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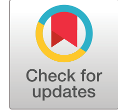

ARTICLE

Effects of Heterologously Overexpressing *PIP5K*-Family Genes in Arabidopsis on Inflorescence Development

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ABSTRACT

Castor is one of the top 10 oil crops in the world and has extremely valuable uses. Castor inflorescences directly affect yield, so the study of inflorescence development is very important in increasing castor yield. Our previous studies have shown that the *PIP5K* gene family (*PIP5Ks*) is associated with inflorescence development. In this study, to determine the function of each *PIP5K* gene in castor, a female Lm-type castor line, aLmAB2, was used to determine the relative expression levels of the *PIP5Ks* in castor inflorescences. Six *PIP5K* genes were heterologously overexpressed in *Arabidopsis thaliana*, the relative expression of each gene and the effect on plants was determined in *A. thaliana*, and the relationships among the *PIP5Ks* in castor were inferred. The expression levels of the *PIP5Ks* in the female Lm-type castor line aLmAB2 were analyzed. The relative expression levels of the *PIP5K9* and *PIP5K11* genes were high ($p < 0.05$) in isofemale inflorescences, and those of *PIP5K1, PIP5K2, PIP5K6*, and *PIP5K8* were high ($p < 0.05$) in female inflorescences but low ($p < 0.05$) in bisexual inflorescences. The *PIP5Ks* were heterologously overexpressed in *A. thaliana*, and T3-generation plants with stable genetic resistance, i.e., AT-*PIP5K*$^+$ plants (AT-*PIP5K1*$^+$, AT-*PIP5K2*$^+$, AT-*PIP5K6*$^+$, AT-*PIP5K8*$^+$, AT-*PIP5K9*$^+$, and AT-*PIP5K11*$^+$ plants), were obtained. Biological tests of the AT-*PIP5K*$^+$ plants showed that the growth of the main stem was significantly delayed in AT-*PIP5K*$^+$ plants compared with Columbia wild-type (WT) *A. thaliana* plants; the *PIP5K1* and *PIP5K2* genes promoted lateral stem growth and flower and silique development; and the *PIP5K6*, *PIP5K8*, *PIP5K9* and *PIP5K11* genes inhibited lateral stem growth and flower and silique development. The correlations among PIP5Ks in castor suggest that there may be a synergistic relationship among *PIP5K1*, *PIP5K2*, and *PIP5K6* in castor inflorescences, and *PIP5K8*, *PIP5K9*, and *PIP5K11* are complementary to the other three genes.

KEYWORDS

Castor; inflorescence development; *PIP5K* gene family; gene overexpression

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1 Introduction

In plants, the phosphatidylinositol (PI) signal transduction pathway is involved in regulating seed germination, growth and development, reproduction, and senescence. Phosphatidylinositol phosphate 5-kinase (PIP5K) is a key enzyme in the PI signal transduction pathway that can catalyze the phosphorylation of PI-4-phosphate (PI4P) and synthesize PI 4,5-biphosphate (PI(4,5)P2) [1]. PI(4,5)P2 is a key component in the generation of second messengers via a variety of pathways (including phosphoinositide phospholipase C, phosphoinositide 3-kinase and phospholipase D (PLD)) [2]. PI(4,5) P2 is involved in regulating the actin cytoskeleton and vesicle transport [3].

Castor is an important oil crop, and the traits of its inflorescences directly affect the entire flowering to fruiting process and determine yield [4–6]. The inflorescence development of castor is extremely complex, and there are two main situations: one is the majority of castor female lines in the world, in which the plants appear monoecious (also called female, with all female flowers on each inflorescence axis of the plant) and hermaphroditic (also called monoecious, with male flowers on the base and female flowers on the top of each inflorescence axis of the plant) inflorescences of two types [7]. The other is the castor Lm-type female line (marker female line) with independent intellectual property rights, in which three types of inflorescences, monoecious, marker female (plants bearing all female flowers on each inflorescence axis, together with willow-like functional leaves) and bisexual, occur in the plants [8]. The contents of endogenous indole-3yl-acetic acid (IAA) and abscisic acid (ABA) in the apical buds also change significantly.

Our previous studies have shown that *PIP5K* family genes (*PIP5Ks*) are associated with the development of castor inflorescences. Therefore, this study used the castor *PIP5Ks* as study subjects and analyzed their expression levels at different developmental stages in different inflorescence types in the female castor line aLmAB$_2$, studied the functions of the *PIP5Ks* through their heterologous overexpression in *Arabidopsis thaliana*, and analyzed the correlations among *PIP5Ks* in castor. This study lays the foundation to study the role of *PIP5Ks* in inflorescence development in the female line aLmAB$_2$ and is of great significance in the breeding of high-yield castor varieties.

2 Materials and Methods

2.1 Materials

The female Lm-type castor line (aLmAB$_2$), Columbia wild-type (WT) *A. thaliana*, and the plant overexpression vector pCAMBIA1390 were provided by the Key Laboratory of Castor Breeding of the State Ethnic Affairs Commission of the Inner Mongolia University for the Nationalities. The full-length coding sequence (CDS) of each *PIP5K* gene (*PIP5K1*, *PIP5K2*, *PIP5K6*, *PIP5K8*, *PIP5K9*, and *PIP5K11*) was synthesized by Beijing Qingke Xinye Biotechnology Co., Ltd. (China); polymerase chain reaction (PCR) product purification kits, plant genomic DNA extraction kits, plant tissue total RNA extraction kits, *Escherichia coli DH5α*, and competent *Agrobacterium* (*GV3101*) cells were purchased from Beijing Zoman Biotechnology Co., Ltd. (China); and complementary DNA (cDNA) synthesis kits, microcolumn concentrated gel DNA extraction kits, fluorescent SYBR dye, *KpnI* restriction endonuclease, and *BamHI* restriction endonuclease were purchased from Beijing Baori Medical Biotechnology Beijing Co., Ltd. (China). The subcellular localization expression vector pCAMBIA1301-*PIP5Ks* was provided by our laboratory.

2.2 Methods

2.2.1 Analysis of the Expression Levels of PIP5Ks in aLmAB$_2$

A total RNA extraction kit was used to extract total RNA from isofemale, female, and bisexual aLmAB$_2$ inflorescences at the four-leaf, five-leaf, main stem spike flowering and secondary branch flowering stages (a total of 12 samples) to synthesize cDNA via reverse transcription using a cDNA synthesis kit. Real-time quantitative PCR (RT-qPCR) was performed using the cDNA as the template and SYBR Green as the fluorescent dye. The primer sequences are shown in the article by one of the authors of this paper,

Liang [9]. The 12 samples were subjected to RT-qPCR using a 20 μL reaction system (2 μL cDNA, 10 μL SYBR Premix Ex *Taq*, 0.4 μL ROX Reference Dye II, 0.4 μL primer S, 0.4 μL primer X, and 6.8 μL ddH_2O). The expression levels of the six genes were analyzed using 18S as an internal reference. The experiment was repeated three times. After data processing, the expression levels of the target genes in different samples were analyzed.

2.2.2 Heterologous Overexpression of the PIP5Ks in A. thaliana

Construction of the Heterologous Overexpression Vector pCAMBIA1390-PIP5Ks

Using SnapGene software (2.3.2), two pairs of primers (*PKsR1* and *PKsF1*; *PKsR2* and *PKsF2*) were designed for the two ends of *PIP5K1*, *PIP5K2*, *PIP5K6*, *PIP5K8*, and *PIP5K9*, and the primer sequences are shown in Table 1 (of the six *PIP5K* genes, the *PIP5K11* gene has *KpnI* and *BamHI* restriction sites at both ends, so there was no need to manually add *KpnI* and *BamHI* restriction sites when designing the primers). The extracted subcellular localization expression vector pCAMBIA1301-*PIP5Ks* was used as the template, and the designed primers were used for PCR (PCR system: 2 μL of recombinant plasmid, 1 μL of PKsR F, 1 μL of PKsF R, 10 μL of PrimeSTAR buffer, 4 μL of dNTP mixture, 0.5 μL PrimeSTAR HS DNA polymerase, and 31.5 μL of ddH_2O). The PCR products were subjected to agarose gel electrophoresis and then purified and extracted using a gel extraction kit. After extraction, digestion was performed at a ratio of 1:1 to obtain two sticky ends, *KpnI* and *BamHI*. The overexpression vector pCAMBIA1390 was extracted using a reagent kit, double digestion was performed with *KpnI* and *BamHI*, and the double digestion products were purified and extracted (double digestion system: 40 μL plasmid, 8 μL *KpnI*, 8 μL *BamHI*, 5 μL 10× K. buffer, and 39 μL ddH_2O). The six genes were ligated into the overexpression vector pCAMBIA1390 overnight at 16°C using T4 DNA ligase (ligation system: 5 μL target gene, 2 μL vector, 1 μL 10× T4 DNA ligase buffer, 1 μL T4 DNA ligase, and 1 μL ddH_2O), and the ligation product was transformed into *E. coli DH5α*. PCR of the transformed products was performed on the bacteria in liquid medium. The PCR primer sequences are shown in Table 2. After validation, the obtained recombinant vector pCAMBIA1390-*PIP5Ks* was transformed into competent *Agrobacterium GV3101* cells.

Table 1: PCR primer sequences for *PIP5Ks* in castor

Primer name	Primer sequence
PK1R1	5′-GTACCATGCGTGAAGGAGTTATACTTG-3′
PK1F1	5′-GATCCCTACTTGTCATCGTCGTCC-3′
PK1R2	5′-CATGCGTGAAGGAGTTATACTTGTTGA-3′
PK1F2	5′-CCTACTTGTCATCGTCGTCCTTGTAA3′
PK2R1	5′-GTACCATGCAAGAGACGCTTCTC-3′
PK2F1	5′-GATCCTCACTTGTCATCGTCGTC-3′
PK2R2	5′-CATGCAAGAGACGCTTCTCCG-3′
PK2F2	5′-CTCACTTGTCATCGTCGTCCTTGTAA3′
PK6R1	5′-GTACCATGAGCAAGGAGCATGGAA-3′
PK6F1	5′-GATCCTTACTTGTCATCGCTGTCCT-3′
PK6R2	5′-CATGAGCAAGGAGCATGGAAGTTTT-3′
PK6F2	5′-CTTACTTGTCATCGTCGTCCTTGTAAT-3′
PK8R1	5′-GTACCATGAAATTAGCCGTTGGAAGT-3′

(Continued)

Table 1 (continued)

Primer name	Primer sequence
PK8F1	5′-GATCCTCACTTGTCATCGTCGTCC-3′
PK8R2	5′-CATGAAATTAGAAGTTGGAAGTGAT-3′
PK8F2	5′-CTCACTTGTCATCGTCGTCCTTGTA-3′
PK9R1	5′-GTACCATGTCTGGTCCTGCG-3′
PK9F1	5′-GATCCTTACTTGTCATCGTCGTCC-3′
PK9R2	5′-CATGTCTGGTCCTGCGGC-3′
PK9F2	5′-CTTACTTGTCATCGTCGTCCTTGT-3′

Table 2: PCR primer sequences for *PIP5Ks* in castor

Primer name	Primer sequence
1390R	5′-AGCTATATGTGGATTTTTTAGCCC-3′
1390F	5′-TGATAATCATCGCAAGACCGGCAAC3′

Genetic Transformation of WT Columbia A. thaliana with Heterologous Overexpression Vectors

Using the method of Jiang et al. [10], the WT *A. thaliana* was genetically transformed, and the obtained T_1 generation *A. thaliana* seedlings were transplanted until they could be harvested. By the T_3 generation, six types of resistant Columbia WT *A. thaliana* plants heterologously overexpressing *PIP5Ks* (AT-*PIP5K*$^+$ plants) were obtained.

Molecular Characterization of A. thaliana Plants with Heterologous Overexpression

Genomic DNA from AT-*PIP5K*$^+$ and WT *A. thaliana* leaves was extracted using a genomic DNA extraction kit.

PCR detection: Genomic DNA from T_3-generation AT-*PIP5K*$^+$ and WT *A. thaliana* leaves were used as templates, the PCR product from the recombinant heterologous overexpression pCAMBIA1390-*PIP5K* vectors were used as positive controls, and the PCR product from genomic DNA from WT *A. thaliana* leaves was used as a negative control. Each sample was subjected to PCR detection using a 50 µL reaction system (2 µL of recombinant plasmid, 1 µL of PKsR F, 1 µL of PKsF R, 10 µL of PrimeSTAR buffer, 4 µL of dNTP mixture, 0.5 µL of PrimeSTAR HS DNA polymerase, and 31.5 µL of ddH_2O). The reaction conditions were as follows: predenaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min 30 s; extension at 72°C for 5 min; and storage at 4°C. The PCR products were detected and observed via agarose gel electrophoresis.

RT-qPCR detection: Total RNA was extracted from inflorescences from T_3-generation AT-*PIP5K*$^+$ and Columbia WT *A. thaliana* plants. The RNA was diluted to approximately 500 ng/µL, the integrity of the obtained RNA was detected via agarose gel electrophoresis, and RNA images were obtained using a gel imaging system. Total RNA from AT-*PIP5K*$^+$ and WT *A. thaliana* inflorescences was reverse transcribed into cDNA according to the cDNA synthesis kit instructions. The *PIP5K* messenger RNA (mRNA) sequences in the National Center for Biotechnology Information (NCBI) database (GenBank accession number: PIP5K 1 (LOC8274135), PIP5K 2 (LOC8287614), PIP5K 6 (LOC8270872), PIP5K 8 (LOC8285184), PIP5K 9 (LOC8283966), PIP5K 11 (LOC8286714)) were searched, and RT-qPCR primers were designed. The primer sequences for each gene, shown in Table 3, were determined based on

preliminary experiments using the same design method. SPSS 19.0 software was used for statistical analysis of the data, and GraphPad was used for data processing and plotting. Independent samples *t* tests were used to compare the averages for significance analyses, and the obtained results are listed in a histogram of relative expression levels.

Table 3: RT-qPCR primer sequences for *A. thaliana* plants overexpressing castor *PIP5Ks*

Primer name	Primer sequence
18S-F	5′-AGGGGATAACCACCCCATGAATCCA-3′
18S-R	5′-TGCATGGTCTCCTGATACGGCCAAG-3′
BM1-S	5′-ATGGCGCGGAGAAGTGATTT-3′
BM1-X	5′-TCGTCGTCCTTGTAATCCCTG-3′
BM2-S	5′-TGAGTTGGATCAGTGCGTGG-3′
BM2-X	5′-CTCACTTGTCATCGTCGTCCT3′
BM6-S	5′-TGACCCGACTTCAATCTCTGC-3′
BM6-X	5′-TCCTTACTTGTCATCGTCGTCC-3′
BM8-S	5′-CGGCAGGAGGTTGATTCCAT-3′
BM8-X	5′-TCCTCACTTGTCATCGTCGT-3′
BM9-S	5′-ACAAATTCCGGGGAGTGAGG-3′
BM9-X	5′-TCCTTACTTGTCATCGTCGTCC-3′
BM11-S	5′-ACCGGAGAGCTTCAATGTGAT-3′
BM11-X	5′-TCACTTGTCATCGTCGTCCTT-3′

Biological Characterization of A. thaliana Plants with Overexpression

WT and AT-*PIP5K*$^+$ plants that grew to the four-leaf stage were transplanted and grown under the same conditions. For each type of heterologous overexpression and control plant, 30 pots were grown, with one plant per pot, for a total of 30 plants. Representative AT-*PIP5K*$^+$ and WT plants were selected for image collection. The phenotypic traits of each type of *A. thaliana* plant were statistically analyzed, and the averages are listed in a table for analysis.

Seeds from WT and T_3-generation AT-*PIP5K*$^+$ plants with stable inheritance were suspended in liquid Murashige and Skoog (MS) medium and inoculated into germination medium. The seeds were cultured under 16 h of light and 8 h of dark per day at 22°C. After 25 d, robust four-leaf *A. thaliana* seedlings were picked for transplantation.

The T_3-generation AT-*PIP5K*$^+$ plants with stable inheritance were biologically characterized, including studying their development, height, and growth status (the number of florets, the bolting time of the main stem, the number of lateral stems, and the numbers of basal leaves and cauline leaves).

Correlation Analysis of PIP5Ks in Castor

RT-qPCR was performed on heterologously overexpressing T_3-generation resistant AT-*PIP5K*$^+$ *A. thaliana* plants (six types). The mRNA sequences of the *A. thaliana PIP5Ks* were queried separately in the NCBI database to determine the primer sequences for each gene, as shown in Table 4. The same design method and experimental procedure were used. RT-qPCR was used to determine the changes in the expression levels of the other five *PIP5K* genes in *A. thaliana* plants caused by the overexpression of

one *PIP5K* gene. Based on sequence homology analysis, the castor *PIP5K1* gene corresponds to the *A. thaliana PIP5K1* gene, the castor *PIP5K2* gene corresponds to the *A. thaliana PIP5K2* and *PIP5K3* genes, the castor *PIP5K6* gene corresponds to the *A. thaliana PIP5K4* and *PIP5K6* genes, the castor *PIP5K8* gene corresponds to the *A. thaliana PIP5K5* gene, the castor *PIP5K9* gene corresponds to the *A. thaliana* accumulation and replication of chloroplasts 3 (*ARC3)* and cytosolic invertase 1 (*CINV1*) genes, and the castor *PIP5K11* gene corresponds to the *A. thaliana PIP5K 9* gene.

Table 4: RT-qPCR primer sequences for *A. thaliana* plants overexpressing AT-*PIP5Ks*

Primer name	Primer sequence
18S-F	5′-AGGGGATAACCACCCCATGAATCCA-3′
18S-R	5′-TGCATGGTCTCCTGATACGGCCAAG-3′
AT1-S	5′-AATGATGCTCTCCGCGAACT-3′
AT1-X	5′-GCCACCAACAGGTTTGACAC-3′
AT2-S	5′-TTCTAAGACCACACCACCGC-3′
AT2-X	5′-TCCAGGCGAAGACAATTCCC-3′
AT3-S	5′-GTGCATTGCGTCAAACCAGT-3′
AT3-X	5′-TGAGTGTTGTGGTCTCGTCG-3′
AT4-S	5′-AGCTCCGGTTGTGTCTCTTG-3′
AT4-X	5′-GATGATAGCTCCCGAAGGGC-3′
AT5-S	5′-AATCGGTGAAGGAGGTGCTG-3′
AT5-X	5′-GATCAAGAGATGCCGCTGGA-3′
AT6-S	5′-ACGCCGAGAAAGCATTACCA-3′
AT6-X	5′-TCGTTCCTTGCCCATGTCTC-3′
AT9-S	5′-TCGGACAGCAGACTTTGGAC-3′
AT9-X	5′-GAACCGATCATCCTGCGACA-3′
ARC3-S	5′-ACCTGTTGGTACTCTTGCCG-3′
ARC3-X	5′-ACCGATCCACACGTTTCTCC-3′
CINV1-S	5′-ACCTGTTGGTACTCTTGCCG-3′
CINVI-X	5′-ACCGATCCACACGTTTCTCC-3′

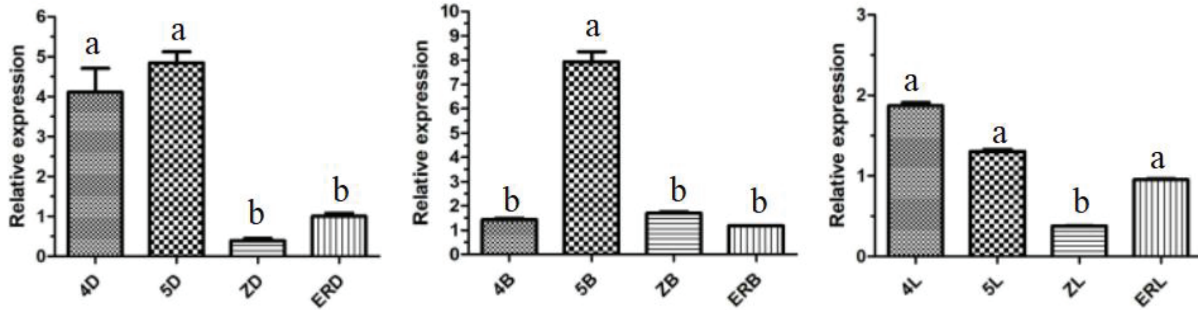
SPSS 19.0 software was used for the statistical analysis of the data, and GraphPad was used for data processing and plotting. Independent samples *t* tests of the means were used for significance analyses, and the obtained results are listed in a histogram of relative expression levels.

3 Results

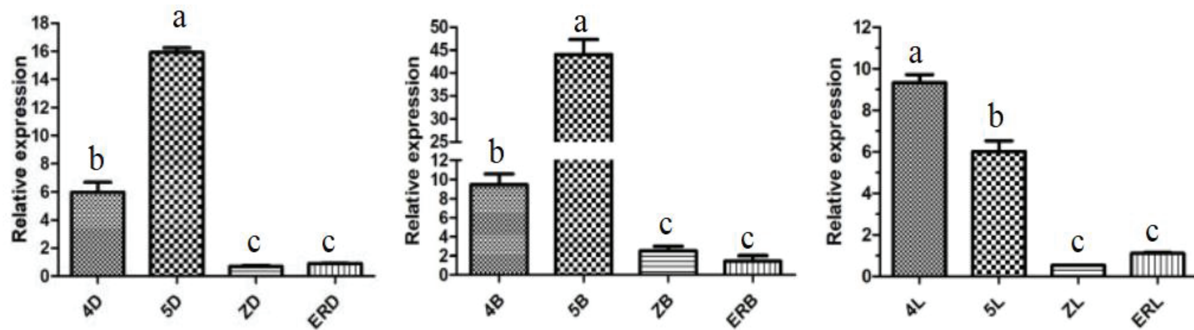
3.1 The Expression Levels of PIP5Ks in Castor Inflorescences

3.1.1 Differential Expression Levels of PIP5Ks in Plants with Different Inflorescence Types at Different Flowering Stages

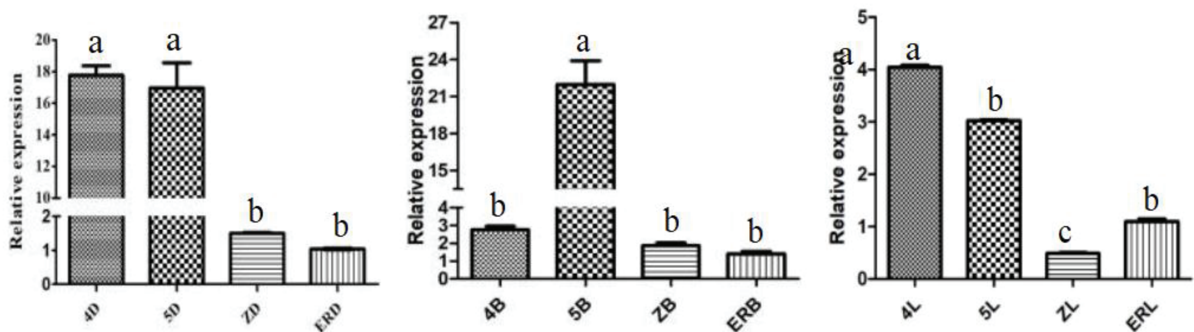
The *PIP5K* RT-qPCR results has been shown in Fig. 1 and include several observations.



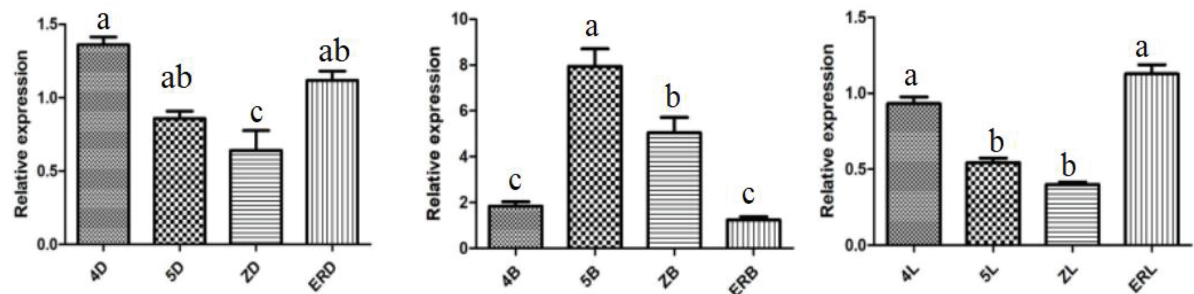
Changes in the relative expression of *PIP5K1*



Changes in the relative expression of *PIP5K2*



Changes in the relative expression of *PIP5K6*



Changes in the relative expression of *PIP5K8*

Figure 1: (Continued)

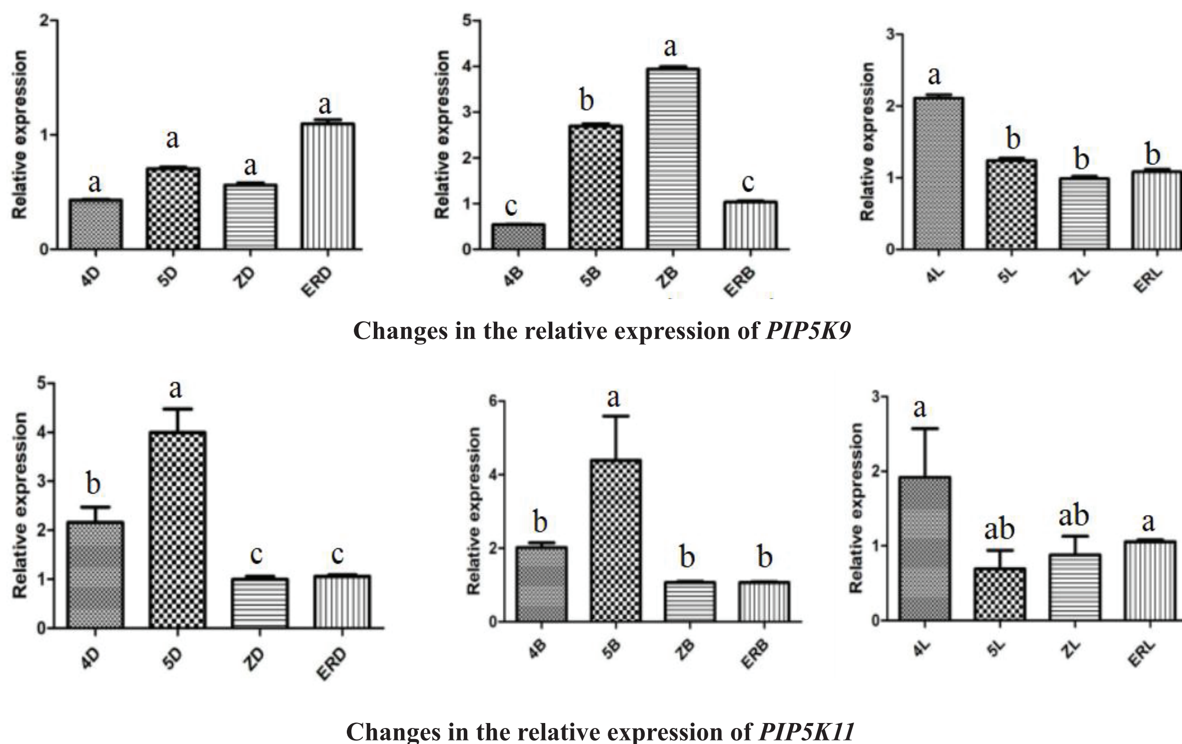


Figure 1: Differential expression levels of *PIP5Ks* at different flowering stages for the three inflorescence types

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 $p < 0.05$.

The relative expression level of *PIP5K1* was high ($p < 0.05$) at the five-leaf stage and low ($p < 0.05$) at the main stem spike flowering stage in all three inflorescence types, namely, isofemale, female and bisexual inflorescences. At the five-leaf stage, it was the highest ($p < 0.05$) in the female inflorescence, and at the main stem spike flowering stage, it was lowest ($p < 0.05$) in the bisexual inflorescence. The highest relative expression level was approximately 16 times the lowest relative expression level.

The relative expression level of *PIP5K2* was high ($p < 0.05$) at the five-leaf stage and low ($p < 0.05$) at the main stem spike flowering stage in all three inflorescence types. At the five-leaf stage, it was highest ($p < 0.05$) in the female inflorescence, and at the main stem spike flowering stage, it was lowest ($p < 0.05$) in the bisexual inflorescence. The highest relative expression level was approximately 80 times the lowest relative expression level.

The relative expression level of *PIP5K6* was high ($p < 0.05$) at the four-leaf and five-leaf stages and low ($p < 0.05$) at the main stem spike flowering and secondary branch flowering stages in all three inflorescence types. At the five-leaf stage, it was highest ($p < 0.05$) in the female inflorescence, and at the main stem spike flowering stage, it was lowest ($p < 0.05$) in the bisexual inflorescence. The highest relative expression level was approximately 46 times the lowest relative expression level.

The relative expression level of *PIP5K8* was high ($p < 0.05$) at the five-leaf and main stem spike flowering stages in the female inflorescence and at the four-leaf and secondary branch flowering stages in

the isofemale and bisexual inflorescences, and it was low ($p < 0.05$) at the four-leaf and secondary branch flowering stages in the female inflorescence and at the five-leaf and main stem spike flowering stages in the isofemale and bisexual inflorescences. It was the highest ($p < 0.05$) at the five-leaf stage in the female inflorescence, and it was the lowest ($p < 0.05$) at the main stem spike flowering stage in the bisexual inflorescence. The highest relative expression level was approximately 16 times the lowest relative expression level.

The relative expression level of *PIP5K9* was high ($p < 0.05$) at the four-leaf, five-leaf, main stem spike flowering and secondary branch flowering stages in the isofemale and bisexual inflorescences and was low ($p < 0.05$) at the five-leaf and main stem spike flowering stages in all three inflorescence types. It was the highest ($p < 0.05$) at the main stem spike flowering stage in the female inflorescence and was the lowest ($p < 0.05$) at the four-leaf stage in the isofemale inflorescence. The highest relative expression level was approximately eight times the lowest relative expression level.

The relative expression level of *PIP5K11* was high ($p < 0.05$) at the five-leaf stage and low ($p < 0.05$) at the main stem spike flowering and secondary branch flowering stages in all three inflorescence types. It was the highest ($p < 0.05$) at the five-leaf stage in the female inflorescence and was the lowest ($p < 0.05$) at the five-leaf stage in the bisexual inflorescence. The highest relative expression level was approximately eight times the lowest relative expression level.

The analysis of the above results showed that in aLmAB_2 plants with different inflorescence types, the expression levels of the six *PIP5K* genes were significantly upregulated and downregulated at different developmental stages. The relative expression levels of *PIP5K1*, *PIP5K2*, *PIP5K6*, and *PIP5K8* were the highest ($p < 0.05$) at the five-leaf stage in the female inflorescence, and those of *PIP5K1*, *PIP5K2*, and *PIP5K6* were the lowest ($p < 0.05$) at the main stem spike flowering stage in the bisexual inflorescence.

3.1.2 *Differential Expression Levels of PIP5Ks in the Same Inflorescence Types at the Same Flowering Stage in Castor*

The RT-qPCR results of the relative expression levels of *PIP5Ks* in the same inflorescence type at the same flowering stage of this part of castor were in article [9] it and yields several observations.

At the four-leaf stage, the relative expression levels of *PIP5K1* and *PIP5K2* were high ($p < 0.05$) for all three inflorescence types, the relative expression levels of *PIP5K8* and *PIP5K9* were medium ($p < 0.05$), and the relative expression levels of *PIP5K6* and *PIP5K11* were low ($p < 0.05$). The relative expression of *PIP5K1* was highest ($p < 0.05$) in the isofemale inflorescence, and the relative expression of *PIP5K11* was lowest ($p < 0.05$) in the bisexual inflorescence.

At the five-leaf stage, the relative expression levels of *PIP5K1* and *PIP5K2* were high ($p < 0.05$) for all three inflorescence types, the relative expression levels of *PIP5K8* and *PIP5K9* were medium ($p < 0.05$), and the relative expression levels of *PIP5K6* and *PIP5K11* were low ($p < 0.05$). The relative expression of *PIP5K2* was highest ($p < 0.05$) in the bisexual inflorescence, and the relative expression of *PIP5K11* was lowest ($p < 0.05$) in the isofemale inflorescence.

At the main stem spike flowering stage, the relative expression levels of *PIP5K1*, *PIP5K8*, and *PIP5K9* were high ($p < 0.05$) for all three inflorescence types, the relative expression of *PIP5K2* was medium ($p < 0.05$), and the relative expression levels of *PIP5K6* and *PIP5K11* were low ($p < 0.05$). The relative expression of *PIP5K9* was highest ($p < 0.05$) in the female inflorescence, and the relative expression of *PIP5K11* was lowest ($p < 0.05$) in the female inflorescence.

At the secondary branch flowering stage, the relative expression levels of *PIP5K1*, *PIP5K8*, and *PIP5K9* were high ($p < 0.05$) for all three inflorescence types, the relative expression of *PIP5K2* was medium ($p < 0.05$), and the relative expression levels of *PIP5K6* and *PIP5K11* were low ($p < 0.05$). The relative expression of *PIP5K9* was highest ($p < 0.05$) in the isofemale inflorescence, and the relative expression of *PIP5K11* was lowest ($p < 0.05$) in the female inflorescence.

In summary, for all three inflorescence types, the expression levels of *PIP5K1* and *PIP5K2* were relatively high ($p < 0.05$) at the four-leaf and five-leaf stages; the expression levels of *PIP5K1*, *PIP5K8*, and *PIP5K9* were relatively high ($p < 0.05$) at the main stem spike flowering and secondary branch flowering stages; and the expression of *PIP5K11* was the lowest ($p < 0.05$) at all four stages.

3.2 Results of Genetically Transforming A. thaliana Plants Using Heterologous Overexpression Vectors

3.2.1 Obtaining Resistant Heterologous Overexpression A. thaliana Plants

Fig. 2 showed the process of resistance screening to obtain T_3-generation AT-*PIP5K*$^+$ plants. Vigorous WT *A. thaliana* plants at the four-leaf stage in medium without MS showed well-developed root systems, and there were no withering phenomena, such as yellowing or transparency. *A. thaliana* plants grown to the two-leaf stage in MS medium supplemented with hygromycin started to yellow and ultimately withered. The transformed *A. thaliana* plants were screened for resistance. When screening the T_1 generation, only 1–2 vigorously growing plants per petri dish reached the four-leaf stage, and most of the plants screened in the T_2 generation reached the four-leaf stage. During the screening of the T_3 generation, vigorously growing plants at the four-leaf stage were obtained. Resistant overexpressing *A. thaliana* plants were shown in Fig. 2. The T_3-generation AT-*PIP5K*$^+$ seeds were germinated to obtain *A. thaliana* plants that were vigorously growing at the four-leaf stage. After transplanting into small pots and growing for 10 d without removing the main stem, the WT plants were approximately 10 cm tall, the resistant AT-*PIP5K1*$^+$ and AT-*PIP5K2*$^+$ plants had a main stem of approximately 3.5 cm, and the remaining four AT-*PIP5K*$^+$ plants had no main stem. The typical growth statuses of the AT-*PIP5K*$^+$ and WT plants were shown in Fig. 3.

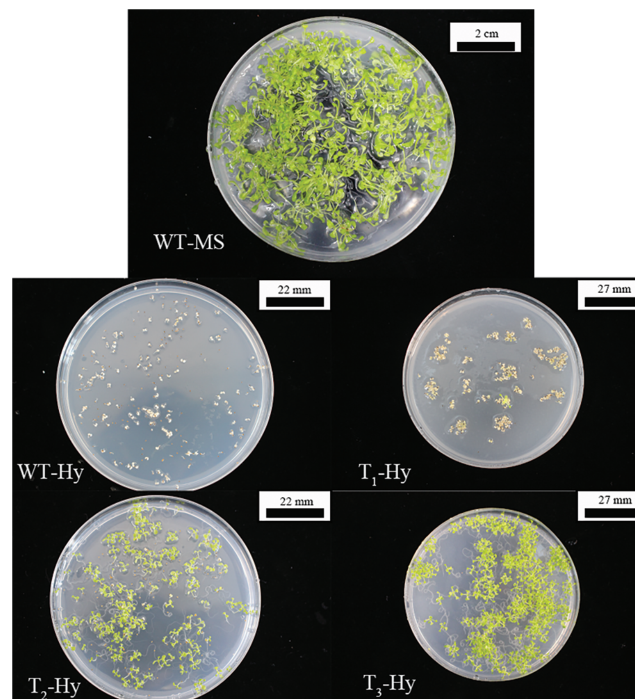


Figure 2: Screening process to identify resistant overexpressing *A. thaliana*

Note: WT-MS: Columbia WT plants germinated on medium without antibiotics; WT-Hy: Columbia WT plants germinated on medium containing hygromycin; T_1-H_y: T1-generation plants germinated on medium containing hygromycin; T_2-Hy: T_2-generation plants germinated on hygromycin-containing medium; T_3-Hy: T_3-generation plants germinated on hygromycin-containing medium.

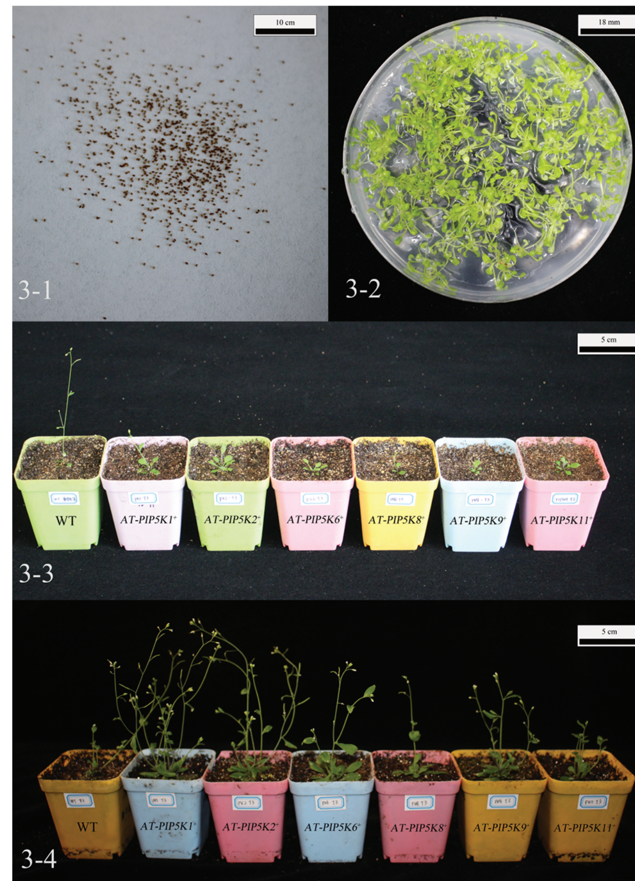


Figure 3: Reproduction and growth processes of transgenic overexpressing *A. thaliana* plants
Note: 3–1: seed stage; 3–2: reaching the four-leaf stage; 3–3: 10 d after transplanting; 3–4: 20 d after transplanting; WT: Columbia WT *A. thaliana* plants.

3.2.2 Molecular Characterization of Resistant Heterologous Overexpression A. thaliana Plants

PCR results: The PCR products were subjected to agarose gel electrophoresis. The PCR results from resistant heterologous overexpression *A. thaliana* plants were shown in Fig. 4. The sizes of the obtained bands were consistent with those of the castor *PIP5Ks*.7

RT-qPCR results: RT-qPCR was performed on AT-*PIP5K*$^+$ plants, and the results were shown in Fig. 5. In AT-*PIP5K*$^+$ plants, the relative expression levels of the castor *PIP5Ks* were all upregulated ($p < 0.05$), indicating successful heterologous overexpression.

3.2.3 Biological Characterization of Resistant Heterologous Overexpression A. thaliana Plants

Phenotypic analysis of *A. thaliana* overexpression plants at 10 d: WT and AT-*PIP5K*$^+$ plants at the four-leaf stage were cultured under the same conditions for 10 d (Fig. 3), and the average phenotypic traits were shown in Table 5. A comparison between the WT and overexpressing *A. thaliana* plants in Fig. 6 and Table 4 showed that when the main stem length of the WT plants was approximately 15 cm, the main stem lengths of the AT-*PIP5K1*$^+$ and AT-*PIP5K2*$^+$ plants were 5 and 3 cm, respectively, and the main stems of the AT-*PIP5K6*$^+$, AT-*PIP5K8*$^+$, AT-*PIP5K9*$^+$, and AT-*PIP5K11*$^+$ plants all tended to sprout but did not bolt, indicating that in the early development of *A. thaliana* seedlings, the bolting time of the main stem was delayed in the AT-*PIP5K*$^+$ plants, and significantly so in the AT-*PIP5K6*$^+$, AT-*PIP5K8*$^+$, AT-*PIP5K9*$^+$, and AT-*PIP5K11*$^+$ plants. The average number of basal leaves was low ($p < 0.05$) in the WT and

AT-*PIP5K9*$^{+}$ plants and was high ($p < 0.05$) in the remaining plants. Cauline leaves grew in the WT, AT-*PIP5K1*$^{+}$, and AT-*PIP5K2*$^{+}$ plants, and in the WT plants, the main stem was relatively long ($p < 0.05$), but there were few ($p < 0.05$) cauline leaves. In contrast, AT-*PIP5K6*$^{+}$, AT-*PIP5K8*$^{+}$, AT-*PIP5K9*$^{+}$, and AT-*PIP5K11*$^{+}$ plants did not bolt, and no cauline leaves were observed. On average, four florets grew on the main stem of WT plants, and one floret grew on the AT-*PIP5K1*$^{+}$ plants. AT-*PIP5K2*$^{+}$ plants grew a main stem but with no florets, and AT-*PIP5K6*$^{+}$, AT-*PIP5K8*$^{+}$, AT-*PIP5K9*$^{+}$, AT-*PIP5K11*$^{+}$ plants showed no bolting or florets. Therefore, the overexpression of the *PIP5Ks* significantly inhibited the early stages of main stem growth in *A. thaliana* plants.

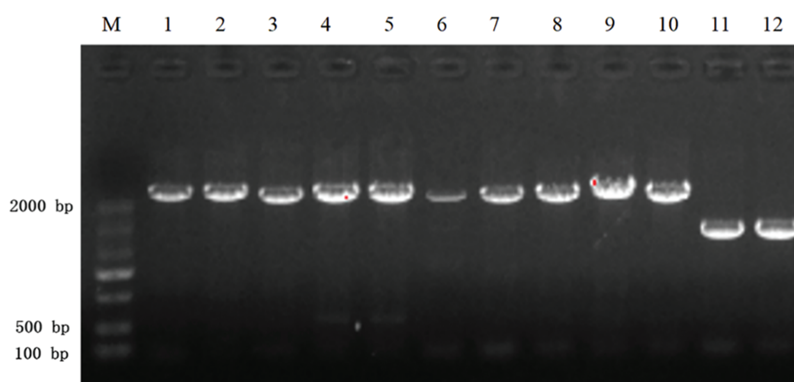


Figure 4: PCR results of transgenic overexpressing A. thaliana plants

Note: M: DL 2000 marker; 1, 2: AT-*PIP5K1*$^{+}$ PCR; 3, 4: AT-*PIP5K2*$^{+}$ PCR; 5, 6: AT-*PIP5K6*$^{+}$ PCR; 7, 8: AT-*PIP5K8*$^{+}$ genomic PCR; 9, 10: AT-*PIP5K9*$^{+}$ PCR; 11, 12: AT-*PIP5K11*$^{+}$ PCR.

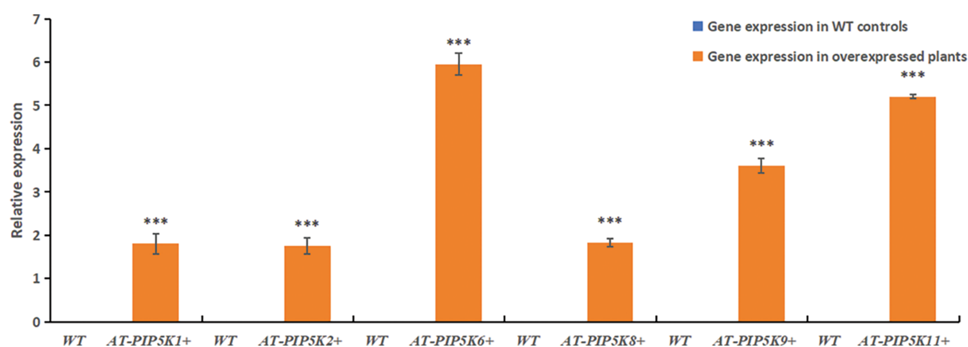


Figure 5: RT-qPCR results of resistant *A. thaliana* plants with heterologous overexpression

Note: *Significant difference, $p < 0.05$, **Significant extremely difference, $p < 0.01$, ***Significant extremely difference, $p < 0.001$.

Table 5: Phenotypic traits of resistant heterologous overexpression *A. thaliana* plants after 10 d of growth

Plant type	Average basal leaves/plant	Average cauline leaves/plant	Average secondary moss/branch	Average florets/flower	Average plant height (cm)	Total plants
WT	3 ± 0.037b	3 ± 0.034a	1 ± 0.011a	4 ± 0.041a	12 ± 0.212a	30
AT-*PIP5K1*$^{+}$	6 ± 0.083a	2 ± 0.082a	1 ± 0.027a	1 ± 0.028b	6 ± 0.124b	30
AT-*PIP5K2*$^{+}$	7 ± 0.056a	2 ± 0.057a	I ± 0.023a	0 ± 0b	4 ± 0.154c	30
AT-*PIP5K6*$^{+}$	6 ± 0.092a	0 ± 0b	0 ± 0b	0 ± 0b	2 ± 0.131c	30

(Continued)

Table 5 (continued)

Plant type	Average basal leaves/plant	Average cauline leaves/plant	Average secondary moss/branch	Average florets/flower	Average plant height (cm)	Total plants
AT-*PIP5K8*$^+$	3 ± 0.117b	0 ± 0b	0 ± 0b	0 ± 0b	2 ± 0.121c	30
AT-*PIP5K9*$^+$	5 ± 0.109ab	0 ± 0b	0 ± 0b	0 ± 0b	2 ± 0.213c	30
AT-*PIP5K11*$^+$	6 ± 0.094a	0 ± 0b	0 ± 0b	0 ± 0b	2 ± 0.123c	30

Note: Different letters indicated different differences, and the significance level was $p < 0.05$.

Figure 6: Phenotypes of resistant heterologous overexpression *A. thaliana* plants after 25 d of growth

Phenotypic analysis of overexpressing *A. thaliana* plants at 25 d: The phenotypic status of the AT-*PIP5K*$^+$ plants grown for 25 d was shown in Fig. 6, and the statistical analyses of the plant traits were shown in Table 6. As shown in Fig. 6 and Table 6, comparison of the length of lateral stems of the AT-*PIP5Ks*$^+$ plants with that of WT plants at 25 d showed the following: 1) the lateral stems of the AT-*PIP5K6*$^+$, AT-*PIP5K8*$^+$, and AT-*PIP5K9*$^+$ plants were the same length as those of the WT plants; 2) the lateral stems of the AT-*PIP5K1*$^+$ and AT-*PIP5K2*$^+$ plants were significantly longer ($p < 0.05$) than those of the WT plants; and 3) the growth of the lateral stems of the AT-*PIP5K11*$^+$ plants was significantly inhibited, thus reducing ($p < 0.05$) length. On average, there were few ($p < 0.05$) basal leaves in WT, AT-*PIP5K1*$^+$, AT-*PIP5K8*$^+$, AT-*PIP5K9*$^+$, and AT-*PIP5K11*$^+$ plants, while there were many ($p < 0.05$) in AT-*PIP5K2*$^+$ and AT-*PIP5K6*$^+$ plants. On average, there were many ($p < 0.05$) lateral stems in AT-*PIP5K1*$^+$ and AT-*PIP5K2*$^+$ plants; a medium ($p < 0.05$) amount in AT-*PIP5K6*$^+$ and AT-*PIP5K11*$^+$ plants; and few ($p < 0.05$) in WT, AT-*PIP5K8*$^+$, and AT-*PIP5K9*$^+$ plants. The average number of florets in was small $p < 0.05$) in WT, AT-*PIP5K6*$^+$, AT-*PIP5K8*$^+$, AT-*PIP5K9*$^+$, and AT-*PIP5K11*$^+$ plants and was large ($p < 0.05$) in AT-*PIP5K1*$^+$ and AT-*PIP5K2*$^+$ plants.

Table 6: Statistics of the phenotypic traits of resistant heterologous overexpression *A. thaliana* plants after 25 d of growth

Plant type	Average basal leaves/plant	Average cauline leaves/plant	Average secondary moss/branch	Average florets/flower	Average plant height (cm)	Total plants
WT	10 ± 0.296c	4 ± 0.147a	2 ± 0.147a	4 ± 0.292d	14 ± 0.421e	30
AT-*PIP5K1*$^+$	12 ± 0.347c	14 ± 0.356b	8 ± 0.411b	26 ± 0.341a	18 ± 0.341b	30
AT-*PIP5K2*$^+$	16 ± 0.242a	12 ± 0.434c	5 ± 0.436cd	18 ± 0.328b	21 ± 0.254a	30
AT-*PIP5K6*$^+$	16 ± 0.256a	7 ± 0.169d	4 ± 0.269cd	12 + 0.223c	15 ± 0.289c	30
AT-*PIP5K8*$^+$	12 ± 0.412c	4 ± 0.099d	2 ± 0.137cd	4 ± 0.132d	15 ± 0.107c	30
AT-*PIP5K9*$^+$	14 ± 0.313b	6 ± 0.526d	3 ± 0.378d	9 ± 0.375d	14 ± 0.336c	30
AT-*PIP5K11*$^+$	11 ± 0.432c	5 ± 0.479d	4 ± 0.424d	4 ± 0.297d	6 ± 0.234d	30

Note: Different letters indicated different differences, and the significance level was $p < 0.05$.

Therefore, in *A. thaliana* plants, overexpressing the *PIP5K1*, *PIP5K2*, and *PIP5K6* genes could significantly promote lateral stem growth and flower and silique development, while overexpressing *PIP5K8*, *PIP5K9*, and *PIP5K11* could significantly inhibit lateral stem growth and flower and silique development.

Phenotypic analysis of overexpression *A. thaliana* plants after 45 d: As shown in Fig. 7 and Table 7, WT, AT-*PIP5K2*$^+$ and AT-*PIP5K6*$^+$ plants had similar heights; however, AT-*PIP5K2*$^+$ was slightly shorter ($p < 0.05$) (growth was slightly inhibited), and AT-*PIP5K6*$^+$ was slightly taller ($p < 0.05$) (growth was slightly promoted) than WT; and the heights of AT-*PIP5K1*$^+$, AT-*PIP5K8*$^+$, AT-*PIP5K9*$^+$, and AT-*PIP5K11*$^+$ were significantly reduced ($p < 0.05$), indicating severe growth inhibition. The AT-*PIP5K1*$^+$, AT-*PIP5K2*$^+$, AT-*PIP5K6*$^+$, AT-*PIP5K8*$^+$, and AT-*PIP5K11*$^+$ plants showed reduced ($p < 0.05$) numbers of basal leaves to varying degrees, while in AT-*PIP5K9* plants, there were slightly more ($p < 0.05$) basal leaves than in WT. The AT-*PIP5K1*$^+$, AT-*PIP5K6*$^+$, AT-*PIP5K8*$^+$, and AT-*PIP5K11*$^+$ plants showed an increase ($p < 0.05$) in the final number of siliques, and AT-*PIP5K2*$^+$ plants had the most ($p < 0.05$) siliques, indicating significant promotion; AT-*PIP5K9*$^+$ plants had the fewest ($p < 0.05$) siliques, indicating a significant inhibition of overall plant growth. The AT-*PIP5K1*$^+$ and AT-*PIP5K11*$^+$ plants matured significantly earlier ($p < 0.05$) than the WT plants. Therefore, in *A. thaliana*, the overexpression of *PIP5K1* and *PIP5K11* could inhibit maturation and lateral stem growth but promote silique development; the overexpression of *PIP5K8* and *PIP5K9* could significantly inhibit growth and development; the overexpression of *PIP5K2* could significantly promote maturation and silique development but inhibit growth to a certain extent; and the overexpression of *PIP5K6* could significantly promote growth and development.

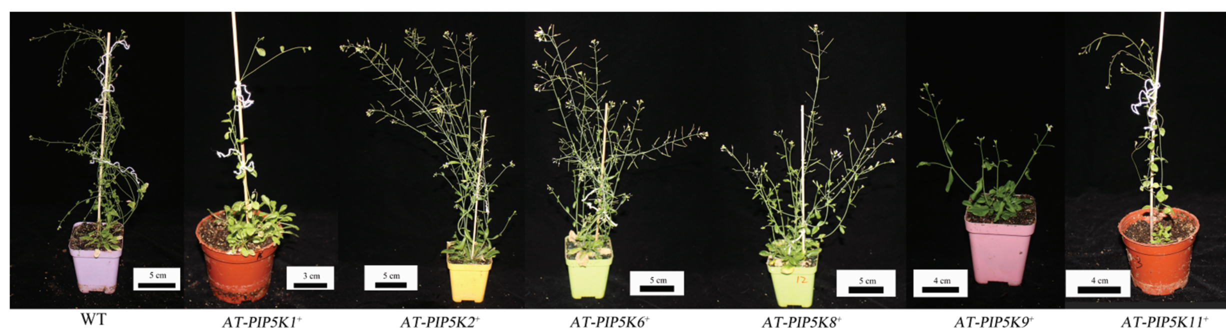


Figure 7: Phenotypes of resistant heterologous overexpression *A. thaliana* plants after 45 d of growth

Table 7: Phenotypic traits of resistant heterologous overexpression *A. thaliana* plants after 45 d of growth

Plant type	Average basal leaves/plant	Average secondary moss/branch	Average plant height (cm)	Average siliques/plant	Total plants
WT	11 ± 0.212b	9 ± 0.212a	38 ± 0.212b	121 ± 0212f	30
AT-*PIP5K1*$^+$	3 ± 0.124d	8 ± 0.132a	24 ± 0.312e	142 ± 0.132d	30
AT-*PIP5K2*$^+$	7 ± 0.154c	9 ± 0.267a	36 ± 0.221c	240 ± 0.267a	30
AT-*PIP5K6*$^+$	9 ± 0.131c	7 ± 0.212a	42 ± 0212a	164 ± 0.212b	30
AT-*PIP5K8*$^+$	3 ± 0.121d	7 ± 0.212a	16 ± 0.124f	123 ± 0.212e	30
AT-*PIP5K9*$^+$	13 ± 0.213a	9 ± 0.113a	14 ± 0.313g	98 ± 0.113g	30
AT-*PIP5K11*$^+$	8 ± 0.123c	7 ± 0.392a	32 ± 0.332d	144 ± 0.392c	30

Note: Different letters indicated different differences, and the significance level was $p < 0.05$.

3.2.4 Correlation Analysis of Castor PIP5Ks

An RT-qPCR analysis was performed on the 11 *A. thaliana PIP5K* genes in the *A. thaliana* AT-*PIP5K*$^+$ plants, as shown in Fig. 8, and yielded several results.

When the castor *PIP5K1* gene was heterologously overexpressed in *A. thaliana*, the expression of the homologous *A. thaliana PIP5K1* gene decreased ($p < 0.05$), and the expression levels of the *A. thaliana PIP5K3*, *PIP5K6* and *PIP5K9* genes increased ($p < 0.05$). Therefore, these results combined with the phenotypic results indicated that the *PIP5K1* gene may have a synergistic relationship with the *PIP5K2* and *PIP5K6* genes in castor inflorescences.

When the castor *PIP5K2* gene was heterologously overexpressed in *A. thaliana*, the expression of the homologous *A. thaliana PIP5K2* gene decreased ($p < 0.05$), and the expression levels of the *A. thaliana PIP5K3*, *PIP5K4, PIP5K6*, and *PIP5K9* genes increased ($p < 0.05$). Therefore, these results combined with the phenotypic results indicated that the *PIP5K2* gene may have a synergistic relationship with the *PIP5K6* gene in castor inflorescences.

When the castor *PIP5K6* gene was heterologously overexpressed in *A. thaliana*, the expression levels of the homologous *A. thaliana PIP5K4* and *PIP5K6* genes decreased ($p < 0.05$), and the expression of the *A. thaliana PIP5K3* gene increased ($p < 0.05$). Therefore, these results combined with the phenotypic results indicated that the *PIP5K6* gene may have a synergistic relationship with the *PIP5K2* gene in castor inflorescences.

When the castor *PIP5K8* gene was heterologously overexpressed in *A. thaliana*, the expression of the homologous *A. thaliana PIP5K5* gene decreased ($p < 0.05$), and the expression levels of the *A. thaliana PIP5K1*, *PIP5K3, PIP5K4, PIP5K6, PIP5K9, ARC3*, and *CINV1* genes also decreased ($p < 0.05$). Therefore, these results combined with the phenotypic results indicated that the *PIP5K8* gene may have a complementary relationship with the *PIP5K1, PIP5K2, PIP5K6*, and *PIP5K9* genes in castor inflorescences.

When the castor *PIP5K9* gene was heterologously overexpressed in *A. thaliana*, the expression levels of the homologous *A. thaliana ARC3* and *CINV1* genes decreased ($p < 0.05$), and the expression levels of the *A. thaliana PIP5K1, PIP5K3, PIP5K4, PIP5K5, PIP5K6*, and *PIP5K9* genes also decreased ($p < 0.05$). Therefore, these results combined with the phenotypic results indicated that the *PIP5K9* gene may have a complementary relationship with the *PIP5K1, PIP5K2*, and *PIP5K6* genes in castor inflorescences.

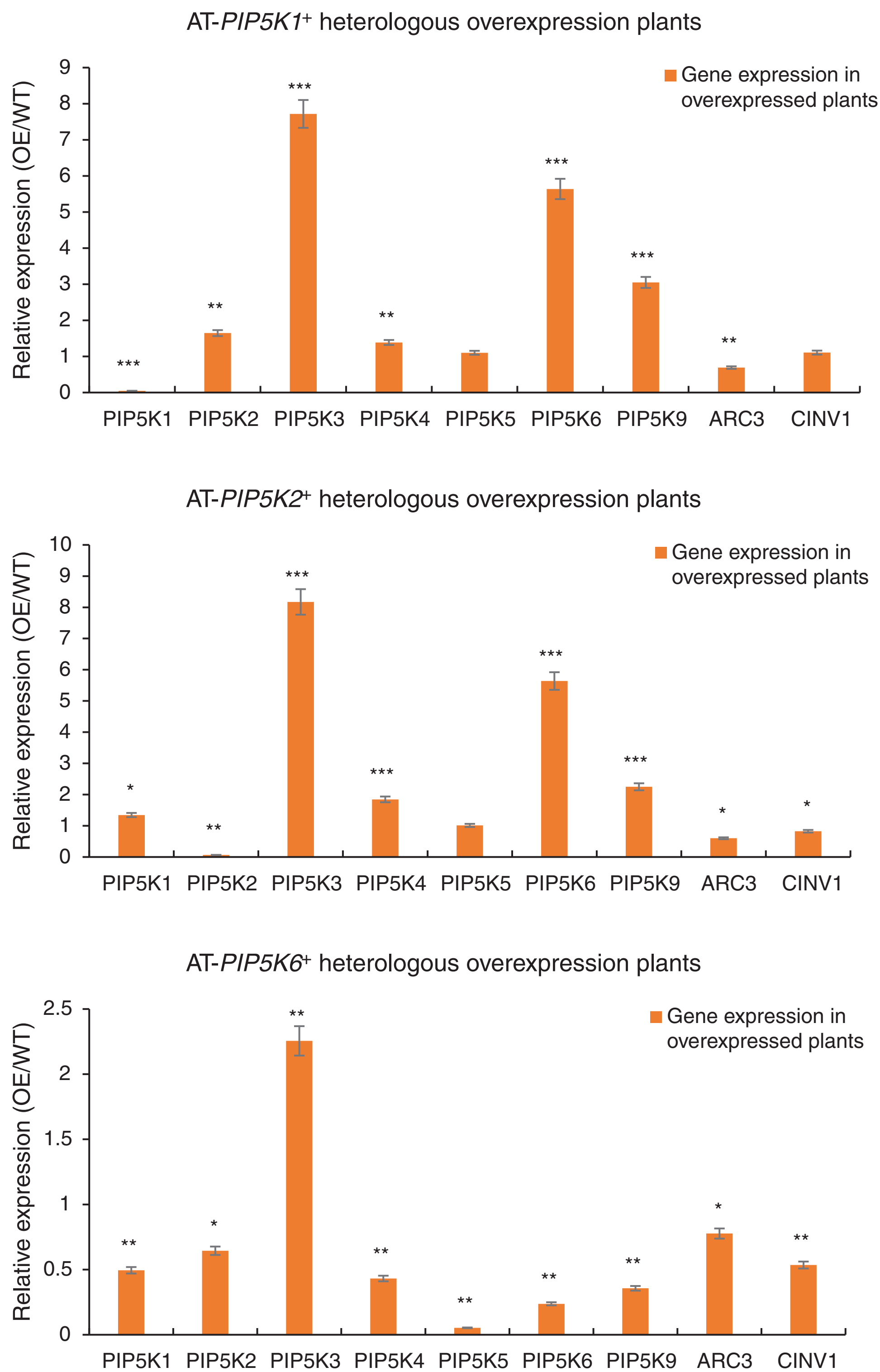


Figure 8: (Continued)

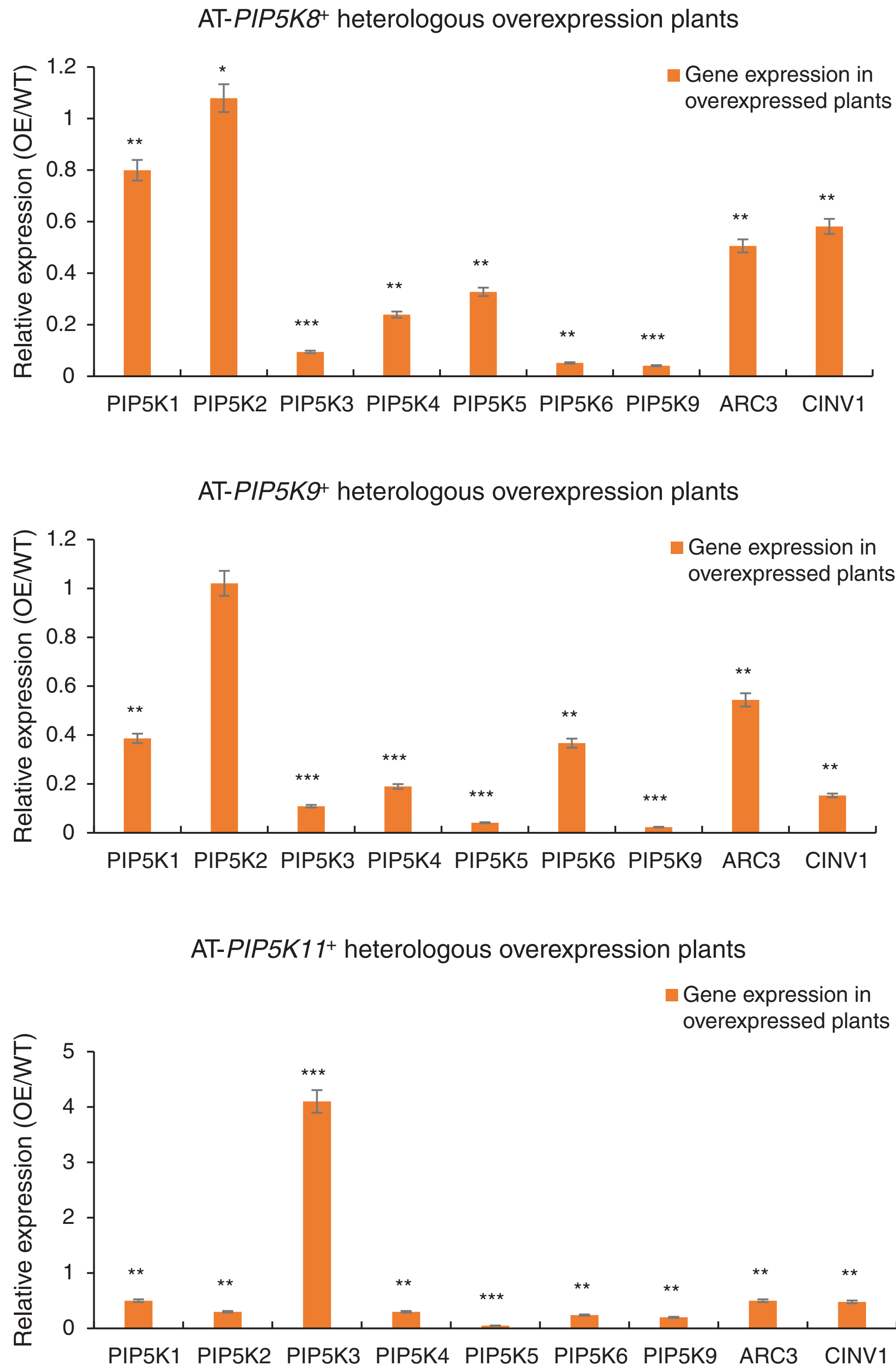


Figure 8: (1) RT-qPCR results from *A. thaliana* plants heterologously overexpressing castor PIP5Ks; (2) RT-qPCR results of *A. thaliana* plants heterologously overexpressing castor PIP5Ks

Note: *Significant difference, $p < 0.05$, **Significant extremely difference, $p < 0.01$, ***Significant extremely difference, $p < 0.001$.

When the castor *PIP5K11* gene was heterologously overexpressed in *A. thaliana*, the expression of the homologous *A. thaliana PIP5K5* gene decreased ($p < 0.05$), and the expression levels of *PIP5K1, PIP5K2, PIP5K4, PIP5K6, PIP5K9, ARC3*, and *CINV1* were also downregulated ($p < 0.05$). Therefore, these results combined with the phenotypic results indicated that the *PIP5K11* gene may have a complementary relationship with the *PIP5K1, PIP5K2, PIP5K6, PIP5K8*, and *PIP5K9* genes in castor inflorescences.

Based on the above results, there may be a synergistic relationship among *PIP5K1, PIP5K2*, and *PIP5K6* in castor inflorescences, and the *PIP5K8, PIP5K9*, and *PIP5K11* genes are complementary to the other three genes.

4 Discussion

In the past few years, the biological functions of some *PIP5K* family members in *A. thaliana* have been reported, including the involvement of AT-*PIP5K1* and AT-*PIP5K2* in pollen development [11–13]. A variety of *PIP5K* isoenzymes had multiple functions in plants. Studies have shown that the lateral roots of AT-*PIP5K2* mutants are reduced, and the gravitational pull on the root system is affected [14]. Other studies have shown that double AT-*PIP5K1* and AT-*PIP5K2* mutations can cause dwarfism and male sterility [15]. In this study, the relative expression levels of *PIP5Ks* in the female Lm-type castor line $aLmAB_2$ showed that the expression levels of *PIP5K1*, *PIP5K2*, *PIP5K6*, *PIP5K8*, *PIP5K9*, and *PIP5K11* in different inflorescence types had certain patterns, such as upregulation and downregulation, at different developmental stages of the inflorescences, and the same patterns were maintained in the expression levels of the *PIP5Ks* in isofemale and bisexual inflorescences at the four-leaf, five-leaf, main stem spike flowering and secondary branch flowering stages. The castor *PIP5Ks* maintained certain patterns at different stages of inflorescence development or participated in the regulation of castor inflorescence development.

Studies have shown that the *PIP5K3* gene is associated with the formation of root hairs in *A. thaliana*, can be specifically expressed in *A. thaliana* roots, and can regulate root hair elongation [16,17]. *PIP5K4* not only regulated stomatal opening [18] but also, together with *PIP5K5*, participated in pollen germination and pollen tube growth [19,20]. The *PIP5K9* gene negatively regulated sugar-mediated root cell elongation by interacting with *CINV1* [21], and RNA interference of the *PIP5K6* gene inhibited the growth and development of pollen tubes [22]. Studies on *PIP5K7-9* have shown that these genes had little effect on plant growth and development under favorable growth conditions, but they were involved in the adaptation of root growth to osmotic stress [23,24]. In this study, when castor *PIP5Ks* were heterologously overexpressed in *A. thaliana*, the relative expression levels of the corresponding *A. thaliana PIP5Ks* were significantly downregulated ($p < 0.05$) due to inhibition. Taking the AT-*PIP5K1*$^+$ plants as an example, when the castor *PIP5K1* gene was heterologously overexpressed, the expression of the *A. thaliana PIP5K1* gene was significantly downregulated ($p < 0.05$), and the relative expression levels of the *A. thaliana PIP5K3* and *PIP5K6* genes were significantly upregulated ($p < 0.05$). These results, combined with the analysis of the phenotypic changes in plants with heterologous overexpression, showed that the *PIP5Ks* jointly regulate *A. thaliana* inflorescence development, and the relative expression of the *A. thaliana PIP5K3* gene was upregulated ($p < 0.05$) when the castor *PIP5Ks* were all heterologously overexpressed. The *PIP5K2* gene corresponded to the *A. thaliana PIP5K2* gene. Therefore, there may be a synergistic relationship among the *PIP5K1, PIP5K2*, and *PIP5K6* genes in castor inflorescences, and *PIP5K8, PIP5K9*, and *PIP5K11* are complementary to the other three genes.

5 Conclusion

An analysis of the expression levels of the *PIP5Ks* in the female Lm-type castor line $aLmAB_2$ showed that except for *PIP5K9* and *PIP5K11*, which had high ($p < 0.05$) relative expression levels in isofemale inflorescences, the other four genes all had the highest ($p < 0.05$) relative expression levels in

female inflorescences. Except for *PIP5K9*, which had a low ($p < 0.05$) relative expression level in isofemale inflorescences, and *PIP5K11*, which had a low ($p < 0.05$) relative expression level in bisexual inflorescences, the other four genes all showed their lowest ($p < 0.05$) relative expression levels in bisexual inflorescences.

PIP5Ks were heterologously overexpressed in *A. thaliana*, and PCR was performed on the AT-*PIP5K* plants, i.e., the six types of T_3-generation *A. thaliana* plants with stable genetic resistance and heterologous overexpression of the *PIP5Ks*. The RT-qPCR results confirmed that the relative expression levels of the six genes in the AT-*PIP5K*$^+$ plants were significantly upregulated ($p < 0.05$) compared to those in Columbia WT plants. The biological characterization of AT-*PIP5K*$^+$ plants showed that main stem growth was significantly delayed in *A. thaliana* plants overexpressing the six genes compared with the control; in the early development of the lateral stems, the overexpression of *PIP5K1* and *PIP5K2* promoted lateral stem growth and flower and silique development, while the overexpression of the other four genes inhibited lateral stem growth and flower and silique development in *A. thaliana*. In the late growth stage of *A. thaliana*, the overexpression of the *PIP5K1* and *PIP5K11* genes significantly promoted maturation; the overexpression of the *PIP5K1, PIP5K2, PIP5K8, PIP5K9* and *PIP5K11* genes inhibited lateral stem growth and flower and silique development; and the overexpression of *PIP5K6* slightly promoted lateral stem growth and flower and silique development. A correlation analysis of the *PIP5Ks* indicated that there may be a synergistic relationship among the *PIP5K1*, *PIP5K2*, and *PIP5K6* genes in castor inflorescences, and the *PIP5K8*, *PIP5K9*, and *PIP5K11* genes are complementary to the other three genes.

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