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Expression, purification, and subcellular localization of phospholipase C in *Dunaliella salina**

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Abstract Plants possess effective mechanisms to respond quickly to the external environment. Rapid activation of phosphatidylinositol-specific phospholipase C (PLC) enzymes occurs after a stimulus. The PLC in *Dunaliella salina* plays important roles in growth and stress responses. However, the molecular basis of PLC action in *D. salina* remains little understood. To gain insight into the potential biological functions of this enzyme, we cloned a phospholipase C gene from *D. salina* in a previous study, named *DsPLC* (GenBank No. KF573428). Here, we present the prokaryotic expression, purification, and characterization of the *DsPLC* gene. The entire coding region of *DsPLC* was inserted into an expression vector pET32a, and the *DsPLC* gene was successfully expressed in *Escherichia coli*. The DsPLC protein was purified and identified using a polyclonal antibody and western blotting. Expressing DsPLC fused with a green fluorescent protein (GFP) in onion showed that DsPLC-GFP was localized to the intracellular membrane. Quantitative real-time PCR analysis revealed that the relative expression of the *DsPLC* gene was induced significantly by 3.0-mol/L NaCl at 4 h. Our results support the importance of PLC enzymes in plant defense signaling. This study provides a basis for further functional studies of the *DsPLC* gene and for additional analysis of the potential roles of PLC enzymes in response to abiotic stress.

Keyword: *Dunaliella salina*; *DsPLC* gene; prokaryotic expression; subcellular localization; salt stress

1 INTRODUCTION

Salt stress is a major abiotic factor limiting the growth, development, and distribution of plants (Zhang et al., 2012). Scientists have long sought to understand and improve the mechanisms of salt tolerance in plants (Chen et al., 2016). As is well known, *Dunaliella salina* is a unicellular, halotolerant green alga; it has the unique ability to survive in a wide range of salt conditions, from 0.05 mol/L NaCl to 5.5 mol/L NaCl. It is a recognized model organism for studying plant adaptation to high salinity (Oren, 2005; Gong et al., 2014; Liu et al., 2015; Arroussi et al., 2018), and previous studies have attempted to reveal the physiological and molecular mechanisms of its salt resistance (Chen et al., 2011; Zhao et al., 2013; Belhaj et al., 2017). To manage high salinity in its living environment, *D. salina* has evolved a significant degree of developmental adaptability, including adaptation by way of molecular networks (Tammam et al., 2011; Liu and Yildiz, 2018). A number of important genes related to salt stress, such as *DsGPI*, *Ds-26-16*, *Dscyp*, and the nitrate reductase (NR) gene, have been cloned, and their functions have been confirmed by genetic transformation (Xie et al., 2007; Cui et al., 2010; Gong et al., 2014; Liu et al., 2015). However, the molecular mechanism of salt stress signaling in *D. salina* is not yet fully clear.

Among abiotic factors, salinity stress affects each aspect of the plant from gene expression to metabolic regulation (Punta et al., 2012). Understanding abiotic stress responses and the signal transduction that controls the adaptive pathways is an important method of determining the resistance of plants exposed to

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adverse environments. In recent years, many studies have reported important research results about osmotic adjustment, salinity tolerance genes (He et al., 2007; Jia et al., 2009; Ramos et al., 2011) and salinity tolerance proteomics (Liska et al., 2004; Katz et al., 2007; Liu et al., 2015) in *D. salina*. The phosphoinositides are phosphorylated derivatives of phosphatidylinositol, and they are a small group of phospholipids with a significant role in lipid signaling in most living organisms. The *PLC* gene, which encodes the phosphoinositide-specific phospholipase C (PLC) enzyme, is involved in a wide variety of stress-induced lipid signaling pathways and plays a prominent role in plant defense signaling (Abd-El-Haliem et al., 2016; Li et al., 2017; Lv et al., 2017).

PLC is a key enzyme in phosphatidylinositol turnover during signal transduction and is widely present in eukaryotes. Phosphatidylinositol-4,5-bisphosphate (PIP2) can be effectively hydrolyzed by PLC into inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG), and these products respectively activate two signal transduction pathways: IP3/Ca^{2+} and DG/PKC (Arz and Grambow, 1994; Liska et al., 2004; Abd-El-Haliem et al., 2016; Cerminati et al., 2017; Lv et al., 2017). Rising IP3 levels cause increased calcium concentration, resulting in the activation of Ca^{2+} binding protein (Hong et al., 2016). Therefore, this process may result in a phosphorylation state change in the key enzyme in the glycerol metabolic pathway (Einspahr et al., 1989; Li et al., 2017). Many *PLC* genes are transcriptionally induced by versatile environmental stimuli such as salt, dehydration, and cold. *PLC1* in *Arabidopsis thaliana* is induced by abscisic acid (ABA), salt stress, dehydration and low temperature (Hirayama et al., 1995). In *Vigna radiata* L. (mung bean), *VrPLC3* is also induced by salt stress in an ABA-independent manner (Kim et al., 2010). *AtPLC2* is the major phospholipase in phosphoinositide metabolism and is involved in seedling growth and the endoplasmic reticulum (ER) stress response in *Arabidopsis thaliana* (Kanehara et al., 2015). The DAG content of *D. salina* has been shown to increase under hypotonic stress by high performance liquid chromatography (Ha and Thompson, 1991). DAG may function as a second messenger in PLC-mediated signal transduction (Hong et al., 2016). Although the biological role and mechanism of action of PLC in *D. salina* have not been fully elucidated, recent studies in *Arabidopsis thaliana* have shown that PLC is associated with abiotic stress responses. In addition, in a previous study, we cloned a full-length phospholipase C cDNA from *D. salina* (GenBank accession No. KF573428). The DNA sequence of DsPLC contained a 1 782-bp open reading frame that encoded a 593-amino-acid protein of 66.0 kDa (Han et al., 2014). Based on these previous studies, we hypothesized that PLC is related to salt stress resistance in *D. salina*.

In the present study, we report that DsPLC was expressed in *E. coli* successfully, and a highly purified fusion protein was obtained. The subcellular localization recombinant vector pMDCG-DsPLC was transformed into onion epidermal cells by an *Agrobacterium tumefaciens*-mediated method, and the cellular distribution of PLC was observed with a reporter gene. In a transient expression assay, DsPLC was observed at the intracellular membrane. Quantitative RT-PCR analysis indicated that the relative expression of *DsPLC* under salt stress (3.0 mol/L NaCl) was almost 100-fold higher than that of the control group (1.5 mol/L NaCl). The DsPLC mRNA level was the highest at 4 h under 3.0 mol/L salt treatment. Our data suggest that DsPLC is a key regulator of salt stress response in *D. salina*.

2 MATERIAL AND METHOD

2.1 Material

The *D. salina* strain CCAP 19/3 was obtained from the Hydrobiology Laboratory of Dalian Ocean University. Cultures of *D. salina* were maintained in an irradiance of 50 μmol photon/(m^2·s) on a 12 h:12 h light:dark cycle. Cells were cultured in f/2 medium for several weeks in 0.5 mol/L NaCl as previously described (Katz and Avron, 1985). Temperature and pH were controlled at 25±1°C and 7.5±0.2, respectively. Cell growth was evaluated by the absorbance of the cultures at 630 nm in a spectrophotometer. Starting culture optical density was adjusted to OD_{630}=0.06–0.08 (approximately 5×10^5 cells/mL). Cells at the logarithmic growth phase, containing $(2–3)\times10^6$ cells/mL, were transferred to fresh medium containing 1.5 mol/L NaCl.

2.2 Method

2.2.1 Extraction of total RNA

Cells were harvested by centrifugation at 8 000×*g* for 5 min. Then, the cell pellets were washed twice with distilled water. Total RNA was extracted from *D. salina* cells using the RNAiso Plus kit (TaKaRa,

Dalian, China) according to the manufacturer's instructions. The RNA quality was examined by 1% agarose gel electrophoresis. The concentration of RNA was evaluated by using a DNA/Protein Analyzer (GeneQuant Pro RNA/DNA, America).

2.2.2 Prokaryotic expression and purification of DsPLC

2.2.2.1 PCR amplification of DsPLC and construction of the prokaryotic expression vector

One microgram of total RNA was converted to cDNA using PrimeScript Reverse Transcriptase (TaKaRa). A pair of primers, S1 (5′-CGAGCTCATGA-AGAACAAGCTCTTTG-3′) and A1 (5′-CAAGCTT-TTAGCAGGAATAAAGGGGC-3′) were designed to amplify the cDNA. The underlined sequences indicate the *Sac I* site in S1 and the *Hind III* site in A1. The cDNA was used as a template, and the PCR conditions included a "hot start" of 94°C for 5 min followed by 35 cycles of 94°C for 0.5 min, 58°C for 0.5 min, and 72°C for 1 min, and then a final extension for 9 min at 72°C. The major products of the PCR were purified using a QIAquick Gel Extraction Kit (Asjswjsh, Hangzhou, China), cloned into T-Vector pMD19 (Simple) (TaKaRa), and then transformed into competent cells of *E. coli* DH5α. The pMD19-DsPLC and pET-32a plasmids were cut with Sac I/Hind III, and then pET-32a-DsPLC was generated by T4 DNA Ligase. The recombinant plasmid pET-32a-DsPLC was identified and sequenced again.

2.2.2.2 Induction of fusion protein expression and solubility analysis

The recombinant plasmid pET-32a-DsPLC was transformed into competent cells of *E. coli* BL21 (Tiangen). The transformed cells were grown at 37°C overnight with shaking in 5 mL Luria-Bertani (LB) medium containing ampicillin at 50 μg/mL. 0.1 mL transformed cells were replaced in 5 mL fresh LB medium with ampicillin. The cells were incubated overnight at 37°C (OD_{600}=0.6–0.8). Subsequently, the culture was induced with 0.8 mmol/L isopropyl β-D-1thiogalactopyranoside (IPTG) and further cultured for an additional 5 h to induce the expression of the fusion protein. A control group was cultured simultaneously. Fifty milliliters of cells from the control group and experimental group were collected separately by centrifugation; 100 μL loading buffer was added, incubated under foil for 5 min, and then centrifuged again. Finally, the supernatant was obtained, and the protein was detected by electrophoresis. In addition, 100 mL of induced cells were added to 10 mL 0.1 mol/L PBS and then disrupted by ultrasonication in an ice bath; the optimal setting for breaking was 10 min (on 9 s, off 9 s) at 30 W. The supernatant and sediment were collected by centrifugation, loading buffer was added, incubated under foil for 5 min, and then the product was monitored by SDS-PAGE.

2.2.3 Expression of *DsPLC* under salt stress

Dunaliella salina was cultured under salt stress conditions (3.0 mol/L NaCl) for 0, 0.5, 1, 2, 3, 4, 6, and 12 h. Total RNA was extracted from each sample and converted to cDNA as described above. To analyze the expression level of *DsPLC* under salt stress (3.0 mol/L NaCl), real-time quantitative PCR was carried out with the DsplcD1 (5′-CAACCACCTCCAGTTTCAATG-3′) and DsplcD2 (5′-CTCCACTGTCTCTCCTTTCTC-3′) primers, following the instructions of the SYBR Premix Ex Taq Kit (TaKaRa) and using an ABI 7300 Fast Real-Time PCR System. Another pair of primers, ActinF (5′-TTGGGTAGTCGGGCTGGTC-3′) and ActinR 5′-CGCTGCGTTCTTCATCGTT-3′), were used as the endogenous control. The reaction was performed in a volume of 25 μL, and cycling conditions were 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, 60°C for 34 s. The specificity of PCR amplification was checked with a melting curve program and electrophoresis on 1% agarose gels. Similar experiments were repeated three times, and the statistical significance of the samples at different treatment times was analyzed using the comparative C_t ($2^{-\Delta\Delta Ct}$) method (Lei et al., 2008).

2.2.4 Western blot analysis of DsPLC

We obtained a large amount of soluble recombinant protein and purified it using a His-Sefinose Kit (Sangon). Rabbits were immunized 4 times with purified recombinant protein (0.01 g/mL) to generate polyclonal antibodies. An enzyme-linked immunosorbent assay (ELISA) showed that the polyclonal antibodies had high antibody titers and good specificity.

Dunaliella salina was cultured under salt stress conditions (3.0 mol/L NaCl) for 0, 2, 3, 4, 6, 12, and 24 h. Cells were harvested by centrifugation for 5 min at 8 000×*g*. Total protein was extracted from each sample using RIPA Lysis Buffer IV (Sangon). The

protein was analyzed by SDS-PAGE and then by electrophoretic transfer onto polyvi-nylidene difluoride (PVDF) membrane (200 mA, 2 h). Protein staining of membranes was performed using Ponceau S solution (Sigma). The PVDF membrane was blocked with 3% BSA for 12 h at 4°C. The membrane was washed 3 times with PBST (0.05% Tween-20 in phosphate-buffered saline (PBS)) then incubated for 1 h at 37°C with the polyclonal antibody (diluted 1:1 000). After the membrane was washed 3 times with PBST, HRP-IgG was applied as the secondary antibody (diluted 1:2 000, Sangon), and specific binding was detected with DAB (Asjswjsh).

2.2.5 Subcellular localization of DsPLC

2.2.5.1 PCR amplification of DsPLC and construction of the subcellular localization vector

Based on the cDNA of *DsPLC* and the site of the pMDCG vector, a pair of primers, S2 (5′-CTCTAGA-ATGAAGAACAAGCTCT-3′) and A2 (5′-GTCGAC-TTAGCAGGAATAAAGG-3′), was used to amplify *DsPLC*. The underlined sequences indicate the *Xba*I site in S2 and *Sal*I site in A2. The cDNA was used as a template, and the PCR conditions included 94°C for 5 min and 34 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 2 min. The amplified DNA cloned in the pMD19-T vector was digested with the *Xba*I and *Sal*I restriction enzymes and then ligated into the pMDCG vector. The pMDCG-DsPLC recombinants were transformed into *E. coli* DH5α. Positive clones were selected by PCR and restriction digestion and then sequenced.

2.2.5.2 Agrobacterium-mediated genetic transformation of onion epidermis

The plant expression vectors pMDCG-DsPLC and pMDCG were transformed into Agrobacterium GV3101. Onion epidermis was cut into 1.0 cm×1.0 cm discs and incubated in the diluted Agrobacterium culture for 20 min, with gentle shaking several times during the co-culture period. After drying the discs with filter paper, the explants were placed on MS medium under 16 h/8 h light/dark conditions at 25°C. Green fluorescence micrographs were obtained using a fluorescence microscope (Carl Zeiss, Germany) at the Key Laboratory of Hydrobiology, Dalian Ocean University. The filter set was 09, and the excitation wavelength was 490 nm. Onion epidermal cells with DsPLC-GFP vector were treated with 3.0 mol/L NaCl for 15 s. In addition, the fluorescence introduced by DsPLC-GFP was directly observed using a confocal microscope. Tissues from nontransgenic onion were also prepared and observed under the same conditions to serve as a control.

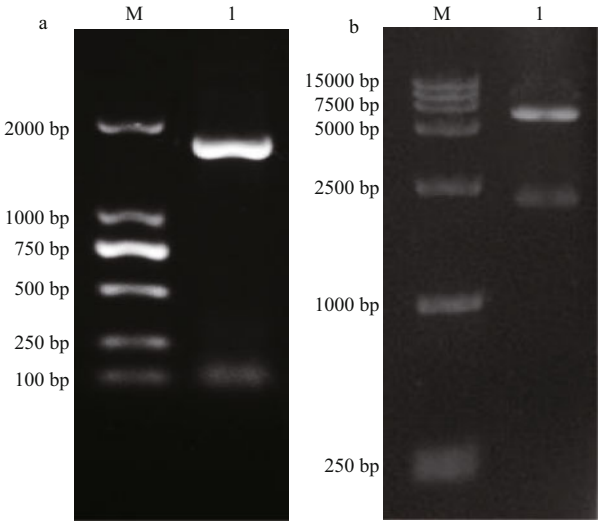

Fig.1 Construction of the recombinant pET-32a-DsPLC plasmid

a. Lane M: DNA marker DL-2000; Lane 1: PCR amplification products; b. Lane M: DNA marker DL-15000; Lane 1: pET-32a-DsPLC (*Sac* I/*Hin*d III) products.

3 RESULT

3.1 Prokaryotic expression and purification of the fusion protein

PCR using the primer pair S1 and A1 generated a *DsPLC* cDNA fragment of 1 782 bp (Fig.1a). The resulting fragment was cloned into pMD-19-T to yield the recombinant plasmid pMD19-DsPLC. The *DsPLC* gene sequence was obtained by restriction endonuclease cleavage and ligation of the recombinant plasmids pMD19-DsPLC and pET-32a, and then pET-32a-DsPLC was transformed into *E. coli* BL21 competent cells. Sequencing of the recombinant plasmid pET-32a-DsPLC showed that the sequence contained no mutations due to cloning or manipulation. The plasmid pET-32a-DsPLC was verified by cutting with *Sac*I/*Hind*III (Fig.1b).

Escherichia coli cells transformed with the gene fusion were induced using IPTG to enhance the transcription of pET-32a-DsPLC. Fusion protein extracts from the supernatant were analyzed by 12% SDS-PAGE. As shown in Fig.2, the induced samples revealed high levels of fusion protein expression (Lanes 1–4) compared to that of the uninduced sample (Lane 6). One distinct band of ~87 kDa was identified

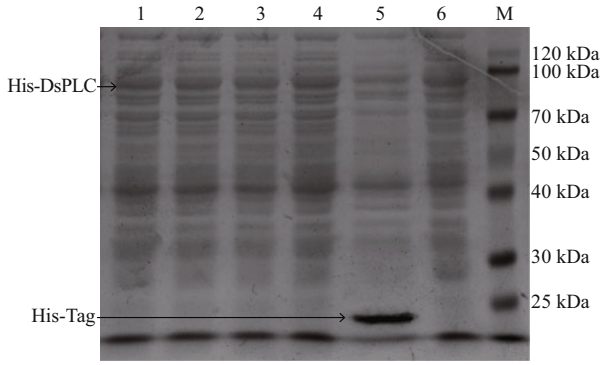

Fig.2 SDS-PAGE analysis of fusion DsPLC protein expressed in *E. coli* at 37°C

Lane M: protein marker; Lanes 1, 2, 3, and 4: samples subjected to IPTG concentrations of 0.2, 0.4, 0.6, and 0.8 mmol/L, respectively; Lane 5: the labeled protein; Lane 6: samples without IPTG induction. Note the target protein at ~87 kDa (arrows).

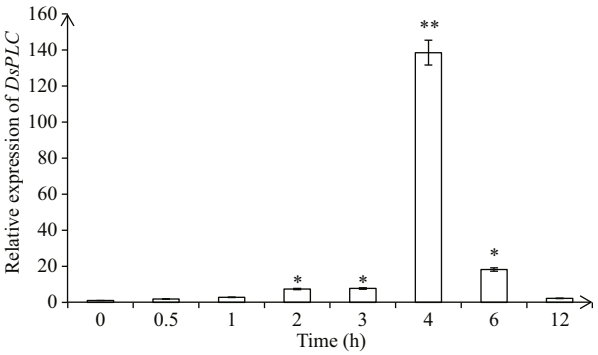

Fig.3 Expression of *DsPLC* in *D. salina* induced by salt stress

The expression level of the *DsPLC* gene at 0 h was used as a control. The relative expression level of *DsPLC* under high-salt stress (3.0 mol/L NaCl) was tested at different salt stress times.

(Fig.2), in agreement with the expected molecular mass of pET-32a-DsPLC, which consists of His (21 Da) and DsPLC (66 kDa). The fusion protein was observed in Lanes 1–4, and His was observed in Lane 5.

3.2 Expression analysis of the *DsPLC* gene

The expression of the *DsPLC* gene was detected at different times under high-salt stress (3.0 mol/L NaCl). *DsPLC* gene expression reached a maximum at 4 h (Fig.3) when the relative expression level of *DsPLC* was almost 100-fold higher than that of the control group (1.5 mol/L NaCl). These results show that the *DsPLC* gene was upregulated under salt stress. The pattern of *DsPLC* expression implied that this gene might play a significant role in the response of *D. salina* to high-salt stress.

3.3 Western blot analysis of DsPLC

The expression of the DsPLC protein at different times under high-salt stress (3.0 mol/L NaCl) was further confirmed by western blot analysis. The results indicated that the DsPLC protein was expressed at 2 h and reached an expression maximum at 4 h (Fig.4); the protein had a relative molecular mass of approximately 66 kDa.

3.4 Subcellular localization of DsPLC

We investigated the subcellular localization of the DsPLC protein with an assay in which the protein was expressed transiently in onion epidermal cells. The control cells showed fluorescence throughout the cells; as shown in Fig.5, the DsPLC-GFP cells displayed signals in the plasma membrane and cytoplasm. When DsPLC-GFP was introduced into onion epidermal cells treated with 3.0 mol/L NaCl salt stress, DsPLC-GFP displayed a high level of the signal in only the cytoplasmic region of the onion cells. Accordingly, these results suggest that DsPLC is localized to the intracellular membrane system of eukaryotic cells.

4 DISCUSSION

Dunaliella salina can survive in extremely variable levels of salinity. It has a rapid and highly effective osmoregulation system, and it has been thoroughly studied as an ideal model organism; many unique genes associated with salt stress responses have been studied already (Oren, 2005; Gong et al., 2014; Belhaj et al., 2017; Fang et al., 2017). The integration of signals with other environmental cues enables plants to adapt their physiology to changing environments (Kanehara et al., 2015; Abd-El-Haliem et al., 2016; Lv et al., 2017). Phosphoinositides are primary lipid-derived signals involved in diverse organism responses to the surrounding environment (Zhai et al., 2005; Kanehara et al., 2015). Membrane-associated PLC enzymes are known to be involved in intracellular signaling in eukaryotes (Kanehara et al., 2015). Many studies to date have investigated the PLC of plants (Hirayama et al., 1995; Kanehara et al., 2015). The *AtPLC6* gene is strongly induced at low temperatures and weakly induced under many stresses, such as ABA, heat and high-salt stress (Xu et al., 2004). The *PtoPLC1* gene is strongly induced under high salt, dehydration, and ABA, and a *PtoPLC1* fusion protein expressed in vitro has enzyme activity (Zhang et al., 2015). We previously cloned a full-length phospholipase C cDNA from *D. salina* (Han et al.,

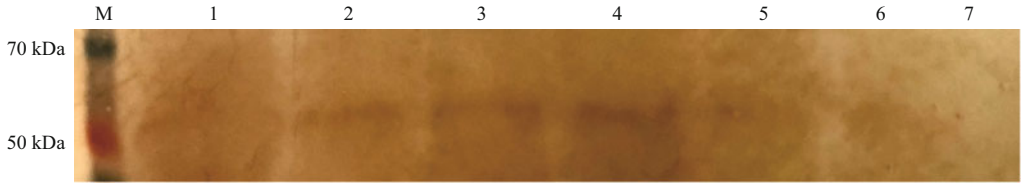

Fig.4 Identification of the DsPLC protein by western blot analysis

Lane M: protein marker; Lanes 1, 2, 3, 4, 5, 6, and 7: expression of DsPLC under high-salt stress (3.0 mol/L NaCl) at 0, 2, 3, 4, 6, 12, and 24 h, respectively.

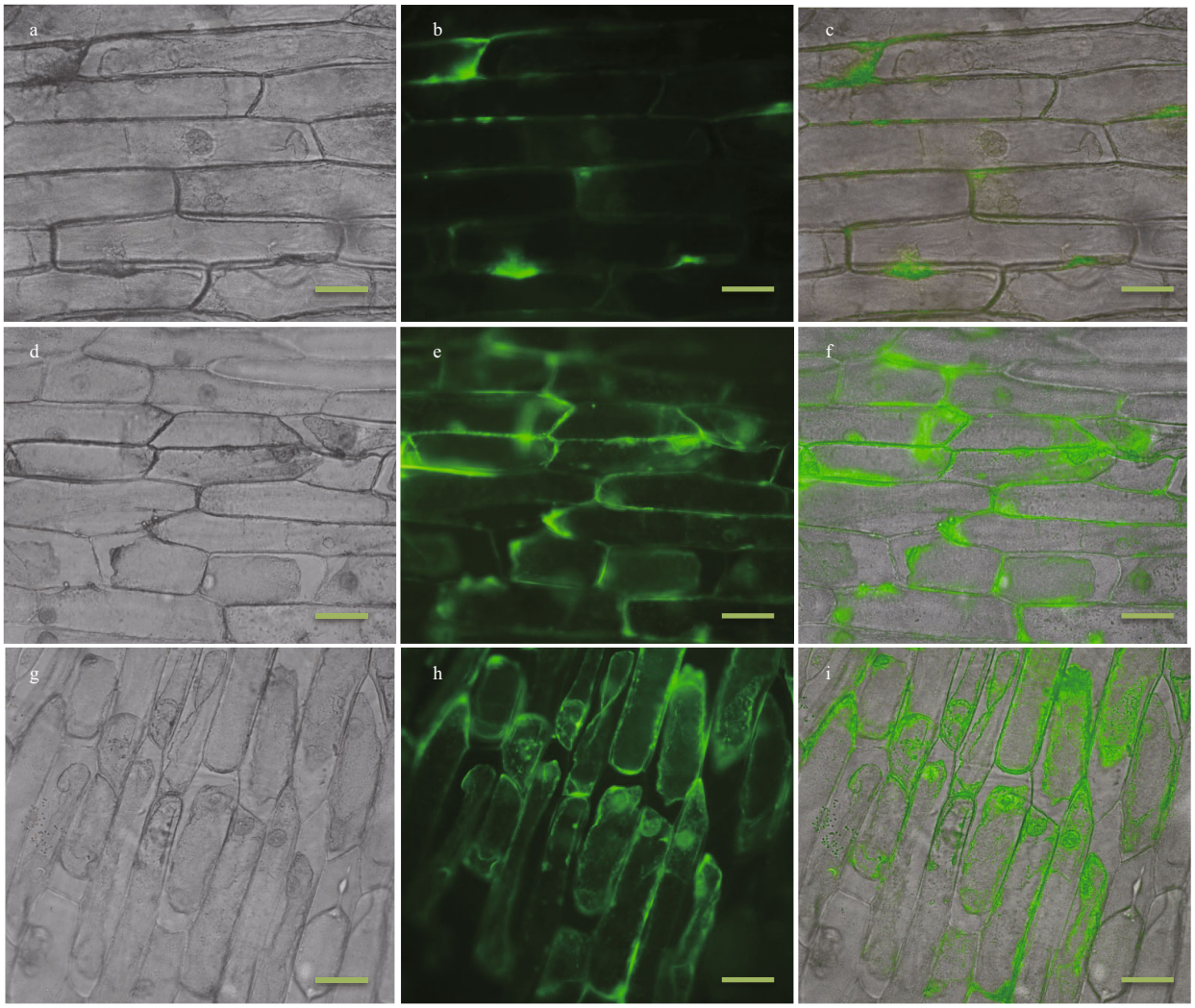

Fig.5 Subcellular localization of the DsPLC protein in onion epidermal cells

Onion epidermal cells were transiently transformed with DsPLC-GFP. The GFP control plasmid (a–c), the fusion construct DsPLC-GFP (d–f) and DsPLC-GFP (g–i) were introduced into onion epidermal cells, which were also treated with 3.0 mol/L salt stress. Images were taken in bright light to display cellular morphology (a, d, g), taken in dark field for green fluorescence (b, e, h) and combined (c, f, i). The scale bar represents 50 μm.

2014). However, the biological role and mechanism of action of DsPLC in salt stress remain unknown.

The coding sequence of PLC was inserted into the expression vector pET-32a, and the protein was expressed in *E. coli* BL21 (DE3). We successfully expressed the DsPLC protein using this prokaryotic expression system, and then, the DsPLC fusion protein was purified by a simple purification system. We also used the purified fusion protein to produce the polyclonal antibody. SDS-PAGE was used for the initial confirmation of fusion protein expression. After induction by IPTG, a protein band of approximately 87 kDa molecular weight emerged, in accord with the expected size (Fig.2). The target DsPLC protein was found both in the precipitate and in the supernatant. Then, we purified the fusion

protein using a His-tag affinity column and the supernatant from the optimal induction time and IPTG concentration.

Quantitative real-time PCR analysis showed upregulation of *DsPLC* under high-salt stress in *D. salina*. The maximum induction was observed after 4 h of 3 mol/L NaCl treatment (Fig.3). Similar findings were reported in *Populus tomentosa*, where transcript levels of PtoPLC1 in the salt treatment were increased by approximately 15-fold compared to those in the control (Zhang et al., 2015). In *Zea*, a role of PLC in mediating the salt stress response has been reported (Zhai et al., 2005). We revealed that the transcriptional expression of the *DsPLC* gene was transiently induced in *D. salina* under a high-salinity (3.0 mol/L NaCl) treatment. This result indicates that the *DsPLC* gene might be related to the molecular regulatory mechanisms of salt stress. The identification of these genes would help us to understand the role of the *DsPLC* gene in response to salt stress.

To gain more insight into the potential biological function of this enzyme in *D. salina*, a routine western blot analysis was used to analyze the expression of the PLC gene by comparing control and salt-stress-treated samples. We obtained total proteins by culturing *D. salina* under salt stress conditions (3.0 mol/L NaCl) for 0, 2, 3, 4, 6, 12, and 24 h. We also used a recombinant DsPLC protein to produce a polyclonal antibody. In the present study, DsPLC protein expression appeared at 2 h and expressed maximum at 4 h. Plant PLC proteins play crucial regulatory roles in many cellular processes and signal transduction networks in response to salt stress (Kocourková et al., 2011; Peters et al., 2014; Singh et al., 2015). Expression of nonspecific phospholipase C4 in *Arabidopsis* was highly induced by NaCl. From 3 h to 6 h after salt treatment, the expression level was at a maximum (Kocourková et al., 2011). The observed correspondences between quantitative real-time PCR and western blot analysis indicated that *DsPLC* was a salt-tolerance gene at both the transcript level and the translation level.

Consistent with the results of previous studies, our subcellular localization experiment showed that the DsPLC protein is localized to the intracellular membrane (Fig.5). PI-PLC1 was localized to the cytosol and plasma membrane in soybean (Shi et al., 1995), while Vr-PLC3 was localized primarily to the plasma membrane in *Arabidopsis thaliana* protoplasts (Kim et al., 2004), and NtPLCδ1 was localized to the cytoplasm of tobacco cells (Tripathy et al., 2012). AtPLC2 was localized to the plasma membrane in different cell types where phosphoinositide signaling occurs, and it showed an obvious tissue-specific expression pattern (Kanehara et al., 2015). Salt stress induced the recruitment of OsPLC1 from the cytoplasm to the plasma membrane (Li et al., 2017). Under 3.0 mol/L NaCl salt stress, DsPLC-GFP showed a strong signal in only the cytoplasmic region of the onion cells (Fig.5). The subcellular localization of the GFP fusion protein in onion epidermal cells leads to the conclusion that DsPLC is localized to the plasma membrane and cytoplasm. Therefore, we suggest that DsPLC is localized to the intracellular membranes of *D. salina* cells.

5 CONCLUSION

We constructed a prokaryotic expression vector of phospholipase C in *D. salina* and *DsPLC* gene was successfully expressed in *E. coli*. The eukaryotic expression vector pMDCG-DsPLC was transformed into onion epidermal cells by *Agrobacterium tumefaciens*-mediated method. We observed that DsPLC-GFP was localized on the intracellular membrane system. The high levels of DsPLC-GFP were also induced when the onion epidermal cells were treated with 3.0 mol/L NaCl for 15 s. Quantitative real-time PCR identified the PLC gene was significantly induced by 3.0 mol/L NaCl at 4h. Western blot analysis further confirmed that the DsPLC protein was expressed maximum at 4 h. Our data suggest that DsPLC is a key regulator in *D. salina* response to abiotic stress.

6 DATA AVAILABILITY STATEMENT

The data used in this study can be shared publicly over a long period. All analysis results mentioned above have been shown in the form of figures in this paper. Please contact the corresponding author on reasonable request.

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