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Research article

Expression and evolution of the phosphoinositide-specific phospholipase C gene family in *Arabidopsis thaliana*

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Abstract

Phosphoinositide-specific phospholipase C cleaves the substrate phosphatidylinositol 4,5-bisphosphate and generates inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both of which are second messengers in the phosphoinositide signal transduction pathways operative in animal cells. Five PI-PLC isoforms, β, γ, δ, ε and ζ, have been identified in mammals. Plant PI-PLCs are structurally close to the mammalian PI-PLC-ζ isoform. The *Arabidopsis* genome contains nine *AtPLC* genes. Expression patterns of all nine genes in different organs and in response to various environmental stimuli were studied by applying a quantitative RT–PCR approach. Multiple members of the gene family were differentially expressed in *Arabidopsis* organs, suggesting putative roles for this enzyme in plant development, including tissue and organ differentiation. This study also shows that a majority of the *AtPLC* genes are induced in response to various environmental stimuli, including cold, salt, nutrients Murashige–Skoog salts, dehydration, and the plant hormone abscisic acid. Results of this and previous studies strongly suggest that transcriptional activation of the *PI-PLC* gene family is important for adapting plants to stress environments. Expression patterns and phylogenetic relationships indicates that *AtPLC* gene members probably evolved through multiple rounds of gene duplication events, with *AtPLC4* and *AtPLC5* and *AtPLC8* and *AtPLC9* being duplicated in tandem in recent times.
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Keywords: Phosphatidylinositol 4,5-bisphosphate; Inositol 1,4,5-trisphosphate; 1,2-Diacylglycerol; *AtPLC* gene family; Abiotic stress; Nutrient induction of *AtPLC*

1. Introduction

Phosphoinositide-specific phospholipase C (PI-PLC) cleaves the substrate phosphatidylinositol 4,5-bisphosphate (PIP_2) and generates inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG), both of which are second messengers in the phosphoinositide signal transduction pathways operative in animals. Five PI-PLC isoforms, named β, γ, δ, ε and ζ, have been identified in mammals [1–5]. The enzymatic activities of each type of PI-PLC are regulated by distinct mechanisms. PI-PLC-β is activated by members of the G protein subfamily Gq, while PI-PLC-γ is activated by phosphorylation of tyrosine residues by receptors with intrinsic tyrosine kinase activity or by nonreceptor tyrosine kinases [6]. The G protein subfamily G_h is involved in the regulation of PI-PLC-δ [7]. PI-PLC-ε is regulated by the heterotrimeric G protein $G\alpha_{12}$ [2,5]. Regulation of PI-PLC-ζ is unknown. It has been shown that this isoform is activated at nanomolar calcium concentrations and subsequently induces calcium oscillation in mouse eggs [8]. IP_3 binds to the IP_3 receptor of the endoplasmic reticulum and releases Ca^{2+} [1], while DAG remains associated with the plasma membrane and activates protein kinase C. IP_3 acts as a second messenger to link cell surface receptors with the intracellular Ca^{2+} concentration to signal physiological functions.

Structural and functional equivalents of the phosphoinositide-signaling pathway are found in plant cells [9–14].

Abbreviations: PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; DAG, 1,2-diacylglycerol; MS, Murashige–Skoog; ABA, abscisic acid; GCR1, G-protein coupled receptor.

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IP_3 was shown to release Ca^{2+} from the vacuoles [9]. Preliminary evidence suggested the existence of IP_3 receptors in higher plants [10,13]. However, efforts to identify this elusive receptor molecule by sequence similarity in the *Arabidopsis thaliana* and rice genomes have not been successful. It is possible that sequences of plant and mammalian IP_3 receptors are so little conserved that computational identification based only on sequence similarity is not possible.

cDNAs encoding PI-PLCs have been isolated from several plant species, including *A. thaliana*, potato (*Solanum tuberosum*), and soybean (*Glycine max*) [11,14–18]. Plant PI-PLC genes are regulated at the transcriptional level [11,16,18]. For example, transcripts of *AtPLC1* and *AtPLC6* (AF434167) are induced in response to abiotic stresses such as dehydration, high salt, and cold treatments [11,18]. The increased *AtPLC* transcript levels are expected to increase the amount of AtPLCs proteins and thereby mediate signal pathways for up- or down-regulation of genes involved in various cellular functions. In fact, *AtPLC1* transcripts, activity, and increased IP_3 levels are necessary for maximal gene expression following abscisic acid (ABA) treatment [19].

There are nine *AtPLC* genes in the *Arabidopsis* genome [20,21]. *AtPLC1* through *AtPLC5* have shown *in vitro* PI-PLC activities, but *AtPLC8* and *AtPLC9* are unlikely to be enzymatically active [18,20]. *AtPLC6* and *AtPLC7* contain necessary domains for active PI-PLC enzymes and are most likely active. Earlier, it was shown that *AtPLC7* transcripts were alternatively spliced and should encode a truncated nonfunctional 30 kDa protein [20]. However, recently a full-length cDNA encoding *AtPLC7* has been reported (GenBank accession no. NM_115452). Therefore, most likely *AtPLC7* encodes a functional PI-PLC protein.

All nine *AtPLC* genes are very similar and belong to single class of plant PI-PLCs that is structurally close to the mammalian PI-PLC-ζ isoform. Plant PI-PLCs and the mammalian PI-PLC-ζ isoform do not contain the pleckstrin homology (PH) domain found in the other four mammalian PI-PLC isoforms. However, at the sequence level the plant PI-PLCs are closer to the mammalian PI-PLC-δ isoform. Expression patterns of *AtPLC1* through *AtPLC5* in response to environmental stresses such as salt, cold and dehydration or drought were presented previously [11,18,20]. By conducting semi-quantitative RT–PCR, Hunt et al. [20] showed that *AtPLC1*, *AtPLC4* and *AtPLC5* were induced in response to cold, drought and salt stresses. In this study, we have applied the quantitative RT–PCR (qRT–PCR) technique in order to determine the expression profiles of all members of this signal transducing gene family and established that most of the *AtPLC* genes are induced in response to various environmental stimuli, including cold, salt, nutrient Murashige–Skoog (MS) salts, dehydration, and the plant hormone abscisic acid. Evolution of the *AtPLC* gene family is also discussed. Results presented in this and previous studies [11,18,20] show the importance of PI-PLCs in the adaptation of plants to changing environmental conditions.

2. Materials and methods

2.1. Plant materials and growth media

A. thaliana ecotype Columbia-0 was grown in Sunshine Mix SB3000 universal soils (Sun Grow Horticulture Inc., Bellevue, WA) under continuous white fluorescent light. Plants were fertilized weekly with the Miracle-Grow Excel water-soluble fertilizer 15-5-15 (Scotts, Marysville, OH). For stress treatment, 3-week-old seedlings were carefully uprooted from the soils to avoid any injury to the roots and washed in water. The seedlings were then maintained in Petri plates containing 15 ml deionized-distilled water (ddH_2O) for 48 h under continuous fluorescent light. After 2 days, the seedlings were exposed to various treatments by displacing the water with specific compounds in 15 ml ddH_2O.

2.2. Preparation of tissues for RNA analysis

Seedlings were treated with 250 mM NaCl, 100 μM ABA (Sigma, St Louis, MO), and 1× MS salts (Life Technologies, Rockville, MD) at room temperature for 10 h under light intensity of approximately 150 $\mu E\ m^{-2}\ s^{-1}$ of cool white fluorescent light. For the cold treatment, seedlings in Petri plates equilibrated for 2 days with ddH_2O were incubated in a cold room at 4 °C under fluorescent light for 10 h with a light intensity of approximately 150 $\mu E\ m^{-2}\ s^{-1}$. The dehydration treatment was conducted at room temperature by placing the seedlings on a dry Whatman 3MM paper for 5 h. In the control treatment, seedlings equilibrated in ddH_2O were kept under fluorescent light at room temperature for 10 h. Following stress treatments, seedlings were harvested, quickly frozen in liquid nitrogen, and stored at −80 °C until their use in RNA preparations.

Leaves, flowers, stems, and roots were harvested from 6-week-old plants. Single leaves or flowers were harvested and chilled in liquid nitrogen individually. The harvested frozen leaves or flowers were then stored as a single sample at −80 °C until their use in RNA preparations. Stems and roots were harvested from those plants that were used to obtain leaves and flowers. Roots were gently uprooted from the soils, quickly and carefully washed in water, blotted on paper towels, and then frozen in liquid nitrogen and stored at −80 °C until their use for RNA preparations.

2.3. RNA extraction

Total RNA was extracted from *Arabidopsis* whole seedlings, leaves, flowers, stems, or roots using the RNeasy Plant Mini Prep kit (Qiagen, Valencia, CA) or according to Bhattacharyya [22]. RNA concentration was determined using a UV-2000 spectrophotometer (Unico, Dayton, NJ).

2.4. cDNA synthesis

Prior to cDNA synthesis RNA samples were treated with RQ1 RNase-free DNase according to the manufacturer

(Promega, Madison, WI). First-strand cDNA synthesis was conducted using a Molony Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Promega, Madison, WI). An RNase-free oligo dT (5′-GGA ATT CGG ATC CTC TAG ACT GCA GAA CCT TTT TTT TTT TTT TTT T-3′) was used to prime cDNA synthesis (Integrated DNA Technologies, Coralville, IA). The cDNA synthesis was conducted as follows: two micrograms of total RNA was mixed with 0.5 μg oligo dT primer to a total of 18 μL, incubated at 70 °C for 5 min, then cooled quickly on ice. A total of 25 μL reaction was used containing 1× M-MLV reaction buffer (50 mM Tris–HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT), 200 μM dNTPs, 25 units RNasin, 200 units M-MLV reverse transcriptase, and RNase-free water to a final volume of 25 μL. cDNA synthesis was conducted at 42 °C for 1 h. The synthesized cDNA was then diluted two-fold in sterile ddH_2O for cDNA derived from stress tissues and was not diluted for cDNA derived from organ tissues. These cDNAs were used as templates in the real time RT–PCR reactions.

2.5. qRT–PCR

The Primer 3 program was used to design PCR primers (Table 1) from cDNA sequences corresponding to each *PLC* gene [23]. The primers have optimal primer annealing temperatures of 60 °C and yield PCR products ranging from 100–150 bp. Primer self complementarity and likelihood of primer dimer formation were analyzed using the Vector NTI program. The secondary structures of the amplified products were evaluated with the DNA *m-fold* server (http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/rna-form1.cgi; [24]) to confirm that the regions did not include stable secondary structure elements such as stem loops. The primers were synthesized by Integrated DNA Technologies (Coralville, IA). PCR reactions of 25 μL containing 1 μL synthesized cDNA or genomic DNA (used for making the standard curves of each gene), 5 pmol of each primer, and iQ SYBR Green supermix were assembled according to the manufacturer (Bio-Rad, Hercules, CA) and analyzed on an iCycler iQ™ Real Time PCR Detection System (Bio-Rad, Hercules, CA). The PCR program was as follows: 95 °C 3 min, 95 °C 15 s, 60 °C 30 s for 45 cycles followed by 80 cycles for melt curves using the starting temperature of 55 °C with 0.5 °C increments every 10 s. Melt curve analyses confirmed that the PCR reactions were free of contaminating PCR products such as primer dimers. We used a 10-fold serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) of Columbia-0 genomic DNA to create standard curves from which mean starting quantity of each gene was extrapolated in each sample. Transcripts of the constitutively expressed *ACTIN 1* gene were also determined. The expression levels of individual *AtPLC* genes were normalized by dividing the mean starting quantity values of each *AtPLC* gene by the mean starting quantity of the Arabidopsis *ACTIN 1* (At2g37620) gene in the corresponding sample [25]. For the stress treatments, data were presented in the form of expression fold changes of each treatment with respect to that of the normalized control.

Table 1
Gene-specific primers for the *AtPLC* gene family and reference gene *Arabidopsis ACTIN 1* used in real-time RT-PCR experiments

Gene	Primer sequences
AtPLC1	Forward: 5′-CGGTTAAGCATGAGTGAGCA-3′
	Reverse: 5′-ATGAATCCACCCAACGAGAG-3′
AtPLC 2	Forward: 5′-CCAATATTCACCGCCTGACT-3′
	Reverse: 5′-AAACACCTCATCCCAAGCTG-3′
AtPLC3	Forward: 5′-CAAGGACATGGGAAGCAACT-3′
	Reverse: 5′-CTTTTGCAAGGGTCGAAGAG-3′
AtPLC4	Forward: 5′-AACTTGCTCTGCTCCGTGTT-3′
	Reverse: 5′-AAGAGTGGAACAGCGCGTAT-3′
AtPLC5	Forward: 5′-CAAAAGACATGGGAGCCATT-3′
	Reverse: 5′-ACCCGAGAAATCGTCCTTCT-3′
AtPLC6	Forward: 5′-TCCCATTAACCGTTCCAGAG-3′
	Reverse: 5′-CACAGATCGAATCCCTGGTC-3′
AtPLC7	Forward: 5′-CAGGGACTTGGACGATCATT-3′
	Reverse: 5′-TACAGCGTTGGATTTCAGCA-3′
AtPLC8	Forward: 5′-CCCCCTATAAACCCCAAAGA-3′
	Reverse: 5′-TCCGGCTTCTTGACATAACC-3′
AtPLC9	Forward: 5′-CCGAATTTCCTTTTGAATGC-3′
	Reverse: 5′-TGAAGTCCACAATCCATCCA-3′
ACTIN 1	Forward: 5′-GGTCGTACTTTGGCGGATTA-3′
	Reverse: 5′-TCAAGGGTGATGGTCTTTCC-3′

2.6. Sequencing of the PCR products

The PCR products of individual genes were sequenced to confirm their identities. About 100 μL PCR product for each gene was purified using a Quick PCR purification kit for sequencing (Qiagen, Valencia, CA). Forward and reverse primers specific to each PCR product were used to perform the sequencing. DNA was sequenced at the Iowa State University DNA Facility. Sequence analysis confirmed that RT–PCR products amplified in real time RT–PCR analyses were indeed individual *AtPLC* genes.

2.7. Sequence analysis

Chromosomal locations of the *AtPLC* genes were established using the GeneSeqer spliced alignment tool [26]. Sequence alignments were generated with ClustalX [27] using default parameters. Protein domains were identified using the NCBI conserved domain search tool (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Phylogenetic analysis of the sequences was conducted using the MEGA software version 2.1 (http://www.megasoftware.net/ [28]). To calculate pairwise distances, all columns in the alignment containing gaps were removed. The phylogenetic tree was obtained using the neighbor-joining method based on γ-distances with parameter $a = 0.65$, corresponding to correction for multiple substitutions at sites with variable substitution rates [29]; see Table 2). Bootstrap confidence percentages for the nodes were calculated based on phylogenetic trees for 1000 random re-samplings (with replacement) of columns from the multiple sequence alignment.

Table 2
Pairwise distances between *AtPLC* gene products

	AtPLC9	*AtPLC8*	*AtPLC2*	*AtPLC7*	*AtPLC6*	*AtPLC5*	*AtPLC4*	*AtPLC3*	*AtPLC1*	*HsPLCd3*
AtPLC9		0.239	1.709	1.651	1.473	1.595	1.165	1.833	1.877	4.107
AtPLC8	0.184		1.729	1.651	1.473	1.577	1.090	1.877	1.833	4.303
AtPLC2	0.567	0.570		0.201	0.543	0.810	0.733	1.021	0.988	3.295
AtPLC7	0.560	0.560	0.161		0.597	0.828	0.749	1.010	0.966	3.075
AtPLC6	0.537	0.537	0.326	0.345		0.654	0.576	1.103	1.043	3.250
AtPLC5	0.553	0.551	0.409	0.414	0.364		0.792	1.392	1.191	3.390
AtPLC4	0.487	0.473	0.388	0.392	0.338	0.404		1.010	1.043	3.342
AtPLC3	0.582	0.586	0.459	0.456	0.475	0.525	0.456		0.543	2.952
AtPLC1	0.586	0.582	0.452	0.447	0.463	0.492	0.463	0.326		3.075
HsPLCd3	0.726	0.733	0.690	0.678	0.688	0.695	0.693	0.671	0.678	

The numbers below the diagonal are the pairwise p-distances (proportion of amino acid site differences). The numbers above the diagonal are the pairwise γ-distances, correcting for multiple substitutions per site (see Section 2 for details). HsPLCd3, human phospholipase C δ3 (GenBank gi number 19115964).

3. Results

3.1. Arabidopsis contains nine AtPLC genes

The eudicot *Arabidopsis thaliana* contains the nine *AtPLC* genes listed in Fig. 1. We have adopted the nomenclature put forward by Mueller-Roeber and Pical [21] and Hunt et al. [20]. *AtPLC1* (previously designated *AtPLC1S* [15,30]), *AtPLC3* (previously designated *AtPLC1F* [15]), *AtPLC2* [15] and *AtPLC5* (previously designated as *AtPLC6* [18]) were reported earlier. *AtPLC4* and *AtPLC8* were previously identified by cDNA sequencing (directly deposited into GenBank, accessions AY053422 and X85973, respectively). *AtPLC5* and *AtPLC9* were obtained from a large-scale cDNA sequencing project (GenBank accessions BT010399 and BX824997, respectively). No expressed sequenced tags or cDNAs have been reported for *AtPLC6* (previously named *AtPLC8*, GenBank accession AY150803). A cDNA for *AtPLC7* has recently been isolated (GenBank accession NM_115452; [20]). *AtPLC8* and *AtPLC9* are located 30 kb apart on Chromosome 3, widely separated from *AtPLC2* and *AtPLC7* on the same chromosome (Fig. 1). *AtPLC1*, *AtPLC5*, and *AtPLC4* are located in a 12 kb DNA fragment on Chromosome 5. *AtPLC3* and *AtPLC6* occur as singlets on Chromosomes 4 and 2, respectively.

A multiple sequence alignment of the deduced AtPLC amino acid sequences with human PLC-δ_3 is displayed in Fig. 2. The N-terminal sequences of the AtPLC proteins are poorly conserved (not shown), although secondary structure prediction, comparison with animal sequences, and deletion mutant analysis indicated that this region may contain an essential EF-hand domain [21,31]. The catalytic domains X and Y and the C2 domain are conserved among all nine gene family members. *AtPLC8* and *AtPLC9* are the most divergent from the other *AtPLC* genes (Table 2) and have long deletions in the Y region. Both of these genes are transcribed (data presented below); but whether they encode functional PI-PLC enzymes will have to be assessed by mutant analyses and *in vitro* PI-PLC activity assays.

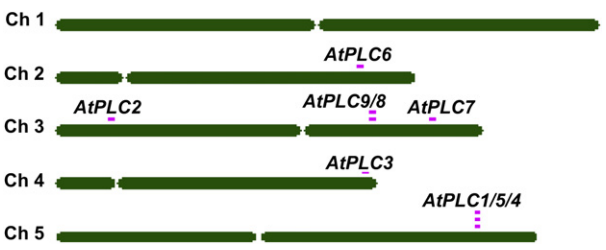

Fig. 1. Chromosomal location of *AtPLC* genes. Gene structures and EST coverage of the genes can be viewed at http://www.plantgdb.org/AtGDB/prj/TBWB08PBB/.

Overall, the predicted amino acid sequences of the *AtPLC* genes are quite divergent, with an average proportion of 39% of the sites differing within the pairwise aligned matching regions of *AtPLC1* to *AtPLC7* (Table 2). Rice, a monocot, contains four *OsPLC* genes. Phylogenetic comparison suggests a single origin of the *AtPLC* and *OsPLC* genes, with subsequent expansion of the gene family by duplications (Fig. 3). The gene pair *AtPLC8* and *AtPLC9* occurs in a tandem array on Chromosome 3 and may represent a relatively recent duplication. However, the evolutionary history of the other genes is less clear. For example, *AtPLC1*, *AtPLC5*, and *AtPLC4* are clustered in an approximate 12 kb region on Chromosome 5, but no two of these genes are closest pairs (Table 2; Fig. 3). Blanc et al. [32] identified large-scale gene duplications in the *Arabidopsis* genome by assigning 3044 gene pairs into collinear blocks. Although some of the *AtPLC* genes are located within duplicated chromosomal segments, none of these genes were conserved in the inferred chromosomal duplications (data shown at http://www.plantgdb.org/AtGDB/prj/TBWB08PBB/), further indicating an early origin of the gene family with much subsequent expansion and rearrangement of chromosomal locations.

The *Arabidopsis* genome does not appear to carry any sequences specific to the other PI-PLC isoforms such as β, γ, and ε found in mammals. Similarly, analysis of the rice genome sequence has also failed to discover homologs of these isoforms. Sequences from other plant species are distributed throughout the plant clade of the phylogenetic tree, with indication of multiple independent within-species duplications (data shown at http://www.plantgdb.org/AtGDB/prj/TBWB08PBB/).

```
AtPLC9          LELPHLNEFLFSTELNP-PISDQVRHRDMNAPLSHYFIHTSLKSYFTGNNVFGRLYSIEP
AtPLC8          LELDQLNEFLFSTKLNP-PIGDQVHH-DMHAPLSHYFIHTSLNSYFTG-NVFG-KYSILP
AtPLC2          LHLDAFFKYLFGDNNPP-LALHKVHH-DMDAPISHYFIFTGHNSYLTG-NQLSSDCSEVP
AtPLC7          LHLDAFFKYLFAVTNSP-LSSLEVH-QDMDAPLSHYFIYTGHNSYLTG-NQLSSDCSELP
AtPLC6          LDLDDFFNFLFYDDLNP-PITPHVH-QDMTAPLSHYFIYTGHNSYLTG-NQLSSDCSEVP
AtPLC5          LNLDDFFSFLFSDDLNH-PIDSKVH-QDMASPLSHYFIYTSHNSYLTG-NQINSECSDVP
AtPLC4          LTLDDFNYYLFSTDLNP-PIADQVH-QNMDAPLSHYFIFTGHNSYLTG-NQLSSNCSELP
AtPLC3          VHLNAFYRYLFSDTNSPLPMSGQVHH-DMKAPLSHYFVYTGHNSYLTG-NQVNSRSSVEP
AtPLC1          IHLEGFYRYLLSDFNSPLPLTREVW-QDMNQPLSHYFLYTGHNSYLTG-NQLNSNSSIEP
HsPLCd3         MTLDGFMMYLLSPEGAALDNTHTCVFQDMNQPLAHYFISSSHNTYLTD-SQIGGPSSTEA
                : *  :  :*:               :*  *::***: :. ::*:*. . ..   *  .
                                         ====================================

AtPLC9          IIDALKQGVRVVELDLLPFGK-DGICVRPKWNFEKPLELQECLDAIKQHAFTPTRSYPVI
AtPLC8          IIEALEQGVRVVELDLWPDGR-GSICVRPSWNFEKPLKLQECLDSIKEHAFT-KCTYPLI
AtPLC2          IIDALKKGVRVIELDIWPNSNKDDIDVLHGMTLTTPVGLIKCLKAIRAHAFD-VSDYPVV
AtPLC7          IIEALKKGVRVIELDIWPNSDEDGIDVLHGRTLTSPVELIKCLRAIREHAFD-VSDYPVV
AtPLC6          VIKALQRGVRVIELDLWPNSTGTDINVLHGRTLTTPVPLMKCLKSIRDYAFS-SSPYPVI
AtPLC5          LIKALKRGVRALELDMWPNSTKDDILVLHGWAWTPPVELVKCLRSIKEHAFY-ASAYPVI
AtPLC4          IADALRRGVRVVELDLWPRGT-DDVCVKHGRTLTKEVKLGKCLESIKANAFA-ISKYPVI
AtPLC3          IVQALRKGVKVIELDLWPNPSGNAAEVRHGRTLTSHEDLQKCLTAIKDNAFH-VSDYPVI
AtPLC1          IVKALRNGVRVIELDLWPNSSGKEAEVRHGGTLTSREDLQKCLNVVKENAFQ-VSAYPVV
HsPLCd3         YVRAFAQGCRCVELDCWEGPG-GEPVIYHGHTLTSKILFRDVVQAVRDHAFT-LSPYPVI
                   *: .* : :***           :           : . :  ::  **     **::
                ================================PLCXc domain=================

AtPLC9          ITIKDSLKPDLQSKVTQMIDQTFGDMVYHEDPQ-QSLEEFPSPAELQNKILISRRPPTKL
AtPLC8          ITFKDGLKPELQSKATQMIQQTFNHMVYHHDP--HSLEVFPSPQQLRNKILISRRPPKEL
AtPLC2          VTLEDHLTPDLQSKVAEMVTEIFGEILFTPPVG-ESLKEFPSPNSLKRRIIISTKPPKEY
AtPLC7          VTLEDHLTPKLQAKVAEMVTDIFGEMLFTPPSG-ECLKEFPSPAFLKKRIMISTKPPKEY
AtPLC6          ITLEDHLTPDLQAKVAEMATQIFGQMLYYPES--DSLLEFPSPASLLHRIIISTKPPKEY
AtPLC5          LTLEDHLTPDLQAKAAEMMKEIFMDMVYFPEA--GGLKEFPSPEDLKYKIVISTKPPKGS
AtPLC4          ITLEDHLTPKLQFKVAKMITQTFGDMLYYHDS--QGCQEFPSPEELKEKILISTKPPKEY
AtPLC3          ITLEDHLPPKLQAQVAKMLTKTYRGMLFRRVS--ESFKHFPSPEELKGKILISTKPPKEY
AtPLC1          LTLEDHLTPILQKKVAKMVSKTFGGSLFQCTD--ETTECFPSPESLKNKILISTKPPKEY
HsPLCd3         LSLENHCGLEQQAAMARHLCTILGDMLVTQALDSPNPEELPSPEQLKGRVLVKGKKLPAA
                :::::      *   :.         :            :***  *  ::::. :
                ========================================================

AtPLC9          LYAK-------------------AVENGV----ELEIQEGSTDKN---------------
AtPLC8          LYAN-------------------DDDGKVGVRNGVEIRQHPADPN---------------
AtPLC2          KEGKDVEVVQKG-KDLGDEEVWGREVPSFIQRNKSEAKDDLDGNDDDDDDDDED------
AtPLC7          KAATDDDLVKKG-RDLGDKEVWGREVPSFIRRDRSVDKNDSNGDDDDDDDDDDDDDDGDD
AtPLC6          LESRNPIVKQKD-NNVSPSSEDETPRTEEIQTLESMLFDQDFESKSDSDQEDEE------
AtPLC5          LR-----------KDKDSESDASGKASSDVSADDEKTEEETSEAKNEEDGFDQE------
AtPLC4          LEANDTKEKDNGEKGKDSDEDVWGKEPEDLISTQSDLDKVTSSVNDLNQDDEERGSCESD
AtPLC3          LES----------KTVH-TTRTPTVKETSWNRVANKILEEYKDMESEAVG----------
AtPLC1          LQT----------QISKGSTTDESTRAKKISDAEEQVQEE--DEESVAIE----------
HsPLCd3         RSED---------------GRALSDREEEEEDDEEEEEEVEAAAQRRLAKQISP------
                                                       .     .

AtPLC9          ---------YQSVVGFHAVEPRGMLQKALT---DDVQQPGWYER-----------DVISN
AtPLC8          ---------YQSLVSFHVVEPRGMLQNVLTGKANKIQRPGWYET-----------DIISF
AtPLC2          KSKINAPPQYKHLIAIHAGKPKGGITECLKVDPDKVRRLSLSEEQLEKAAEKYAKQIVRF
AtPLC7          KIKKNAPPEYKHLIAIEAGKPKGGITECLKVDPDKVRRLSLSEEQLEKASEKYAKQIVRF
AtPLC6          -ASEDQKPAYKRLITIHAGKPKGTVKEEMKVVVDKVRRLSLSEQELDRTCSSNSQDVR--
AtPLC5          -SSNLDFLTYSRLITIPSGNAKNGLKEALTIDNGGVRRLSLREQKFKKATEMYGTEVIKF
AtPLC4          TSCQLQAPEYKRLIAIHAGKPKGGLRMALKVDPNKIRRLSLSEQLLEKAVASYGADVIRF
AtPLC3          ---------YRDLIAIHAANCKDPSKDCLSDDPEKPIRVSMDEQWLDTMVRTRGTDLVRF
AtPLC1          ---------YRDLISIHAGNRKGGLKNCLNGDPNRVIRLSMSEQWLETLAKTRGPDLVKF
HsPLCd3         ---------ELSALAVYCHATRLRTLHPAPNAPQPCQVSSLSERKAKKLIREAGNSFVRH
                            : .     :                .  *            ..
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Fig. 2. Multiple sequence alignment of *AtPLC* gene products. Non-matching N-terminal regions were omitted. The conserved PLCXc (http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=smart00148&version=v1.62), PLCYc (http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=smart00149&version=v1.62) and C2_2 domains (http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=cd00275&version=v1.62) are indicated by underlining. HsPLCd3, human phospholipase C δ3 (GenBank gi number 19115964). "*" represents identical residues in all sequences; ":" means conserved substitutions between similar residues have occurred; "." indicates the semi-conserved substitutions between similar residues.

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AtPLC9          -----------------PPYKPQRAWMHGAQMIALSRQDDKEKLWLMQGMFRANGGCGYV
AtPLC8          TQKRFLRTRPQRKLLIYAPYKPQRAWMHGAQLIALSRKEEKEKLWLMQGMFRANGGCGYV
AtPLC2          TQHNLLRIYPKGTRVTSSNYNPLVGWSHGAQMVAFNMQGYGRSLWLMQGMFRANGGCGYI
AtPLC7          TQRNLLRVYPKGTRITSSNYNPLIAWSHGAQMVAFNMQGLGRSLWVMQGMFRGNGGCGYI
AtPLC6          ---NLLRIYPKGTRFNSSNYKPLIGWTHGAQMIAFNMQGYGKSLWLMHGMFRANGGCGYV
AtPLC5          TQKNLLRIYPKATRVNSSNYRPYNGWMYGAQMVAFNMQGYGRALWMMHGMFRGNGGCGYV
AtPLC4          TQKNFLRIYPKGTRFNSSNYKPQIGWMSGAQMIAFNMQGYGRALWLMEGMFRANGGCGYV
AtPLC3          TQRNLVRIYPKGTRVDSSNYDPHVGWTHGAQMVAFNMQGHGKQLWIMQGMFRGNGGCGYV
AtPLC1          TQRNLLRIFPKTTRFDSSNYDPLVGWIHGAQMVAFNMQSHGRYLWMMQGMFKANGGCGYV
HsPLCd3         NARQLTRVYPLGLRMNSANYSPQEMWNSGCQLVALNFQTPGYEMDLNAGRFLVNGQCGYV
                                . * *   *  *.*::*:. :     : :  * *  ** ***:
                ====================PLCYc domain============================

AtPLC9          KKPNFLLNAG-SSGVFYPTEN-PVVVKTLKVKIYMGDGWIVDFKKR-IGRLSKPDLYVRI
AtPLC8          KKPDFLLNAG-PSGVFYPTVN-PVVVKILKVKIYMGDGWIVDFKKR-IGRLSKPDLYVRI
AtPLC2          KKPDLLLKSGSDSDIFDPKAT-LPVKTTLRVTVYMGEGWYFDFRHTHFDQYSPPDFYTRV
AtPLC7          KKPDLLLKS---NAVFDPEAT-LPVKTTLRVTIYMGEGWYYDFPHTHFDRYSPPDFYTRV
AtPLC6          KKPNFLMKKGFHDEVFDPRKK-LPVK------VYMGDGWRMDFSHTHFDAYSPPDFYTKM
AtPLC5          KKPDFMMNNNLSGEVFNPKAK-LPIKKTLKVKVYMGKGWDSGFQRTCFNTWSSPNFYTRV
AtPLC4          KKPDFLMDASPNGQDFYPKDN-SSPKKTLKVKVCMGDGWLLDFKKTHFDSYSPPDFFVRV
AtPLC3          KKPRILLD---EHTLFDPCKR-FPIKTTLKVKIYTGEGWDLDFHHTHFDQYSPPDFFVKI
AtPLC1          KKPDVLLSNGPEGEIFDPCSQNLPIKTTLKVKIYTGEGWNMDFPLDHFDRYSPPDFYAKV
HsPLCd3         LKPACLRQPD---STFDPEYP-GPPRTTLSIQVLTAQQLP---KLNAEKPHSIVDPLVRI
                 **  : .       * *              :  ..              *  :  .::
                =======                  ==================================

AtPLC9          SIAGVPHDEKIMNTTVKNNE-WKPTWGE-EFTFPLTYPDLALISFEVYDYEVSTPDYFCG
AtPLC8          SIAGVPHDENIMKTTVKNNE-WTPTWGE-EFTFPLTYPDLALISFEVYDYEVSTADAFCG
AtPLC2          GIAGVPGDTVMKKTKTLEDN-WIPAWDE-VFEFPLTVPELALLRLEVHEYDMSEKDDFGG
AtPLC7          GIAGVPADTVMKKTKTLEDN-WIPAWDE-VFEFPLTVPELALLRIEVHEYDMSEKDDFGG
AtPLC6          FIVGVPADNAKKKTKIIEDN-WYPIWDE-EFSFPLTVPELALLRIEVREYDMSEKDDFGG
AtPLC5          GITGVRGDKVMKKTKKEQKT-WEPFWNE-EFEFQLTVPELALLRIEVHDYNMPEKDDFSG
AtPLC4          GIAGAPVDEVMEKTKIEYDT-WTPIWNK-EFTFPLAVPELALLRVEVHEHDVNEKDDFGG
AtPLC3          GIAGVPRDTVSYRTETAVDQ-WFPIWGNDEFLFQLSVPELALLWFKVQDYDNDTQNDFAG
AtPLC1          GIAGVPLDTASYRTEIDKDE-WFPIWDK-EFEFPLRVPELSLLCITVKDYDSNTQNDFAG
HsPLCd3         EIHGVPADCARQETDYVLNNGFNPRWGQ-TLQFQLRAPELALVRFVVEDYDATSPNDFVG
                 * *.  *    .*    .  : * *.:  : * *  *:*:*: . * :::    : * *
                ==============================C2_2 domain====================

AtPLC9          QTCLPVSELIEGIRAVPLYDERGKACSSTMLLTRFKWS------
AtPLC8          QTCLPVSELIEGIRAVPLYDERGKACSSTMLLTRFKWS------
AtPLC2          QTCLPVWELSEGIRAFPLHSRKGEKYKSVKLLVKVEFV------
AtPLC7          QICLPVWELRQGIRAVPLRNQDGVKCRSVKLLVRLEFV------
AtPLC6          QTCLPVAELRPGIRSVPLYDKKGEKMKSVRLLMRFIFE------
AtPLC5          QTCLPVSELRQGIRSVPLYDRKGERLVSVTLLMRFHFL------
AtPLC4          QTCLPVSEIRQGIRAVPLFNRKGVKYSSTRLLMRFEFV------
AtPLC3          QTCLPLPELKSGVRAVRLHDRTGKAYKNTRLLVSFALDPPYTFR
AtPLC1          QTCFPLSEVRPGIRAVRLHDRAGEVYKHVRLLMRFVLEPR----
HsPLCd3         QFTLPLSSLKQGYRHIHLLSKDGASLSPATLFIQIRIQRS----
                *  :*: .:  * * . * .. *     . *:  .
                ========================================
```

Fig. 2. (*Continued*)

3.2. Expression patterns of AtPLC genes in various organs

A quantitative RT–PCR (qRT–PCR) approach was applied to investigate the relative expression patterns of each *AtPLC* gene in different organs. Our data show that *AtPLC2* is constitutively expressed in a variety of *Arabidopsis* tissues (Fig. 4), which is in agreement with a previous study [15]. The other *AtPLC*s have more variable expression profiles, and most are preferentially expressed in a subset of *Arabidopsis* organs (Fig. 4). *AtPLC1* and *AtPLC7* are expressed at higher levels in stems than in the other organs. However, their profiles are distinct, because transcript levels of *AtPLC7* are very low in the other tissues. *AtPLC3* expressed to higher transcript levels in leaves and stems as compared to flowers and roots. The patterns of gene expression among the proximal genes *AtPLC8* and *AtPLC9* are very similar. *AtPLC1*, *AtPLC4*, and *AtPLC5* are clustered on Chromosome 5; however, only *AtPLC4* and *AtPLC5* share similar expression profiles.

For *AtPLC6*, no cDNA sequences are reported. Our qRT–PCR data (Fig. 4) as well as other recent RT–PCR data [20] demonstrate that this gene is transcribed. Expression patterns presented in Fig. 4 are normalized transcript levels against that of the constitutively expressed *Arabidopsis ACTIN 1* gene. Therefore, expression data indicate the relative steady state transcript levels of *AtPLC* gene members and are comparable. Transcripts of *AtPLC2* are most abundant among all organs except roots, in which the level

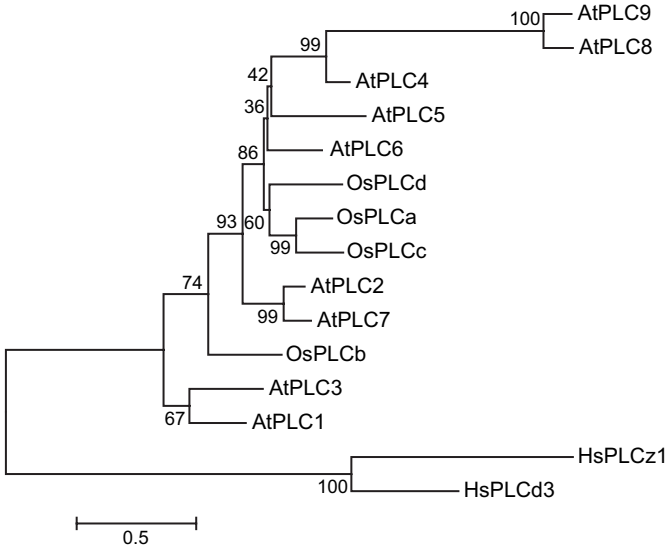

Fig. 3. Phylogenetic analysis of AtPLC and OsPLC proteins. The phylogenetic tree was derived with the neighbor-joining method based on pairwise γ-distances as explained in Section 2. HsPLCd3 and HsPLCz1 refer to human phospholipase C δ3 and ζ_1, respectively (GenBank gi numbers 19115964 and 25188201). Numbers indicate bootstrap confidence percentages.

of *AtPLC4* transcripts is about two-fold higher than that of the *AtPLC2* transcripts. Among the nine genes, transcripts of both *AtPLC6* and *AtPLC7* were the least abundant in all organs investigated.

3.3. Expression patterns of AtPLC genes in stressed tissues

Steady state expression profiles of all nine *AtPLC* genes in *Arabidopsis* seedlings in response to various stress conditions were investigated in two independent experiments (Fig. 5). RNA was extracted from whole seedlings treated with 250 mM salt, 100 μM ABA, 1× MS salts, 4 °C, or dehydration and used in qRT–PCR assays [11]. The fold changes in AtPLC expression were determined relative to non-treated control seedlings (Fig. 5). *AtPLC1* was induced in response to salt, ABA, cold and dehydration, as in the study by Hirayama et al. [11]. The constitutively expressed gene *AtPLC2* was not responsive to the abiotic stresses, which is also in agreement with a previously published report [15]. Apart from *AtPLC2*, all other eight *AtPLC* genes were induced in response to various environmental stimuli. When compared to the non-treated controls, over two-fold increases in transcript levels were recorded for four genes in response to salt, three in response to ABA, four in response to cold, one in response to MS salts, and six in response to dehydration. For *AtPLC8* and *AtPLC9*, the transcript levels were induced less than two-fold of that in controls in response to any of the environmental stimuli. In response to several environmental stimuli, over two-fold induction in transcript levels was recorded for *AtPLC6* for which no cDNA has been reported.

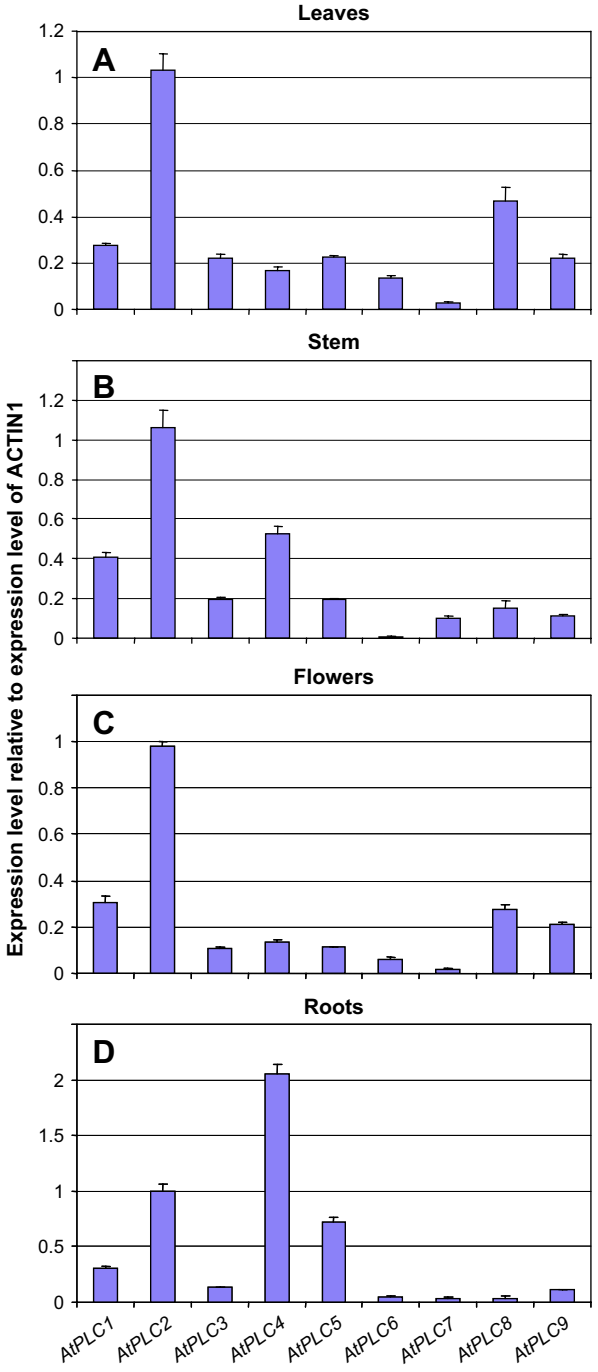

Fig. 4. Relative expression levels of *AtPLC* genes in *Arabidopsis* organs. Relative expression levels for each gene were calculated by normalizing the raw expression levels to the expression levels of *Arabidopsis ACTIN 1* in various organs. A, leaves; B, stems; C, flowers; D, roots. The error bars indicate the standard errors of two independent biological replicates. Total RNA was extracted from *Arabidopsis* organs (ecotype Columbia), and first-strand cDNA was synthesized. Expression of each *AtPLC* gene was determined by quantitative real-time RT–PCR using SYBR Green I detection (see Section 2 for details).

3.4. Association analyses of expression patterns and evolution of AtPLC genes

The steady state *AtPLC* transcript levels, normalized against that of *ACTIN 1*, were used to determine the correlation

Table 3
Association between expression levels of *AtPLC* gene members

	AtPLC1	*AtPLC2*	*AtPLC3*	*AtPLC4*	*AtPLC5*	*AtPLC6*	*AtPLC7*	*AtPLC8*	*AtPLC9*
AtPLC1	1	−0.79*	0.84*	−0.25	0.23	0.5	−0.49	0.09	0.27
AtPLC2		1	−0.59	0.26	−0.18	−0.56	0.66	−0.06	−0.28
AtPLC3			1	−0.3	0.17	0.67	−0.35	0.41	0.4
AtPLC4				1	0.69*	−0.32	0.57	−0.52	−0.54
AtPLC5					1	0.11	0.19	−0.36	−0.22
AtPLC6						1	−0.5	0.73*	0.79*
AtPLC7							1	−0.1	−0.29
AtPLC8								1	0.87*
AtPLC9									1

*The correlation coefficients are significant at $p = 0.001$, d.f. = 18. Normalized expression data for the tissues and treatments (Figs. 4 and 5) were used collectively to calculate the r values shown here.

co-efficient between the expression patterns of pairs of *AtPLC* genes (Table 3). Statistically significant correlation coefficients were recorded between *AtPLC1* and *AtPLC3*, *AtPLC4* and *AtPLC5*, and among *AtPLC6*, *AtPLC8* and *AtPLC9*. In general, association of expression patterns parallel the phylogenetic relationships observed for AtPLC proteins (Fig. 3). In particular, the phylogenetically close tandem gene pairs *AtPLC4*/*AtPLC5* and *AtPLC8*/*AtPLC9* have highly correlated expression patterns (Table 3). This suggests that these gene pairs represent recent duplications. *AtPLC1* is clustered with *AtPLC4*/*AtPLC5* on Chromosome 5 but is most similar to *AtPLC3* (located on Chromosome 4) in both sequence and expression pattern (Table 3; Fig. 3).

Significant similarities in the expression patterns of certain pairs of *AtPLC* gene members led us to investigate the possible role of common *cis*-regulatory elements in transcription of those genes. Gene pairs with significant association for their expression patterns also contained common *cis*-regulatory elements in their promoter regions. For example, *AtPLC1* and *AtPLC3* had significant positive association for their expression patterns ($r = 0.84$), and their promoter sequences contain the same nine *cis*-regulatory elements. There was only one exception for the *AtPLC3* promoter that contains an additional *cis*-regulatory element (Table 4). Similar patterns of *cis*-regulatory elements were also observed for gene pairs *AtPLC4* and *AtPLC5* and *AtPLC8* and *AtPLC9* showing significant associations for their steady-state transcript levels (Table 3). Although *AtPLC4*, *AtPLC5* and *AtPLC7* showed similar stress-related induction patterns (Fig. 5), there was no significant association of *AtPLC7* with either *AtPLC4* or *AtPLC5* at $p = 0.001$ (Table 3).

ClustalW alignment of the promoters of gene pairs showing significant association for their expression patterns revealed high levels of sequence conservation. The promoters of *AtPLC4* and *AtPLC5* contain two putative *cis*-regulatory elements (Fig. 6). In the *AtPLC4* promoter these two sequence motifs are 10 nucleotides apart. This distance is equivalent to one complete turn of the DNA double-helix, placing both conserved motifs into adjacent locations presumably for binding transcription factors.

4. Discussion

To date cloned plant PI-PLC sequences, including those from moss, form a single class of proteins with structural similarity to the mammalian PI-PLC-ζ isoform, which also does not contain the PH domain [4,33]. Alignment of all nine *AtPLC* genes with mammalian PLC isoforms PLC-δ and ζ revealed that although structurally PLC-ζ is closest to plant PI-PLCs, at the sequence level PLC-δ is closest to *AtPLC* genes

Table 4
Common putative *cis*-acting regulatory[a] elements of the *AtPLC* gene family

Binding site name	Binding site sequence	Transcription factor	*AtPLC* gene[b]
RAV1-A binding site motif	caaca	ABI3VP1	1, 2, 3, 4, 5, 6, 8, 9
ARF1 binding site motif	Tgtctc	ARF	1, 2, 3, 4, 5, 8
AtMYC2 BS in RD22	cacatg	BHLH	1, 3, 8, 9
ATB2/AtbZIP53/AtbZIP44/GBF5 BS in ProDH	acacatg	bZIP	1, 3, 4, 5, 6, 8, 9, 7
ATHB6 binding site motif	caattatta	HB	5, 9
Bellringer/replumless/pennywise BS1 IN AG	aaattaaa	Homeobox	1, 3, 4, 8, 9
LFY consensus binding site motif	ccaatg	LFY	1, 3, 4, 8, 9
CArG promoter motif	ccaaaaatgg	MADS	3, 5
MYB4 binding site motif	aactaac	MYB	1, 2, 3, 4, 5, 8
CCA1 binding site motif	aaaaatct	MYB related	1, 3, 6, 8
W-box promoter motif	ttgact	WRKY	1, 3, 4, 8, 9

[a] The cis-acting regulatory elements for the *AtPLC* gene members were obtained from http://arabidopsis.med.ohio-state.edu/ and links presenting information of individual *AtPLC* members are included at http://www.plantgdb.org/AtGDB/prj/TBWB08PBB/.
[b] Genes that showed significant associations for their expression patterns (Table 2) are underlined.

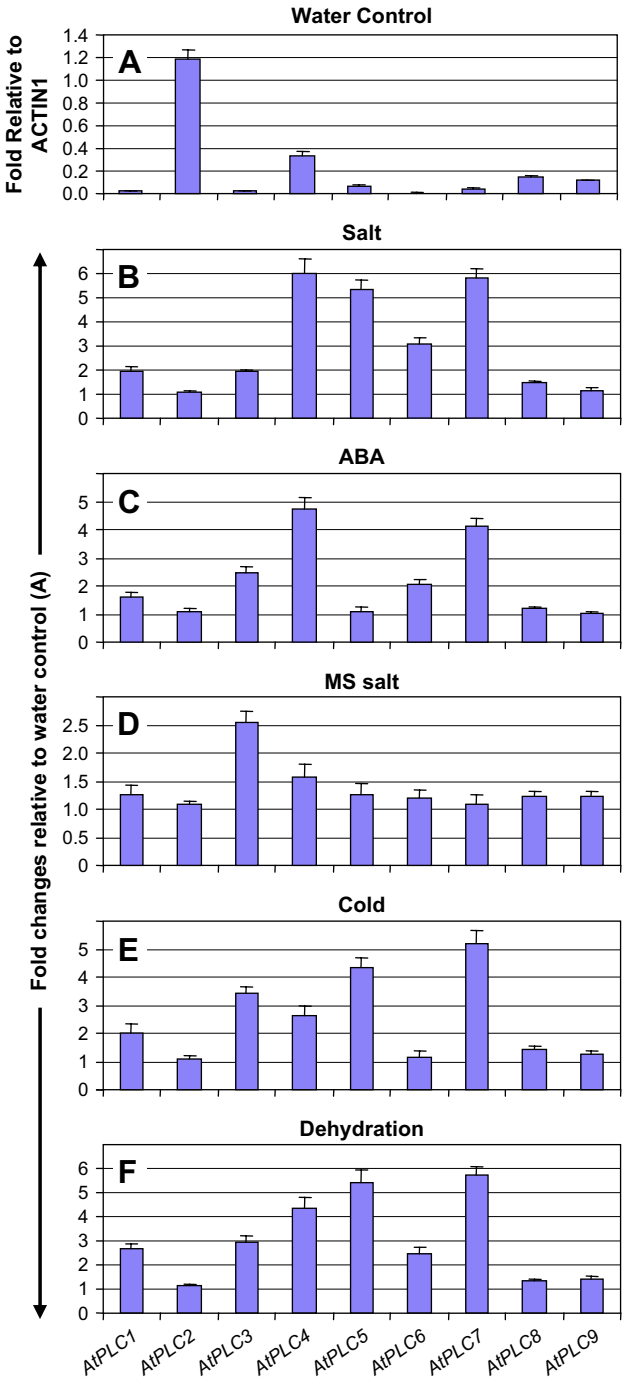

Fig. 5. Relative expression levels of *AtPLC* gene family in response to environmental stimuli. (A) Relative expression levels of "water control" seedlings (fed with water for 10 h) were calculated relative to expression level of *ACTIN 1*. (B–F) Fold changes relative to water controls were calculated as follows. First, expression levels of all nine *AtPLC* genes relative to expression levels of *ACTIN 1* in individual treatments were calculated. The normalized data of all nine genes were then used to calculate their fold changes relative to their normalized transcript levels in water control shown in A. Seedling in B (Salt), fed with 250 mM NaCl for 10 h; C (ABA), 100 μM ABA for 10 h; D (MS salt), 1× MS salt for 10 h; E (Cold), exposed to 4 °C under light for 10 h; F (Dehydration), dehydrated on dry Whatman 3MM papers for 5 h. The error bars indicate the standard errors of two independent biological replicates. Total RNA was extracted from 3-week-old seedlings of the ecotype Columbia following various treatments for qRT–PCR experiments.

(Fig. 2 http://www.plantgdb.org/AtGDB/prj/TBWB08PBB/). The phylogenetic history of the eukaryotic PI-PLCs is complex, apparently involving multiple domain gain or loss events and gene duplications.

Evolution of the members of the *AtPLC* gene family probably occurred in multiple steps. The tandem genes *AtPLC8* and *AtPLC9* presumably evolved through local duplication in more recent times. *AtPLC1*, *AtPLC4* and *AtPLC5* are tandem genes organized in a small DNA fragment. It is possible that the progenitors of *AtPLC4*/*AtPLC5* and *AtPLC1*/*AtPLC3* arose from a single gene by a duplication event on Chromosome 5, with subsequent duplications and relocation of *AtPLC3* to Chromosome 4 responsible for the extant gene arrangement. Knowledge of the sequence and organization of *PI-PLC* genes present in the genomes of other members of the *Brassicaceae* will be required to clarify the complex evolution of this gene family.

In the present study, transcript profiles of *AtPLC1* are in agreement with the observations by Hirayama et al. [11], who also reported induction of the gene in response to ABA, salt stress, low temperature and dehydration. In general, the quantitative expression patterns for *AtPLC1* through *AtPLC5* in response to salt, cold and dehydration or drought were comparable to the corresponding qualitative expression patterns reported earlier by Hunt et al. [20]. Similarly, overall quantitative expression patterns in leaves, flowers and roots (Fig. 4) were comparable to the qualitative expression patterns for these three organs reported by Hunt et al. [20]. In our quantitative PCR, the amounts of *AtPLC7* transcripts in all three organs were similar and very low (Fig. 4). Hunt et al. [20] also observed similar expression patterns of *AtPLC7* in these three organs. Likewise, very little expression levels of *AtPLC6* and *AtPLC8* in roots were observed in this and the study by Hunt et al. [20]. Lack of responses of *AtPLC5* to ABA (Fig. 5) was comparable to the corresponding qualitative expression pattern of the same gene (termed earlier as *AtPLC6*) in ABA-treated seedlings studied by Xu et al. [18].

Steady-state transcripts of most of the *AtPLC* genes were induced in response to the environmental stimuli considered in our investigation. Environmental factors such as salt, cold, and dehydration enhanced transcript accumulation of multiple members of the *AtPLC* gene family. Steady-state transcript levels of all nine genes except *AtPLC2*, *AtPLC8* and *AtPLC9* increased two or more fold over that in the water controls in response to at least one of the environmental stimuli. Some of the treatments such as salt, ABA, cold, and dehydration caused transcript levels of three to six *AtPLC* genes to increase two or more fold over that in the water controls. An increase in transcript levels of *AtPLC* genes is expected to correlate with greater accumulation of AtPLC proteins resulting in enhanced PI-PLC activities and higher accumulation of IP_3 molecules. Increased IP_3 levels over a sustained period have been reported during gravistimulated growth as well as in responses to nutrients and salts [34–36].

PI-PLCs most likely play an important role in regulating growth. Perera et al. [34] reported increases in IP_3 levels for a sustained period preceding the gravistimulated growth of maize pulvini. We have observed that soybean PI-PLC activity was induced by MS salts. By using a PI-PLC-specific

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At5g58690.1_AtPLC5      GCGTGTATCCATCTAGATTTATATCTTCTTACTTTATTCGATATGCTGTGTTTTATGTGT 74
At5g58700.1_AtPLC4      ATAGCCGAACACTTTATTTGTTGTCGCTTTGCTAT-TGCAACATATCTAATGTTATCTGT 719
                                ** *  **  * **   ** ** * * * * **      * **** ***

At5g58690.1_AtPLC5      CTTATGCTTCTTCATTGATGA-ATAAAATTTGATCTTTACATGAGA--AATATATAGATC 131
At5g58700.1_AtPLC4      TTTTTTTTTTCTTTTGGCCGACATCTGAAGTATTTCAAAAATTAAATTGAATTTCGCATT 779
                         ** *  **  *  * *  ** **   *  *  *    * ** * *   *  *    **

At5g58690.1_AtPLC5      TTCTGAAATTTCCTTTATGG--TTTCATTTA-TATATGTTACCAGTTTCTTGGTC-TTGG 187
At5g58700.1_AtPLC4      TCTTGTTTTTTCCTACATAAAACATCATTTAATAGATAATTAAAGAAATTCAACCACTGA 839
                        *  **   ******  **      ******* ** **  *   **    *    *  **

At5g58690.1_AtPLC5      GATGAAGTTTTGTTAAATAAGA----ACCAAAACCAAAGAACTTATGATAACATTAAT-- 241
At5g58700.1_AtPLC4      TAAATAGTGCAAAAATACAAATTGTTACAAACATCAAATAATTTGGGGGAAAAATAATTC 899
                         *   ***      * * **      ** ** * **** ** **  *  ** * ****

At5g58690.1_AtPLC5      --AAGCATCTCTCATCTTATCATAAAAACATA--ACCT--CTTTATTCTTTTT--CTGGA 293
At5g58700.1_AtPLC4      CCAAGCATGCTATAAAATAAAATGGAAATATATGAATTAATTTTACTATGAATAACAAAA 959
                          ******     *   **  **  *** ***  *  *   **** * *   *  *   *
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Fig. 6. Identification of a pair of putative *cis*-acting regulatory elements. ClustalW analysis of the promoters of *AtPLC4* and *AtPLC5* (http://www.ebi.ac.uk/clustalw/index.html) revealed the putative *cis*-regulatory elements that are underlined.

inhibitor, we had shown that a sustained increase in PI-PLC activity caused by MS salts was correlated with DNA synthesis [35]. Transcripts of *AtPLC3* were induced to more than two-fold over that in control in response to the MS treatment (Fig. 5). Therefore, MS-induced growth responses are most likely regulated, in part, by increased accumulation of PI-PLC transcripts. Study of the *atplc3* knock-out mutants should enable us to establish the role of PI-PLC in plant growth.

Recently, through use of a PI-PLC-specific inhibitor, the role of this signaling enzyme in proline accumulation has been established [37]. In plants, proline accumulates in response environmental stresses such as salinity and water stresses. Parre et al. [37] have shown that IP_3-regulated Ca^{2+} is required for salt-induced proline accumulation.

The plant hormone ABA plays important roles in embryogenesis, seed dormancy, root and shoot growth, transpiration, and stress tolerance. Importance of phosphoinositides in ABA-induced signaling has been established through genetic experiments [19,38]. Results presented here showed that at least three *AtPLC* genes (*AtPLC3*, *AtPLC4*, and *AtPLC7*) could be important for the ABA-induced signal pathway.

Results from previous and this study have showed increased transcript accumulation for most members of the *AtPLC* gene family under stress conditions [11,18,20]. Investigation of null *atplc* mutants should confirm the importance of this candidate signal transducing gene family in dealing with environmental stresses. Salt stress, dehydration, and cold stress are some of the environmental stresses that plants face during their life cycles. Transcriptional activation of *PI-PLC* genes in *Arabidopsis* and other plant species in response various environmental stresses strongly suggests the importance of this signal transducing enzyme for adapting plants to stress environmental conditions [11,16,20,39].

Acknowledgments

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