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ARTICLE

Functional Studies of Castor (*Ricinus communis* L.) PLC Family Genes in Arabidopsis Inflorescence Development

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ABSTRACT

Castor (*Ricinus communis* L.) is one of the top 10 oil crops in the world, and inflorescence is a trait that directly affects its yield. Phospholipase C (PLCs) is involved in many plant activities and metabolic processes. To study the functions of *PLC* family genes in the regulation of the inflorescence development of the female line of Lm-type castor aLmAB2, we determined the expression levels of six *PLC* family genes of three types of inflorescences of aLmAB2 (isofemale line, female line, bisexual line) at different developmental stages. The results showed that the 6 genes of the castor *PLC* family had relative expression levels at different developmental stages of the three types of inflorescences. The subcellular location of all six protein products was the cell membrane. The six genes were heterologously overexpressed in *Arabidopsis thaliana* to obtain the T3 generation-resistant *Arabidopsis thaliana* plants. The results showed that the overexpression of six genes significantly promoted the maturation of *Arabidopsis thaliana*, the growth of lateral moss, and the development of flowers and pods, but the development of basal leaves and stem leaves of *Arabidopsis thaliana* was significantly inhibited. According to homology analysis, it is speculated that *PLC2*, *PLC2M*, *PLC2N*, *PLC4*, *PLC4X2*, and *PLC6* genes have the same regulatory function.

KEYWORDS

Castor; *PLC* family genes; the female line of Lm-type castor; inflorescence development

1 Introduction

Phospholipases (PL) are the main enzymes that catalyze the catabolism of membrane phospholipids and can produce a variety of lipid-derived second messengers in this process. According to the hydrolysis

sites of phosphate ester bonds, PLs are divided into PLA1, PLA2, PLC, and PLD. Each group of PLs has different enzyme families or subfamilies, which differ in structure, substrate selectivity, cofactor requirements, and reaction conditions [1]. According to their hydrolysis substrate, PLCs can be divided into phosphatidylinositol-specific phospholipase C (PI-PLC) and nonspecific phospholipase C (NPC). The latter acts on common phospholipids, such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, while the former only acts on phosphatidylinositol [2]. Five different types of PI-PLCs, i.e., β, γ, δ, ε, and ζ, have been identified in animal cells.

PLC is an important regulatory enzyme involved in a variety of lipid- and Ca^{2+}-dependent signal transduction pathways. In animals, PLC selectively catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) on the glycerol side of a phosphodiester bond [3], which reaction produces two important secondary messengers, diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3). DAG regulates the activities of many important enzymes and structural proteins by binding to the conserved C1 domain, while IP3 binds to and controls the gating of endoplasmic reticulum Ca^{2+} channels to finely regulate the cytoplasmic Ca^{2+} concentration. In mammals, G protein-coupled receptors receive ligand signals, activate PI-PLC through G proteins, and hydrolyze PIP2 to produce DAG and IP3. DAG and IP3 separately bind to and activate protein kinase C (PKC) and IP3 receptors (Ca^{2+} channel proteins on the endoplasmic reticulum membrane), and the latter releases Ca^{2+} into the cells [4]. Plants do not have IP3 receptors but have unique phosphoinositide signaling components and pathways [5].

Plant *PLC* genes are involved in cell growth and differentiation, hormone signal transduction, response to biotic and abiotic stresses, and regulation of polar growth, and Ca^{2+} plays an important role in various signal transduction pathways [6]. In plant cells, after entering the cytoplasm, water-soluble IP3 can be rapidly converted to IP6 under the action of a series of phosphokinases to promote Ca^{2+} influx into the intracellular calcium pool, thereby regulating the Ca^{2+} concentration and the activity of Ca^{2+}-dependent enzymes or channels [6]. IP3 produced by the hydrolysis of PIP2 by PI-PLC can induce the activity of voltage-dependent Ca^{2+} channels on the plasma membrane of pollen tubes and rapidly increase the cytoplasmic Ca^{2+} concentration of pollen tubes, and the released Ca^{2+} can activate PLC to increase IP3 production. IP3 causes a second peak of cytoplasmic Ca^{2+} concentration, which in turn promotes the release of other Ca^{2+} ions from the calcium pool, forms a Ca^{2+} flow to generate a current, and continuously induces the activity of Ca^{2+} channels in pollen. Therefore, different Ca^{2+} gradients can accumulate at the top of the tube, where they regulate the polar growth of the pollen tube [7–10]. Overall, *PLC* family genes play an important role in the development of plant inflorescences.

Castor (*Ricinus communis* L.) is an annual or perennial herbaceous plant with high economic and ecological value. Because castor is rich in oils, it is known as one of the top 10 most important oil crops in the world [11–15]. Castor oil has a variety of industrial uses and is widely used in fields, such as agriculture, medicine, chemical engineering, aviation, navigation, electronics, and machinery manufacturing [16–18]. With the acceleration of economic globalization and international trade liberalization, the international competition for agricultural products is becoming increasingly fierce, and the demand for castor seeds/oil and their products in China and abroad is growing [19–21]. The growth and development of castor inflorescences is a key factor affecting its yield, so studying the influencing factors of castor inflorescence development can greatly improve its yield.

There are generally two types of inflorescences in the female lines of castor, namely, isofemale and bisexual lines [22]. The female line of Lm-type castor, i.e., the female castor line with iconic traits, was developed by Zhu, a researcher at the Tongliao Academy of Agricultural Sciences. Zhu treated the dry seeds of castor line Yong283 with ^{60}Co γ-rays in 1985 and discovered the mutant in the M_2 generation in 1986. There are three types of inflorescences in the female line of Lm-type castor, i.e., isofemale line, female line, and bisexual line [23–25].

There are few relevant studies on the *PLC* gene family in regulating castor inflorescence development. The purpose of this study is to clarify the functions of *PLC* family genes in regulating the inflorescence

development of female lines of Lm-type castor and analyze the relationships between the six *PLC* family genes.

2 Materials and Methods

2.1 Materials

In this study, the gene and protein sequences of the castor *PLC* gene family were obtained from the NCBI database, and the gene nucleic acid and protein sequences of the Arabidopsis *PLC* gene family were obtained from the TAIR database (Table 1). The materials used in this study were the female line of Lm-type castor aLmAB$_2$ (Fig. 1), the Colombia wild-type (WT) *Arabidopsis thaliana*, the overexpression vector pC1390U, the subcellular localization binary expression vector pBI121-GFP, and the PAD$_{62}$ *Agrobacterium*, which were provided by the Key Laboratory of Castor Breeding of State Ethnic Affairs Commission of the Inner Mongolia University for Nationalities. The full-length coding sequence of each *PLC* family gene was synthesized by Beijing Qingke Xinye Biotechnology Co., Ltd. (China), and the restriction endonucleases *Bam*HI and *Kpn*I, a complementary DNA (cDNA) synthesis kit, and the fluorescent dye SYBR were purchased from Baori Medical Biotechnology Beijing Co., Ltd. (China). The GV3101 *Agrobacterium* strain and plant genomic DNA and RNA extraction kits were purchased from Beijing Zhuangmeng International Bio-Gene Technology Co. Ltd. (China).

Table 1: Accession number of *PLC* gene family of castor and *Arabidopsis thaliana*

Castor *PLC* family genes	Registration number	Arabidopsis PLC family genes	Registration number
*PLC*2	XP_025014303.1	*PLC1*	NP_568881.1
PLC2M	XP_015579164.1	*PLC2*	NP_187464.1
*PLC*2N	XP_002533650.1	*PLC3*	NP_191153.1
*PLC*4	XP_002525652.2	*PLC4*	NP_001318832.1
*PLC*4X2	XP_025014307.1	*PLC5*	NP_001332555.1
*PLC*6	XP_002533649.1	*PLC6*	NP_850327.2
–	–	*PLC7*	NP_195565.2
–	–	*PLC8*	NP_190313.1
–	–	*PLC9*	NP_190306.2

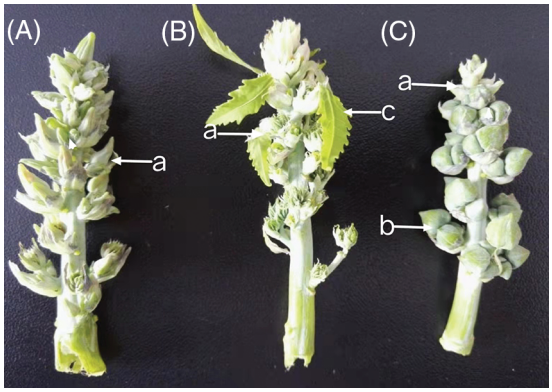

Figure 1: Inflorescence development of the female line of Lm-type castor
Note: A: isofemale inflorescence; B: female inflorescence; C: bisexual inflorescence; a: female flowers; b: male flowers; c: willow-like functional leaves.

2.2 Analysis of the Expression Levels of PLCs in Castor Inflorescences

The plant total RNA extraction kit was used to extract the total RNA from the isofemale, female, and bisexual lines of $aLmAB_2$ at the four-leaf stage, five-leaf stage, main stem spike flowering stage, and secondary branch flowering stage (a lot of 12 samples), in order to synthesize cDNA by reverse transcription. With cDNA as the template, reverse transcription–quantitative polymerase chain reaction (RT-qPCR) was performed using SYBR Green as the fluorescent dye and the primers listed in Table 2. The relative expression levels of *PLC* family genes in the Lm-type castor inflorescences were detected. The expression levels of the six genes were analyzed using the 18S rRNA gene as an internal reference. The experiment was repeated three times. The $2^{-\Delta\Delta Ct}$ method was used for data processing, i.e., the expression of each target gene in different samples was investigated by calculating the F value of the gene's relative expression level.

Table 2: Sequences of qRT-PCR primers for *PLCs* in castor inflorescences

Primer name	Primer sequence (5′-3′)
18s-F	AGGGGATAACCACCCCATGAATCCA
18s-R	TGCATGGTCTCCTGATACGGCCAAG
PLC2-1R	ATGCTCTTCTGTCCCAAA
PLC2-1F	CTCATCCTCCGACTGTTC
PLC4-1R	ACTCCTCCAACTACAAGCC
PLC4-1F	ACATAGCCACACCCACCAT
PLC4x2-1R	CGGAAGTTGTGGTTATGT
PLC4x2-1F	GCTGGCACTCCTGCTATG
PLC6-1R	GAATGGAGGGTGTGGTTA
PLC6-1F	TTTCGTTTTTCTCTTGGC
PLC2M-1R	GCTCCTCTGTCTCATTATT
PLC2M-1F	TCACTCTCACACCTCTCTT
PLC2N-1R	GTTCCCATCATACAAGCACT
PLC2N-1F	TCATCTCAGCCACTTTAGCC

2.3 Subcellular Localization

2.3.1 Cloning of the Target Genes and Construction of the Plant Binary Expression Vector pBI121-GFP-PLCs

The coding sequence of each *PLC* gene was downloaded from the National Center for Biotechnology Information (NCBI) database, and the primers for the seamless cloning of the gene sequences were designed using CE Design V1.03 software (Table 3). The cDNA of castor leaves was used as the template for PCR amplification. The restriction enzymes *Bam*H I and *Kpn* I were used to linearize pBI121-GFP, and the reaction condition was 37°C for 3 h. Double-digestion products and amplification products were purified and recovered through gel excision. The target gene fragment was ligated to the expression vector using the seamless cloning method to obtain the fused expression vector. The obtained recombinant vector was transformed into *Agrobacterium GV3101* competent cells.

Table 3: Sequences of primers for seamless cloning

Primer name	Primer sequence (5′-3′)
PLC2-A	ATGTCCGACTCTAAGGGACTCAAC
PLC2-B	AACAATCTCAAATTCCATTAGAAGCC
PLC2M-A	ATGTCAACGAAGGAGGCGG
PLC2M-B	AACAAAATCGAAATGCATAAGCAG
PLC2N-A	ATGTCCAAACAGACATACAGAGTGTG
PLC2N-B	AACAAAATCAAAACGGACGAGG
PLC4-A	ATGAAGATGTTCTTTAGTGGGAAGTTT
PLC4-B	AAGAAACTCGAATCTCATGAGAAGTCG
PLC4*2-A	ATGGGGAGCTATAGGATGTGTATGT
PLC4*2-B	AACAAACTCAAACCGCATCAGAA
PLC6-A	ATGGGAAGCTACAGGAAAGAACG
PLC6-B	TACAAACTCAAACTTCATTAGAAGCCT

2.3.2 Transient Transformation and Observation of Onion Epidermal Cells

To produce the fusion protein of green fluorescent protein (GFP) with each *PLC* gene product, the gene downstream of the CaMV35S promoter was cloned in the pBI121-GFP vector. The plasmid pBI121-GFP-*PLCs* was confirmed by sequencing. The successfully constructed subcellular localization expression vector was used to transform onion epidermal cells using the *Agrobacterium*-mediated method. The cultured onion epidermal cells were observed under an inverted fluorescence microscope and photographed under natural light and fluorescence. The subcellular locations of the PLC proteins in castor cells were determined as described [26].

2.4 Heterologous Overexpression of Castor PLC Family Genes in A. thaliana

The overexpression vector pCAMBIA1390-*PLCs* was constructed by the method described in Section 2.3.1, and the obtained recombinant overexpression vector was transformed into *Agrobacterium* GV3101 competent cells. WT *A. thaliana* plants were genetically transformed with the *Agrobacterium* overexpression vectors of the six target genes using the *A. thaliana* floral dip transformation method. The T_3 generation *A. thaliana* plants was obtained by inoculation, transplantation, dipping, resistance screening, and domestication, and the resistant *A. thaliana* plants expressing the six *PLC* family genes were obtained, i.e., *AtPLCs*$^+$, *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, *AtPLC6*$^+$ plants (for the specific steps, please refer to the literature [27]).

2.5 Molecular Level Identification of AtPLCs$^+$ *Plants*

2.5.1 PCR Identification

Genomic DNA was extracted from the T_3 generation *AtPLCs*$^+$ and WT *A. thaliana* inflorescences. The recombinant plasmid of the plant overexpression vector pCAMBIA1390-PLC_S was used as a positive control, the genomic DNA of the Columbia WT *A. thaliana* leaves was used as a negative control, and PCR analysis was performed. The reaction products were separated by agarose gel electrophoresis.

2.5.2 RT-qPCR Identification

Total RNA of from the T_3 generation *AtPLCs*$^+$ and WT *A. thaliana* inflorescences was extracted and reverse-transcribed into cDNA. The cDNA samples of the T_3 generation *AtPLCs*$^+$ and WT *A. thaliana* inflorescences were used as templates, and SYBR Green was used as the fluorescent dye in RT-qPCR to detect the relative expression levels of *PLC* family genes in *A. thaliana* plants. The primer sequences are shown in Table 4. SPSS 19.0 software and GraphPad Prism were used for data processing and plotting, respectively. The significance analysis was performed by the independent-sample T-test on the averages, and the results are presented aa histograms of the relative expression levels.

Table 4: RT-qPCR primer sequences of castor *PLCs* in *AtPLCs*$^+$ plants

Primer name	Primer sequence
AtActin-F	GATCCATGTTTGGCTCCTTG
AtActin-R	TGGTGGAAGCACAGAAGTTG
NOEP2-S	TCGCCATGGAGGGACTCTTA
NOEP2-X	TATTGGCCACCTTTGCCTGA
NOEP2M-S	CGTCAGGGTAGGGATTGCTG
NOEP2M-X	TCATTCCAAACGGGTGTCCA
NOEP2N-S	ACTCCGTCGATTTCTGGTGG
NOEP2N-X	TCATGATGCACCCCACGTTT
NOEP4-S	GGAAATGGATGGCACCTGGA
NOEP4-X	CTGCCATGCCAACCCTGATA
NOEP4X2-S	GCCGTCCCTCTTTTTGATCG
NOEP4X2-X	CCACCGGCCTTCATGGACTA
NOEP6-S	ACATGCAGGGATACGGCAAA
NOEP6-X	GTGGCTAAAATCCAACCGCC

2.6 Biological-Level Identification of AtPLCs$^+$ Plants

The T_3-generation *AtPLCs*$^+$ plants were identified at the biological level. The growth and development of the plants were observed and measured, including the plant height, the number of florets, the bolting time of the main stem, the number of lateral stems, and the number of basal leaves and cauline leaves.

Both the WT *A. thaliana* plants and *A. thaliana* plants with heterologous overexpression (*AtPLCs*$^+$, *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, *AtPLC6*$^+$ plants) at the four-leaf stage in the Murashige and Skoog (MS) solid medium were transplanted and cultured under the same conditions. For each type of plant (a total of seven types), the plants were cultured in 30 pots, and pictures of the representative plants with the similar growth state were taken. For the 30 plants of each type, phenotypic trait statistics were calculated, and the averages were used for statistical analysis.

2.7 Correlation Analysis of the Castor PLC Family Genes

Fluorescence quantification was performed on the T_3-generation *AtPLCs*$^+$ *A. thaliana* plants with stable heterologous overexpression of the six *PLC* family genes in order to determine the induced expression levels of the other five *A. thaliana PLC* family genes when one castor *PLC* gene was overexpressed. According to

the homology comparison results between the castor and *A. thaliana PLC* gene sequences, the relationship between the *PLC* family genes was predicted.

The messenger RNA (mRNA) sequences of *A. thaliana PLC*s were obtained from NCBI. The cDNA samples of the T_3-generation *AtPLCs*$^+$ and WT *A. thaliana* inflorescences were used as templates and SYBR Green was used as the fluorescent dye in the RT-qPCR to detect the relative expression levels of *PLC* family genes in *A. thaliana* plants. SPSS 19.0 software and GraphPad Prism were used for data processing and plotting, respectively. The significance analysis was performed by the independent-sample T-test on the means, and the results are presented as histograms of the relative expression levels. The primer sequences are shown in Table 5.

Table 5: RT-qPCR primer sequences of *AtPLCs* in *AtPLCs*$^+$ plants

Primer name	Primer sequence
AtActin-F	GATCCATGTTTGGCTCCTTG
AtActin-R	TGGTGGAAGCACAGAAGTTG
NP1-S	CGGCGTTTTTCATCCTCGTG
NP1-X	CGTCCCTCCATGACGAACTT
NP2-S	CCAGCTTGGGATGAGGTGTT
NP2-X	TGTGGCACTCAAACCTACCA
NP3-S	CTTGATCGCAATCCACGCTG
NP3-X	TCCAACCAACATGGGGATCG
NP4-S	AATCGGCGATCAGGTCCATC
NP4-X	TACCACGTGGCCAAAGATCC
NP5-S	CATGGGCAAAGGATGGGACT
NP5-X	CTCAGCTCTGACACAGGCAA
NP6-S	AAGCGAGCAGGAACTTGACA
NP6-X	AAACATGCCGTGCATCAACC
NP7-S	CAGAGGAACTTGCTTCGGGT
NP7-X	TAGCCACATCCTCCATTGCC
NP8-S	GAGCCAAGAGGCATGCTACA
NP8-X	TTGACATAACCGCACCCTCC
NP9-S	AGACGGCATATGCGTTAGGC
NP9-X	TCCTCCAAGCTTTGTTGGGG

3 Results

3.1 Analysis of the Expression Levels of PLC Family Genes at Different Developmental Stages of Different Types of Castor Inflorescences

The results of the RT-qPCR analysis of *PLC2*, *PLC2M*, *PLC2N*, *PLC4*, *PLC4X2*, and *PLC6* in the three types of inflorescences of $aLmAB_2$ (isofemale line, female line, bisexual line) at the four-leaf stage, five-leaf stage, main stem spike flowering stage, and secondary branch flowering stage are shown in Fig. 2.

The *PLC2* gene was highly expressed in the five-leaf stage of the isofemale inflorescence and the secondary branch flowering stage of the bisexual inflorescence. It had low expression in the four-leaf stage of the isofemale inflorescence, the main stem spike flowering stage of the female inflorescence, and the four-leaf stage of the bisexual inflorescence. Its relative expression level in the five-leaf stage of the female inflorescence was approximately 87.43 times that in the four-leaf stage of the bisexual inflorescence.

PLC2M was highly expressed in the five-leaf stage of the isofemale and female inflorescences and the main stem spike flowering stage of the bisexual inflorescence. Its relative expression was low in the secondary branch flowering stage of isofemale inflorescence, the main stem spike flowering stage of female inflorescence, and the four-leaf stage of bisexual inflorescence. Its relative expression level in the five-leaf stage of the isofemale inflorescence was approximately 32.45 times that in the four-leaf stage of the bisexual inflorescence.

PLC2N was highly expressed in the main stem spike flowering stage of the isofemale inflorescence and the secondary branch flowering stage of the female and bisexual inflorescences. It had low relative expression in the five-leaf stage of the three types of inflorescences. Its relative expression level in the main stem spike flowering stage of the isofemale inflorescence was approximately 126.24 times that in the main stem spike flowering stage of the bisexual inflorescence.

PLC4 was highly expressed in the secondary branch flowering stage of the isofemale inflorescence and the main stem spike flowering stage of the female and bisexual inflorescences. Its relative expression was low in the five-leaf stage of the three types of inflorescences. Its relative expression level in the main stem spike flowering stage of female inflorescence was approximately 202.25 times that in the five-leaf stage of the isofemale inflorescence.

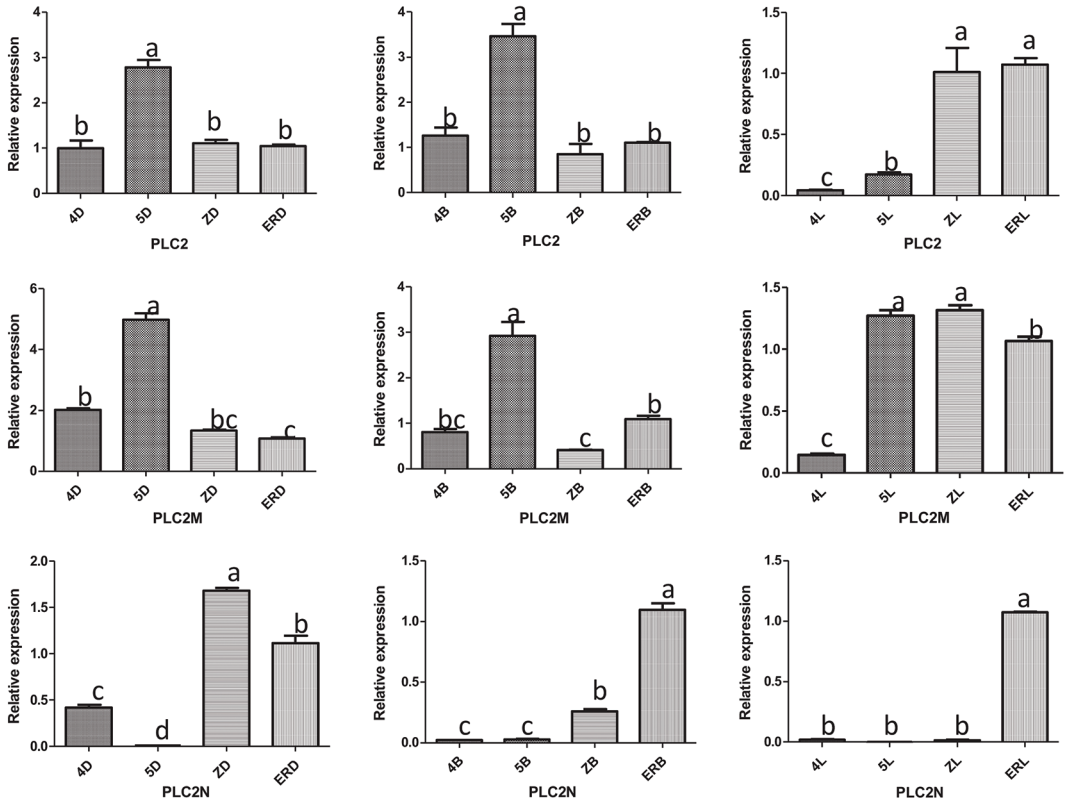

Figure 2: (Continued)

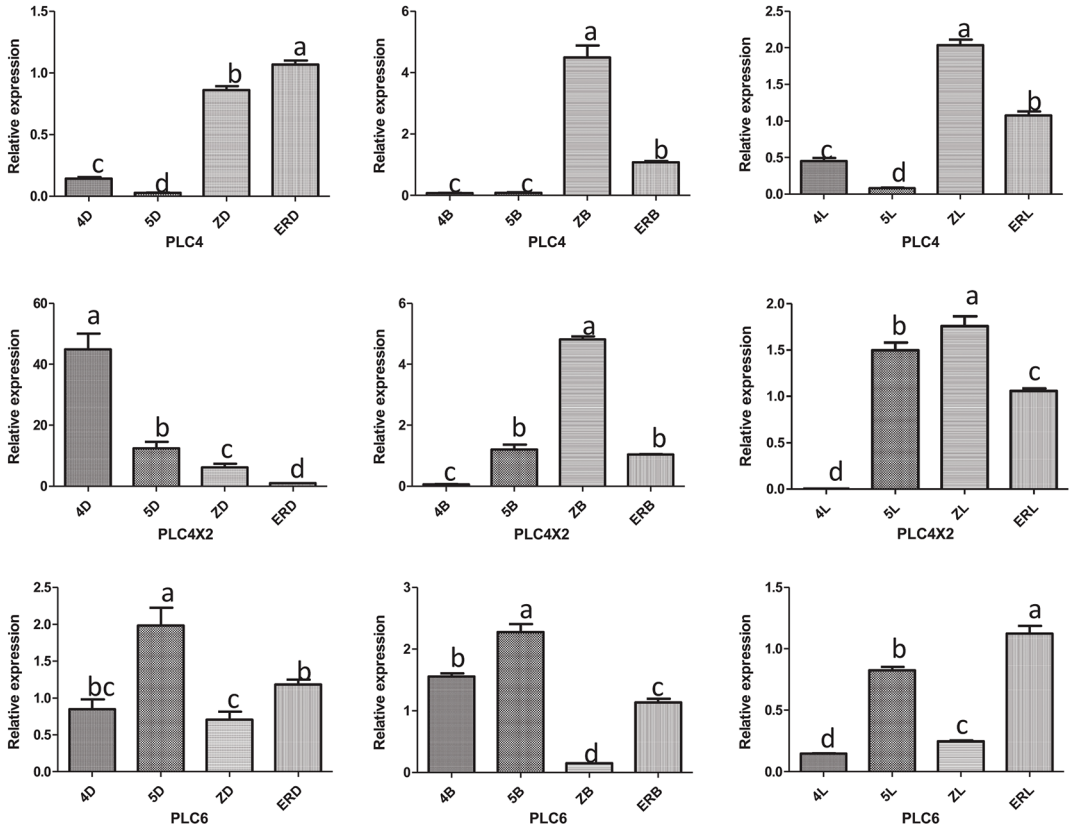

Figure 2: Analysis of the expression levels

Note: 4D: isofemale four-leaf stage; 5D: isofemale five-leaf stage; ZD: isofemale main stem spike flowering stage; ERD: isofemale secondary branch flowering stage; 4B: female four-leaf stage; 5B: female five-leaf stage; ZB: female main stem spike flowering stage; ERB: female secondary branch flowering stage; 4L: bisexual four-leaf stage; 5L: bisexual five-leaf stage; ZL: bisexual main stem spike flowering stage; ERL: bisexual secondary branch flowering period. Different letters indicated different differences, and the significance level was $p < 0.05$.

PLC4X2 was highly expressed in the four-leaf stage of the isofemale inflorescence and the main stem spike flowering stage of the female and bisexual inflorescences but had low expression in the secondary branch flowering stage of the isofemale inflorescence and the four-leaf stage of the female and bisexual inflorescences. Its relative expression level in the four-leaf stage of the isofemale inflorescence was approximately 11190.6 times that in the four-leaf stage of the bisexual inflorescence.

PLC6 was highly expressed in the five-leaf stage of the isofemale and female inflorescences and the secondary branch flowering stage of the bisexual inflorescence, but it had low expression in the main stem spike flowering stage of isofemale and female inflorescences and the four-leaf stage of the bisexual inflorescence. Its relative expression level in the five-leaf stage of the female inflorescence was approximately 15.14 times that in the four-leaf stage of the bisexual inflorescence.

Overall, the *PLC2*, *PLC4*, and *PLC6* genes showed relatively high expression levels in the female inflorescence; the *PLC2M*, *PLC2N*, and *PLC4X2* genes showed relatively high expression in the isofemale inflorescence, while *PLC4* showed relatively low expression in isofemale inflorescence; the *PLC2*, *PLC2M*, *PLC2N*, *PLC4X2*, and *PLC6* genes showed relatively low expression levels in the bisexual inflorescence.

3.2 Comparison of the Expression Levels of Six PLC Family Genes in the Same Developmental Stage of the Same Type of Inflorescence

The expression levels of the six *PLC* family genes in the same developmental stage of the same type of inflorescence were analyzed. It can be seen from Fig. 3.

In the isofemale inflorescence at the four-leaf stage, the expression levels of *PLC2M* and *PLC4X2* were relatively high, and those of the *PLC2N* and *PLC6* genes were relatively low. In the five-leaf stage, *PLC2M* and *PLC2* were relatively high, and *PLC2N* and *PLC4* were relatively low. In the main stem spike flowering stage, *PLC2M* and *PLC4* were relatively high, and *PLC6* and *PLC2N* were relatively low. In the secondary branch flowering stage, *PLC2M* and *PLC4* were relatively high, and *PLC4X2* and *PLC6* were relatively low.

In the female inflorescence in the four-leaf and five-leaf stages, *PLC2M* and *PLC2* genes were relatively highly expressed, and *PLC4X2* and *PLC2N* were relatively low. In the main stem spike flowering stage, *PLC4* and *PLC2M* were relatively high, and *PLC6* and *PLC2N* were relatively low. In the secondary branch flowering stage, *PLC2M* and *PLC2N* were relatively high, and *PLC6* and *PLC4X2* were relatively low.

In the bisexual inflorescence at the four-leaf stage, the expression levels of the *PLC2M* and *PLC2N* genes were relatively high, and those of the *PLC4X2* and *PLC6* genes were relatively low. In the five-leaf stage, *PLC2M* and *PLC2* were relatively high, and *PLC2N* and *PLC4* were relatively low. In the main stem spike flowering stage, *PLC2M* and *PLC4* were relatively high, and *PLC6* and *PLC4X2* were relatively low. In the secondary branch flowering stage, *PLC2N* and *PLC2M* were relatively high, and *PLC6* and *PLC4X2* were relatively low.

3.3 Subcellular Localization of the Castor PLC Family Genes

The expression of *PLC* family genes in the onion epidermis was observed under a fluorescence microscope. It can be seen from Fig. 4 that under the condition of bright field, the complete cell structure can be observed, and the expression of green fluorescent protein in the cell membrane and nucleus can be seen in the pBI121-GFP no-load plasmid control (Fig. 4A). Under the condition of green fluorescence, PLC family proteins are mainly accumulated on the cell membrane (Fig. 4B), indicating that castor PLC family genes are expressed in the cell membrane of onion epidermis cells.

3.4 Results of Genetic Transformation of A. thaliana Plants with Plant Overexpression Vectors

The screen of the T_3 generation *AtPLCs*$^+$ resistant plants is depicted in Fig. 5. The WT plants only grew to the two-leaf stage in MS medium supplemented with 50 mg/mL hygromycin, the root system was short, and the plants showed wilting signs, such as yellowing and transparency. Compared with WT plants, the T_3-generation resistant *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, and *AtPLC6*$^+$ plants grew to the four-leaf stage in the MS medium supplemented with hygromycin, and the resistant plants showed a well-developed root system, with no wilting signs, such as yellowing and transparency.

3.5 Molecular-Level Examination of the Castor PLC Family Genes in AtPLCs$^+$ *Resistant Plants*

3.5.1 PCR Results

The genomic DNAs of the T_3-generation *AtPLCs*$^+$ resistant plants with the six genes were used as templates for PCR detection. The results of agarose gel electrophoresis are shown in Fig. 6. The size of the target bands was still approximately 1700 bp, which are consistent with the size of the castor *PLC2*, *PLC2M*, *PLC2N*, *PLC4*, *PLC4X2*, and *PLC6* genes, indicating that the successful heterologous overexpression of the castor *PLC* genes in *A. thaliana* plants was preliminarily verified.

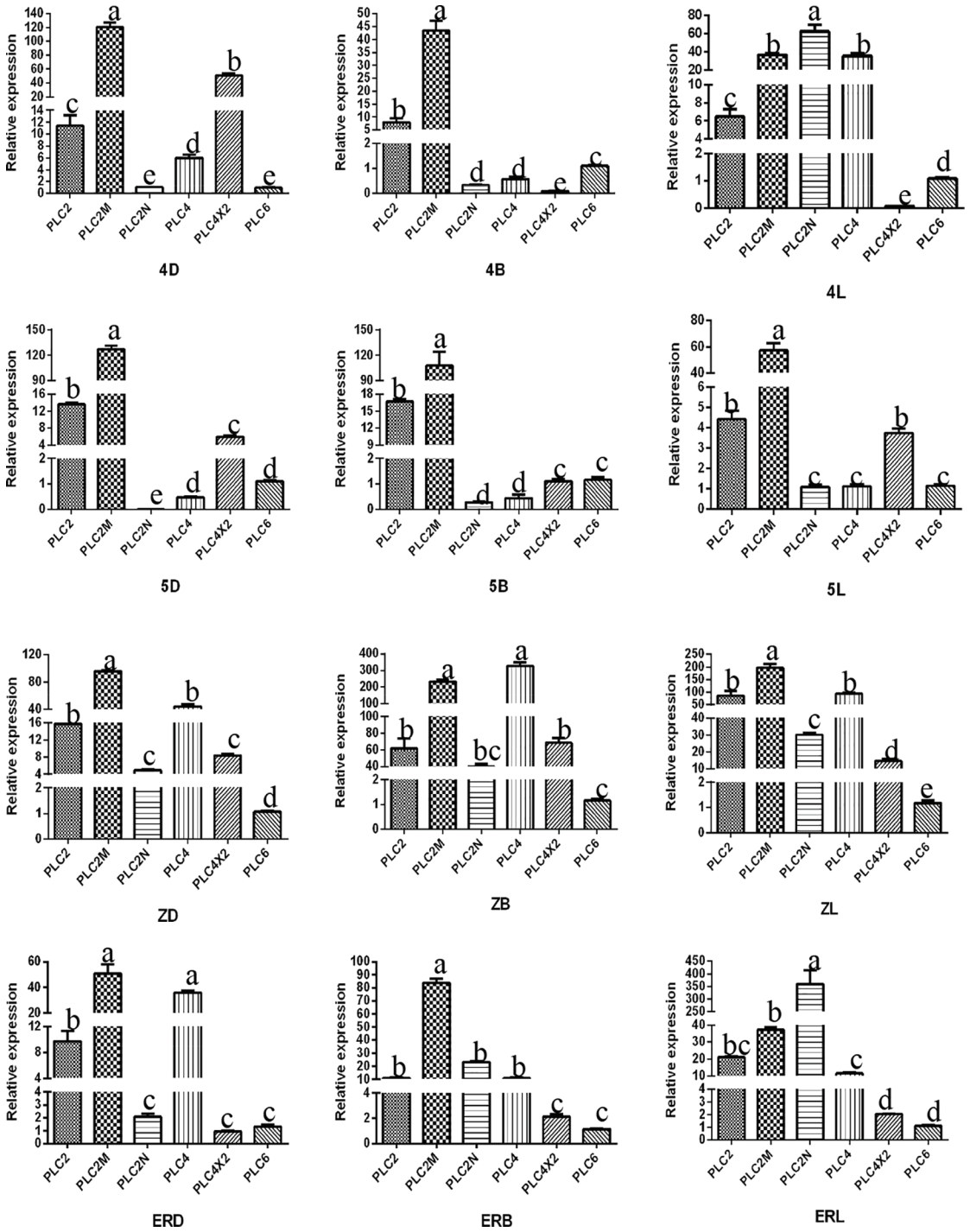

Figure 3: Analysis of the expression amount of the same type of inflorescence development time

Note: 4D: isofemale four-leaf stage; 5D: isofemale five-leaf stage; ZD: isofemale main stem spike flowering stage; ERD: isofemale secondary branch flowering stage; 4B: female four-leaf stage; 5B: female five-leaf stage; ZB: female main stem spike flowering stage; ERB: female secondary branch flowering stage; 4 L: bisexual four-leaf stage; 5 L: bisexual five-leaf stage; ZL: bisexual main stem spike flowering stage; ERL: bisexual secondary branch flowering period. Different letters indicated different differences, and the significance level was $p \leq 0.05$.

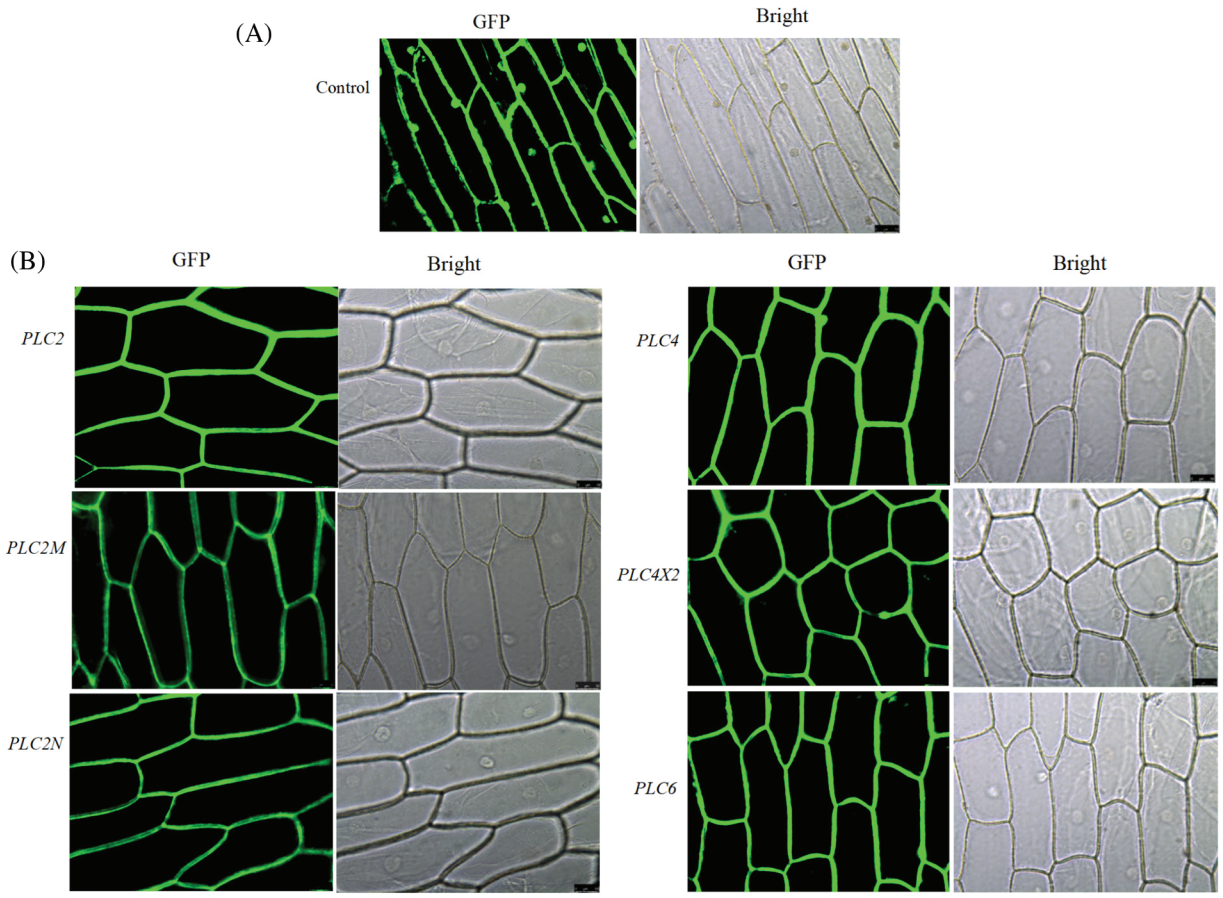

Figure 4: Subcellular localization of the castor *PLC* family genes
Note: A: Empty vector control transformation under bright field and fluorescent conditions B: Protein transformation of castor *PLC* family genes under bright field and fluorescent conditions.

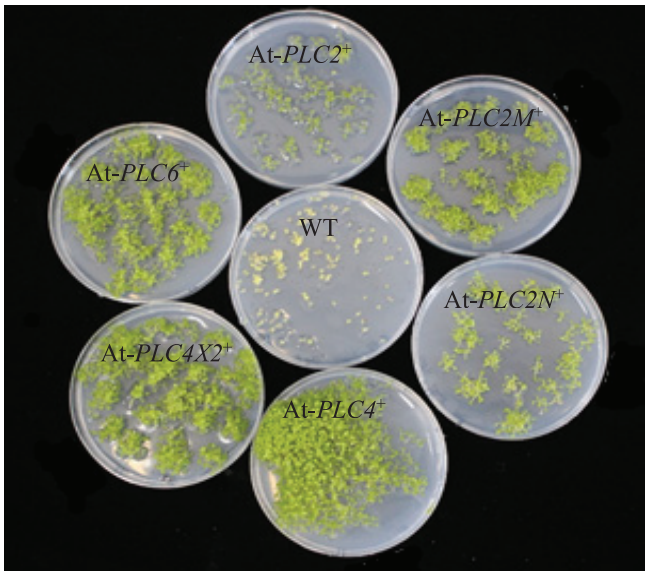

Figure 5: Screening of T_3-generation *AtPLCs*$^+$ resistant plants
Note: Comparison of T_3 generation *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, and *AtPLC6*$^+$ plants with WT plants.

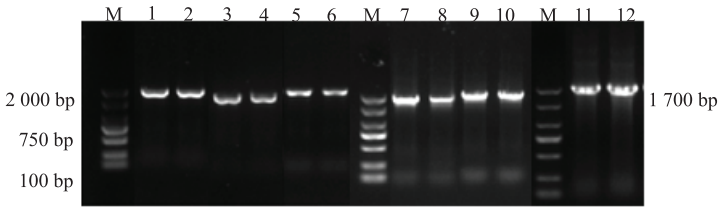

Figure 6: The PCR results in T_3-generation *AtPLCs*$^+$ resistant plants
Note: M: DL 2000 Marker; 1, 2: *AtPLC2*$^+$ PCR products; 3, 4: *AtPLC2M*$^+$ PCR products; 5, 6: *AtPLC2N*$^+$ PCR products; 7, 8: *AtPLC4* $^+$ PCR products; 9, 10: *AtPLC4X2*$^+$ PCR products; 11, 12: *AtPLC6*$^+$ PCR products.

3.5.2 RT-qPCR Results

Fig. 7 shows that the castor *PLC* family genes were not expressed in the WT plants but were all upregulated in the inflorescence samples of *AtPLCs*$^+$ plants (*AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, and *AtPLC6*$^+$), indicating that the transgene was successfully integrated.

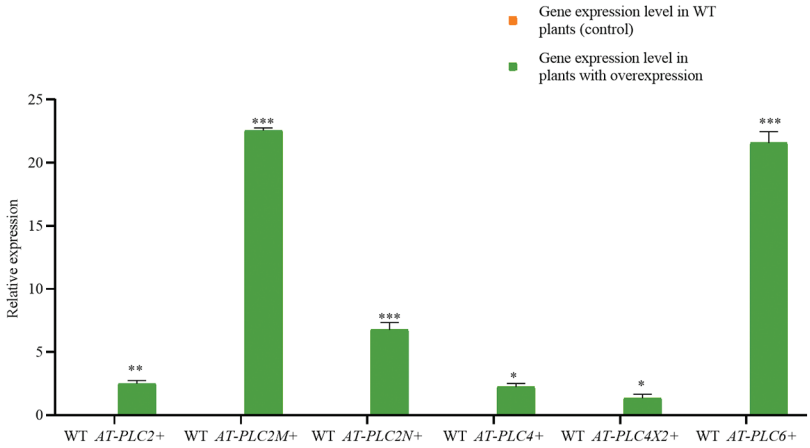

Figure 7: RT-qPCR RESULTS of *AtPLCs*$^+$ resistant plants
Note: In the T_3-generation *A. thaliana* resistant plants with heterologous overexpression (*AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, and *AtPLC6*$^+$), the RT-qPCR detection of castor *PLC* family genes was performed. *significant difference, $p \leq 0.01$; **significant difference, $p \leq 0.05$; ***difference is extremely significant, $p \leq 0.01$.

3.6 Biological-Level Examination of AtPLCs$^+$ *Resistant Plants*

3.6.1 Phenotypes of AtPLCs$^+$ *Resistant Plants before Transplanting*

Compared with the WT plants in Fig. 8, the *AtPLC2*$^+$ and *AtPLC4*$^+$ plants, i.e., the two *A. thaliana* resistant plants with heterologous overexpression, had significantly greater root length, while *AtPLC2N*$^+$ and *AtPLC6*$^+$ plants had significantly shorter root length, and *AtPLC2M*$^+$ and *AtPLC4X2*$^+$ plants had no difference.

3.6.2 Analysis of the Phenotypic Results of AtPLCs$^+$ *Resistant Plants at 15 d*

After the *AtPLCs*$^+$ resistant plants had been cultured under the same conditions for approximately 8 d, the main stem was removed to promote the growth of the lateral stems. The phenotypic results of these plants and WT at 15 d are shown in Fig. 9, and the plant traits are listed in Table 6.

The average plant height of WT plants was approximately 8 cm. The average plant height of *AtPLC2*$^+$ plants was significantly higher than that of WT plants. The plant height of *AtPLC4*$^+$ plants (approximately 9 cm) was slightly higher than that of WT plants. The plant height of *AtPLC4X2*$^+$ and *AtPLC6*$^+$ plants was basically the same as that of WT plants. The plant height of *AtPLC2M*$^+$ and *AtPLC2N*$^+$ plants was significantly lower than that of WT plants.

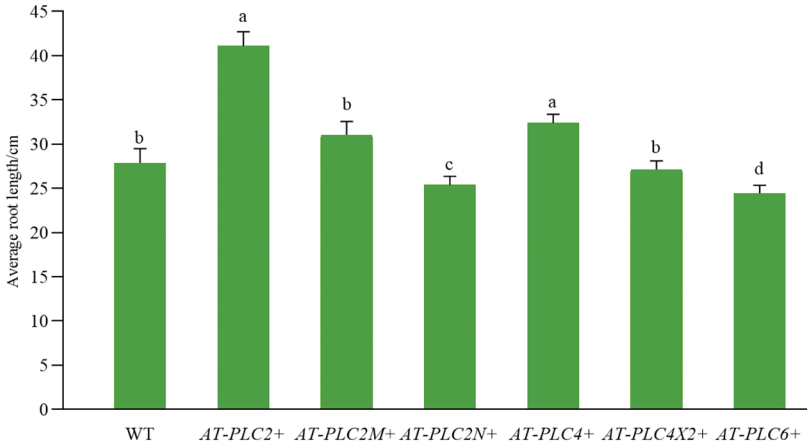

Figure 8: Root length statistics of *AtPLCs*$^{+}$ resistant plants before transplanting
Note: For WT plants and six T_3-generation *AtPLCs*$^{+}$ resistant plants in the four-leaf stage (*AtPLC2*$^{+}$, *AtPLC2M*$^{+}$, *AtPLC2N*$^{+}$, *AtPLC4*$^{+}$, *AtPLC4X2*$^{+}$, *AtPLC6*$^{+}$), the root length was statistically analyzed.

Figure 9: *AtPLCs*$^{+}$ resistant plants at day 15 after transplanting scale

Table 6: Statistics of the phenotypic traits of *AtPLCs*$^{+}$ and WT plants at day 15

Plant type	Average number of basal leaves/ piece	Average number of cauline leaves/ piece	Average number of lateral stems/ branch	Average floret number/piece	Average plant height/ cm	Average silique number/piece
WT	11 ± 0.288^{a}	10 ± 0.082^{a}	3 ± 0.028^{c}	7 ± 0.142^{b}	8 ± 0.541^{b}	0 ± 0.371^{d}
AtPLC2^{+}	7 ± 0.247^{c}	3 ± 0.155^{cd}	5 ± 0.105^{b}	24 ± 0.231^{a}	13 ± 0.311^{a}	2 ± 0.251^{c}
AtPLC2M^{+}	8 ± 0.232^{bc}	6 ± 0.086^{b}	4 ± 0.098^{bc}	5 ± 0.438^{c}	5 ± 0.284^{d}	0 ± 0.464^{d}
AtPLC2N^{+}	6 ± 0.252^{d}	4 ± 0.032^{c}	6 ± 0.068^{a}	5 ± 0.343^{c}	6 ± 0.389^{c}	0 ± 0.239^{d}
AtPLC4^{+}	9 ± 0.312^{b}	2 ± 0.163^{d}	5 ± 0.143^{b}	5 ± 0.156^{c}	9 ± 0.247^{b}	3 ± 0.147^{b}
AtPLC4X2^{+}	8 ± 0.313^{bc}	6 ± 0.122^{b}	5 ± 0.132^{b}	4 ± 0.465^{c}	7 ± 0.436^{bc}	0 ± 0.336^{d}
AtPLC6^{+}	8 ± 0.232^{bc}	2 ± 0.074^{d}	2 ± 0.094^{d}	7 ± 0.237^{b}	7 ± 0.624^{bc}	4 ± 0.245^{a}

WT plants had a high average number of basal leaves. *AtPLC4*$^+$ plants had slightly fewer basal leaves on than WT plants on average. *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4X2*$^+$, and *AtPLC6*$^+$ plants had significantly fewer basal leaves than WT plants, and among them, *AtPLC2N*$^+$ had the lowest average (approximately six leaves).

The average number of cauline leaves in *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, and *AtPLC6*$^+$ plants was significantly lower than that of WT plants, while *AtPLC2M*$^+$ and *AtPLC4X2*$^+$ had about the same number as WT. *AtPLC4*$^+$ had the fewest on average (approximately two leaves).

The average number of lateral stems in *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, *AtPLC4X2*$^+$, and *AtPLC6*$^+$ plants was higher than that of WT plants, *AtPLC2*$^+$ plants having the most. *AtPLC2N*$^+$ and *AtPLC4X2*$^+$ plants had about the same number as WT, while *AtPLC2M*$^+$, *AtPLC4*$^+$, and *AtPLC6*$^+$ plants had fewer.

There was no significant difference in the average floret number between WT and *AtPLC6*$^+$ plants; the average floret number of *AtPLC2*$^+$ plants was higher than that of WT plants; and the average floret number of *AtPLC2M*$^+$, *AtPLC2N*$^+$, *AtPLC4*$^+$, and *AtPLC4X2*$^+$ plants was lower than that of WT plants.

The WT, *AtPLC2M*$^+$, *AtPLC2N*$^+$, and *AtPLC4X2*$^+$ plants did not grow siliques. Although the *AtPLC2*$^+$, *AtPLC4*$^+$, and *AtPLC6*$^+$ plants all grew siliques, they only grew 2–4.

Statistical analysis showed that the overexpression of the *PLC2*, *PLC2M*, *PLC2N*, *PLC4*, *PLC4X2*, and *PLC6* genes significantly promoted the growth of lateral stems and the development of flowers and siliques in *A. thaliana*.

3.6.3 Analysis of the Phenotypic Results of AtPLCs$^+$ *Resistant Plants at Day 35*

AtPLCs$^+$ resistant plants are compared with WT plants at 35 d in Table 7. The phenotypic results of these plants and WT at 35 d are shown in Fig. 10.

Table 7: Statistics of phenotypic traits of *AtPLCs*$^+$ and WT plants at day 35

Plant type	Average number of basal leaves/piece	Average number of cauline leaves/piece	Average number of lateral stems/branches	Average floret number/piece	Average plant height/cm	Average silique number/piece
WT	13 ± 0.258^{a}	9 ± 0.415^{a}	3 ± 0.027^{d}	10 ± 0.512^{ab}	18 ± 0.361^{c}	18 ± 0.541^{g}
AtPLC2$^+$	8 ± 0.436^{d}	4 ± 0.417^{cd}	7 ± 0.081^{a}	6 ± 0.091^{cd}	27 ± 0.4221^{b}	93 ± 0.191^{a}
AtPLC2M$^+$	8 ± 0.601^{d}	7 ± 0.247^{b}	4 ± 0.046^{cd}	9 ± 0.218^{b}	29 ± 0.354^{a}	32 ± 0.354^{f}
AtPLC2N$^+$	8 ± 0.346^{d}	5 ± 0.369^{c}	6 ± 0.072^{b}	5 ± 0.231^{d}	18 ± 0.869^{c}	73 ± 0.239^{b}
AtPLC4$^+$	12 ± 0.445^{b}	3 ± 0.258^{d}	4 ± 0.157^{cd}	7 ± 0.157^{c}	25 ± 0.153^{b}	53 ± 0.215^{c}
AtPLC4X2$^+$	10 ± 0.373^{c}	8 ± 0.431^{b}	5 ± 0.101^{c}	11 ± 0.3341^{a}	18 ± 0.3464^{c}	41 ± 0.342^{d}
AtPLC6$^+$	8 ± 0.573^{cd}	7 ± 0.421^{b}	4 ± 0.064^{cd}	9 ± 0.209^{b}	29 ± 0.235^{a}	22 ± 0.253^{e}

The plant height of WT, *AtPLC2N*$^+$, and *AtPLC4X2*$^+$ plants was similar, while the overall height of *AtPLC2*$^+$, *AtPLC2M*$^+$, *AtPLC4*$^+$, and *AtPLC6*$^+$ plants was greater. WT plants still had the highest average number of basal leaves, but all *AtPLCs*$^+$ plants had more basal leaves than they had at day 15. The average number of basal leaves of *AtPLC4*$^+$ plants increased the most. The average number of lateral stems in *AtPLCs*$^+$ plants was higher than that of WT plants, and the average number of lateral stems in *AtPLC2*$^+$ plants was the highest. With the increase in the number of lateral stems, the average number of cauline leaves in *AtPLCs*$^+$ also increased but was still less than that of WT plants. Compared with 15 d, all *AtPLCs*$^+$ groups had more florets and siliques at 35 d. In addition to the average floret number of

AtPLCs^{+} plants was slightly higher than that of WT plants, the average floret number of *AtPLC2N*$^{+}$, *AtPLC4*$^{+}$, and *AtPLC6*$^{+}$ plants was less than that in WT plants. The average silique number of *AtPLC2*$^{+}$, *AtPLC2M*$^{+}$, *AtPLC2N*$^{+}$, *AtPLC4*$^{+}$, *AtPLC4X2*$^{+}$, and *AtPLC6*$^{+}$ plants was higher than that of WT plants, and the *AtPLCs*$^{+}$ plants matured significantly earlier than WT plants.

Figure 10: *AtPLCs*$^{+}$ resistant plants at day 35 after transplanting

Statistical analysis showed that the overexpression of the *PLC2*, *PLC2M*, *PLC2N*, *PLC4*, *PLC4X2*, and *PLC6* genes significantly promoted the maturation of *A. thaliana* and the growth of lateral stems but significantly inhibited the development of basal leaves and cauline leaves of *A. thaliana*.

3.7 Correlation Analysis of Castor PLC Family Genes

The homologous relationship between castor PLC family genes and Arabidopsis PLC family genes is shown in Fig. 11, and the results of fluorescence quantitative analysis are as shown in Fig. 12.

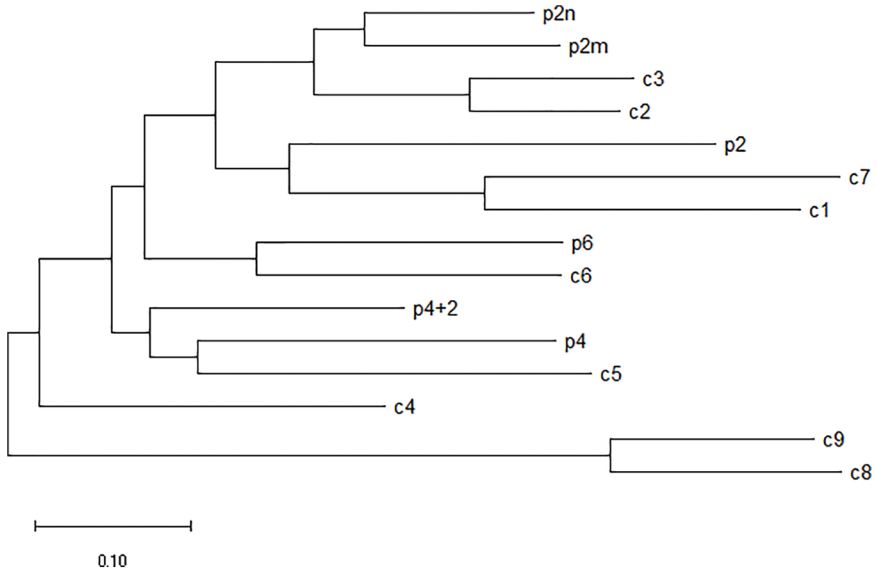

Figure 11: Homologous relationship between castor *PLC* family genes and *Arabidopsis thaliana* PLC family genes

Note: p2: Castor PLC2 (XP_025014303.1); p2m: Castor PLC2M (XP_015579164.1); p2n: Castor PLC2N (XP_002533650.1); p4: Castor PLC4 (XP_002525652.2); p4+2: Castor PLC4X2 (XP_025014307.1); p6: Castor PLC6 (XP_002533649.1); c1: Arabidopsis PLC1 (NP_568881.1); c2: Arabidopsis PLC2 (NP_187464.1); c3: Arabidopsis PLC3 (NP_191153.1). c4: Arabidopsis PLC4 (NP_001318832.1); c5: Arabidopsis PLC5 (NP_001332555.1); c6: Arabidopsis PLC6 (NP_850327.2); c7: Arabidopsis PLC7 (NP_195565.2); c8: Arabidopsis PLC8 (NP_190313.1); c9: Arabidopsis PLC9 (NP_190306.2).

When the *PLC2* gene of castor bean was heterologous overexpressed in *Arabidopsis thaliana* (Fig. 12A), the expression levels of *PLC3* and *PLC8* genes in *Arabidopsis thaliana* were significantly up-regulated compared with the wild type. The gene expressions of *PLC1*, *PLC2*, *PLC4*, *PLC5*, *PLC6*, *PLC7* and *PLC9* in *Arabidopsis thaliana* were significantly down-regulated.

When the *PLC2M* gene of castor bean was overexpressed heterogeneously in *Arabidopsis thaliana* (Fig. 12B), the expressions of *PLC4* and *PLC9* genes in *Arabidopsis thaliana* were down-regulated compared with the wild type, but the expressions of *PLC1*, *PLC2*, *PLC3*, *PLC5*, *PLC6* and *PLC8* genes in *Arabidopsis thaliana* were up-regulated.There was no significant change in *PLC7* gene expression in *Arabidopsis thaliana*.

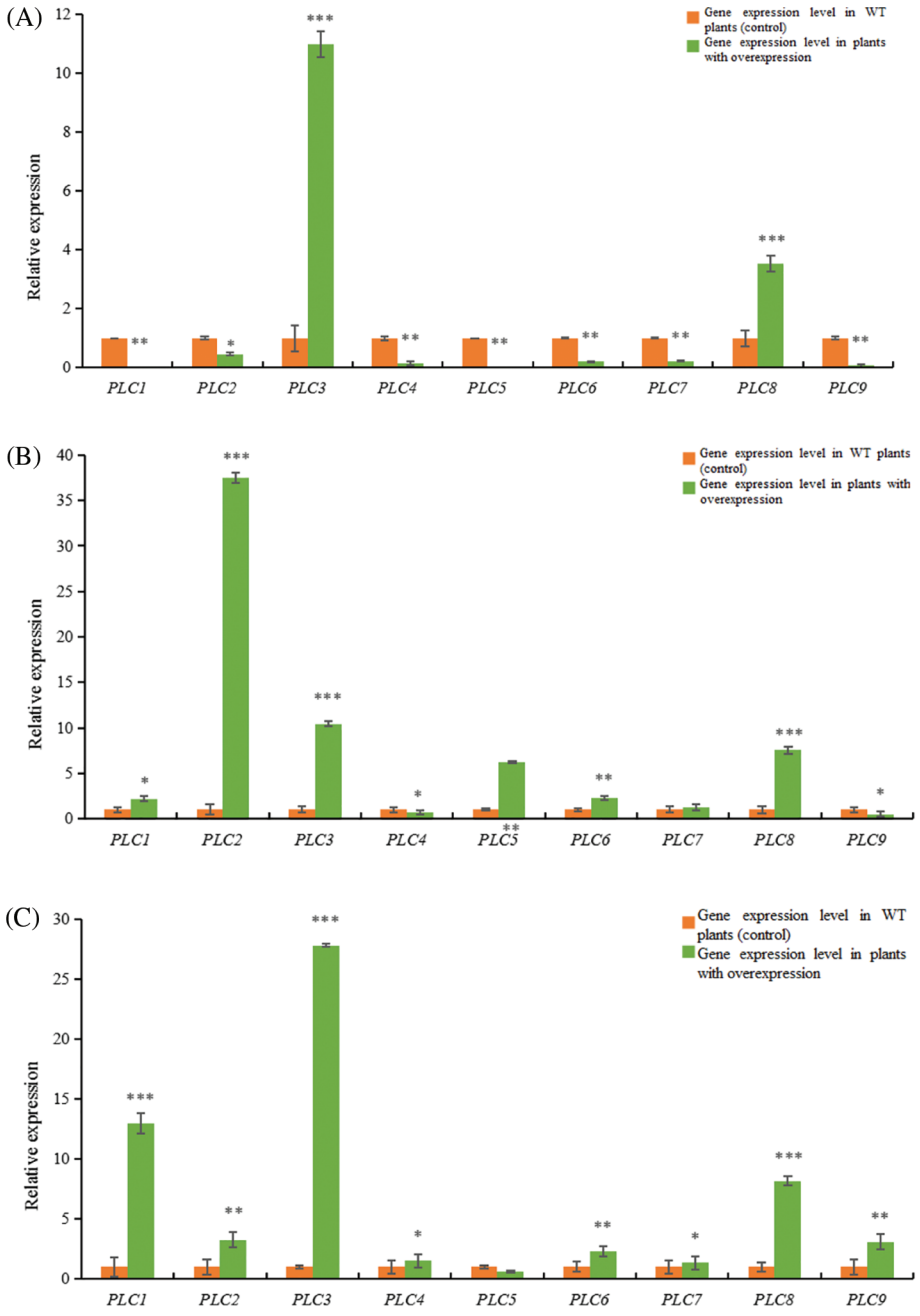

Figure 12: (Continued)

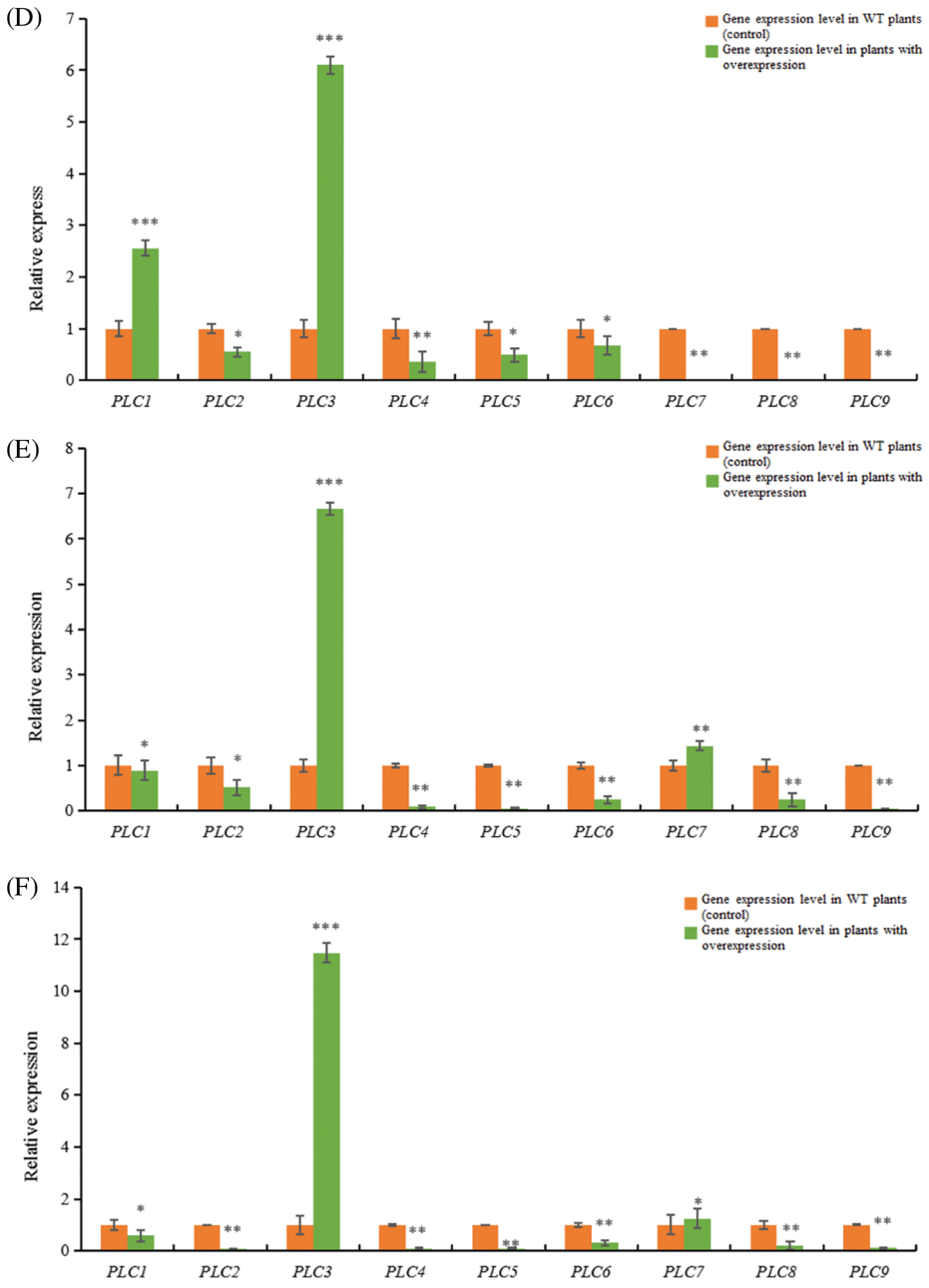

Figure 12: RT-qPCR results of *A. thaliana* plants with heterologous overexpression of the castor *PLC* family genes

Note: (A) *AtPLC2*$^{+}$ plants with heterologous overexpression; (B) *AtPLC2M*$^{+}$ plants with heterologous overexpression; (C) *AtPLC2N*$^{+}$ plants with heterologous overexpression; (D) *AtPLC4*$^{+}$ plants with heterologous overexpression; (E) *AtPLC4X2*$^{+}$ plants with heterologous overexpression; (F) *AtPLC6*$^{+}$ plants with heterologous overexpression; *PLC1*, *PLC2*, *PLC3*, *PLC4*, *PLC5*, *PLC6*, *PLC7*, *PLC8*, and *PLC9* are *thaliana PLC* family genes; *significant difference, $p \leq 0.01$; **significant difference, $p \leq 0.05$; ***difference is extremely significant, $p \leq 0.01$.

When castor *PLC2N* gene was overexpressed heterogeneously in *Arabidopsis thaliana* (Fig. 12C), the expression levels of *PLC1*, *PLC2*, *PLC3*, *PLC4*, *PLC6*, *PLC7*, *PLC8*, *PLC9* genes in *Arabidopsis thaliana* were significantly up-regulated compared with the wild type, while the expression levels of *PLC5* gene in *Arabidopsis thaliana* were not significantly changed.

When the *PLC4* gene of castor was overexpressed heterogeneously in *Arabidopsis thaliana* (Fig. 12D), the expressions of *PLC1*, *PLC2*, *PLC3* genes in *Arabidopsis thaliana* were significantly up-regulated compared with the wild type, while the expressions of *PLC4*, *PLC5*, *PLC6*, *PLC7*, *PLC8*, *PLC9* genes were down-regulated.

When castor *PLC4X2* gene was overexpressed heterogeneously in *Arabidopsis thaliana* (Fig. 12E), the expressions of *PLC3* and *PLC7* genes in *Arabidopsis thaliana* were significantly up-regulated compared with the wild type, while the expressions of *PLC1*, *PLC2*, *PLC4*, *PLC5*, *PLC6*, *PLC8* and *PLC9* genes in *Arabidopsis thaliana* were down-regulated.

When castor *PLC6* gene was heterologous overexpressed in *Arabidopsis thaliana* (Fig. 12F), the expressions of *PLC3* and *PLC7* genes in *Arabidopsis thaliana* were significantly up-regulated compared with the wild type, while the expressions of *PLC1*, *PLC2*, *PLC4*, *PLC5*, *PLC6*, *PLC8* and *PLC9* genes in *Arabidopsis thaliana* were down-regulated.

In summary, according to homology analysis, it is speculated that *PLC2*, *PLC2M*, P*LC2N*, *PLC4*, *PLC4X2* and *PLC6* genes of castor bean have the same regulatory function.

4 Discussion

*PLC*s play key roles in the phosphatidylinositol signaling pathway. In recent years, an increasing number of plant *PLC* functions have been reported. Several *PLC* members are involved in the signaling events triggered by different abiotic stresses in many plants. The overexpression of PLCs in maize and tobacco brings increased drought resistance and salt tolerance to transgenic plants [28,29]. *PLC*s also play a role under heat stress in different plant species [30]. The PI-PLC protein can accumulate in pea plants under heat stress, and the activity of PI-PLC protein in the membrane increases within 40 min of heat treatment [31]. Similarly, the *A. thaliana* PI-PLC and PLC9 proteins are involved in the heat stress response. Genetic analysis has shown that the AtPLC9 mutant exhibits a hyperthermia phenotype after heat stress [32]. A similar and important role of *AtPLC3* has been confirmed in the thermotolerance of *A. thaliana*, i.e., a heat-induced reduction in Ca^{2+} level in the *AtPLC3* mutant has been detected, which could suggest a possible fundamental mechanism [33]. PLC is a multifunctional enzyme in plants and is also involved in different plant growth and development processes [34]. Pollen tube growth is an event of asymmetric cell division, and some key processes in cells include Ca^{2+} signal transduction, vesicle transport, and cytoskeletal rearrangement [35]. PI-PLC localizes to the plasma membrane of pollen tube, and during the process of pollen tube elongation, it is preferentially localized at the edge of the tip, and its substrate PIP2 always accumulates at the top of the pollen tube [36,37]. The catalytically inactive form of petunia PLC1 is expressed in the pollen tube and competes with its natural form for membrane localization, which results in changes in the Ca^{2+} gradient and cytoskeletal reorganization and causes the delocalized growth and enlarged pollen tube apex [36], thus showing that PLCs can regulate the pollen tube elongation. In addition, growing evidence indicates that PLCs are involved in the response to biotic and abiotic stresses. The promoter of the *A. thaliana PLC* genes [38] has cis-regulatory elements that can respond to external stress. The expression level of PI-PLC in rice and *A. thaliana* is increased under salt stress [39,40].

There have been many studies on plant *PLC*s, but they mainly focus on the relevant functions of *PLC* genes and their products, and their mechanisms of action still need in-depth study. Plant PLC proteins lack the pH domain, which plays an important role in the specific binding of enzymes to the substrate on the

membrane [41,42]. Therefore, whether the specific binding of plant PLCs to membrane phospholipids is realized by other domains and the specific mechanisms need to be discovered.

5 Conclusions

This study determined the expression levels of six castor *PLC* family genes at different developmental stages in different types of castor inflorescences. The results showed that the *PLC2*, *PLC4*, and *PLC6* genes had relatively high expression in the female inflorescence; the *PLC2M*, *PLC2N*, and *PLC4X2* genes had relatively high expression in the isofemale inflorescence, while *PLC4* a relatively low expression level in the isofemale inflorescence; and the *PLC2*, *PLC2M*, *PLC2N*, *PLC4X2*, and *PLC6* genes had relatively low expression levels in the bisexual inflorescence. The subcellular localization results showed that the proteins of the six genes were all expressed on the cell membrane. Heterologous overexpression of the six genes in *A. thaliana* plants significantly promoted the maturation of *A. thaliana*, the growth of lateral stems, and the development of flowers and siliques. According to homology analysis, *PLC2*, *PLC2M*, *PLC2N*, *PLC4*, *PLC4X2*, and *PLC6* genes were speculated to have the same regulatory function.

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Author Contributions: Fenglan Huang designed this research and guided data analysis and writing. Yanpeng Wen, Rui Luo, Lili Li, and Xiaotian Liang performed the experiments. Rui Luo and Lili Li guided the experimental process. Yanpeng Wen, Xuemei Hu, Ruxin Li, and Zhiyan Wang completed the data analysis and the writing of the first draft of the paper. All the authors read and agreed to the final text.

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Hong, Y., Zhao, J., Guo, L., Kim, S. C., Deng, X. et al. (2016). Plant phospholipases D and C and their diverse functions in stress responses. *Progress in Lipid Research, 62,* 55–74.
2. Wang, X. (2001). Plant phospholipases. *Annual Review of Plant Physiology and Plant Molecular Biology, 52,* 211–231.

3. Kadamur, G., Ross, E. M. (2013). Mammalian phospholipase C. *Annual Review of Physiology, 75,* 127–154.
4. Berridge, M. J. (1993). Inositol trisphosphate and calcium signalling. *Nature, 361,* 315–325.
5. Li, L. (2015). Signal transduction by phosphoinositides and phospholipase C in plant cells. *Journal of Plant Physiology, 51,* 1590–1596.
6. Munnik, T., Testerink, C. (2009). Plant phospholipid signaling: "in a nutshell". *Journal of Lipid Research, 50,* 260–265.
7. Li, H., Yang, N., Liu, R. R. (2020). The role of phosphoinositide-specific phospholipase C in plant growth and development. *Chinese Journal of Cell Biology, 42,* 2215–2226.
8. Huang, D., Wu, Y. (2017). Research progress on the function of plant phospholipase C. *Life Science, 29,* 575–581.
9. Sun, D. Q., Dong, J. Y., Li, Y. (2017). Research progress of phosphatidylinositol-specific phospholipase C gene. *Biotechnology, 27,* 92–97.
10. Zhao, A. H., Wang, X. G., Dong, J. (2021). Research progress of plant phospholipase C in response to stress. *Chinese Journal of Biotechnology, 37,* 154–164.
11. Zhang, Z. L., Wang, W. J., Ding, B. H. (2016). Identification and bioinformatic analysis of *Riciuns communis* L. steroleosin. *Hubei Agricultural Sciences, 55,* 2930–2933.
12. de Sousa Natália, L., Cabral Glaucia, B., Aragão Francisco, J. L. (2022). Herbicide tolerance and gene silencing stability over generations in the ricin bio-detoxicated castor bean. *Journal of Genetic Engineering and Biotechnology, 20,* 16.
13. Sánchez-Álvarez, A., Ruíz-López, N., Moreno-Pérez, A. J., Venegas-Calerón, M., Martínez-Force, E. et al. (2022). Meta-bolism and accumulation of hydroxylated fatty acids by castor (*Ricinus comunis*) seed microsomes. *Plant Physiology and Biochemistry, 170,* 266–274.
14. Huang, F., Peng, M., Chen, X., Li, G., Di, J. et al. (2018). Comparison of DNA methylation in developing seeds of *Ricinus communis* L. using MSAP analysis. *Biotechnology & Biotechnological Equipment, 32,* 858–864.
15. Chen, X., Peng, M., Huang, F., Luo, R., Zhao, Y. et al. (2016). A quantitative assay for fatty acid composition of castor seed in different developmental stages. *Molecular Plant Breeding, 7,* 1–8.
16. Wei, H. L., Li, X. M., Wang, X. H., Yang, J., Hang, H. (2019). Study on the relation between morphological structure and yield of castor. *Hunan Forestry Science and Technology, 46,* 65–71.
17. Zhao, X. P., Li, G. R., Di, J. J., Hang, F., Chen, Y. et al. (2019). Bioinformatic analysis of the castor LRR-RKsII family and LRR-RKsX family. *Journal of Inner Mongolia University for Nationalities (Natural Science Edition), 34,* 221–230.
18. Han, B., Wu, D., Zhang, Y., Li, D. Z., Xu, W. et al. (2022). Epigenetic regulation of seed-specific gene expression by DNA methylation valleys in castor bean. *BMC Biology, 20,* 1–18.
19. Chen, M., Fan, T., Zhang, L. L., Dai, J. J., Zhao, P. et al. (2018). Research advances in comprehensive utilization of *Ricinus communis* resources. *Journal of Agriculture, 8,* 58–63.
20. Tripathi, A. C., Gupta, R., Saraf, S. K. (2011). Phytochemical investigation characterisation and anticonvulsant activity of *Ricinus communis* seeds in mice. *Natural Product Research, 25,* 1881–1884.
21. Ferraz, A. C., Angelucci, M. E., da Costa, M. L., Batista, I. R., De Oliveira, B. H. et al. (1999). Pharmacological evaluation of ricinine, a central nervous system stimulant isolated from *Ricinus communis*. *Pharmacology, Biochemistry and Behavior, 63,* 367–375.
22. Tan, M., Xue, J., Wang, L., Huang, J., Fu, C. et al. (2015). Transcriptomic analysis for different sex types of *Ricinus communis* L. during development from apical buds to inflorescences by digital gene expression profiling. *Frontiers in Plant Science, 6,* 1208.
23. Huang, F., Peng, M., Chen, X., Li, G., Di, J. et al. (2018). cDNA-AFLP analysis of transcript derived fragments during seed development in castor bean (*Ricinus communis* L.). *Biotechnology & Biotechnological Equipment, 32,* 1119–1125.
24. Huang, F., Bao, C., Peng, M., Zhu, G., He, Z. et al. (2015). Chromatographic analysis of fatty acid composition in differently sized seeds of castor accessions. *Biotechnology & Biotechnological Equipment, 29,* 892–900.

25. Huang, F., Zhu, G., Chen, Y., Meng, F., Peng, M. et al. (2016). Seed characteristics and fatty acid composition of castor (*Ricinus communis* L.) varieties in Northeast China. *Phyton-International Journal of Experimental Botany, 84,* 26–33. https://doi.org/10.32604/phyton.2015.84.026
26. Zhu, D., Wang, X., Zhu, Y., Chen, C., Li, Y. et al. (2011). Construction and application of a plant subcellular localization vector box of pCEG. *Journal of Northeast Agricultural University, 42,* 83–87.
27. Ugalde, J. M., Rodriguez-Furlán, C., Rycke, R., Norambuena, L., Friml, J. et al. (2016). Phosphatidylinositol 4-phosphate 5-kinases 1 and 2 are involved in the regulation of vacuole morphology during *Arabidopsis thaliana* pollen development. *Plant Science, 250,* 10–19.
28. Dowd, P. E., Gilroy, S. (2010). The emerging roles of phospholipase C in plant growth and development. *Lipid Signaling in Plants, 16,* 23–37.
29. Zhai, S. M. (2005). Gene cloning and functional identification of enzymes in the maize phosphatidylinositol metabolic pathway. *Shandong University, 7,* 1–117.
30. Singh, A., Bhatnagar, N., Pandey, A., Pandey, G. K. (2015). Plant phospholipase C family: Regulation and functional role in lipid signaling. *Cell Calcium, 58,* 139–146.
31. Vermeer, J. E. M., van, L. W., Tobeña-Santamaria, R., Laxalt, A. M., Jones, D. R. et al. (2016). Visualization of PtdIns3P dynamics in living plant cells. *The Plant Journal, 47,* 687–700.
32. Zheng, S. Z. (2011). *Role of phosphatidylinositol specific phospholipase C 9 (PI-PLC9) in heat tolerance and heat shock signal transduction in Arabidopsis thaliana*, pp. 1–122. Hebei Normal University, China. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=1016313485.nh&DbName=CDFDTEMP
33. Suh, P. G., Park, J. I., Manzoli, L., Cocco, L., Peak, J. C. et al. (2008). Multiple roles of phosphoinositide-specific phospholipase C isozymes. *BMB Reports, 41,* 415–434.
34. Wang, X., Wang, C., Sang, Y., Qin, C., Welti, R. (2001). Networking of phospholipases in plant signal transduction. *Physiologia Plantarum, 115,* 331–335.
35. Gao, K., Du, J., Hou, M., Zhao, J., Pan, Y. (2013). Research progress of inositol phospholipid signal components regulating pollen development and pollen tube growth. *Journal of Botany, 48(2),* 210–218.
36. Arisz, S. A., Testerink, C., Munnik, T. (2009). Plant PA signaling via diacylglycerol kinase. *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids, 1791,* 869–875.
37. Qu, H., Guan, Y., Wang, Y., Zhang, S. (2017). PLC-mediated signaling pathway in pollen tubes regulates the gametophytic self-incompatibility of pyrus species. *Frontiers in Plant Science, 8,* 1164. https://doi.org/10.3389/fpls.2017.01164
38. van Leeuwen, W., Vermeer, J. E. M., Gadella, T. W. J., Munnik, T. (2007). Visualization of phosphatidylinositol 4,5-bisphosphate in the plasma membrane of suspension-cultured tobacco BY-2 cells and whole Arabidopsis seedlings. *The Plant Journal, 52,* 1014–1026.
39. Li, L. (2015). Study on the mechanism of transduction of OsPLCl ginseng and salt stress signal in paddy rice. *South Beijing Agricultural University, 12,* 1–134.
40. Xia, K. (2016). Mechanism of *Arabidopsis thaliana* phosphatidylinositol specific phospholipase C 4 regulating seedling response to salt stress. *China Agricultural University, 1,* 1–98.
41. Otterhag, L., Sommarin, M., Pical, C. (2001). N-terminal EF-hand-like domain is required for phosphoinositide-specific phospholipase C activity in *Arabidopsis thaliana*. *FEBS Letters, 497,* 165–170.
42. Falasca, M., Logan, S. K., Lehto, V. P., Baccante, G., Lemmon, M. A. et al. (1998). Activation of phospholipase Cγ by PI 3-kinase-induced PH domain-mediated membrane targeting. *The EMBO Journal, 17,* 414–422.