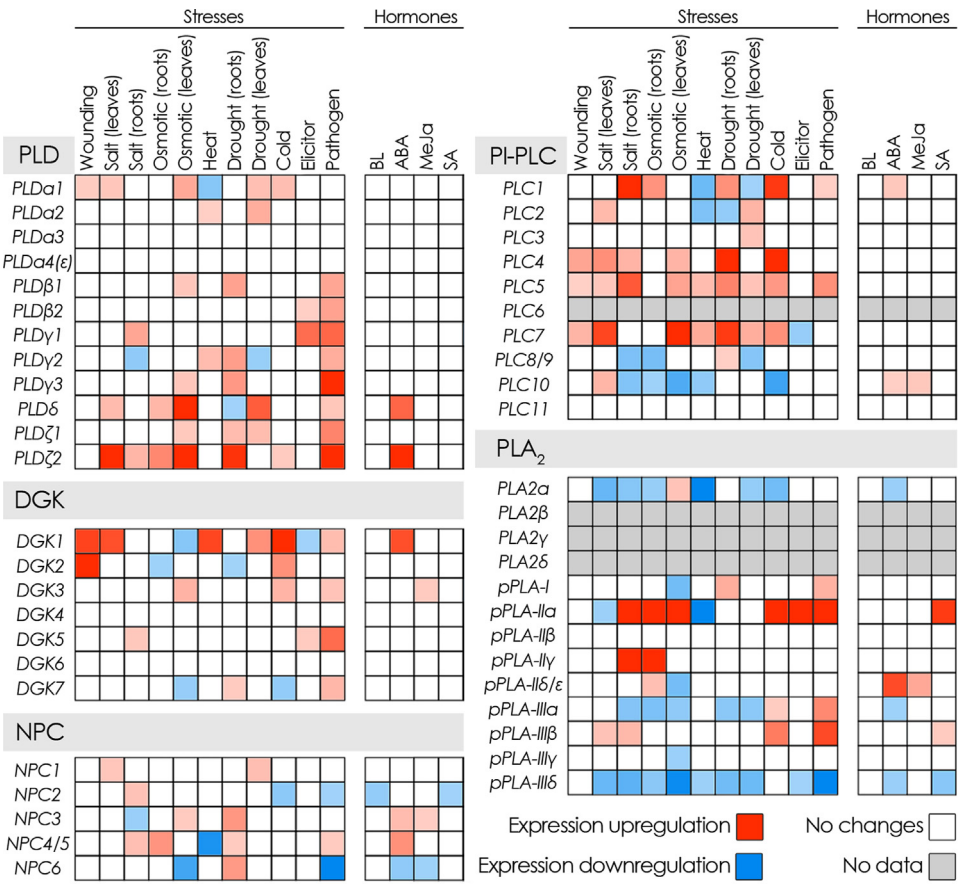

Fig. 4. Transcriptional responses of lipid-processing enzymes to environmental cues and hormones (Hruz et al., 2008). Colour saturation corresponds to the level of upregulation (red) and downregulation (blue) of gene expression in designated conditions. Expression changes of five-fold or more are given as maximum color saturation. Expression changes that are lower than 1.5-fold are assumed insignificant and marked white. ABA, abscisic acid; BL, brassinolide; MeJA, methyl jasmonate SA, salicylic acid. Gene Expression Omnibus (GEO) codes for each set of experimental data are as follows: wounding, salt (roots), osmotic (leaves), osmotic (roots), cold – AT-00120; salt (leaves) – AT-00262; heat – AT-00387; drought (leaves), drought (roots) – AT-00626; elicitor (flagellin 22 peptide) – AT-00107; pathogen (*P. syringae* pv. Maculicola) – AT-00406; BL, ABA, MeJA – AT-00110; SA – AT-00113 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

in *pldδ* mutants (Pinosa et al., 2013). In pepper, silencing of the *CaPLP1* gene, encoding a $pPLA_2$, activity resulted in loss of resistance to *Xanthomonas campestris* pv. *Vesicatoria* (Kim et al., 2014). The expression of *CaPLP1* in *A. thaliana* (under the CaMV 35S promoter) has rendered plants more tolerant to the pathogen infection. This effect was accompanied by an increased ROS generation and an enhanced expression of several defence-related genes upon infection (Kim et al., 2014). In contrast, the activity of some of the plant phospholipases seems to favour the resting state of plant defences. Suppression of *PLDβ1* expression in rice resulted in the activation of defence-like reactions that include accumulation of ROS and phytoalexins and expression of defence-related genes, contributing to elevated resistance to pathogenic fungi. These defence reactions are thus constitutively repressed by a basal PLDβ1 activity (Yamaguchi et al., 2009).

Stress-induced accumulation of hormones constitutes an essential part of plant defences (Derksen et al., 2013). Interestingly, the well-known antagonism between the signalling pathways that control plant resistance and implicate salicylic acid (SA) and jasmonic acid (JA), may be implemented at the level of phospholipase-dependent signalling. The positive contribution of *A. thaliana* PLDβ1 to plant JA-dependent responses to the necrotrophic fungi *Botrytis cinerea* has to be opposed to its negative role in plant SA-dependent response to biotrophic Pst DC3000 (Zhao et al., 2013). Moreover, a negative correlation between PLDβ1 activity and lysophospholipid content has been reported in the same study. Taken together with the data from another study where the activity of a Suppressor of AvrBsT Elicited Resistance1 (SOBER1) PLA_2 was antagonistic to PLD-dependent PA production in *A. thaliana* elicited with AvrBsT (Kirik and Mudgett, 2009), a tight interplay between PLA and PLD in plant defence signalling may be suggested.

The downstream mechanisms of metabolism regulation by phospholipases, including those in biotic stress conditions, are discussed below.

3.1.2. Salt and osmotic stresses

Salt stress is a frequent environmental situation caused by both natural and anthropogenic factors. High osmolarity constitutes an intrinsic, rapid and dominant component of salt stress. It is accompanied by a long-lasting ionic component mainly caused by the toxicity of Na^+ ions. Salt stress has a great impact on plants, affecting photosynthesis and resulting in growth inhibition or complete growth arrest. Different phospholipases are involved in plant response to salinity. The expression of *A. thaliana AtPLC1, N. tobacco NtPLCδ1* and *Vr-PLC3* from *Vigna radiate*, all coding for PI-PLCs, was greatly induced in salt conditions (Hirayama et al., 1995; Kim et al., 2004; Tripathy et al., 2012). The same reaction was observed for *AtPLDδ* (Katagiri et al., 2001). A post-translational response was also elicited, as a strong increase in IP_3 production resulting from PI-PLC activation, observed in the first minutes of plant exposure to salt or hyperosmotic stress (DeWald et al., 2001; Drøbak and Watkins, 2000). This response was coupled to an increase in intracellular Ca^{2+} (DeWald et al., 2001). The implication of PLC/DGK pathway to

rapid PA production in salt-treated rice has also been demonstrated (Darwish et al., 2009). With respect to NPC, *AtNPC4* expression was induced after 6 h in salt-treated roots of *A. thaliana*, while the induction of NPC activity was detected as early as after 30 min of salt exposure (Kocourková et al., 2011). It is important to mention that plants overexpressing phospholipase genes frequently display higher tolerance to salt stress. This is the case of NPC4-OE *A. thaliana* (Peters et al., 2010) and PLCδ1-OE tobacco (Tripathy et al., 2012).

Many studies imply PA as a key signalling molecule in plant responses to salinity. A critical role in salt-induced PA production in *A. thaliana* has been assigned to PLDα1. It has been reported that PA content was significantly induced by salt treatment in WT plants. However, this effect was almost negligible in PLDα1-deficient mutants (Yu et al., 2010). The importance of PA production in response to salinity is further supported by the fact that PLDα1-deficient (Yu et al., 2010) and PLDα3-deficient *A. thaliana* mutants had severely lowered salt stress resistance (Hong et al., 2008a).

PA has an impact on cytoskeleton organisation under salt stress. *Pldα1* mutants have irreversibly disordered microtubule structure in stress conditions, contrary to WT plants. This observation may be explained, at least in part, by PA-binding to MAP65-1 – a microtubule-associated protein (Zhang et al., 2012). PA can also stimulate activity of protein kinases in salt-stressed plants, for instance that of MPK6 in *A. thaliana* (Yu et al., 2010). PA is also thought to mediate accumulation of soybean MAPK in salt stress conditions (Im et al., 2012). Interestingly, DGK-derived PA can be a source of LPA produced by PLA_2 in *Chlamydomonas* following salt stress (Arisz and Munnik, 2011). In this regard, a novel role for mitochondrial PLA_2 in responses to hyperosmotic treatment was recently reported in wheat. The FA production by PLA_2 has been proposed to influence ion channels, resulting in the quenching of transmembrane electric potential in mitochondria (Trono et al., 2013). These events result in a stabilisation of respiratory chain and prevent stress-induced ROS accumulation.

Lipid signalling pathways can also be involved in the regulation of the level of proline (an osmolyte) in *Thellungiella halophila* under salt stress. Depending on the severity of the stress, the activity of either PLD or PI-PLC was essential for proline accumulation as suggested by inhibitor studies (Ghars et al., 2012). Similarly, PI-PLC specifically stimulated proline accumulation under ionic, but not non-ionic, hyperosmotic stress in *A. thaliana* (Parre et al., 2007).

Interestingly, other lipid molecules are also implicated in salt-stress responses. Phosphatidylinositol-3-phosphate or phosphatidylinositol-4-phosphate were demonstrated to mediate a defensive ROS production in salt-stressed *A. thaliana* (Leshem et al., 2007).

3.1.3. Drought stress

Drought stress results in loss of turgor, inhibition of metabolic activities, and most often in stomata closure. Both PLCs and PLDs are involved in cell responses to dehydration. *Zea mays* plants overexpressing PI-PLC1 (*ZmPLC1*) had better adaptation to drought, while the corresponding antisense line has significantly impaired growth parameters (Wang et al., 2008). In transgenic tobacco plants, expression of *ZmPI-PLC1*, under the CaMV 35S promoter, also conferred drought resistance (Zhai et al., 2013). Overexpression of PI-PLC2 in canola plants resulted in drought tolerance accompanied by an improved photosynthetic rate (Georges et al., 2009). This is also true in canola expressing *A. thaliana PLDα1* under a guard cell-specific promoter (Lu et al., 2013). On the other hand, antisense *pldα1 A. thaliana* plants were more sensitive to drought, had lower leaf water potential and had their photosynthesis more severely affected compared to WT plants under the same conditions (Mane et al., 2007). These effects can be explained by the acute requirement for an up-regulation of phospholipase activity during stress. In durum wheat, the abundance of four secreted PLA_2 transcripts, as well as measured PLA_2 activity *in vitro* and the increase in free FA content, positively correlated with a better resistance to drought (Verlotta et al., 2013). A patatin-like protein, with galactolipid and phospholipid acyl hydrolase activities, is also expressed in response to drought in *A. thaliana* (Matos et al., 2008). The expression of *AtPLDδ* was significantly induced and PA was accumulated in *A. thaliana* tissues in response to dehydration. Interestingly, the PA accumulation was significantly hampered in *AtPLDδ*-antisense lines, suggesting an essential role of *AtPLDδ* in drought stress responses (Katagiri et al., 2001). It was also reported that under water deficit, a significant amount of produced PA is phosphorylated to DGPP in the leaves of *Craterostigma plantagineum* (Munnik et al., 2000). The same effect has been observed in *Chlamydomonas moewusii* green alga and in cells of tomato and alfalfa in response to water deficit induced by NaCl treatment (Munnik et al., 2000).

Besides a role as signalling enzymes, PLDs can also act as catabolic enzymes, participating in a slow membrane degradation process. It is therefore not uncommon to assign this dual role to PLDs during a stress. For instance, *PLDα1* mediates guard cells closure at the early stages of drought, thus contributing to short-term plant tolerance. However, plants overexpressing *PLDα1* were more sensitive to prolonged stress. The latter effect can be explained by the membrane degradation as confirmed by the analysis of ion leakage. This suggests that both catabolic and signalling roles of phospholipases in plant metabolism should always be considered in parallel (Hong et al., 2008b)

Apart from phospholipases, the contribution of other lipid-processing enzymes to plant resistance has also been reported. Overexpression of phosphatidylinositol synthase from *Zea mays* (ZmPIS) in tobacco plants led to drought stress tolerance (Zhai et al., 2012), tentatively explained by an induced phosphoinositide signalling. Indeed, the over-expression of *ZmPIS* in maize was accompanied by an enhanced content of major phospholipids (including phosphoinositides) together with a higher level of transcripts encoding DGKs (ZmDGK1, ZmDGK3), PI-PLCs (ZmPLC) and PLDs (ZmPLDα1). Genes involved in ABA biosynthesis were also up-regulated in ZmPIS-overexpressing plants (Liu et al., 2013a).

3.1.4. Temperature stress

In plants, acute temperature shifts represent a major stress. Some plants, according to the species and cultivars, will be able to trigger a response to help them cope with this stress. Lipid signalling is a key part of temperature responses (Ruelland and Zachowski, 2010).

We can distinguish heat stress, from chilling stress and freezing stress. Heat stress leads to an early activation of PLD in BY-2 tobacco cells. In parallel, $PI(4,5)P_2$ accumulated at the plasma membrane and nucleus levels, indicating a stimulation of (a) PIP kinase(s) (Mishkind et al., 2009). In *S. Lycopersicum* (a tomato) *PLC3* and *PLC6* genes were up-regulated in response to high temperature exposure. It is important to mention that an increased PA level in such conditions was accompanied by decreased levels of PI and PIP *in vivo*, suggesting an activation of PLC/DGK pathway (Abd-El-Haliem et al., 2012). In *A. thaliana*, AtPLC9 expression tightly correlated with plant resistance to heat stress. *Atplc9* mutant plants became thermosensitive compared with wild-type plants after high temperature stress. Complementation of *atplc9* mutants by *AtPLC9* restored a thermotolerance. Moreover thermotolerance was further improved in overexpressor lines. This effect was explained by altering level of IP_3 accumulation and Ca^{2+} influx in mutant plants. In addition, *AtPLC9* expression has also correlated with the accumulation of two small heat shock proteins, HSP18.2 and HSP25.3 (Zheng et al., 2012). A complementary implication of *AtPLC3* to heat stress repsonses in *A. thaliana* has also been revealed (Gao et al., 2014).

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

In response to chilling temperatures, a rapid phosphoinositide turnover and IP_3 production has been observed to occur within minutes in maize (Kravets and Nokhrina, 1998) and *A. thaliana* cell suspensions (Ruelland et al., 2002). A burst of PA production associated both with PI-PLC/DGK and PLD activities has also been observed in these conditions (Ruelland et al., 2002). The PI-PLC/DGK pathway was also involved in early PA generation in cold-stressed *A. thaliana* plantlets or leaf disks (Arisz et al., 2013; Delage et al., 2012). The proposed origin of PI-PLC activation is the cold-induced membrane rigidification that might activate mechanosensitive calcium channels, leading to calcium entry (Vaultier et al., 2006). The activity of PI-PLC is also dependent on the availability of its main substrate, $PI(4,5)P_2$, that is generated by the combined action of PI 4-kinases and PI4P 5-kinases. During a cold response, PI-PLC does not only uses pre-existent phosphoinositides; a *de novo* production, that occurs in parallel to PI-PLC activity, has been monitored. Mutations in two type III phosphatidylinositol 4-kinases (β1 and β2) led to lower PIP and $PI(4,5)P_2$ levels and to a decrease in PLC/DGK mediated PA production in response to cold in *A. thaliana* plantlets. This implies that PI4KIIIβ1, PI4KIIIβ2 and PI4KIIIα1 all participate in providing the substrates to the PI-PLC activity during cold stress (Delage et al., 2012). Several DGK isoforms are probably at play to transform DAG into PA at low temperatures, as PA production was not significantly affected in various *dgk* single mutants (Arisz et al., 2013). As for the role of PLD in response to chilling stress, genes downstream of the cold-activated PLD activity have been identified in *A. thaliana* suspension cells. They include the CBF transcription factors and their target genes (Ruelland et al., 2009; Vergnolle et al., 2005).

Response to freezing stress is more complex than evidenced in chilling stress. In addition to induced temperature stress, freezing is accompanied with ice formation in the apoplast. This can lead to water efflux from the cytosol, essentially effecting subcellular dehydration or desiccation and loss of cellular integrity (Ruelland and Zachowski, 2010). The analysis of the lipid composition of cold-tolerant *Thellungiella salsuginea* plant showed that PA content was low under normal growth conditions. In contrast, it increased almost 10-fold at low but positive temperature (chilling) and 100-fold in response to negative temperature (freezing). The membrane PC content was also reduced, suggesting an activation of PLD(s) or NPC(s) (Zhang et al., 2013). In *Celtis bungeana* callus, a rise in PLD activity, as measured by choline release *in vitro*, in both microsomal and mitochondrial membrane fractions was also reported in response to freezing. This effect correlated to the elevated expression of *CbPLD* noticed after 6-days of stress (Yang et al., 2013). Therefore a PLD is activated during freezing stress. But, similarly to what is discussed with drought stress, the PLD function might be more attributable to its catabolic role rather than to a signalling one. Moreover, the functions of PLD during freezing are likely to be isoform-dependent as suggested by the discrepancy in the role of PLDδ (a positive role)(Li et al., 2004) and PLDα1 (a negative role) (Rajashekar et al., 2006) in *A. thaliana* freezing tolerance.

3.1.5. Nutrient deficiency and toxic metals

Plants obtain all of the required essential nutrients from the soil. However, they frequently find themselves in a situation when the soil nutrient composition is unbalanced of deficient. These conditions require rearrangements of metabolism that are facilitated, at least in part, by the activity of lipid-processing enzymes. Overexpression of PLDε had a positive effect on *A. thaliana* root growth in conditions of nitrogen, phosphorous or potassium deprivation and affected the activity of several enzymes employed in nitrogen assimilation (Hong et al., 2009). Membrane phospholipids can be considered as a source of phosphate. The functions of NPCs were associated with phosphoglycerolipid turnover in phosphate-deprived plants. In those conditions, membrane phosphoglycerolipids are hydrolysed by NPCs, releasing DAG which is used for the synthesis of digalactosyldiacylglycerol, which in turn substitutes for phosphoglycerolipids in extra-plastidial membranes. The released phosphorylated head group is catabolised to recycle the phosphate (Gaude et al., 2008; Nakamura et al., 2005). An implication of PLDζ2 (Cruz-Ramírez et al., 2006) to plant responses to low phosphate has also been reported. These effects are to be associated with metabolic, rather than signalling, functions of phospholipases. Many phospholipids contain nitrogen in their structure (*e.g.* PC, PE). Despite this, no significant changes to phospholipid contents were reported following nitrogen deprivation in soybean (Narasimhan et al., 2013).

Toxic metals (such as Al^{3+} or Cd^{2+}) are common, though unwelcome, constituent of soils. They can drastically affect essential plant enzymes and metabolism by forming chelates or antimetabolites. The induced Al resistance in PLDγ-suppressed *A. thaliana* plants was ascribed to the alleviation of ROS damage and lipid remodelling (Zhao et al., 2011). An inhibition of NPC activity has been monitored as early as after 10 min in aluminium-treated tobacco BY-2 cells (Pejchar et al., 2010). The physiological importance of the observed effects is yet to be explained.

3.2. Hormonal responses

Hormones govern plant life, from seed germination to senescence. They also constitute an intrinsic part of plant adaptation. Both stress-related hormones (*e.g.* salicylic acid, abscisic acid) and some of the growth-promoting hormones (*e.g.* brassinosteroids) may actively participate in plant responses to the environment. Indeed, lipid signalling has been revealed to be an essential part of hormonal signal transduction (Janda et al., 2013). Therefore, hormones could interfere on the lipid signalling pathways activated by environmental stresses. This interference could be positive or negative.

3.2.1. Brassinosteroids

Connections between phospholipid and brassinosteroid signalling pathways in plants are now documented. Brassinosteroids stimulate NPC activity as early as 15 min after hormone application (Wimalasekera et al., 2010). Moreover, the expression of the negatively-regulated brassinosteroid biosynthetic *CPD* gene was not repressed in *npc3* and *npc4 A. thaliana* mutants treated by a brassinolide (a brassinosteroid) (Wimalasekera et al., 2010). The stimulatory role of a brassinosteroid on the post-translational activation of PLD activity was observed in *Brassica napus* (Pokotylo et al., 2014b). A functional connection between brassinosteroid and lipid signalling pathways in plants may rely on the activity of protein phosphatase 2A (PP2A). PP2A is a multifunctional heterotrimeric enzyme. For instance, PP2A dephosphorylates Brassinazole-Resistant 1 (BZR1) – a transcriptional repressor that is active in a dephosphorylated state (Tang et al., 2011). Together with BRI1-EMS-Suppressor 1 (BES1), BZR1 is tightly connected with brassinosteroid-induced regulation of gene expression (Ryu et al., 2007). In turn, the activity of BRI1 receptor kinase (a brassinosteroid receptor), is negatively regulated by PP2A at the plasma membrane (Wu et al., 2011). PA recruits and activates PP2A specifically in membrane fractions presumably via binding to its A1 subunit. At the same time, a depletion of total (cytosolic) PP2A activity following PA treatment has been reported (Gao et al., 2013). Therefore, PA as a mediator of lipid signalling could act on brassinosteroid perception, through BRI1, and its transduction, through BZR1. However, it is becoming apparent that different pools of PA and PP2A may be involved in these processes. In this manner, a negative effect of PA on BZR1 phosphorylation has been reported. This effect is likely to be exerted by PLD-derived PA, since treatment with R59022 (an inhibitor of DGK) did not affect

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

the phosphorylation of BZR1 while treatment with *n*-butanol (an inhibitor of PA production by PLD) reversed the effect. In addition, the *pldζ2* mutant (PA-deficient) was characterised by the constitutively activated brassinosteroid signalling as suggested by the repressed expression of *CPD* and *DWF4* genes implicated in brassinosteroid biosynthesis (Wu et al., 2014). The existence of a PP2A-independent mechanism of PA-mediated regulation of the BZR1 phosphorylation and BR-signalling as a whole should also be considered.

Expression of genes encoding the enzymes implicated in the lipid-signalling pathways, such as PLDα1, PLDα2, PLDγ1, PLDδ, DGKs (Wu et al., 2014) and AtPLC1 (Sun et al., 2010) are upregulated by brassinosteroids in *A. thaliana*. This might point out at a feedback regulatory loop between a PA-mediated brassinosteroid signalling and an expression of PA-generating enzymes.

Other phospholipids act as mediators of brassinosteroid in plants. For example, lyso-phosphatidylethanolamine, a product of PLA activity, takes part in the control of brassinosteroid-mediated root elongation in *A. thaliana* (yoon Jeong et al., 2012).

3.2.2. ABA

Abscisic acid (ABA) is a key hormone implicated, among other mechanisms, in plant stomatal responses to water loss. In barley protoplasts, ABA activated PLD and thus led to a rise in PA content, while the addition of *n*-butanol inhibited ABA responses (Ritchie and Gilroy, 1998). ABA-induced PLD activation was mediated by G-proteins and localised at the plasma membrane (Ritchie and Gilroy, 2000). PLDδ and PLDα1 were shown to redundantly mediate ABA-induced stomata closure in *A. thaliana*. A double mutant *pldα1pld*δ had no stomatal response to ABA. However, both single mutants had retained their sensitivity to ABA treatment, even though ABA-triggered PA accumulation was significantly reduced (Uraji et al., 2012).

Recent *in vitro* experiments using isotopic labelling have revealed that PA produced by PLD in response to ABA treatment in barley aleurone layers was likely to be rapidly processed. This conclusion has been drawn from the fact that activities of both PA kinase and PA phosphatase 1 (that is *N*-ethylmaleimide-sensitive) were induced by ABA (Villasuso et al., 2013). This suggests a transient nature of PA-signalling in response to ABA.

Besides their signalling role, PLDs could act as catabolising enzymes during ABA-responses. One of the PLD isoforms activated by ABA in *A. thaliana*, PLDδ, was shown to be responsible for the ABA-induced senescence mediated by a PLD-assisted degradation of membrane PC (Jia et al., 2013). In another study, a metabolic-wise PA production has also been observed in response to long-term (12 h) ABA exposure in *A. thaliana* (Katagiri et al., 2005). Interestingly, in the latter study, ABA sensitivity of *A. thaliana* plants deficient in the lipid phosphate phosphatase gene *AtLPP2* has also been investigated. These plants had a constitutively higher PA content and were hypersensitive to germination inhibition by exogenous ABA. This confirms a positive role of PA in ABA signal transduction. This signalling pathway apparently implicates ABA-insensitive 4 (ABI4) transcription factor as suggested by epistasis mutant analysis. However, owing to the fact that germination of *AtLPP2*-deficient mutants was not affected in the absence of ABA (Katagiri et al., 2005), one can conclude that PA accumulation alone is not sufficient to mimic the effect of ABA on seed germination.

Apart from PA, DGPP accumulation in response to ABA treatment has also been observed (Katagiri et al., 2005; Zalejski et al., 2005). DGPP could be a biologically active molecule. But it could also serve to eliminate PA as a signal. It has been shown in *A. thaliana* cells that the signal was effectively borne by DGPP, while PA did not transduce the signal (Zalejski et al., 2005). If this applies to all situations where DGPP is produced remains unanswered.

The implication of PI-PLC in ABA signalling is also evident. Transgenic tobacco plants with depleted PI-PLC content in guard cells were impaired in ABA-induced stomata movements (Hunt et al., 2003). However, contrary to PLDs, PI-PLCs contribute to ABA signalling by inhibiting ABA-induced stomata opening rather than promoting ABA-induced closure (Mills et al., 2004). This effect is probably mediated by Ca^{2+} as evoked by the production of soluble inositol phosphates.

3.2.3. Salicylic acid

Salicylic acid (SA) activated PLD after 45 min of incubation in *A. thaliana* cells (Krinke et al., 2009; Rainteau et al., 2012). This SA-stimulated activity was upstream of *PR1* expression, since diversion of PLD activity with primary alcohols led to a lower *PR1* transcript content (Krinke et al., 2009). PI-PLC response to SA is also well known. IP_3 content decreased greatly in *Capsicum chinense* J. cells under SA treatment, suggesting a negative regulation of PI-PLC. An increased $PI(4,5)P_2$ content, observed in the same conditions can be explained by the PI-PLC inhibition, together with the activation of PI-kinases (Altúzar-Molina et al., 2011). An increase in $PI(4,5)P_2$ has also been reported in *A. thaliana* suspension cells (Krinke et al., 2007b) and it has been proposed that $PI(4,5)P_2$ would act as a cofactor for the PLD activity responsible for *PR1* expression in response to SA (Krinke et al., 2009).

3.2.4. Jasmonates

Jasmonates are hormones implicated in various plant stress responses (Wasternack and Hause, 2013). They are classified as lipid molecules (oxylipins) derived from oxidised FA. This postulates an involvement of phospholipases (notably a PLA) in their biosynthesis (Wasternack and Hause, 2013). Moreover, transduction of the jasmonate signal in cells will also involve enzymes of the lipid-signalling pathway. In *Capsicum chinense* J. cells, jasmonate was reported to stimulate PI-PLC and PLD activities, as measured *in vitro*, as well as that of PI kinases, in a biphasic dose-dependent manner (Armando Muñoz-Sánchez et al., 2012). In *A. thaliana*, leaf treatment by methyl-jasmonate (a volatile form of the hormone) stimulated *in vitro* measured activity of PI-PLC and also that of three different PLDs: α and δ isoforms and PI(4,5)P2-dependent isoforms *in vitro* (Profotová et al., 2006).

4. Molecular mechanisms of phosphoglycerolipid signalling in plants

4.1. Phosphatidic acid molecular targets in plant cells

Several mechanisms have been described unfolding downstream mechanisms of PA signalling in plants (Liu et al., 2013b). One of the most important features of PA is its ability to bind proteins. This interaction is pH-dependent (Loew et al., 2013) and typically results in protein recruiting to membranes, accompanied by changes of protein conformation (Kooijman and Testerink, 2010). When PA-target proteins are enzymes, these changes correspond to an activation or an inhibition of their activity. PA-binding has also been shown to promote nuclear localisation of a MYB family transcriptional factor (Yao et al., 2013).

Several PA-binding proteins are involved in the regulation of plant responses to hormones. PA binds to and inhibits the activity of ABI1, a member of the protein phosphatase 2C (PP2C) family and a key component of ABA signal transduction network (Mishra et al., 2006). ROS production required for ABA-dependent stomata closure is also directly controlled by PA. This lipid indeed binds to and activates two NADPH oxidases (RbohD and RbohF) both *in vivo* and *in vitro* (Zhang et al., 2009). Sphingosine kinases are responsible for the production of biologically active phytosphingosine-1-phosphate and implicated in plant responses to ABA (Guo et al.,

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

2012b) and chilling (Dutilleul et al., 2012). PA binds to two sphingosine kinases in *A. thaliana*, resulting in the induction of their activity (Guo et al., 2011). This provides another interface for PA to regulate cell responses.

PA furthermore binds to the scaffolding A1 subunit of Protein Phosphatase 2A (PP2AA1) from *A. thaliana in vitro* (Gao et al., 2013). This mechanism should be responsible for PP2AA1 translocation and activation *in vivo*. This process would have a role in the regulation of auxin response (via the control of the dephosphorylation of PIN1, an auxin transporter (Michniewicz et al., 2007)) and of the brassinosteroid mediated signalling pathway (*e.g.* via the dephosphorylation of the brassinosteroid receptor BRI1 (Tang et al., 2011).

PA is also able to bind to CTR1 kinase in *A. thaliana*, resulting in inhibition of its activity. This process could contribute to the activation of ethylene signalling pathway in the absence of ethylene itself (Testerink et al., 2007). Although, provided that CTR1 is co-localised with ethylene receptor at the ER, an allocation of different pool of PA (comparing to plasma membrane PA) to this process should be considered.

More PA target proteins involved in its mediating role in plant responses to abiotic stresses are now described. PA directly stimulates the activity of salt stress-induced MPK6 kinase from *A. thaliana in vitro*. This interaction is considered to be important, since a Salt Overly Sensitive 1 (SOS1), a key agent of plant tolerance to salinity, is a downstream target of MPK6 (Yu et al., 2010). Other PA-binding proteins implicated in plant responses to salinity were identified in cell cultures of tomato and *A. thaliana*, such as SnRK2 protein kinase, RCN1 (a regulatory subunit of phosphatase 2A), and DRG (a GTP-binding protein) (Testerink et al., 2004). AtPTEN2, a protein/phosphoinositide phosphatase from *A. thaliana* is another target of PA (Pribat et al., 2012). PA can bind to and activate 3'-phosphoinositide-dependent kinase-1 (PDK1) in *A. thaliana* that, in turn, controls the activity of AGC2-1 protein kinase (Anthony et al., 2004), a regulator of plant metabolism and defence reactions (Garcia et al., 2012).

DHN1, a dehydrin from maize, also binds to PA (Koag et al., 2009). In *C. plantagineum*, a so-called "resurrection plant" capable of surviving severe dehydration, a CDeT11-24 protein, a late embryogenesis abundant-like and intrinsically disordered, binds to PA. This association is an important regulatory event for plant survival following desiccation (Petersen et al., 2012).

PA binding to 14-3-3 proteins in maize resulted in the downstream regulation of plasma membrane H^+-ATPase activity (Camoni et al., 2012). In this regard it would be of great interest to know if PA is a modulator of the effect of 14-3-3 proteins on their numerous protein targets, including those implicated in plant growth regulation and hormonal signalling (for review see Boer et al., 2013).

PA-binding to various proteins/enzymes appears to be similarly important for re-programming a lipid metabolism in plants. The detailed PA-binding characteristics of a trigalactosyldiacylglycerol 4 – a lipid trafficking protein – has been recently reported (Wang et al., 2013b) indicating a potential role for PA to shift main metabolic fluxes in response to environmental stimuli. Similar conclusion can be drawn from the fact that PA also binds to such metabolically important enzymes as acyl-CoA-binding protein (Du et al., 2010).

The molecular role of PA in the regulation of cytoskeletal dynamics has been recently reviewed (Pleskot et al., 2013). Among the mechanisms involved, there is a direct PA binding to microtubule-bundling protein MAP65-1 (Zhang et al., 2012) and capping protein (Li et al., 2012). These interactions are also thought to promote stress tolerance in *A. thaliana*.

A link between PA and clathrin-mediated endocytosis has been demonstrated in animals (Antonescu et al., 2010). In plants, a similar relationship is likely to be present. Two clathrin heavy chain proteins and two putative clathrin assembly proteins from *A. thaliana* bind to PA (McLoughlin et al., 2013). More important, it was shown that upon salt stress, these proteins are recruited to the plasma membrane in a PA-dependent manner. This may be important for stress-induced vesicle-mediated transport aimed at rapid turnover of membrane components. One of the mechanism, describing the role of clathrin-mediated endocytosis in plant stress reactions, was characterised, suggesting that clathrin-mediated endocytic recycling of PIN2 results in subsequent auxin-dependent growth that facilitates negative root salt tropism (Galvan-Ampudia et al., 2013).

Despite the number of identified PA-binding proteins, no consensus for the PA-binding site could be identified in such proteins. This hampers a bioinformatics research of PA-binding proteins in databases. Not surprisingly, a critical role of positively charged amino acids in mediating electrostatic interactions between PA and targeted proteins was proposed (Kooijman and Testerink, 2010; Liu et al., 2013b).

4.2. Molecular targets of diacylglycerol and diacylglycerol pyrophosphate in plant cells

Little is known about the molecular targets of diacylglycerol pyrophosphate (DGPP) and diacylglycerol (DAG) in plants. In contrast to animal systems, where DAG is a ubiquitous activator of PKC via its binding to C1 domain (Leonard and Hurley, 2011), the signalling role of DAG in plants is still questionable (Dong et al., 2012). Even though several putative C1 domain-containing proteins are encoded by plant genomes (Janda et al., 2013), the molecular targets of DAG in plants await for experimental verification. A majority of DAG-dependent signalling is ascribed to DAG as a PA precursor (Arisz et al., 2013). Recently, we showed that the basal expression of genes could be affected in the presence of PI-PLC inhibitors, but also of DGK inhibitors (Djafi et al., 2013). Interestingly, there is an over representation of genes for which the effect (inducing or inhibiting) of PI-PLC inhibitors and of DGK inhibitor is the same: the basal expression of those genes is controlled by PA synthesised through a PI-PLC pathway. Amongst genes negatively controlled are some *DREB2* genes. The fact that PA, and not DAG, is the active signalling molecule can also be concluded from reverse genetic approach. The knockdown of *DS1*, a PA phosphatase gene, resulted in an accumulation of PA in plant tissues and induced resistance of *Nicotiana benthamiana* plants to the infection by *Ralstonia Solanacearum*. In contrast, *DS1*-overexpressing plants that had low levels of PA (and presumably high levels of DAG) had weakened defence-related responses and higher susceptibility to the pathogen (Nakano et al., 2013). Nevertheless, DAG appears to have its role in particular plant activities. The formation of lateral roots was impaired in *npc5-1* knockout mutants of *A. thaliana* under salt stress. This effect had correlated with the diminished content of DAG in roots of such plants. Intriguingly, the supplementation of DAG, but not PA, to the cultivation media has reverted the effect of salt on root development in *npc5-1* plants (Peters et al., 2014).

DGPP is an almost undetectable lipid of cell membranes under normal growth conditions. In contrast, it can be detected in plants in response to some stimuli including hyperosmotic stress and elicitor treatment (Jeannette et al., 2010). In plants the production of DGPP is always related to accumulation of PA. One can assume that DGPP could represent a PA signal attenuation. However, DGPP is likely to have a proper signalling role, as it is the case in ABA signal transduction; exogenous DGPP, and not PA, stimulated RAB18 expression (Zalejski et al., 2005). DGPP and PA have also been shown to have considerably different biophysical properties. The presence of two phosphate groups on DGPP, instead of one on PA, will affect the interaction mode with putative targets. Electrostatic forces (attractive towards positively charged residues, repulsive

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

against negatively charged ones) will be stronger with DGPP. On the other hand, the distance of the phosphate group to the bilayer interface will be longer for DGPP than for PA (Strawn et al., 2012).

4.3. Molecular targets of phosphoinositides and inositolphosphates

Phosphoinositides compose a divergent group of phospholipids derived from phosphatidylinositol. Despite their low content in plant membranes, phosphoinositides participate in regulation of numerous cell activities. Some of their regulatory functions are implemented via binding/recruiting of protein targets. These interactions are mediated by PH, PX, FYVE domains and other structural motifs. Particular isoforms of PLD were shown to bind polyphosphoinositides (*e.g.* PI(4,5)P_2) resulting in their activation (Wang et al., 2012). This is considered as an important link between two branches of lipid signalling. Among other polyphosphoinositide-binding proteins are those related to the regulation of actin cytoskeleton (Pleskot et al., 2014). The presence of dissimilar intracellular pools and molecular species of phosphoinositides with distinct regulatory functions has been suggested (Heilmann and Heilmann, 2013). It is also important to mention that different phosphoinositides can be easily interconverted in plant cells. This raises the question of the role of corresponding lipid kinases and phosphatases in cell regulation (Delage et al., 2012).

Inositol-phosphates arise from the hydrolysis of phosphoinositides by PI-PLC. The mechanisms behind a inositol-phosphate dependent signalling in plants are dissimilar to those in animals (Pokotylo et al., 2014a). However, a rapid accumulation of IP_3 in plants is similarly associated with Ca^{2+} release (Zheng et al., 2012).

4.4. Role of phosphoglycerolipids in modulation of membrane properties

Phospholipids constitute a major part of cell membrane as intrinsic structural components. When produced, the majority of the phospholipid signalling molecules still reside in cell membranes. This postulates their contribution to complex changes of their physical properties. The diminishing levels of phospholipids that are processed by phospholipases as substrates may also have a regulatory effect *per se* (Pokotylo et al., 2014a). That is why the downstream effects of lipid signalling can be achieved by affecting the membrane-associated activities. Among them are cell vesicular trafficking, endocytosis, secretion and other processes associated with changes to membrane dynamics (Galvan-Ampudia et al., 2013; Li and Xue, 2007). However the molecular role of lipid mediators in these processes is difficult to generalise.

In plant cells, the composition and structure of various cell membranes are highly dynamic. In quiescent cells, the plasma membrane is predominantly found in a stable bilayer state. However, upon stimulation, it could rapidly produce non-bilayer phases provided that lipid molecules favoring this organisation are present at a high enough molecular fraction. Phosphoinositides, DAG, and PA belong to this lipid group (Kooijman et al., 2003). Unlike most phospholipids that has a cylinder "shape", DAG and PA are cone-shaped molecules. In fact, for PA this shape exists at conditions typical of those in Golgi vesicles, but under cytoplasmic conditions, PA behaves as a cylindrical lipid (Kooijman et al., 2003).

Hence, the general notion suggests that, when introduced into lipid bilayer, these molecules loosen the membrane and excite membrane curvature rendering it prone for fusion/fission activities. As an example it is long known that PA stimulates vesicle budding from Golgi apparatus in animals (Siddhanta and Shields, 1998). Similar conclusions were later reported for DAG (Fernández-Ulibarri et al., 2007). However, the general pattern of the impact of such lipid mediators on cell membranes in plants is still unclear. Current clues are pointing at their unspecific role in rendering a cell membrane less stable ready for rapid turnover required for perception and efficient implementation of environmental cues on the cellular level. However, signalling lipids are far from being abundant, and unless being locally enriched (by interaction with proteins, for instance) their potential physical effect can be buffered by the other (dominant) lipids.

5. Conclusions and open questions

Phosphoglycerolipid-based signalling is a ubiquitous mechanism employed for transduction of environmental cues. Such diversity of functions is implemented via a limited set of lipid and soluble messengers that raises the question of the specificity of these signals. Many researchers agree that spatial (*i.e.* in which cellular compartment) and temporal (*i.e.* when and how long the production is sustained) features of lipid signalling are important (*e.g.* biphasic PA accumulation following stress responses (Andersson et al., 2006)). In addition, most lipid mediators are composed of several molecular species owing to differences in their FA composition. This factor is now being considered as an important criterion when assaying the cellular effects of lipid mediators (Peters et al., 2010; Rainteau et al., 2012). Different molecular species of PA had markedly variable affinity towards certain protein targets (Zhang et al., 2009).

In support of this suggestion, the phosphatidylbutanol (PBut) produced by PLD in response to SA treatment in *A. thaliana* cell has the same FA composition as PBut produced in untreated cells (Rainteau et al., 2012). So it might be not the FA composition that is important for the activation of hormone-triggered responses but the level of the active molecule(s). In the same manner, it can be expected that dissimilarities in FA composition of DAG produced by NPCs and PI-PLCs do not impede its conversion by DGK thus contributing to PA-signalling.

So far the multiplicity of regulatory effects exerted by lipid-signalling is commonly explained by protein binding partners that downstream mediate signalling on the whole-cell level. On this basis, the role of PA in osmotic/salt stressed plants has been recently summarised (McLoughlin and Testerink, 2013). More and more novel lipid-binding proteins are now being discovered. However, the results of *in vitro* binding assays, as well as studies exploiting exogenous application of lipid mediators, should be taken with caution since they may not fully correspond to the processes that occur *in planta*. In addition, researches should not restrict their attention to PA, since other lipid (*e.g.* DAG, lysophospholipids) may be equally effective in binding plant proteins.

Several other concepts regarding functioning of lipid signalling in plants should be carefully considered. First, "lipid mediator signature" represents the pattern of lipid messenger production, conversion and decay which is notably specific to a particular stimulus. As an example, PA phosphorylation into DGPP has been observed in response to elicitors (van der Luit et al., 2000), ABA (Zalejski et al., 2005) and salt stress (Darwish et al., 2009) but not in response to cold stress (Ruelland et al., 2002).

It has been found that some phospholipases themselves are able to regulate cell activities via protein-protein binding. PLDδ was shown to bind two glyceraldehyde-3-phosphate dehydrogenases of *A. thaliana* – GAPC1 and GAPC2 – implicated in cell energy metabolism (Guo et al., 2012a). Such interaction was promoted by H_20_2 and resulted in PLDδ activation. Intriguingly, it was later revealed that PA also binds glyceraldehyde-3-phosphate dehydrogenases in *Camelina sativa* and *A. thaliana*. It has been observed that PA induce proteolytic cleavage of GAPC2 in *A. thaliana in vivo*, suggesting a feedback regulatory loop (Kim et al., 2013). This suggests that studies aimed at identification of protein-protein interaction

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

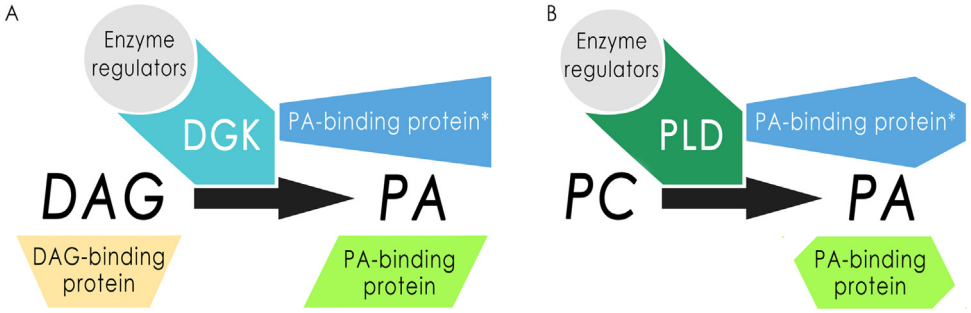

Fig. 5. Models demonstrating PA-producing enzymes, DGK (A) or PLD (B), as a part of supramolecular complexes. DGK or PLD can be involved in protein-protein interaction with proteins that will regulate their activity (enzyme regulators). The lipid they produce (PA) can be bound by proteins. Some PA-binding proteins might also bind PA-producing enzymes, as shown by (Du et al., 2013, 2010). DAG, diacylglycerol; DGK, diacylglycerol kinase; PA, phosphatidic acid; PC, phosphatidylcholine; PLD, phospholipase D; *, proteins with the dual capability for binding PA and PA-producing enzymes.

implicating PA-producing enzymes (DGK, PLD) can at the same time be a strategy for identifying PA binding proteins. For instance, ACBP1 interacts with both PLDα1 and PA (Du et al., 2013; Du et al., 2010). On this basis a concept of "dynamic lipid signalling platform" can be proposed (Fig. 5.). It can thus be assumed that a particular PA-binding protein may first bind a PA-producing enzyme. This interaction may facilitate a production of PA that further mediates the activation or translocation of the very same protein. This may also suggests a feedback connection between lipid mediators and lipid-generation enzymes.

Rendering lipid signalling even more complex is the fact that changes in the levels of phospholipases substrates have a regulatory role (*e.g.* stress-regulated phosphoinositides) (Delage et al., 2013; Oxley et al., 2013). In addition, a tight interplay of lipid signalling with lipid metabolism as well as with other cell signalling systems (*e.g.* Ca^{2+}, NO, ROS) is also evident.

Another exciting question is about the possibility that lipid rafts, *i.e.* the detergent-insoluble membrane fraction extracted from a plasma membrane, might be a lipid signalling platform. Phosphoinositides are enriched in plant membrane rafts where up to a half of all cell phosphoinositides are present. PLD, PI4 kinase and PI4P5 kinase activities are also detected in this membrane subfraction. Nevertheless, their activity is not enriched in lipid rafts when compared to the control plasma membrane fraction (Furt et al., 2010). More experimental data are necessary to establish the role of detergent-insoluble membranes or other isolated pools of signalling lipids in cell regulation.

Finally, a biotechnological implementation of lipid signalling is another important task offering great benefits. Numerous data suggest possibilities for increasing plant endurance in stress conditions achieved via manipulations with lipid-signalling (Georges et al., 2009; Peters et al., 2014; Wang et al., 2008). However, induced resistance of crops may commonly come at the price of reduced yield. Therefore a delicate balancing is required.

Acknowledgements

This work was supported by grants from the NAS of Ukraine (2.1.10.32-10 and 9.1-12.06), the Czech Science Foundation (P501/11/1654), the Centre National de la Recherche Scientifique (CNRS) and the Université Paris Est Créteil (UPEC). We would like to thank Prof. Jill Farrant (Department of Molecular and Cell Biology, University of Cape Town, South Africa) for carefully reading the manuscript.

References

Abd-El-Haliem, A., Meijer, H.J.G., Tameling, W.I.L., Vossen, J.H., Joosten, M.H.A.J., 2012. Defense activation triggers differential expression of *phospholipase-C (PLC)* genes and elevated temperature induces phosphatidic acid (PA) accumulation in tomato. Plant Signal. Behav. 7, 1073–1078.

Altúzar-Molina, A.R., Muñoz-Sánchez, J.A., Vázquez-Flota, F., Monforte-González, M., Racagni-Di Palma, G., Hernández-Sotomayor, S.M.T., 2011. Phospholipidic signaling and vanillin production in response to salicylic acid and methyl jasmonate in *Capsicum chinense* J. cells. Plant Physiol. Biochem. 49, 151–158.

Andersson, M.X., Kourtchenko, O., Dangl, J.L., Mackey, D., Ellerström, M., 2006. Phospholipase-dependent signalling during the AvrRpm1- and AvrRpt2-induced disease resistance responses in *Arabidopsis thaliana*. Plant J. 47, 947–959.

Anthony, R.G., Henriques, R., Helfer, A., Meszaros, T., Rios, G., Testerink, C., Munnik, T., Deak, M., Koncz, C., Bogre, L., 2004. A protein kinase target of a PDK1 signalling pathway is involved in root hair growth in Arabidopsis. EMBO J. 23, 572–581.

Antonescu, C.N., Danuser, G., Schmid, S.L., 2010. Phosphatidic acid plays a regulatory role in clathrin-mediated endocytosis. Mol. Biol. Cell 21, 2944–2952.

Arisz, S.A., Munnik, T., 2011. The salt stress-induced LPA response in *Chlamydomonas* is produced via PLA2 hydrolysis of DGK-generated phosphatidic acid. J. Lipid Res. 52, 2012–2020.

Arisz, S.A., Testerink, C., Munnik, T., 2009. Plant PA signaling via diacylglycerol kinase. Biochim. Biophys. Acta (BBA) – Mol. Cell Biol. Lipids 1791, 869–875.

Arisz, S.A., van Wijk, R.v., Roels, W., Zhu, J.-K., Haring, M.A., Munnik, T., 2013. Rapid phosphatidic acid accumulation in response to low temperature stress in Arabidopsis is generated through diacylglycerol kinase. Front. Plant Sci., 4.

Armando Muñoz-Sánchez, J., Altúzar-Molina, A., Teresa Hernández-Sotomayor, S.M., 2012. Phospholipase signaling is modified differentially by phytoregulators in *Capsicum chinense* J. cells. Plant Signal. Behav. 7, 1103–1105.

Bargmann, B.O.R., Laxalt, A.M., Riet, B.T., Testerink, C., Merquiol, E., Mosblech, A., Leon-Reyes, A., Pieterse, C.M.J., Haring, M.A., Heilmann, I., Bartels, D., Munnik, T., 2009. Reassessing the role of phospholipase D in the Arabidopsis wounding response. Plant, Cell Environ. 32, 837–850.

Boer, A., Kleeff, P.M., Gao, J., 2013. Plant 14-3-3 proteins as spiders in a web of phosphorylation. Protoplasma 250, 425–440.

Camoni, L., Di Lucente, C., Pallucca, R., Visconti, S., Aducci, P., 2012. Binding of phosphatidic acid to 14-3-3 proteins hampers their ability to activate the plant plasma membrane H^+-ATPase. IUBMB Life 64, 710–716.

Canonne, J., Froidure-Nicolas, S., Rivas, S., 2011. Phospholipases in action during plant defense signaling. Plant Signal. Behav. 6, 13–18.

Chen, G., Greer Michael, S., Weselake Randall, J., 2013. Plant phospholipase A: advances in molecular biology, biochemistry, and cellular function. Biomol. Concepts, 527.

Chen, G., Greer, M.S., Lager, I., Lindberg Yilmaz, J., Mietkiewska, E., Carlsson, A.S., Stymne, S., Weselake, R.J., 2012. Identification and characterization of an LCAT-like *Arabidopsis thaliana* gene encoding a novel phospholipase A. FEBS Lett. 586, 373–377.

Cruz-Ramírez, A., Oropeza-Aburto, A., Razo-Hernández, F., Ramírez-Chávez, E., Herrera-Estrella, L., 2006. Phospholipase DZ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. Proc. Natl. Acad. Sci. U. S. A. 103, 6765–6770.

D'Angelo, G., Vicinanza, M., Di Campli, A., De Matteis, M.A., 2008. The multiple roles of PtdIns(4)P – not just the precursor of PtdIns(4,5)P2. J. Cell Sci. 121, 1955–1963.

Darwish, E., Testerink, C., Khalil, M., El-Shihy, O., Munnik, T., 2009. Phospholipid signaling responses in salt-stressed rice leaves. Plant Cell Physiol. 50, 986–997.

Delage, E., Puyaubert, J., Zachowski, A., Ruelland, E., 2013. Signal transduction pathways involving phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate: convergences and divergences among eukaryotic kingdoms. Prog. Lipid Res. 52, 1–14.

Delage, E., Ruelland, E., Guillas, I., Zachowski, A., Puyaubert, J., 2012. Arabidopsis type-III phosphatidylinositol 4-kinases β1 and β2 are upstream of the phospholipase C pathway triggered by cold exposure. Plant Cell Physiol 53, 565–576.

Derksen, H., Rampitsch, C., Daayf, F., 2013. Signaling cross-talk in plant disease resistance. Plant Sci. 207, 79–87.

DeWald, D.B., Torabinejad, J., Jones, C.A., Shope, J.C., Cangelosi, A.R., Thompson, J.E., Prestwich, G.D., Hama, H., 2001. Rapid accumulation of phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate correlates with

calcium mobilization in salt-stressed Arabidopsis. Plant Physiol. 126, 759–769.
Djafi, N., Vergnolle, C., Cantrel, C., Wietrzynski, W., Delage, E., Cochet, F., Puyaubert, J., Soubigou-Taconnat, L., Gey, D., Collin, S., Balzergue, S., Zachowski, A., Ruelland, E., 2013. The Arabidopsis DREB2 genetic pathway is constitutively repressed by basal phosphoinositide-dependent phospholipase C coupled to diacylglycerol kinase. Front. Plant Sci. 4.
Dong, W., Lv, H., Xia, G., Wang, M., 2012. Does diacylglycerol serve as a signaling molecule in plants? Plant Signal. Behav. 7, 472–475.
Drøbak, B.K., Dewey, R.E., Boss, W.F., 1999. Phosphoinositide kinases and the synthesis of polyphosphoinositides in higher plant cells. In: Kwang, W.J. (Ed.), International Review of Cytology. Academic Press, New York, pp. 95–130.
Drøbak, B.K., Watkins, P.A.C., 2000. Inositol(1,4,5)trisphosphate production in plant cells: an early response to salinity and hyperosmotic stress. FEBS Lett. 481, 240–244.
Du, Z.-Y., Chen, M.-X., Chen, Q.-F., Xiao, S., Chye, M.-L., 2013. Arabidopsis acyl-CoA-binding protein ACBP1 participates in the regulation of seed germination and seedling development. Plant J. 74, 294–309.
Du, Z.-Y., Xiao, S., Chen, Q.-F., Chye, M.-L., 2010. Depletion of the membrane-associated acyl-coenzyme A-binding protein ACBP1 enhances the ability of cold acclimation in Arabidopsis. Plant Physiol. 152, 1585–1597.
Dutilleul, C., Benhassaine-Kesri, G., Demandre, C., Rézé, N., Launay, A., Pelletier, S., Renou, J.-P., Zachowski, A., Baudouin, E., Guillas, I., 2012. Phytosphingosine-phosphate is a signal for AtMPK6 activation and Arabidopsis response to chilling. New Phytol. 194, 181–191.
Effendi, Y., Radatz, K., Rietz, S., Labusch, C., Wimalasekera, R., Zeidler, M., Scherer, G.F.E., 2014. Mutants of phospholipase A (pPLA-I) have a red light and auxin phenotype. Plant Cell Environ. 37, 1626–1640.
Elmore, J.M., Liu, J., Smith, B., Phinney, B., Coaker, G., 2012. Quantitative proteomics reveals dynamic changes in the plasma membrane during *Arabidopsis* immune signaling. Mol. Cell. Proteomics 11, M111.014555.
Exton, J.H., 2002. Regulation of phospholipase D. FEBS Lett. 531, 58–61.
Fernández-Ulibarri, I., Vilella, M., Lázaro-Diéguez, F., Sarri, E., Martínez, S.E., Jiménez, N., Claro, E., Mérida, I., Burger, K.N.J., Egea, G., 2007. Diacylglycerol is required for the formation of COPI vesicles in the golgi-to-ER transport pathway. Mol. Biol. Cell 18, 3250–3263.
França, M.G.C., Matos, A.R., D'Arcy-Lameta, A., Passaquet, C., Lichtlé, C., Zuily-Fodil, Y., Pham-Thi, A.T., 2008. Cloning and characterization of drought-stimulated phosphatidic acid phosphatase genes from *Vigna unguiculata*. Plant Physiol. Biochem. 46, 1093–1100.
Furt, F., König, S., Bessoule, J.J., Sargueil, F., Zallot, R., Stanislas, T., Noirot, E., Lherminier, J., Simon-Plas, F., Heilmann, I., Mongrand, S., 2010. Polyphosphoinositides are enriched in plant membrane rafts and form microdomains in the plasma membrane. Plant Physiol. 152, 2173–2187.
Galvan-Ampudia, Carlos S., Julkowska, Magdalena M., Darwish, E., Gandullo, J., Korver, Ruud A., Brunoud, G., Haring, Michel A., Munnik, T., Vernoux, T., Testerink, C., 2013. Halotropism is a response of plant roots to avoid a saline environment. Curr. Biol. 23, 2044–2050.
Gao, H.-B., Chu, Y.-J., Xue, H.-W., 2013. Phosphatidic acid (PA) binds PP2AA1 to regulate PP2A activity and PIN1 polar localization. Mol. Plant 6, 1692–1702.
Gao, K., Liu, Y.-L., Li, B., Zhou, R.-G., Sun, D.-Y., Zheng, S.-Z., 2014. Phosphoinositide-specific phospholipase C isoform 3 (AtPLC3) and AtPLC9 function additionally to each other in thermotolerance in *Arabidopsis thaliana*. Plant Cell Physiol., http://dx.doi.org/10.1093/pcp/pcu116.
Garcia, A., Al-Yousif, M., Hirt, H., 2012. Role of AGC kinases in plant growth and stress responses. Cell. Mol. Life Sci. 69, 3259–3267.
Gaude, N., Nakamura, Y., Scheible, W.R., Ohta, H., Dörmann, P., 2008. Phospholipase C5 (NPC5) is involved in galactolipid accumulation during phosphate limitation in leaves of Arabidopsis. Plant J. 56, 28–39.
Ge, H., Chen, C., Jing, W., Zhang, Q., Wang, H., Wang, R., Zhang, W., 2012. The rice diacylglycerol kinase family: functional analysis using transient RNA interference. Front. Plant Sci., 3.
Georges, F., Das, S., Ray, H., Bock, C., Nokhrina, K., Kolla, V.A., Keller, W., 2009. 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, 1664–1681.
Ghars, M.A., Richard, L., Lefebvre-De Vos, D., Leprince, A.-S., Parre, E., Bordenave, M., Abdelly, C., Savouré, A., 2012. 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, 183–192.
Gómez-Merino, F.C., Arana-Ceballos, F.A., Trejo-Téllez, L.I., Skirycz, A., Brearley, C.A., Dörmann, P., Mueller-Roeber, B., 2005. 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, 34888–34899.
Gómez-Merino, F.C., Brearley, C.A., Ornatowska, M., Abdel-Haliem, M.E.F., Zanor, M.-I., Mueller-Roeber, B., 2004. AtDGK2, a novel diacylglycerol kinase from *Arabidopsis thaliana*, phosphorylates 1-stearoyl-2-arachidonoyl-sn-glycerol and 1,2-dioleoyl-sn-glycerol and exhibits cold-inducible gene expression. J. Biol. Chem. 279, 8230–8241.
Guo, L., Devaiah, S.P., Narasimhan, R., Pan, X., Zhang, Y., Zhang, W., Wang, X., 2012a. Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in the Arabidopsis response to stress. Plant Cell Online 24, 2200–2212.
Guo, L., Mishra, G., Markham, J.E., Li, M., Tawfall, A., Welti, R., Wang, X., 2012b. Connections between sphingosine kinase and phospholipase D in the abscisic acid signaling pathway in Arabidopsis. J. Biol. Chem. 287, 8286–8296.
Guo, L., Mishra, G., Taylor, K., Wang, X., 2011. Phosphatidic acid binds and stimulates Arabidopsis sphingosine kinases. J. Biol. Chem. 286, 13336–13345.
Heilmann, M., Heilmann, I., 2013. Arranged marriage in lipid signalling? The limited choices of PtdIns(4,5)P2 in finding the right partner. Plant Biol. 15, 789–797.
Heinze, M., Herre, M., Massalski, C., Hermann, I., Conrad, U., Roos, W., 2013. Signal transfer in the plant plasma membrane: phospholipase A2 is regulated via an inhibitory Gα protein and a cyclophilin. Biochem. J. 450, 497–509.
Hirayama, T., Ohto, C., Mizoguchi, T., Shinozaki, K., 1995. A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A. 92, 3903–3907.
Hong, Y., Devaiah, S.P., Bahn, S.C., Thamasandra, B.N., Li, M., Welti, R., Wang, X., 2009. Phospholipase Dε and phosphatidic acid enhance Arabidopsis nitrogen signaling and growth. Plant J. 58, 376–387.
Hong, Y., Pan, X., Welti, R., Wang, X., 2008a. Phospholipase Dα3 Is Involved in the hyperosmotic response in Arabidopsis. Plant Cell Online 20, 803–816.
Hong, Y., Zheng, S., Wang, X., 2008b. Dual functions of phospholipase Dα1 in plant response to drought. Mol. Plant 1, 262–269.
Horváth, I., Glatz, A., Nakamoto, H., Mishkind, M.L., Munnik, T., Saidi, Y., Goloubinoff, P., Harwood, J.L., Vigh, L., 2012. Heat shock response in photosynthetic organisms: membrane and lipid connections. Prog. Lipid Res. 51, 208–220.
Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Widmayer, P., Gruissem, W., Zimmermann, P., 2008. Genevestigator V3: a reference expression database for the meta-analysis of transcriptomes. Adv. Bioinform. 2008, 5.
Hunt, L., Mills, L.N., Pical, C., Leckie, C.P., Aitken, F.L., Kopka, J., Mueller-Roeber, B., McAinsh, M.R., Hetherington, A.M., Gray, J.E., 2003. Phospholipase C is required for the control of stomatal aperture by ABA. Plant J. 34, 47–55.
Hunt, L., Otterhag, L., Lee, J.C., Lasheen, T., Hunt, J., Seki, M., Shinozaki, K., Sommarin, M., Gilmour, D.J., Pical, C., Gray, J.E., 2004. Gene-specific expression and calcium activation of Arabidopsis thaliana phospholipase C isoforms. New Phytol. 162, 643–654.
Im, J., Lee, H., Kim, J., Kim, H., An, C., 2012. Soybean MAPK, GMK1 is dually regulated by phosphatidic acid and hydrogen peroxide and translocated to nucleus during salt stress. Mol. Cells 34, 271–278.
Janda, M., Planchais, S., Djafi, N., Martinec, J., Burketova, L., Valentova, O., Zachowski, A., Ruelland, E., 2013. Phosphoglycerolipids are master players in plant hormone signal transduction. Plant Cell Rep. 32, 839–851.
Jeannette, E., Paradis, S., Zalejski, C., 2010. Diacylglycerol pyrophosphate, a novel plant signaling lipid. In: Munnik, T. (Ed.), Lipid Signaling in Plants. Springer Verlag, Berlin, pp. 263–276.
Jia, Y., Tao, F., Li, W., 2013. Lipid profiling demonstrates that suppressing *Arabidopsis* phospholipase Dδ retards ABA-promoted leaf senescence by attenuating lipid degradation. PLoS ONE 8, e65687.
Katagiri, T., Ishiyama, K., Kato, T., Tabata, S., Kobayashi, M., Shinozaki, K., 2005. An important role of phosphatidic acid in ABA signaling during germination in *Arabidopsis thaliana*. Plant J. 43, 107–117.
Katagiri, T., Takahashi, S., Shinozaki, K., 2001. Involvement of a novel *Arabidopsis* phospholipase D, AtPLDδ, in dehydration-inducible accumulation of phosphatidic acid in stress signalling. Plant J. 26, 595–605.
Kim, D., Jeun, Y., Hwang, B., 2014. The pepper patatin-like phospholipase CaPLP1 functions in plant cell death and defense signaling. Plant Mol. Biol. 84, 329–344.
Kim, S.-C., Guo, L., Wang, X., 2013. Phosphatidic acid binds to cytosolic glyceraldehyde-3-phosphate dehydrogenase and promotes its cleavage in *Arabidopsis*. J. Biol. Chem. 288, 11834–11844.
Kim, Y.J., Kim, J.E., Lee, J.-H., Lee, M.H., Jung, H.W., Bahk, Y.Y., Hwang, B.K., Hwang, I., Kim, W.T., 2004. 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, 127–136.
Kirik, A., Mudgett, M.B., 2009. SOBER1 phospholipase activity suppresses phosphatidic acid accumulation and plant immunity in response to bacterial effector AvrBsT. Proc. Natl. Acad. Sci. U. S. A. 106, 20532–20537.
Koag, M.-C., Wilkens, S., Fenton, R.D., Resnik, J., Vo, E., Close, T.J., 2009. The K-Segment of maize DHN1 mediates binding to anionic phospholipid vesicles and concomitant structural changes. Plant Physiol. 150, 1503–1514.
Kocourková, D., Krčková, Z., Pejchar, P., Veselková, Š., Valentová, O., Wimalasekera, R., Scherer, G.F.E., Martinec, J., 2011. The phosphatidylcholine-hydrolysing phospholipase C NPC4 plays a role in response of *Arabidopsis* roots to salt stress. J. Exp. Bot. 62, 3753–3763.
Kolesnikov, Y.S., Nokhrina, K.P., Kretynin, S.V., Volotovski, I.D., Martinec, J., Romanov, G.A., Kravets, V.S., 2012. Molecular structure of phospholipase D and regulatory mechanisms of its activity in plant and animal cells. Biochemistry (Moscow) 77, 1–14.
Kooijman, E., Testerink, C., 2010. Phosphatidic acid: an electrostatic/hydrogen-bond switch? In: Munnik, T. (Ed.), Lipid Signaling in Plants. Springer Verlag, Berlin, pp. 203–222.
Kooijman, E.E., Chupin, V., de Kruijff, B., Burger, K.N.J., 2003. Modulation of membrane curvature by phosphatidic acid and lysophosphatidic acid. Traffic 4, 162–174.
Kravets, V.S., Nokhrina, K.P., 1998. Reaction of polyphosphatidylinositol metabolism to cold shock treatment. Rep. Natl. Acad. Sci. Ukr. 11, 166–169.

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

Krinke, O., Flemr, M., Vergnolle, C., Collin, S., Renou, J.P., Taconnat, L., Yu, A., Burketová, L., Valentová, O., Zachowski, A., Ruelland, E., 2009. Phospholipase D activation is an early component of the salicylic acid signaling pathway in Arabidopsis cell suspensions. Plant Physiol. 150, 424–436.
Krinke, O., Novotná, Z., Valentová, O., Martinec, J., 2007a. Inositol trisphosphate receptor in higher plants: is it real? J. Exp. Bot. 58, 361–376.
Krinke, O., Ruelland, E., Valentová, O., Vergnolle, C., Renou, J.-P., Taconnat, L., Flemr, M., Burketová, L., Zachowski, A., 2007b. Phosphatidylinositol 4-kinase activation is an early response to salicylic acid in Arabidopsis suspension cells. Plant Physiol. 144, 1347–1359.
Labusch, C., Shishova, M., Effendi, Y., Li, M., Wang, X., Scherer, G.F.E., 2013. Patterns and timing in expression of early auxin-induced genes in phospholipase A (pPLA) T-DNA insertion mutants reveal a function in auxin signaling. Mol. Plant 6, 1473–1486.
Lanteri, M.L., Laxalt, A.M., Lamattina, L., 2008. Nitric oxide triggers phosphatidic acid accumulation via phospholipase D during auxin-induced adventitious root formation in cucumber. Plant Physiol. 147, 188–198.
Lee, Y., Kim, E.-S., Choi, Y., Hwang, I., Staiger, C.J., Chung, Y.-Y., Lee, Y., 2008. The Arabidopsis phosphatidylinositol 3-kinase is important for pollen development. Plant Physiol. 147, 1886–1897.
Lemtiri-Chlieh, F., MacRobbie, E.A.C., Webb, A.A.R., Manison, N.F., Brownlee, C., Skepper, J.N., Chen, J., Prestwich, G.D., Brearley, C.A., 2003. Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc. Natl. Acad. Sci. U. S. A. 100, 10091–10095.
Leonard, T.A., Hurley, J.H., 2011. Regulation of protein kinases by lipids. Curr. Opin. Struct. Biol. 21, 785–791.
Leshem, Y., Seri, L., Levine, A., 2007. Induction of phosphatidylinositol 3-kinase-mediated endocytosis by salt stress leads to intracellular production of reactive oxygen species and salt tolerance. Plant J. 51, 185–197.
Li, G., Lin, F., Xue, H.-W., 2007. Genome-wide analysis of the phospholipase D family in *Oryza sativa* and functional characterization of PLD[beta]1 in seed germination. Cell Res. 17, 881–894.
Li, G., Xue, H.-W., 2007. Arabidopsis *PLDζ2* regulates vesicle trafficking and is required for auxin response. Plant Cell Online 19, 281–295.
Li, J., Henty-Ridilla, J.L., Huang, S., Wang, X., Blanchoin, L., Staiger, C.J., 2012. Capping protein modulates the dynamic behavior of actin filaments in response to phosphatidic acid in Arabidopsis. Plant Cell Online 24, 3742–3754.
Li, W., Li, M., Zhang, W., Welti, R., Wang, X., 2004. The plasma membrane-bound phospholipase D[delta] enhances freezing tolerance in *Arabidopsis thaliana*. Nat. Biotechnol. 22, 427–433.
Liu, Q., Zhang, C., Yang, Y., Hu, X., 2010. Genome-wide and molecular evolution analyses of the phospholipase D gene family in poplar and grape. BMC Plant Biol. 10, 117.
Liu, X., Zhai, S., Zhao, Y., Sun, B., Liu, C., Yang, A., Zhang, J., 2013a. Overexpression of the phosphatidylinositol synthase gene (ZmPIS) conferring drought stress tolerance by altering membrane lipid composition and increasing ABA synthesis in maize. Plant Cell Environ. 36, 1037–1055.
Liu, Y., Su, Y., Wang, X., 2013b. Phosphatidic acid-mediated signaling. In: Capelluto, D.G.S. (Ed.), Lipid-mediated Protein Signaling. Springer, Netherlands, pp. 159–176.
Loew, S., Kooijman, E.E., May, S., 2013. Increased pH-sensitivity of protein binding to lipid membranes through the electrostatic-hydrogen bond switch. Chem. Phys. Lipids 169, 9–18.
Lu, S., Bahn, S.C., Qu, G., Qin, H., Hong, Y., Xu, Q., Zhou, Y., Hong, Y., Wang, X., 2013. Increased expression of phospholipase Dα1 in guard cells decreases water loss with improved seed production under drought in *Brassica napus*. Plant Biotechnol. J. 11, 380–389.
Mackey, D., Holt, B.F., Wiig, A., Dangl, J.L., 2002. RIN4 Interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell 108, 743–754.
Mane, S.P., Vasquez-Robinet, C., Sioson, A.A., Heath, L.S., Grene, R., 2007. Early PLDα-mediated events in response to progressive drought stress in *Arabidopsis*: a transcriptome analysis. J. Exp. Bot. 58, 241–252.
Matos, A.R., Gigon, A., Laffray, D., Pêtres, S., Zuily-Fodil, Y., Pham-Thi, A.-T., 2008. Effects of progressive drought stress on the expression of patatin-like lipid acyl hydrolase genes in *Arabidopsis* leaves. Physiol. Plant. 134, 110–120.
McLoughlin, F., Arisz, S.A., Dekker, H.L., Kramer, G., de Koster, C.G., Haring, M.A., Munnik, T., Testerink, C., 2013. Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of *Arabidopsis thaliana* roots. Biochem. J. 450, 573–581.
McLoughlin, F., Testerink, C., 2013. Phosphatidic acid, a versatile water-stress signal in roots. Front. Plant Sci., 4.
Michniewicz, M., Zago, M.K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I., Heisler, M.G., Ohno, C., Zhang, J., Huang, F., Schwab, R., Weigel, D., Meyerowitz, E.M., Luschnig, C., Offringa, R., Friml, J., 2007. Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. Cell 130, 1044–1056.
Mills, L.N., Hunt, L., Leckie, C.P., Aitken, F.L., Wentworth, M., McAinsh, M.R., Gray, J.E., Hetherington, A.M., 2004. The effects of manipulating phospholipase C on guard cell ABA-signalling. J. Exp. Bot. 55, 199–204.
Mishkind, M., Vermeer, J.E.M., Darwish, E., Munnik, T., 2009. Heat stress activates phospholipase D and triggers PIP2 accumulation at the plasma membrane and nucleus. Plant J. 60, 10–21.
Mishra, G., Zhang, W., Deng, F., Zhao, J., Wang, X., 2006. A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in Arabidopsis. Science 312, 264–266.
Monaghan, J., Zipfel, C., 2012. Plant pattern recognition receptor complexes at the plasma membrane. Curr. Opin. Plant Biol. 15, 349–357.
Munnik, T., Arisz, S.A., De Vrije, T., Musgrave, A., 1995. G protein activation stimulates phospholipase D signaling in plants. Plant Cell Online 7, 2197–2210.
Munnik, T., Laxalt, A., 2013. Measuring PLD activity *in vivo*. In: Munnik, T., Heilmann, I. (Eds.), Plant Lipid Signaling Protocols. Humana Press, New York, pp. 219–231.
Munnik, T., Meijer, H.J.G., Ter Riet, B., Hirt, H., Frank, W., Bartels, D., Musgrave, A., 2000. Hyperosmotic stress stimulates phospholipase D activity and elevates the levels of phosphatidic acid and diacylglycerol pyrophosphate. Plant J. 22, 147–154.
Munnik, T., Testerink, C., 2009. Plant phospholipid signaling: "In a nutshell". J. Lipid Res. 50, S260–S265.
Nakamura, Y., Awai, K., Masuda, T., Yoshioka, Y., Takamiya, K.I., Ohta, H., 2005. A novel phosphatidylcholine-hydrolyzing phospholipase C induced by phosphate starvation in Arabidopsis. J. Biol. Chem. 280, 7469–7476.
Nakano, M., Nishihara, M., Yoshioka, H., Takahashi, H., Sawasaki, T., Ohnishi, K., Hikichi, Y., Kiba, A., 2013. Suppression of DS1 phosphatidic acid phosphatase confirms resistance to *Ralstonia solanacearum* in *Nicotiana benthamiana*. PLoS ONE 8, e75124.
Narasimhan, R., Wang, G., Li, M., Roth, M., Welti, R., Wang, X., 2013. Differential changes in galactolipid and phospholipid species in soybean leaves and roots under nitrogen deficiency and after nodulation. Phytochemistry 96, 81–91.
Nováková, P., Hirsch, S., Feraru, E., Tejos, R., van Wijk, R., Viaene, T., Heilmann, M., Lerche, J., De Rycke, R., Feraru, M.I., Grones, P., Van Montagu, M., Heilmann, I., Munnik, T., Friml, J., 2014. SAC phosphoinositide phosphatases at the tonoplast mediate vacuolar function in *Arabidopsis*. Proc. Natl. Acad. Sci. 111, 2818–2823.
Novotná, Z., Linek, J., Hynek, R., Martinec, J., Potocký, M., Valentová, O., 2003. Plant PIP2-dependent phospholipase D activity is regulated by phosphorylation. FEBS Lett. 554, 50–54.
Oxley, D., Ktistakis, N., Farmaki, T., 2013. Differential isolation and identification of PI(3)P and PI(3,5)P2 binding proteins from Arabidopsis thaliana using an agarose-phosphatidylinositol-phosphate affinity chromatography. J. Proteomics 91, 580–594.
Pappan, K., Zheng, S., Wang, X., 1997. Identification and characterization of a novel plant phospholipase D that requires polyphosphoinositides and submicromolar calcium for activity in Arabidopsis. J. Biol. Chem. 272, 7048–7054.
Paradis, S., Villasuso, A.L., Aguayo, S.S., Maldiney, R., Habricot, Y., Zalejski, C., Machado, E., Sotta, B., Miginiac, E., Jeannette, E., 2011. *Arabidopsis thaliana* lipid phosphate phosphatase 2 is involved in abscisic acid signalling in leaves. Plant Physiol. Biochem. 49, 357–362.
Parre, E., Ghars, M.A., Leprince, A.-S., Thiery, L., Lefebvre, D., Bordenave, M., Richard, L., Mazars, C., Abdelly, C., Savouré, A., 2007. Calcium signaling via phospholipase C is essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in Arabidopsis. Plant Physiol. 144, 503–512.
Pejchar, P., Potocký, M., Novotná, Z., Veselková, Š., Kocourková, D., Valentová, O., Schwarzerová, K., Martinec, J., 2010. Aluminium ions inhibit the formation of diacylglycerol generated by phosphatidylcholine-hydrolysing phospholipase C in tobacco cells. New Phytol. 188, 150–160.
Peters, C., Kim, S.-C., Devaiah, S., Li, M., Wang, X., 2014. Non-specific phospholipase C5 and diacylglycerol promote lateral root development under mild salt stress in Arabidopsis. Plant, Cell Environ., http://dx.doi.org/10.1111/pce.12334.
Peters, C., Li, M., Narasimhan, R., Roth, M., Welti, R., Wang, X., 2010. Nonspecific phospholipase C NPC4 promotes responses to abscisic acid and tolerance to hyperosmotic stress in Arabidopsis. Plant Cell 22, 2642–2659.
Petersen, J., Eriksson, S.K., Harryson, P., Pierog, S., Colby, T., Bartels, D., Röhrig, H., 2012. The lysine-rich motif of intrinsically disordered stress protein CDeT11-24 from *Craterostigma plantagineum* is responsible for phosphatidic acid binding and protection of enzymes from damaging effects caused by desiccation. J. Exp. Bot. 63, 4919–4929.
Pierrugues, O., Brutesco, C., Oshiro, J., Gouy, M., Deveaux, Y., Carman, G.M., Thuriaux, P., Kazmaier, M., 2001. Lipid phosphate phosphatases in Arabidopsis. Regulation of the AtLPP1 gene in response to stress. J. Biol. Chem. 276, 20300–20308.
Pinosa, F., Buhot, N., Kwaaitaal, M., Fahlberg, P., Thordal-Christensen, H., Ellerström, M., Andersson, M.X., 2013. *Arabidopsis* phospholipase Dδ is involved in basal defense and nonhost resistance to powdery mildew fungi. Plant Physiol. 163, 896–906.
Pleskot, R., Li, J., Žárský, V., Potocký, M., Staiger, C.J., 2013. Regulation of cytoskeletal dynamics by phospholipase D and phosphatidic acid. Trends Plant Sci. 18, 496–504.
Pleskot, R., Pejchar, P., Staiger, C.J., Potocký, M., 2014. When fat is not bad: the regulation of actin dynamics by phospholipid signaling molecules. Front. Plant Sci., 5.
Pokotylo, I., Kolesnikov, Y., Kravets, V., Zachowski, A., Ruelland, E., 2014a. Plant phosphoinositide-dependent phospholipases C: variations around a canonical theme. Biochimie 96, 144–157.
Pokotylo, I.V., Kretynin, S.V., Khripach, V.A., Ruelland, E., Blume, Y.B., Kravets, V.S., 2014b. Influence of 24-epibrassinolide on lipid signalling and metabolism in *Brassica napus*. Plant Growth Regul. 73, 1–9.
Pokotylo, I., Pejchar, P., Potocký, M., Kocourková, D., Krčková, Z., Ruelland, E., Kravets, V., Martinec, J., 2013. The plant non-specific phospholipase C gene family. Novel competitors in lipid signalling. Prog. Lipid Res. 52, 62–79.
Pribat, A., Sormani, R., Rousseau-Gueutin, M., Julkowska, M.M., Testerink, C., Joubès, J., Castroviejo, M., Laguerre, M., Meyer, C., Germain, V., Rothan, C., 2012. A novel class of PTEN protein in Arabidopsis displays unusual phosphoinositide phosphatase activity and efficiently binds phosphatidic acid. Biochem. J. 441, 161–171.

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

Profotová, B., Burketová, L., Novotná, Z., Martinec, J., Valentová, O., 2006. Involvement of phospholipases C and D in early response to SAR and ISR inducers in *Brassica napus* plants. Plant Physiol. Biochem. 44, 143–151.
Qin, C., Wang, X., 2002. The *Arabidopsis* phospholipase D family. Characterization of a calcium-independent and phosphatidylcholine-selective PLDζ1 with distinct regulatory domains. Plant Physiol. 128, 1057–1068.
Raho, N., Ramirez, L., Lanteri, M.L., Gonorazky, G., Lamattina, L., ten Have, A., Laxalt, A.M., 2011. Phosphatidic acid production in chitosan-elicited tomato cells, via both phospholipase D and phospholipase C/diacylglycerol kinase, requires nitric oxide. J. Plant Physiol. 168, 534–539.
Rainteau, D., Humbert, L., Delage, E., Vergnolle, C., Cantrel, C., Maubert, M.-A., Lanfranchi, S., Maldiney, R., Collin, S., Wolf, C., Zachowski, A., Ruelland, E., 2012. Acyl chains of phospholipase D transphosphatidylation products in Arabidopsis cells: a study using multiple reaction monitoring mass spectrometry. PLoS ONE 7, e41985.
Rajashekar, C.B., Zhou, H.-E., Zhang, Y., Li, W., Wang, X., 2006. Suppression of phospholipase Dα1 induces freezing tolerance in Arabidopsis: response of cold-responsive genes and osmolyte accumulation. J. Plant Physiol. 163, 916–926.
Ritchie, S., Gilroy, S., 1998. Abscisic acid signal transduction in the barley aleurone is mediated by phospholipase D activity. Proc. Natl. Acad. Sci. U. S. A. 95, 2697–2702.
Ritchie, S., Gilroy, S., 2000. Abscisic acid stimulation of phospholipase D in the barley aleurone is G-protein-mediated and localized to the plasma membrane. Plant Physiol. 124, 693–702.
Ruelland, E., Cantrel, C., Gawer, M., Kader, J.C., Zachowski, A., 2002. Activation of phospholipases C and D is an early response to a cold exposure in Arabidopsis suspension cells. Plant Physiol. 130, 999–1007.
Ruelland, E., Vaultier, M.-N., Zachowski, A., Hurry, V., 2009. Chapter 2 Cold signalling and cold acclimation in plants. In: Jean-Claude, K., Michel, D. (Eds.), Advances in Botanical Research. Academic Press, New York, pp. 35–150.
Ruelland, E., Zachowski, A., 2010. How plants sense temperature. Environ. Exp. Bot. 69, 225–232.
Rupwate, S., Rajasekharan, R., 2012. C2 domain is responsible for targeting rice phosphoinositide specific phospholipase C. Plant Mol. Biol. 78, 247–258.
Ryu, H., Kim, K., Cho, H., Park, J., Choe, S., Hwang, I., 2007. Nucleocytoplasmic shuttling of BZR1 mediated by phosphorylation is essential in Arabidopsis brassinosteroid signaling. Plant Cell 19, 2749–2762.
Ryu, S.B., Wang, X., 1996. Activation of phospholipase D and the possible mechanism of activation in wound-induced lipid hydrolysis in castor bean leaves. Biochim. Biophys. Acta (BBA) – Lipids and Lipid Metab. 1303, 243–250.
Saavedra, L., Mikami, K., Malhó, R., Sommarin, M., 2012. PIP kinases and their role in plant tip growing cells. Plant Signal. Behav. 7, 1302–1305.
Scherer, G.E., 2010. Phospholipase A in plant signal transduction. In: Munnik, T. (Ed.), Lipid Signaling in Plants. Springer Verlag, Berlin, pp. 3–22.
Scherer, G.F.E., Paul, R.U., Holk, A., Martinec, J., 2002. Down-regulation by elicitors of phosphatidylcholine-hydrolyzing phospholipase C and up-regulation of phospholipase A in plant cells. Biochem. Biophys. Res. Commun. 293, 766–770.
Senthil-Kumar, M., Mysore, K.S., 2013. Nonhost resistance against bacterial pathogens: retrospectives and prospects. Annu. Rev. Phytopathol. 51, 407–427.
Siddhanta, A., Shields, D., 1998. Secretory vesicle budding from the trans-Golgi network is mediated by phosphatidic acid levels. J. Biol. Chem. 273, 17995–17998.
Singh, A., Kanwar, P., Pandey, A., Tyagi, A.K., Sopory, S.K., Kapoor, S., Pandey, G.K., 2013. Comprehensive genomic analysis and expression profiling of phospholipase C gene family during abiotic stresses and development in Rice. PLoS ONE 8, e62494.
Strawn, L., Babb, A., Testerink, C., Kooijman, E.E., 2012. The physical chemistry of the enigmatic phospholipid diacylglycerolpyrophosphate. Front. Plant Sci., 3.
Sun, Y., Fan, X.Y., Cao, D.M., Tang, W., He, K., Zhu, J.Y., He, J.X., Bai, M.Y., Zhu, S., Oh, E., Patil, S., Kim, T.W., Ji, H., Wong, W.H., Rhee, S.Y., Wang, Z.Y., 2010. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev. Cell 19, 765–777.
Sun, Y., Li, L., Macho, A.P., Han, Z., Hu, Z., Zipfel, C., Zhou, J.-M., Chai, J., 2013. Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. Science 342, 624–628.
Tang, W., Yuan, M., Wang, R., Yang, Y., Wang, C., Oses-Prieto, J.A., Kim, T.-W., Zhou, H.-W., Deng, Z., Gampala, S.S., Gendron, J.M., Jonassen, E.M., Lillo, C., DeLong, A., Burlingame, A.L., Sun, Y., Wang, Z.-Y., 2011. PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. Nat. Cell Biol. 13, 124–131.
Testerink, C., Dekker, H.L., Lim, Z.-Y., Johns, M.K., Holmes, A.B., de Koster, C.G., Ktistakis, N.T., Munnik, T., 2004. Isolation and identification of phosphatidic acid targets from plants. Plant J. 39, 527–536.
Testerink, C., Larsen, P.B., van der Does, D., van Himbergen, J.A.J., Munnik, T., 2007. Phosphatidic acid binds to and inhibits the activity of Arabidopsis CTR1. J. Exp. Bot. 58, 3905–3914.
Tripathy, M., Tyagi, W., Goswami, M., Kaul, T., Singla-Pareek, S., Deswal, R., Reddy, M., Sopory, S., 2012. Characterization and functional validation of tobacco PLC delta for abiotic stress tolerance. Plant Mol. Biol. Rep. 30, 488–497.
Trono, D., Soccio, M., Laus, M.N., Pastore, D., 2013. The existence of phospholipase A2 activity in plant mitochondria and its activation by hyperosmotic stress in durum wheat (*Triticum durum* Desf.). Plant Sci. 199–200, 91–102.
Uraji, M., Katagiri, T., Okuma, E., Ye, W., Hossain, M.A., Masuda, C., Miura, A., Nakamura, Y., Mori, I.C., Shinozaki, K., Murata, Y., 2012. Cooperative function of PLDδ and PLDα1 in abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol. 159, 450–460.
van der Luit, A.H., Piatti, T., van Doorn, A., Musgrave, A., Felix, G., Boller, T., Munnik, T., 2000. Elicitation of suspension-cultured tomato cells triggers the formation of phosphatidic acid and diacylglycerol pyrophosphate. Plant Physiol. 123, 1507–1516.
van Schooten, B., Testerink, C., Munnik, T., 2006. Signalling diacylglycerol pyrophosphate, a new phosphatidic acid metabolite. Biochim. Biophys. Acta – Mol. Cell Biol. Lipids 1761, 151–159.
Vaultier, M.-N., Cantrel, C., Vergnolle, C., Justin, A.-M., Demandre, C., Benhassaine-Kesri, G., Çiçek, D., Zachowski, A., Ruelland, E., 2006. Desaturase mutants reveal that membrane rigidification acts as a cold perception mechanism upstream of the diacylglycerol kinase pathway in Arabidopsis cells. FEBS Lett. 580, 4218–4223.
Vergnolle, C., Vaultier, M.-N., Taconnat, L., Renou, J.-P., Kader, J.-C., Zachowski, A., Ruelland, E., 2005. 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, 1217–1233.
Verlotta, A., Liberatore, M., Cattivelli, L., Trono, D., 2013. Secretory phospholipases A2 in durum wheat (*Triticum durum* Desf.): gene expression, enzymatic activity, and relation to drought stress adaptation. Int. J. Mol. Sci. 14, 5146–5169.
Viehweger, K., Dordschbal, B., Roos, W., 2002. Elicitor-activated phospholipase A2 generates lysophosphatidylcholines that mobilize the vacuolar H^+ pool for pH signaling via the activation of Na^+-dependent proton fluxes. Plant Cell 14, 1509–1525.
Villasuso, A.L., Di Palma, M.A., Aveldaño, M., Pasquaré, S.J., Racagni, G., Giusto, N.M., Machado, E.E., 2013. Differences in phosphatidic acid signalling and metabolism between ABA and GA treatments of barley aleurone cells. Plant Physiol. Biochem. 65, 1–8.
Vossen, J.H., Abd-El-Haliem, A., Fradin, E.F., Van Den Berg, G.C.M., Ekengren, S.K., Meijer, H.J.G., Seifi, A., Bai, Y., Ten Have, A., Munnik, T., Thomma, B.P.H.J., Joosten, M.H.A.J., 2010. 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, 224–239.
Wang, C.-R., Yang, A.-F., Yue, G.-D., Gao, Q., Yin, H.-Y., Zhang, J.-R., 2008. Enhanced expression of phospholipase C 1 (ZmPLC1) improves drought tolerance in transgenic maize. Planta 227, 1127–1140.
Wang, G., Ryu, S., Wang, X., 2012. Plant phospholipases: an overview. In: Sandoval, G. (Ed.), Lipases and Phospholipases. Humana Press, New York, pp. 123–137.
Wang, L., Zhu, X., Liu, J., Chu, X., Jiao, J., Liang, Y., 2013a. Involvement of phospholipases C and D in the defence responses of riboflavin-treated tobacco cells. Protoplasma 250, 441–449.
Wang, Z., Anderson, N.S., Benning, C., 2013b. The phosphatidic acid binding site of the Arabidopsis trigalactosyldiacylglycerol 4 (TGD4) protein required for lipid import into chloroplasts. J. Biol. Chem. 288, 4763–4771.
Wasternack, C., Hause, B., 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann. Bot. 111, 1021–1058.
Wimalasekera, R., Pejchar, P., Holk, A., Martinec, J., Scherer, G.F.E., 2010. Plant phosphatidylcholine-hydrolyzing phospholipases C NPC3 and NPC4 with roles in root development and brassinolide signaling in *Arabidopsis thaliana*. Mol. Plant 3, 610–625.
Wirthmueller, L., Maqbool, A., Banfield, M.J., 2013. On the front line: structural insights into plant-pathogen interactions. Nat. Rev. Microbiol. 11, 761–776.
Wu, G., Wang, X., Li, X., Kamiya, Y., Otegui, M.S., Chory, J., 2011. Methylation of a phosphatase specifies dephosphorylation and degradation of activated brassinosteroid receptors. Sci. Signal. 4, ra29.
Wu, P., Gao, H.-B., Zhang, L.-L., Xue, H.-W., Lin, W.-H., 2014. Phosphatidic acid regulates BZR1 activity and brassinosteroid signal of Arabidopsis. Mol. Plant 7, 445–447.
Yamaguchi, T., Kuroda, M., Yamakawa, H., Ashizawa, T., Hirayae, K., Kurimoto, L., Shinya, T., Shibuya, N., 2009. Suppression of a phospholipase D gene, *OsPLDβ1*, activates defense responses and increases disease resistance in rice. Plant Physiol. 150, 308–319.
Yamaguchi, T., Minami, E., Ueki, J., Shibuya, N., 2005. Elicitor-induced activation of phospholipases plays an important role for the induction of defense responses in suspension-cultured rice cells. Plant Cell Physiol. 46, 579–587.
Yang, N., Yue, X.L., Zhang, H., Wu, G.F., Ding, F.X., Zhang, T.G., An, L.Z., 2013. Characterization of phospholipase D from *Chorispora bungeana* callus in response to freezing stress. Biol. Plant. 57, 113–120.
Yao, H., Wang, G., Guo, L., Wang, X., 2013. Phosphatidic acid interacts with a MYB transcription factor and regulates its nuclear localization and function in Arabidopsis. Plant Cell Online 25, 5030–5042.
yoon Jeong, S., Park, C., Kim, M.-K., jun Nam, S., Hong, J., Kim, S.-K., 2012. Effect of lysophosphatidylethanolamine and brassinosteroids on development of Arabidopsis roots. J. Plant Biol., 1–7.
Yu, L., Nie, J., Cao, C., Jin, Y., Yan, M., Wang, F., Liu, J., Xiao, Y., Liang, Y., Zhang, W., 2010. Phosphatidic acid mediates salt stress response by regulation of MPK6 in *Arabidopsis thaliana*. New Phytol. 188, 762–773.
Zalejski, C., Zhang, Z., Quettier, A.-L., Maldiney, R., Bonnet, M., Brault, M., Demandre, C., Miginiac, E., Rona, J.-P., Sotta, B., Jeannette, E., 2005. Diacylglycerol pyrophosphate is a second messenger of abscisic acid signaling in *Arabidopsis thaliana* suspension cells. Plant J. 42, 145–152.
Zhai, S.-M., Gao, Q., Xue, H.-W., Sui, Z.-H., Yue, G.-D., Yang, A.-F., Zhang, J.-R., 2012. Overexpression of the phosphatidylinositol synthase gene from *Zea mays* in

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009

tobacco plants alters the membrane lipids composition and improves drought stress tolerance. Planta 235, 69–84.
Zhai, S., Gao, Q., Liu, X., Sui, Z., Zhang, J., 2013. Overexpression of a *Zea mays* phospholipase C1 gene enhances drought tolerance in tobacco in part by maintaining stability in the membrane lipid composition. Plant Cell, Tissue Organ Cult. 115, 253–262.
Zhang, Q., Lin, F., Mao, T., Nie, J., Yan, M., Yuan, M., Zhang, W., 2012. Phosphatidic acid regulates microtubule organization by interacting with MAP65-1 in response to salt stress in Arabidopsis. Plant Cell Online 24, 4555–4576.
Zhang, X.D., Wang, R.P., Zhang, F.J., Tao, F.Q., Li, W.Q., 2013. Lipid profiling and tolerance to low-temperature stress in *Thellungiella salsuginea* in comparison with *Arabidopsis thaliana*. Biol. Plant. 57, 149–153.
Zhang, Y., Zhu, H., Zhang, Q., Li, M., Yan, M., Wang, R., Wang, L., Welti, R., Zhang, W., Wang, X., 2009. Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in *Arabidopsis*. Plant Cell Online 21, 2357–2377.
Zhao, J., Devaiah, S.P., Wang, C., Li, M., Welti, R., Wang, X., 2013. *Arabidopsis* phospholipase Dβ1 modulates defense responses to bacterial and fungal pathogens. New Phytol. 199, 228–240.
Zhao, J., Wang, C., Bedair, M., Welti, R., Sumner, L.W., Baxter, I., Wang, X., 2011. Suppression of phospholipase Dγs confers increased aluminum resistance in *Arabidopsis thaliana*. PLoS ONE 6, e28086.
Zhao, J., Zhou, D., Zhang, Q., Zhang, W., 2012. Genomic analysis of phospholipase D family and characterization of GmPLDαs in soybean (*Glycine max*). J. Plant Res. 125, 569–578.
Zheng, S.-Z., Liu, Y.-L., Li, B., Shang, Z.-l., Zhou, R.-G., Sun, D.-Y., 2012. Phosphoinositide-specific phospholipase C9 is involved in the thermotolerance of Arabidopsis. Plant J. 69, 689–700.

Please cite this article in press as: Ruelland, E., et al., Role of phospholipid signalling in plant environmental responses. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.08.009