Phosphatidic acid, a versatile water-stress signal in roots

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Adequate water supply is of utmost importance for growth and reproduction of plants. In order to cope with water deprivation, plants have to adapt their development and metabolism to ensure survival. To maximize water use efficiency, plants use a large array of signaling mediators such as hormones, protein kinases, and phosphatases, Ca\(^{2+}\), reactive oxygen species, and low abundant phospholipids that together form complex signaling cascades.

Phosphatidic acid (PA) is a signaling lipid that rapidly accumulates in response to a wide array of abiotic stress stimuli. PA formation provides the cell with spatial and transient information about the external environment by acting as a protein-docking site in cellular membranes. PA reportedly binds to a number of proteins that play a role during water limiting conditions, such as drought and salinity and has been shown to play an important role in maintaining root system architecture. Members of two osmotic stress-activated protein kinase families, sucrose non-fermenting 1-related protein kinase 2 and mitogen activated protein kinases were recently shown bind PA and are also involved in the maintenance of root system architecture and salinity stress tolerance. In addition, PA regulates several proteins involved in abscisic acid-signaling. PA-dependent recruitment of glyceraldehyde-3-phosphate dehydrogenase under water limiting conditions indicates a role in regulating metabolic processes. Finally, a recent study also shows the PA recruits the clathrin heavy chain and a potassium channel subunit, hinting toward additional roles in cellular trafficking and potassium homeostasis. Taken together, the rapidly increasing number of proteins reported to interact with PA implies a broad role for this versatile signaling phospholipid in mediating salt and water stress responses.

Keywords: phosphatidic acid, Arabidopsis thaliana, roots, salt, drought, protein kinase

**INTRODUCTION**

Plants have to adapt to various changes in their environment and signals from the outside have to pass the membrane in order for the cell to respond. Environmental stress causes changes in the phospholipid composition of cellular membranes. Several lipids, which are only present in small amounts under normal conditions, are synthesized rapidly and transiently in response to stress. They act as a lipid second messenger and can form docking sites that bind different proteins and thus provide spatial and transient signals needed to adequately respond to external stimuli (Meijer and Munnik, 2005; Wang, 2004; Munnik and Testerink, 2009; Xue et al., 2009; Munnik and Vermeer, 2010). The phospholipase D (PLD) enzyme hydrolyses primarily structural lipids such as phosphatidylcholine (PC), and phosphatidylethanolamine (PE), resulting in formation of PA and the remaining headgroup triphosphate (IP\(_2\), IP\(_3\)) and diacylglycerol (DAG), which remains in the membrane (Munnik and Vermeer, 2010). DAG can be subsequently phosphorylated to PA by DAG kinase (DGK; Arisz et al., 2009).

Twelve PLDs have been identified in the model plant species *Arabidopsis*, which were initially classified in two groups based on their N-terminal lipid-binding domain. These domains consist either of a pleckstrin homology (PH) and PHOX (PX) or a calcium dependent-lipid binding (C2) domain (Elia et al., 2002). Later, these classes were further subdivided into six classes based on sequence homology and in vitro enzymatic activity: three α-, two β-, three γ-, one δ-, and one ε- PLD with a C2 domain and two ε-PLD with a C2 domain and two β-PLD with a C2 domain and two γ-PLD with C2 domains (Qin and Wang, 2002; Bargmann and Munnik, 2006; Li et al., 2009).

In the *Arabidopsis* genome, nine PLCs and seven DGK genes have been identified. Initially, PLC/DGK derived PA was primarily
Phosphatidic acid (PA) is a signaling lipid that plays a central role in the regulation of various cellular processes in plants. PA is involved in responses to biotic stress, drought, and low nutrient availability. To cope with these conditions, plants adapt the growth and morphology of their roots. Several PLD isoforms were found to be involved in adjusting root system architecture during abiotic stress (Galvan-Ampudia and Testerink, 2011; Table 1).

**PLDδ** is involved in directional root growth in saline conditions. Exposing one side of the root to salt increased pin-formed (PIN)2 internalization, effectively redistributing auxin in the root tip. This redistribution resulted in bending away from saline conditions, named halotropism. A **pldδ**-KO (knock-out) mutant showed reduced clathrin-dependent PIN2 internalization and reduced primary root bending (Galvan-Ampudia et al., 2013). Expression of **PLDδ** increased under low phosphate availability (Orozco-Sandoval et al., 2011) and the **pldδ**-KO showed increased root hair growth when deprived of phosphate (Cruz-Ramirez et al., 2006). In accordance, less PA was formed in low phosphate conditions (Wang et al., 2007). In the **pldδ**-KO double mutant (**pldεζδ**-KO mutant) also exhibited decreased sensitivity to auxin and a reduced root gravitropic response (Li and Xue, 2007).

Nitrogen is another important nutrient for plants and *Arabidopsis* PLDα-DE lines displayed an increased lateral root and root hair elongation and primary root growth in low nitrogen conditions. This effectively increased the dry weight of the plant under these conditions and indicated an important role for PLDαs in growth and nitrogen signaling (Hong et al., 2009).

**PLDα1** and **PLDα5** are involved in different responses to abiotic stress including reactive oxygen species (ROS) signaling in response to ABA (Sang et al., 2001; Zhang et al., 2003; Zhang et al., 2009; Uraji et al., 2012) studied in stomata and leaves. The same phospholipases were also shown to play distinct roles in freezing tolerance (Welti et al., 2002; Li et al., 2006b). Expression of **PLDα5** was elevated in response to dehydration and high salt stress (Katagiri et al., 2003). Salt stress induced formation of PA through **PLDα5** and **PLDδ**, where both single mutants showed a reduction in primary root growth in saline and hyperosmotic stress conditions. This effectively resulted in the plant being more salt intolerant. A central mediator in metabolism, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is targeted to PA in response to salt in *Arabidopsis* roots (McLoughlin et al., 2013). The best-described role of GAPDH is the conversion of glyceraldehyde-3-phosphate to di-glycerate 1,3-bisphosphate in the glycolytic breakdown of glucose. PA-binding does not alter the activity of GAPDH dramatically in vitro but adding PA to seedlings did induce proteolytic cleavage of glyceraldehyde-3-phosphate dehydrogenase C2 (GAPC2; Kim et al., 2013). Adding exogenous PA also reduced primary root growth, which was more severe when GAPDH was over-expressed whilst knock-out mutants showed less reduction in growth (Kim et al., 2013) indicating that the effect of PA on root growth was partially mediated by proteolytic degradation of GAPDH. Although GAPDH has been described to be involved in different non-metabolic processes, promoting its degradation might mediate energy conservation and arrest of root growth, which are immediate and relevant responses to any osmotic stress including saline conditions (Munnis and Tester, 2008).

**PA PROTEIN TARGETS INVOLVED IN OSMOTIC/SALT STRESS SIGNALING AND ROOT SYSTEM ARCHITECTURE**

A central mediator in metabolism, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is targeted to PA in response to salt in *Arabidopsis* roots (McLoughlin et al., 2013). The best-described role of GAPDH is the conversion of glyceraldehyde-3-phosphate to di-glycerate 1,3-bisphosphate in the glycolytic breakdown of glucose. PA-binding does not alter the activity of GAPDH dramatically in vitro but adding PA to seedlings did induce proteolytic cleavage of glyceraldehyde-3-phosphate dehydrogenase C2 (GAPC2; Kim et al., 2013). Adding exogenous PA also reduced primary root growth, which was more severe when GAPDH was over-expressed whilst knock-out mutants showed less reduction in growth (Kim et al., 2013) indicating that the effect of PA on root growth was partially mediated by proteolytic degradation of GAPDH. Although GAPDH has been described to be involved in different non-metabolic processes, promoting its degradation might mediate energy conservation and arrest of root growth, which are immediate and relevant responses to any osmotic stress including saline conditions (Munnis and Tester, 2008).

**CYTOSKELETON AND MEMBRANE CELLULAR TRAFFICKING**

Phosphatidic acid has also emerged as an important regulator of microtubules and actin organization and re-organization. Microtubule reorganization is crucial for plants to adapt to saline conditions (Wang et al., 2007). In the **pldα1**/A double mutant (Bargmann et al., 2009). A similar reduction in root growth was observed in **pldα3**, which was more susceptible to salinity and water deficiency. In hyperosmotic-stress conditions, the **pldα3** mutant displays a reduction in primary root growth and a reduction in lateral roots (Hong et al., 2008).

Together, these studies show that PLDs are important for maintaining root growth in saline and hyperosmotic stress conditions amongst other functions. PA is not limited to its function as a signaling lipid; it is also an important intermediate during lipid-turnover. Therefore it is hard to separate the role of PA in lipid-turnover from its role in signaling and protein recruitment (Testerink and Munnik, 2011). To discern between the different roles of PA it is important to identify which proteins interact with PA and how these mediate the response that eventually leads to the acclimation to different stresses (Testerink and Munnik, 2005). In contrast to other signaling lipids such as phosphoinositides, no consensus PA-binding domain has been identified, which hampers the identification of new PA targets. A number of PA binding proteins have been identified involved in diverse cellular processes (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>PA-protein targets</th>
<th>Role in osmotic/salt stress signaling and root system architecture</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Key regulator in metabolism, involved in proteolytic cleavage</td>
</tr>
<tr>
<td>Microtubules</td>
<td>Reorganization critical for adaptation to saline conditions</td>
</tr>
<tr>
<td>Actin</td>
<td>Important for the behavior of actin filaments through their regulation of actin capping proteins</td>
</tr>
<tr>
<td>Microfilaments</td>
<td>interferes with actin capping protein</td>
</tr>
<tr>
<td>Clathrin</td>
<td>Important for actin capping protein</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>Interacts with actin capping protein</td>
</tr>
</tbody>
</table>

Together, these studies show that PLDs are important for maintaining root growth in saline and hyperosmotic stress conditions amongst other functions. PA is not limited to its function as a signaling lipid; it is also an important intermediate during lipid-turnover. Therefore it is hard to separate the role of PA in lipid-turnover from its role in signaling and protein recruitment (Testerink and Munnik, 2011). To discern between the different roles of PA it is important to identify which proteins interact with PA and how these mediate the response that eventually leads to the acclimation to different stresses (Testerink and Munnik, 2005). In contrast to other signaling lipids such as phosphoinositides, no consensus PA-binding domain has been identified, which hampers the identification of new PA targets. A number of PA binding proteins have been identified involved in diverse cellular processes (Table 1).
FIGURE 1 | (A) PA derived from different PLDs involved in the maintenance of root system architecture during abiotic stress. PLDs regulate downstream targets through producing PA. Although all PLD isoforms hydrolyse structural lipids and generate PA in vitro, they have been identified to be involved in different processes and signaling cascades in vivo. (B) Preliminary network of osmotic stress-induced PA-SnRK2 signaling cascades in roots. This model is based on data obtained on the class 1 SnRK2 members in different plant species. SnRK2, sucrose non-fermenting 1-related protein kinase 2; SCS, SnRK2-interacting calcium sensor; PA, phosphatidic acid; NO, nitric oxide; SNO, S-nitrosylated; PP, protein phosphatase; GAPDH, glyceraldehyde-3-phosphate; PLDδ, phospholipase D δ. Solid lines indicate an activation or inhibitory effect, dashed lines show which proteins/lipids interact without a direct change in activity.

which prevents actin filament from annealing and elongating. PA-binding reduced the activity of CP, effectively promoting actin reorganization and promoting cytoskeleton dynamics which are required for adaptation to adverse conditions.

In mammalian cells, PA and PA-generating enzymes such as PLD and DGK have been implicated in various aspects of vesicle transport (Manifava et al., 2001; Corda et al., 2002; Jang et al., 2012), but so far, little evidence is present that suggest a similar role in plants. Recently, clathrin heavy chain and clathrin assembly units were shown to recruit to the membrane in Arabidopsis roots in response to salt and to bind to PA-beads (McLoughlin et al., 2013). This likely represents an important aspect of the molecular mechanism of salt-induced PIN2 internalization which controls directional root bending in saline conditions (Galvan-Ampudia et al., 2013).

CELLULAR SIGNALING AND DEVELOPMENT
The Arabidopsis phosphoinositide-dependent kinase 1 (PDK1) binds several phosphoinositides and PA through its PH domain (Dxak et al., 1999). PA activates PDK1 and indirectly, its downstream target AGC2-1 (OXI1) (Anthony et al., 2004, 2006). This signaling cascade induces a respiratory burst required for the
Table 1: An overview of PA targets identified in plants.

<table>
<thead>
<tr>
<th>PA targets</th>
<th>Function</th>
<th>Role in root growth?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDK1</td>
<td>Root hair development, defense to pathogens</td>
<td>Yes</td>
<td>Deak et al. (1999), Anthony et al. (2004, 2006)</td>
</tr>
<tr>
<td>ABI1</td>
<td>ABA-signaling</td>
<td>Yes</td>
<td>Zhang et al. (2004)</td>
</tr>
<tr>
<td>Dehydrins</td>
<td>Protection during abiotic stress</td>
<td>Yes</td>
<td>Kooi et al. (2003, 2009), Eriksson et al. (2011)</td>
</tr>
<tr>
<td>SnRK2.4</td>
<td>Salt stress signaling</td>
<td>Yes</td>
<td>Testerink et al. (2004), McLoughlin et al. (2012)</td>
</tr>
<tr>
<td>RCN1</td>
<td>Auxin transport, ethylene signaling</td>
<td>Yes</td>
<td>Testerink et al. (2004), Gao et al. (2013)</td>
</tr>
<tr>
<td>PID</td>
<td>PIN localization</td>
<td>Yes</td>
<td>Zegzouti et al. (2008)</td>
</tr>
<tr>
<td>CP</td>
<td>Actin polymerization</td>
<td>Yes</td>
<td>Huang et al. (2008), Li et al. (2012), Pleasot et al. (2013)</td>
</tr>
<tr>
<td>TGD2</td>
<td>Lipid transport</td>
<td>Not reported</td>
<td>Awai et al. (2006)</td>
</tr>
<tr>
<td>AGD7</td>
<td>ER/Golgi trafficking</td>
<td>Not reported</td>
<td>Min et al. (2007)</td>
</tr>
<tr>
<td>CT1*</td>
<td>Ethylene signaling</td>
<td>Yes</td>
<td>Testerink et al. (2007)</td>
</tr>
<tr>
<td>TaPEAMT1/2</td>
<td>Lipid metabolism</td>
<td>Not reported</td>
<td>Jost et al. (2009)</td>
</tr>
<tr>
<td>Raet/DeF*</td>
<td>Oxidative stress</td>
<td>Yes</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td>PEPC</td>
<td>Metabolism</td>
<td>Not reported</td>
<td>Testerink et al. (2004), Monreal et al. (2010)</td>
</tr>
<tr>
<td>MPK6</td>
<td>Abiotic and biotic stress signaling</td>
<td>Yes</td>
<td>Yu et al. (2010)</td>
</tr>
<tr>
<td>MGD1</td>
<td>Lipid metabolism</td>
<td>Not reported</td>
<td>Dubots et al. (2010)</td>
</tr>
<tr>
<td>ZmCPK11</td>
<td>Protein kinase</td>
<td>Not reported</td>
<td>Klimeda et al. (2011)</td>
</tr>
<tr>
<td>SPHK1</td>
<td>Sphingosine kinase</td>
<td>Yes</td>
<td>Guo et al. (2011)</td>
</tr>
<tr>
<td>TGD4</td>
<td>Lipid transport</td>
<td>Not reported</td>
<td>Wang et al. (2012, 2013)</td>
</tr>
<tr>
<td>PTKL25A</td>
<td>Lipid phosphate activity</td>
<td>Not reported</td>
<td>Prabat et al. (2012)</td>
</tr>
<tr>
<td>CsH13-24</td>
<td>Protein protection</td>
<td>Not reported</td>
<td>Peteren et al. (2012)</td>
</tr>
<tr>
<td>14-3-3-protein</td>
<td>Protein binding</td>
<td>Not reported</td>
<td>Camoni et al. (2012)</td>
</tr>
<tr>
<td>MAP65-1</td>
<td>Microtubule organization</td>
<td>Yes</td>
<td>Zhang et al. (2012)</td>
</tr>
<tr>
<td>GAPC</td>
<td>Metabolism</td>
<td>Yes</td>
<td>McLoughlin et al. (2013), Kim et al. (2013)</td>
</tr>
</tbody>
</table>

Abbreviations of all PA targets identified in different parts of the plant are given (left column) with their putative function (2nd column), whether there is a known effect on root growth (3rd column) and references for the PA-binding evidence (right column). PDK1, phosphoinositide-dependent kinase 1; ABI1, ABA insensitive 1; SnRK2.4, sucrose non-fermenting 1-related protein kinase 2; RCN1, roots curl in NPA 1; PID, PINOID; CP, Ca2+/calmodulin-dependent protein kinase; TGD2, trigalactosyldiacylglycerol 2; AGD7, Arf gap domain 7; CTR1, constitutive triple response 1; TaPEAMT1/2, phosphoethanolamine N-methyltransferase; SnRK2.10, sucrose non-fermenting 1-related protein kinase 2; PID, PINOID; MAP65-1, microtubule-associated proteins 65-1; GAPC, glyceraldehyde-3-phosphate dehydrogenase C. *Evidence for a role in root growth: PDK1, phosphoinositide-dependent kinase 1; ABI1, ABA insensitive 1; SnRK2.4, sucrose non-fermenting 1-related protein kinase 2; PID, PINOID; CP, Ca2+/calmodulin-dependent protein kinase; TGD2, trigalactosyldiacylglycerol 2; AGD7, Arf gap domain 7; CTR1, constitutive triple response 1; TaPEAMT1/2, phosphoethanolamine N-methyltransferase; SnRK2.10, sucrose non-fermenting 1-related protein kinase 2; PID, PINOID; MAP65-1, microtubule-associated proteins 65-1; GAPC, glyceraldehyde-3-phosphate dehydrogenase C. *Evidence for a role in root growth: PDK1, phosphoinositide-dependent kinase 1; ABI1, ABA insensitive 1; SnRK2.4, sucrose non-fermenting 1-related protein kinase 2; PID, PINOID; CP, Ca2+/calmodulin-dependent protein kinase; TGD2, trigalactosyldiacylglycerol 2; AGD7, Arf gap domain 7; CTR1, constitutive triple response 1; TaPEAMT1/2, phosphoethanolamine N-methyltransferase; SnRK2.10, sucrose non-fermenting 1-related protein kinase 2; PID, PINOID; MAP65-1, microtubule-associated proteins 65-1; GAPC, glyceraldehyde-3-phosphate dehydrogenase C. *Evidence for a role in root growth: PDK1, phosphoinositide-dependent kinase 1; ABI1, ABA insensitive 1; SnRK2.4, sucrose non-fermenting 1-related protein kinase 2; PID, PINOID; CP, Ca2+/calmodulin-dependent protein kinase; TGD2, trigalactosyldiacylglycerol 2; AGD7, Arf gap domain 7; CTR1, constitutive triple response 1; TaPEAMT1/2, phosphoethanolamine N-methyltransferase; SnRK2.10, sucrose non-fermenting 1-related protein kinase 2; PID, PINOID; MAP65-1, microtubule-associated proteins 65-1; GAPC, glyceraldehyde-3-phosphate dehydrogenase C.
of KAT1 by PA opens the possibility that PA might function as a regulator of potassium homeostasis in different parts of the plant.

The sucrose non-fermenting 1-related protein kinase 2 proteins (SnRK2s), is a family of osmotic stress-activated protein kinases (Kulik et al., 2011). SnRK2.10, a member of the class 1 subfamily, which is activated by salt and water stress but not by ABA, was identified in a PA affinity screen (Testerink et al., 2004). More recently, both SnRK2.10 and its close homolog SnRK2.4 were shown to bind PA directly. Arabidopsis snrk2.1 and 2.10 KO mutants exhibited reduced primary root length and lateral root density in saline conditions, respectively (McLoughlin et al., 2012). SnRK2.4 was shown to be involved in regulating the ROS levels in roots in response to cadmium, but remarkably snrk2.4 KO mutants displayed an increase in primary root length when exposed to cadmium (Kulik et al., 2012). Overexpression of the SnRK2.4 wheat ortholog (TaSnRK2.4) in Arabidopsis caused an increase in primary root growth and resulted in more drought tolerant plants. This was explained by stronger water retention ability in these plants compared to wild-type (Mao et al., 2010). Overexpression of SAPK4 (a rice class 1 SnRK2 ortholog) increased tolerance to salinity and oxidative stress (Diedhiou et al., 2008).

CONCLUSION AND OUTLOOK

In conclusion, PA is a central phospholipid intermediate which is rapidly and transiently formed during salt and osmotic stress. PA accumulation is used as an appropriate and general sensor to monitor the extracellular environment, which could explain the versatile role of this phospholipid. The specificity in which phospholipases transduce diverse signals remains largely unknown, although differences can be expected through abundance, localization, and substrate preference of the different phospholipases. Besides evidence that PA acts as a docking site for protein recruitment to the membrane, an increasing amount of papers indicate post-translational modifications, activation/inactivation or proteolytic cleavage upon binding either directly (solely in the presence of PA) or indirectly (orchestrated with different proteins), profoundly increasing the complexity of its role. The localization of the phospholipases and the effect of PLD-KO and OE lines on the functionality and localization of PA-targets have to be studied to verify their exact relation, which will ultimately result in the understanding of how PA-signaling mediates root proliferation in adverse conditions.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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