Preliminary Study Mechanism of Ethylene Induced Abscission Zone Formation and Function of *GhArfGAP* in Upland Cotton Boll Stalk

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Research Article

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Abstract

Background: With the continuous growth of population, the demand for fiber is also rising sharply. As one of the main fiber plants in the world, cotton fiber yield of upland cotton is affected by boll abscission, which is related to the formation of abscission zone. Therefore, we explored the formation of the abscission zone of upland cotton.

Result: The formation of abscission layer of cotton boll stalk was promoted by exogenous ethylene. It was found that both the number of Golgi apparatus and the number of stacking layers increased in the dissociated cells. The *GhArfGAP* gene family in upland cotton was screened by bioinformatics method, and the species and evolutionary relationship of *GhArfGAP* gene family were analyzed. qRT-PCR showed that the expression patterns of *GhArfGAP13*,*GhArfGAP15*, *GhArfGAP25* and *GhArfGAP34* in cotton were spatiotemporal specific. Subcellular localization suggested that *GhArfGAP25* played a role in Golgi apparatus . The expression of *GhArfGAP25* in transgenic *Arabidopsis* increased in the root, stem and leaf.

Conclusions: Ethylene could induce the formation of abscission zone in upland cotton. *GhArfGAP13,GhArfGAP15,GhArfGAP25,GhArfGAP34* might regulate the changes of Golgi apparatus in abscisson zone.Taken together the findings provide new ideas for the study of cotton abscission formation.

Background

Upland cotton (*Gossypium hirsutum L*.) is a major cotton cultivar in China and even in the world. It ccupies an important place in the national economy. The main product of cotton is cotton fiber, which is an important raw material of textile industry. natural cotton fiberhas many excellent characteristics that chemical cotton fiber does not have, such as good heat preservation, no pollution, strong moisture absorption, easy dyeing, beautiful and comfortable products. cottonseed as by-product can produce oil. Therefore, the demand for cotton is increasing year by year. However in the actual production of cotton, in many factors affecting cotton yield, the shedding of buds and bolls is an important factor, which seriously restricts the yield of cotton. According to relevant reports, the shedding rate of upland cotton is generally 60% ~ 70% [1], and the shedding rate of cotton in Northern Xinjiang is even more than 80% [2]. The shed of buds and bolls is related to the formation of abscission zone,and ethylene plays an important role in this process. The tissue area and adjacent layers of cells in which plant organ abscission occurs are called abscission zone[3].Abscission layer is composed of several layers of cells separated from adjacent cells in the abscission zone, which can receive abscission signals and make abscission layer cells separate from surrounding cells, thus leading to abscission of plant organs [4].Many $ArfGAP_S$ have already been reported,for example Age2[5] ,GCS1[6],GCO3[7].They have been shown to play an important role in the transport of Golgi vesicles.In the model plant *Arabidopsis thaliana*, a typical ArfGAP protein is ZAC. ZAC protein has been proved to be related to Golgi apparatus.,and analysis of its expression pattern revealed that it has different expression patterns in different organs[8].But there is few study about ArfGAP and the relationship between ArfGAP and abscission zone formation in upland

cotton.So we carried out this study in time.In this study, ethephon was used to induce abscission formation in cotton petioles. Morphological and microscopic observation and molecular experiment were carried out to preliminary explore mechanism of boll stalk abscission formation and the function of GhArfGAP in upland cotton, and lay a foundation for reducing boll abscission in advance.

Materials And Methods

Plant Materials

Ekangmian No.9 (Jing55173) that formal identified Hubei Jingzhou Academy of Agricultural Sciences was selected as experimental variety,Seedlings acquired from institute of cotton research of CAAS.and were grown in Anhui Agricultural University high-tech agricultural park,HeFei,China. Cotton plants with consistent growth and good condition were selected as the experimental materials.These samples can be collected without permission.

Bioinformatics analysis of *GhArfGAP* gene family and GhArfGAP25 protein

The TblastN [9] sequence was aligned between the amino acid sequence of ArfGAP characteristic domain and the whole genome sequence database of upland cotton, and the protein sequence of the candidate gene of *GhArfGAP* was screened. Then Pfam and SMART program [10] were used to test whether it contains GhArfGAP domain. The *GhArfGAP* gene sequence was aligned by using the ClustalW tool [11] of MEGA6.0 software to construct the phylogenetic tree. The exons and introns of *GhArfGAP* gene family in upland cotton were analyzed by GSDs [12]. MEME online analysis tool [13] was used to analyze the conserved motifs of GhArfGAP protein in upland cotton. MapInspect software was used to generate the distribution of all *GhArfGAP* genes on chromosomes.TMHMM, SignalP4.1 Server,NPS and SWISS-MODELwere used to analyze the structure of GhArfGAP25 protein.

Screening of ethephon concentration and obtaining of Boll stalk abscission layer

This experimental method refered to MA Nagao[14] and was adjusted appropriately.Ethephon with concentrations of 200mg•L^{-1}、400mg•L^{-1}、800mg•L^{-1} was applied to the cotton petiole abscission area. The control group was treated with water for 24h, and the abscission layer formation was observed in the field to determine the concentration used in the experiment. At 8:00 on the day of flowering, immediately after treatment with ethephon, the detached area of flower stalk was cut with a clean and sharp blade, which was recorded as $ET_{0h.}$ Treating another flower with water, the detached area of flower stalk was cut as control, which was recorded as CK_{0h}. The rest were divided into treatment group (ET) and control group (CK), treated with ethephon and water respectively, and samples were taken every 4 hours. The materials were fixed with FAA, glutaraldehyde and liquid nitrogen respectively according to the different follow-up experiments, and then stored in - 80 ℃ refrigerator.

qRT-PCR analysis

RNA was extracted from ET and CK using RNAprep Pure Plant Kit(TIANGEN,Beijing China).Total RNA was reversed to cDNA using a FastQuant RT Kit(TIANGEN,Beijing China). qRT-PCR was carried out on an Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) in a 20 μl volume containing 2 μl of cDNA, 0.8 μl of each primer, 10 μl of SYBR®Premix Ex Taq™ II(2×),and 6.4 μl ddH_2O. The PCR conditions were as follows: primary denaturation at 50 °C for 2 min followed by 40 amplification cycles of 30s at 95 °C, 5s at 95 °C, 20s at 60 °C,After the last cycle, the reaction was maintained at 72 ℃ for 10 minutes. Melting curve analysis was performed to ensure there was no primer-dimer formation. Information on the qRT- PCR primers for gene expression analysis is listed in Table S1. Three replicate assays were performed with independently isolated RNAs, and each RT reaction was loaded in triplicate. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [15]. Statistical analysis of the number of rosette leaves was performed using one-way ANOVA. Statistically significant differences ($P<0.05$) are indicated by different lower-case letters.was used for fluorescence quantitative PCR. *UBQ7* (GenBank accession number: DQ116441) as internal reference gene.

Construction of expression vector

GhArfGAP25 gene was ligated into pMD-18T plasmid to form pMD-18T-GhArfGAP25 plasmid, and then pCambia1304 plasmid and pMD-18T-GhArfGAP25 plasmid were ligated to form pCambia1304-GhArfGAP25 recombinant plasmid.Then the recombinant plasmid pCambia1304-GhArfGAP25 and empty vector pCambia1304 were transformed into competent cells of agrobacterium EHA105 by electroporation method.

Subcellular localization

The agrobacterium that containing recombinant plasmid and empty vector was coated on double antibody LB solid medium with rifampicin and kanamycin to an OD_{600} value of the bacterial solution was about 1.5, it was put into a centrifuge at 4000rpm and separated from the center for 10min to collect the bacteria. Then the bacteria were resuspended with the infection solution to make the OD_{600} value of the bacterial solution 0.8-1.0,and the infection solution was put into 4 ℃ refrigerator for 3 hours.Subsequently the infection liquid was injected into tobacco leaves(*Nicotiana benthamiana*) with sterile syringe, and dark culture was carried out in light incubator. After 3 days, the infected tobacco leaves were placed under laser confocal microscope to observe.The tobacco was acquired from Anhui province academy of agricultural sciences tobacco institute.

Genetic transformation of *GhArfGAP25* in *Arabidopsis thaliana*

Arabidopsis is a wild type of Columbia, which is planted on pot containing nutrient soil and vermiculite(1:2)and cultured in an artificial climate room with light cycle of 16h/8h and temperature of 22 °C.Transgenic plants were generated by the agrobacterium transformation method[16].The agrobacterium was used containing recombinant plasmid mentioned above.Transgenic plants were selected on plate culture medium containing 50 mg · L^{-1} hygromycin.The hygromycin-resistant plants were transplanted

and subsequently monitored for growth.Positive lines showed normal growth and true leaves, while the non positive lines showed short plants, no euphyllas, or even death.

GUS histochemical analysis

Plant tissues were from *GhArfGAP25* transgenic arabidopsis lines.The tissues were placed in 1.5ml centrifuge tube, and the pre cooled 90% acetone was added to completely cover the material, and the leaves were treated at room temperature for 20 min.Then rinse the material with distilled water, place it in a 1.5 ml centrifuge tube, add appropriate amount of GUS staining solution(100 mmol L^{-1} NaH_2 PO_4 buffer pH 7.0, 0.5% Triton X-100, 0.5 mg mL^{-1}X-Gluc and 20 % methanol) to completely cover the material, wrap it in tin foil paper, and place it at 37 ℃ overnight.Tissues were subsequently rinsed by 95% ethanol and mounted on slides, and photographed using a stereo microscope (Leica MZ95, Nussloch, Germany).

Results

Acquisition and observation of boll stalk abscission zone

Apply ethephon 200 mg · L^{-1}, 400 mg · L^{-1} and 800 mg · L^{-1} to cotton petioles with the same growth and at full flowering stage.After 24 hours, the effects of different concentrations of ethrel on the formation of abscission layer of flower stalks were observed (Fig. 1). It can be seen that with the increase of ethrel concentration, the effect of abscission layer formation was more obvious, but no abscission layer was found in water treatment . After treated with 800 mg · L^{-1} for 24 hours, most of petioles had fallen off. The effect of 200 mg · L^{-1} treatmentfor 24 hours on abscission formation was not obvious compared with 400 mg · L^{-1} treatment. Therefore, The cotton petiole treated with 400mg.L^{-1} ethephon was selected as the experimental material.

After treatment with 400 mg · L^{-1} ethrel, the change of abscission zone at different time after treatment was observed (Fig. 2). There was no significant change in the abscission zone of boll stalk before 8 h after treatment, but at 12 h, the change of abscission zone could be observed obviously. There were broken marks between boll stalk and stem, and the abscission zone began to form. After 12 h, the broken marks gradually expanded, and finally petiole falled off.

The changes of abscission layer cells were observed by microscope.In the control group (Fig. 3), it can be observed that with the passage of time, there is no obvious morphological difference between the cells in the abscission zone of the boll stalk and the surrounding cells, and the cells are closely arranged, indicating that water treatment has no effect on the abscission cells. However, the treatment group (Fig. 3), around 12h-16h after treatment, the cells became loose, the cell walls of the exfoliated cells began to disintegrate, and obvious tissue fracture marks appeared. As time goes on, the fracture marks became larger and larger until petiole exfoliated. It can also be seen from the figure that the formation of abscission layer starts from the epidermal cells and extends to the vascular bundles of the boll stalk.

Scanning electron microscopy of treatment group showed that the number of Golgi apparatus and the number of stacking layers of Golgi apparatus were significantly more than those in the control group(Fig. 4).It indicated that the number and structure of Golgi apparatus changed during the formation of ionosphere induced by ethrel. The increased number of Golgi apparatus and stacking layers is related to a large number of physiological and biochemical reactions in the process of abscission formation.

Bioinformatics analysis of *GhArfGAP* gene family in upland cotton

The characteristic domain of *GhrfGAP* gene was obtained by Pfam protein database. TblastN sequence comparison was carried out in the whole genome of upland cotton by using "DNATOOLS" software. The *GhArfGAP* gene was compared by cluster multi sequence comparison tool in MEGA6.0 software, and repeated and redundant *GhArfGAP* gene was deleted, and then the residual base was tested by Pfam and SMART.The amino acid sequence contains the GhArfGAP conserved domain. Finally, 35 candidate genes of *GhArfGAP* are obtained. According to their location on chromosome, they are named GhGrfGAP1-GhGrfGAP35. The molecular weight and isoelectric point of protein amino acids encoded by the ExPasy proteomics server online tool were predicted in TableS2. The results showed that the length of 35 GhArfGAP proteins was 275-867 amino acid residues, and the longest and shortest was GhArfGAP27. The largest molecular weight is GhArfGAP20, 94.1KD, the smallest is GhGrfGAP17, 30.9KD. The maximum isoelectric point is GhArfGAP20, 9.38, and the smallest is GhArfGAP11, which is 4.96.

MEME online analysis tool was used to analyze the conserved motifs of GhArfGAP protein in upland cotton (Fig. 5). The results show that motif 1 exists in every GhArfGAP, which indicates that motif 1 may be necessary for GhArfGAP to play its function. Some motifs exist among some families, and others do not, such as motif 2 and motif 4, which indicates that these motifs are related to some functions of the family proteins. At the same time, we can find that the same family has the same motif type and order, but different families have differences. At present, the functions of motifs in the *GhArfGAP gene* family of upland cotton are not clear, but they may be necessary for these proteins to perform their functions.

The distribution of 35 *GhArfGAP* genes on chromosomes was generated by MapInspect software(Fig. 6). the results show that 35 *GhArfGAP* genes were distributed on 16 of 26 chromosomes of upland cotton, and the gene distribution was relatively uniform in at At and Dt genomes of upland cotton. The most *GhArfGAPs* distributed on chromosome 9 of DT genome, 5 *GhArfGAPs* were located on it, while only one GhArfGAP gene was found on chromosome 7, 13 and chromosome 10 and 12 of At genome. It is believed that more than 3 genes are contained in the nucleotide units of about 200 kb, which is called gene cluster [17-18], but no gene cluster has been found on the chromosome of upland cotton.

Relative expression analysis of *GhArfGAPgene*

The phylogenetic tree analysis showed that *GhArfGAP13* , G*hArfGAP15*, G*hArfGAP25* and G*hArfGAP34* were clustered into one group(Fig.S1),and suggested these genes might be related to the formation of abscission layer. Therefore, qRT- PCR was used to analyze the expression of these genes. The results (Fig.7) showed that *GhArfGAP13* was expressed in root, stem and leaf, and the highest expression level

was found in leaves, about 2 times higher than that in roots, and the expression level in stem was about 1.5 times of that in root; *GhArfGAP15* was the highest expression in stem, but almost no expressing in root; *GhArfGAP25* expression was relatively high in stem and leaf, and also almost no expressing in root; the expression level of *GhArfGAP34* was the highest in roots, relatively low in leaves, and almost no in stems.These data indicated that *GhArfGAP13*, *GhArfGAP15*, *GhArfGAP25* and *GhArfGAP34* were expressed in all tissues of upland cotton, but the expression levels in different tissues were different, which might be related to the functions of these genes in upland cotton.

Furthermore, the expression of these four genes in different time periods of ethrel induced abscission formation was detected (Fig. 8). In general, the expression levels of *GhArfGAP15* and *GhArfGAP25* genes increased first and then decreased, and both showed a downward trend at 12 h. The difference was that the peak of *GhArfGAP15* expression appeared at 8 h, and the peak of *GhArfGAP25* expression appeared at 16 h; the expression levels of *GhArfGAP13* showed an alternating rise and fall, and the peak appeared at 4 h; The expression level of *GhArfGAP34* increased first, then decreased, and then increased, and the peak appeared at 4 h. except that the expression level of *GhArfGAP13* at 0 h was lower than that of the control group, the other expression levels were higher than that of the corresponding control group. There was no significant change in the expression of the four genes in the control group. The changed expression of *GhArfGAP13*, *GhArfGAP15*, *GhArfGAP25* and *GhArfGAP34* indicated that these four genes all played a certain role in the formation of abscission layer.

Structural analysis of GhArfGAP25 protein

According to the results of qRT-PCR, we cloned *GhArfGAP25* and expected to study its role in the formation of abscission layer.The structure of the protein expressed by this gene was analyzed.TMHMM tool analysis showed that the protein had no transmembrane domain(Fig. 9A).Online tool signalP 4.1 server predicted that GhArfGAP25 protein does not contain signal peptide sequence,and it is a non secretory protein(Fig. 9B). Studies have shown that ArfGAP protein is involved in intracellular material transport, which indirectly proves that the prediction is reliable[19-20].Analysis of secondary structure of GhArfGAP25 protein by NPS showed(Fig. 9C) that the secondary structure of GhArfGAP25 protein is mainly composed ofα- helix and random coil, in addition to someβ-turn and extended chain. There are 158α- helices, accounting for 33.83% of the total protein; 237 irregular coils, accounting for 50.75% of the total protein; 25β-turns, accounting for 5.35% of the total protein; 47 extended chains, accounting for 10.06% of the total protein.SWISS-MODEL [21-24] was used to predict the tertiary structure of GhArfGAP25 protein. The results are shown in the (Fig. 9D). The main structure wasα-helix, irregular curl and a small amount ofβ-angle.

Analysis of expression in transgenic *Arabidopsis* plants

In order to further study *GhArfGAP25*, we transferred *GhArfGAP25* into arabidopsis thaliana by agrobacterium transformation and obtained verified by GUS staining(Fig. 10).Fortunately, we got transgenic arabidopsis.

We analyzed the expression of *GhArfGAP25 gene* in different tissues of wild-type and transgenic arabidopsis by qRT-PCR(Fig. 11) .The results showed that the expression of *GhArfGAP25* in roots, stems and leaves of transgenic Arabidopsis was higher than that of wild type. Compared with different tissue parts of cotton, the expression of *GhArfGAP25 gene* was the lowest in *arabidopsis* stem and the highest in root, while the expression of *GhArfGAP25 gene* was the lowest in cotton root and the highest in leaf, indicating that the expression of the same gene was different in different tissue parts of different species.

The function of *GhArfGAP25* is located in Golgi apparatus and endoplasmic reticulum

To investigate the functional localization of *GhArfGAP25* gene, tobacco was used for subcellular localization(Fig. 12). The results showed that the green fluorescence was the fluorescence emitted by GFP and surrounded the tobacco epidermal cells. The green fluorescence emitted by GhArfGAP25-GFP not only appeared around the tobacco leaf epidermal cell membrane, but also appeared in the cells. Therefore, it was speculated that the function of *GhArfGAP25* gene was located in Golgi apparatus and endoplasmic reticulum, which was consistent with previous studies.

Discussion

Formation of abscission layer of cotton boll stalk induced by ethephon

Ethylene is an essential hormone in plants, which plays an momentous role in the whole process of plant growth and development[25].At the same time, ethylene can also induce the formation of abscission zone and cause the abscission of flowers and fruits.In mango,after ethylene induction,*MiIDA1* and *MiIDA2* expression had upregulated,then promoted the formation of abscission zone[26]. In *Camellia oleifera,CoIDAs* are abscission

associated genes which can be induced by ethphon and push forward the formation of abscission zone[27].Our previous studies have shown that ethephon treatment simulates abscission layer formation in cotton,and

Genome-wide analysis showed that it was related to miRNA[28]. In this experiment, cotton boll stalk abscission zone was treated with 200 mg· L^{-1}, 400 mg·L^{-1} and 800 mg·L^{-1} ethrel, and abscission layer was found after 24 hours, which indicated that the abscission layer of cotton boll stalk could be induced by ethrel above 200 mg·L^{-1}. Whether low concentration ethrel could promote the growth and development of boll stalk might need further screening.After 12 hours of treatment with 400 mg·L^{-1} ethrel, the morphology of abscission layer cells changed, the arrangement of cells became loose, the cell wall began to degrade and separate from the surrounding cells, and the abscission layer began to form.During the formation of abscission layer, a series of physiological and biochemical reactions occur in cells, and a large number of substance molecules need to be synthesized and transported. Compared with the control, the number of Golgi apparatus and the number of abscission layers were increased.Studies have shown that the main function of Golgi apparatus is to process, classify and

package proteins synthesized by endoplasmic reticulum, and then send them to specific parts of cells or secrete them out of cells[29]. The increase in the number of Golgi apparatus and stacking layers may be closely related to a large number of physiological and biochemical reactions in the process of abscission formation.

GhArfGAP **Gene function may be related to Golgi apparatus and vesicle trafficking**

Small GTP binding protein family is a kind of GTP binding protein family, which is ubiquitous in eukaryotic cells. Its molecular weight is about 20–30 kDa and exists in monomer form. According to its protein structure and function, it can be divided into five subfamilies: Ras, rho, RAB, ARF / SAR and ran 5 [30–31].ArfGAP is as a key protein regulating ARF activity.Up to now,there is no clear definition of the function of ARF gene,however,a number of reports support the hypothesis that ARF-GAPs are involved in vesicle trafficking at Golgi apparatus.In yeast, ArfGAP Age1 is regulated by phospholipase D for post-Golgi vesicular transport[32].In rice over-expression of *OsAGAP* caused pattern changes in vesicle traffificking[33].*OsAGAP* can also recovered the defect of vesicular transport in the yeast ARF-GAP double mutant *gcs1Dglo3D* [34].In this paper, 35 members of *GhArfGAP* family in upland cotton were identified as 4 families(Fig.S3). The gene structure of each family is similar, mainly in the structure of exon and intron [35–37]. These *GAP* genes all have conserved motifs.qRT-PCR analysis of the expression of *GhArfGAP13*,*GhArfGAP15*,*GhArfGAP25*,*GhArfGAP34* levels, of which *GhArfGAP13* and *GhArfGAP34* expression levels in cotton root expression are higher, and *GhArfGAP15* and *GhArfGAP25* expression levels in cotton root are low.At the same time, the expression level of *GhArfGAP25* gene in transgenic *arabidopsis* thaliana was the highest in roots. Based on these results and related literature has to do with the previously reported ArfGAP influence root has certain relevance. After ethylene induction, the number of isolated Golgi apparatus and stacking layers increased significantly, and the expression levels of these four genes were higher than those of the control group.Based on these results and related literatur, we speculated that *GhArfGAP*, in particular *GhArfGAP25* is invovled in Golgi apparatus vesicular transport and root growth and development in upland cotton, but further research is needed.

Conclusions

In this study,ethylene could induce the formation of abscission zone in *Gossypium hirsutum* and then promote boll stalk detaching, and the number and structure of Golgi apparatus in abscission zone also changed.*GhArfGAP13,GhArfGAP15,GhArfGAP25,GhArfGAP34* might regulate the changes of Golgi apparatus.Our study provides a new insight for the regulatory mechanisms of abscission zone formation. and will be helpful for improving cotton production.

Abbreviations

ArfGAP,ADP ribosylation factor GTPase-activating proteins;qRT-PCR,Quantitative Real-time PCR;UTR,Untranslated Regions;miRNA,microRNAs;GFP,Green fluorescent protein.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors agreed to publish.

Availability of data and material

There is no sequencing data generated in this study.

We downloaded the whole genome of *Gossypium hirsutum* from (https://www.ncbi.nlm.nih.gov/assembly/?term=Gossypium+hirsutum),and this database are open.

Competing interests

The authors have no competing interest to declare.

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Authors contributions

Conceived and designed the experiments:NG, SHY.Performed the experiments:AFL, LC, ZWG.Analyzed the data:HJ, LL, DHL,JSG.Wrote the paper: LC.All authors have read and approved the manuscript.

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Figures

Figure 1

Effects of different concentrations of ethephon for 24 h on bascission zone in upland cotton pedicle A、B、C、D shows treatment with water、200mg.L-1、400mg.L-1and 800mg.L-1 ethephon, respectively、AZ: abscission zone

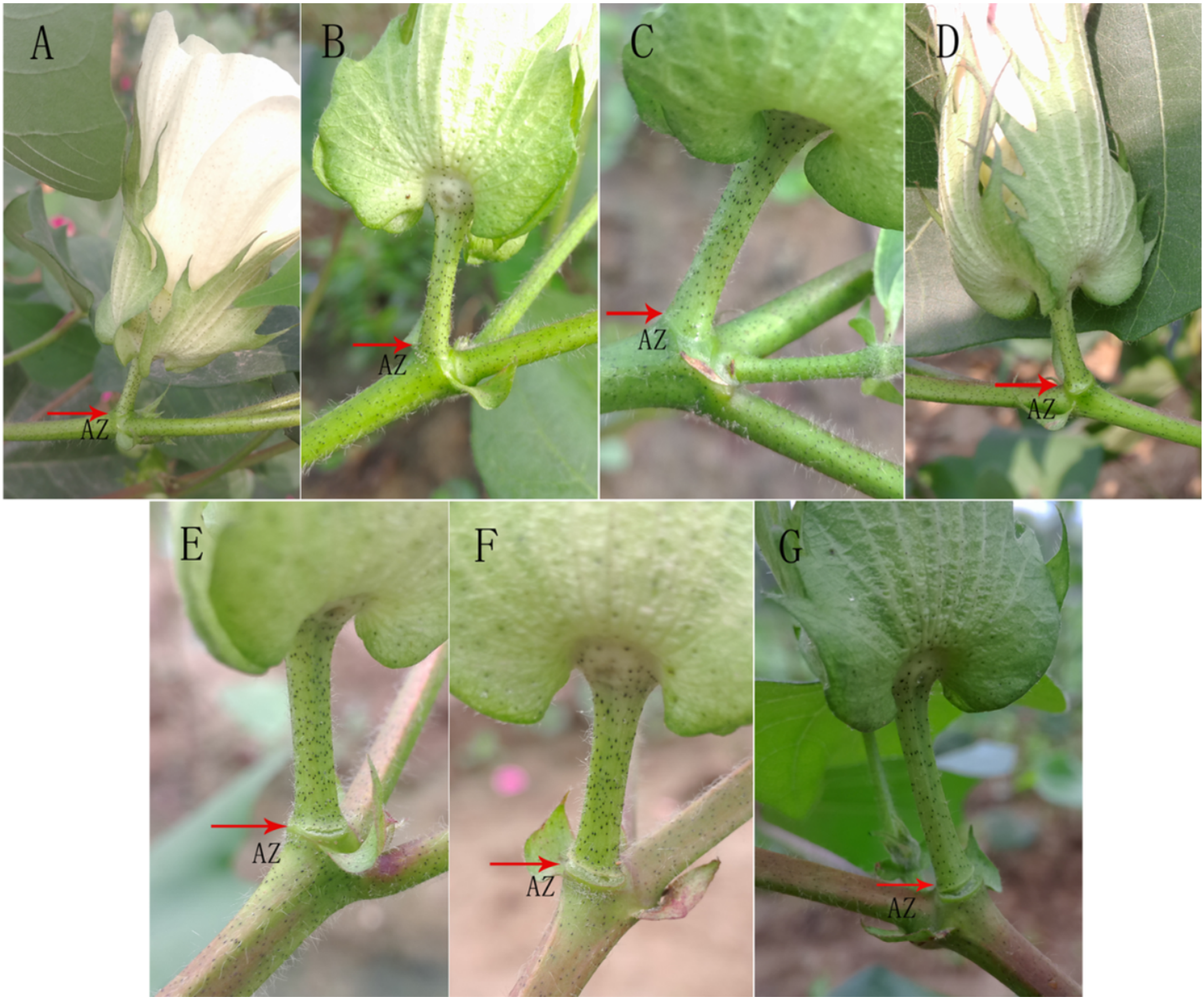

Figure 2

Effect of ethephon treatment at different times on of bascission zone in upland cotton pedicle A-G shows treatment 0h、4h、8h、12h、16h、20h、24h, respectively; AZ: abscission zone

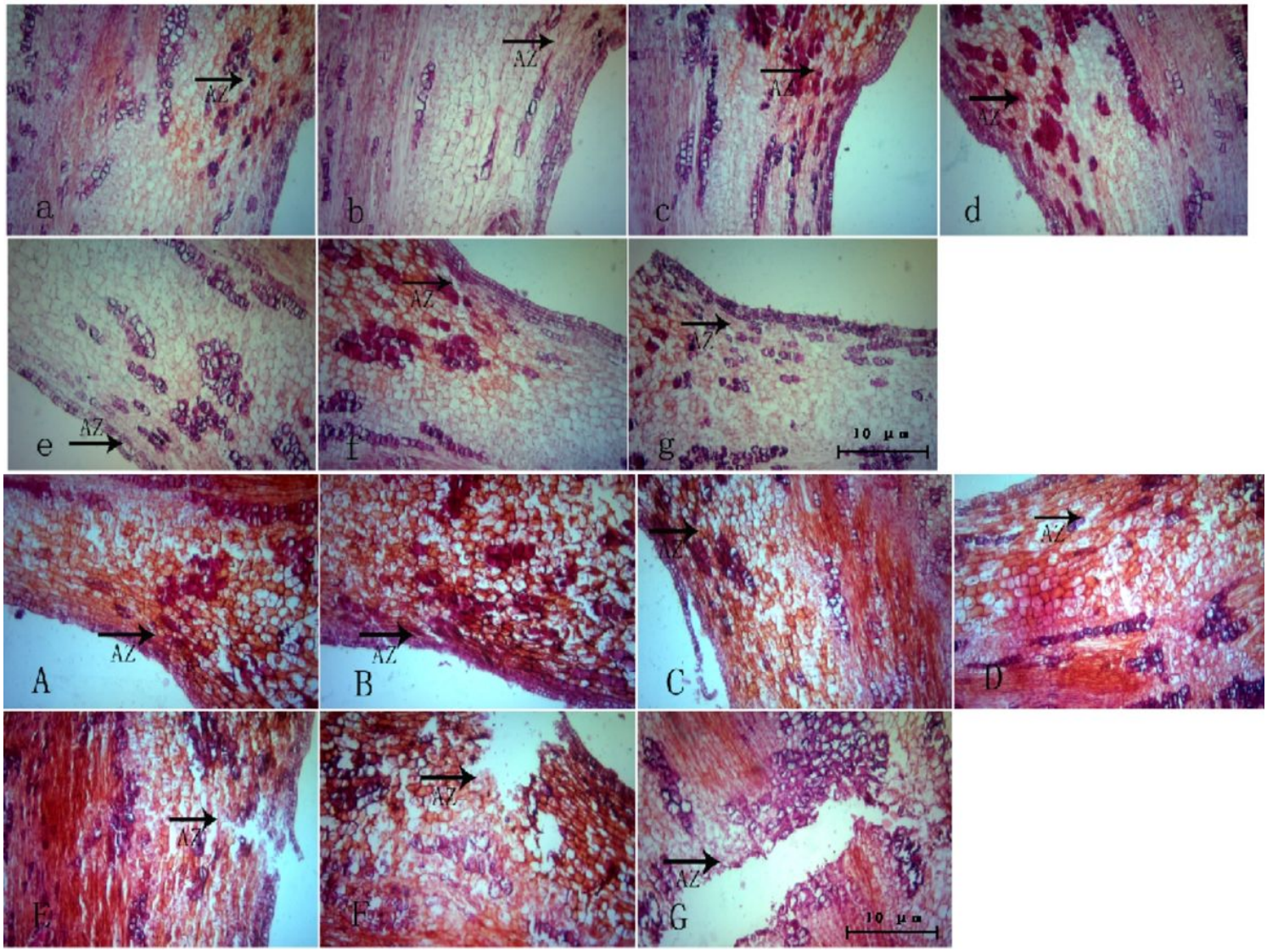

Figure 3

different treatment at different times on of bascission zone in upland cotton pedicle a-g shows treatment with water 0h、4h、8h、12h、16h、20h、24h, respectively A-G shows treatment withe thephon 0h、4h、8h、12h、16h、20h、24h, respectively; AZ: abscission zone

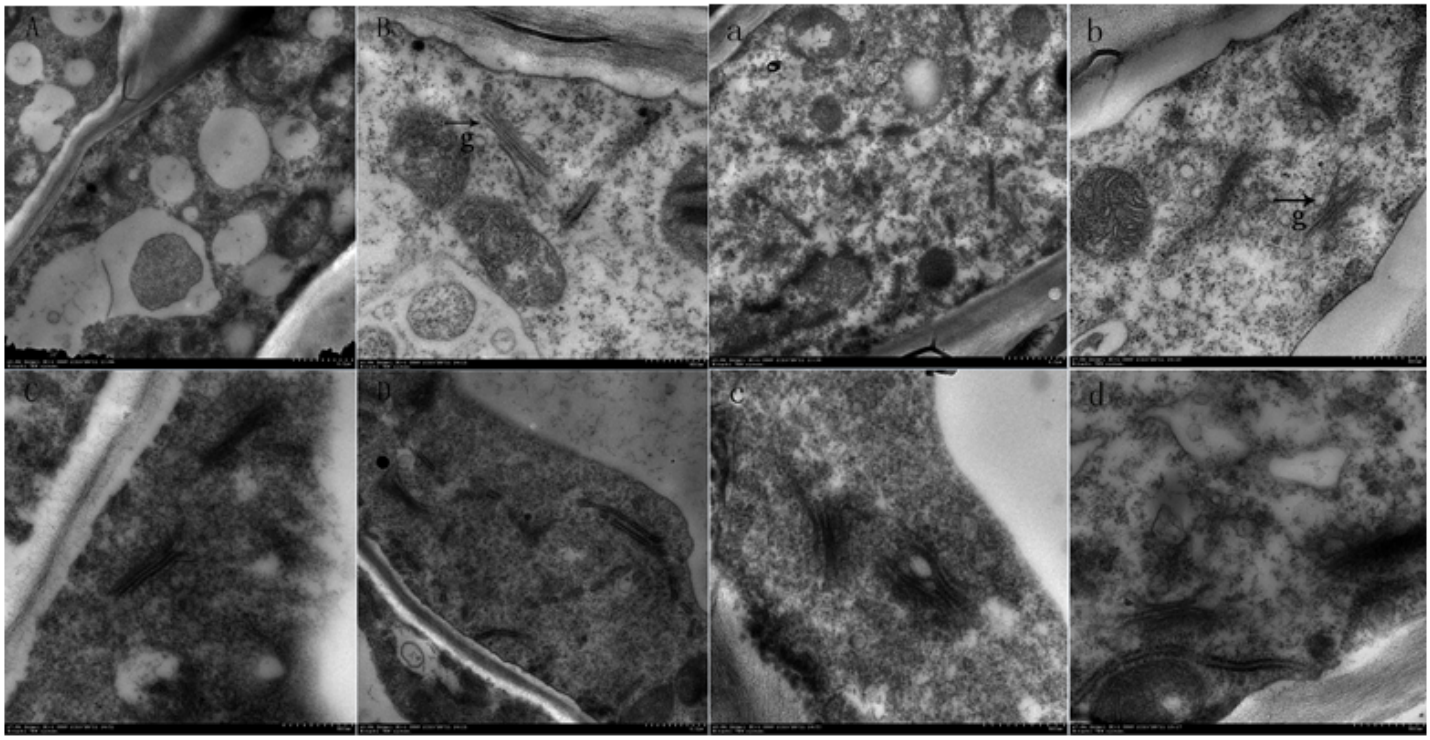

Figure 4

Electron microscopic observation on the abscission zone at different time A,B, C,D shows treatment with water 0h□8h□16h□24h ,respectively; a,b,c,d shows after treatment with ethephon 0h, 8h, 16h, 24h, respectively; g□Golgi boides

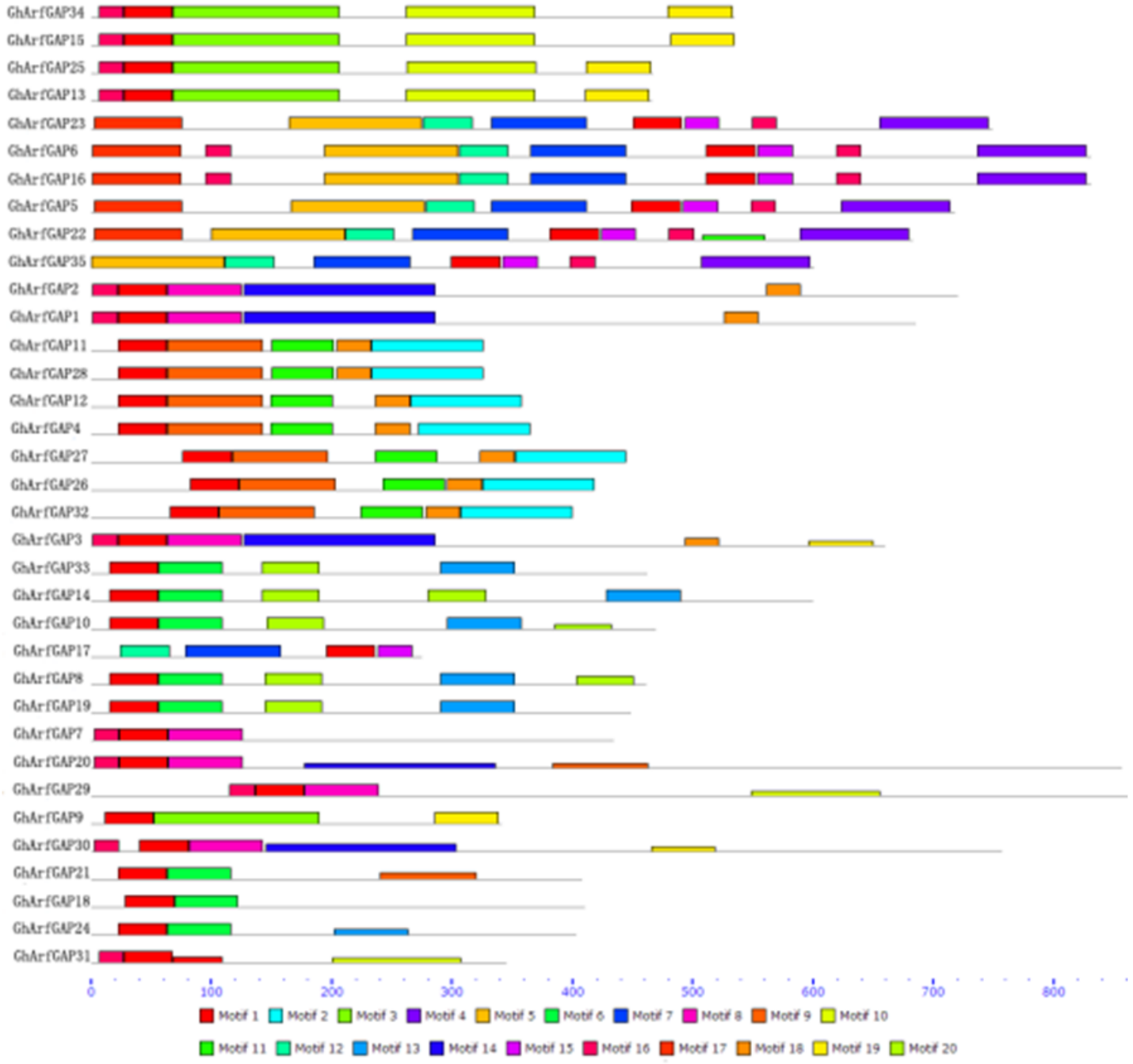

Figure 5

The Motif distribution of GhArfGAP protein sequences in upland cotton

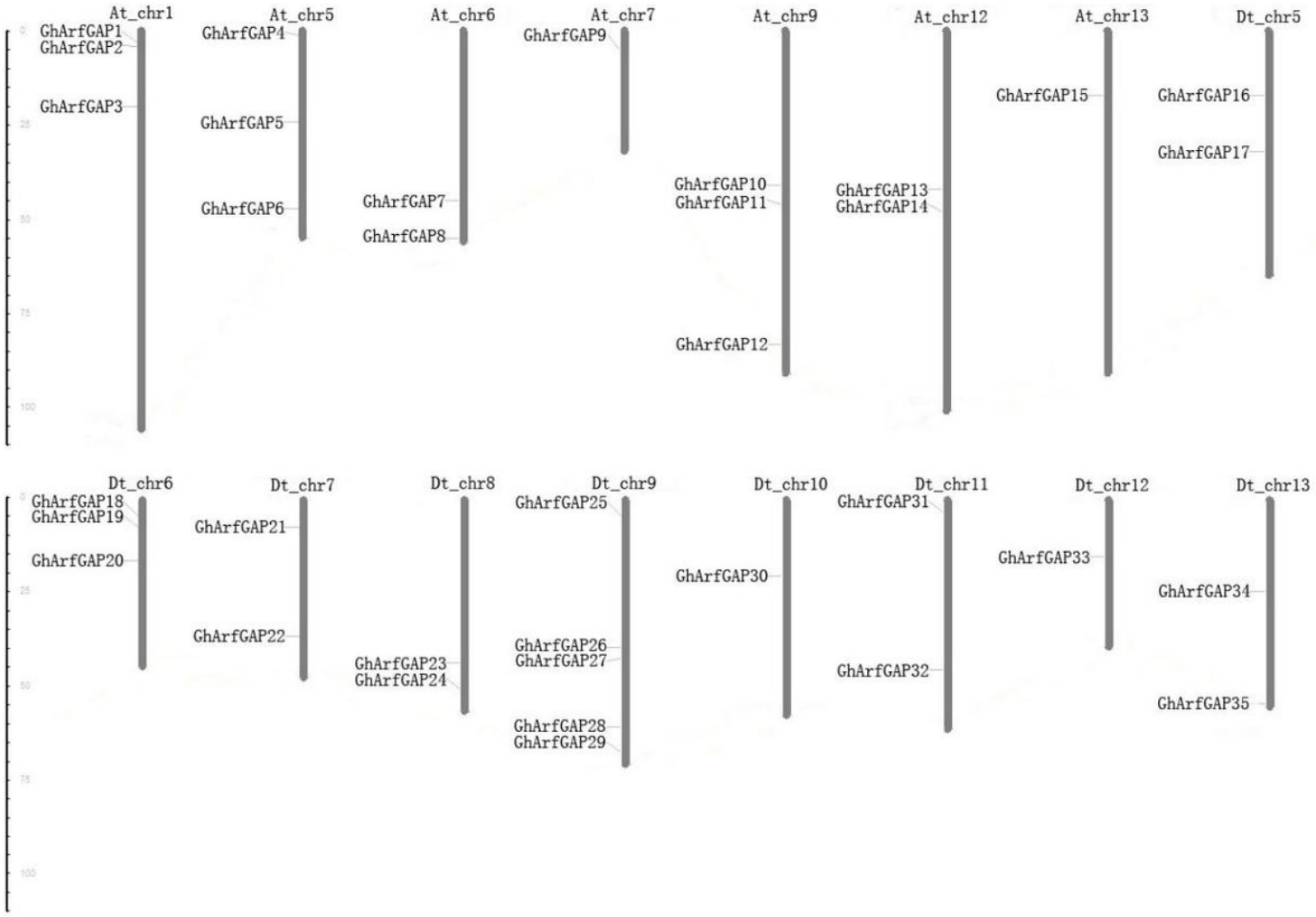

Figure 6

Chromosomal location of 35GhArfGAP genes in upland cotton

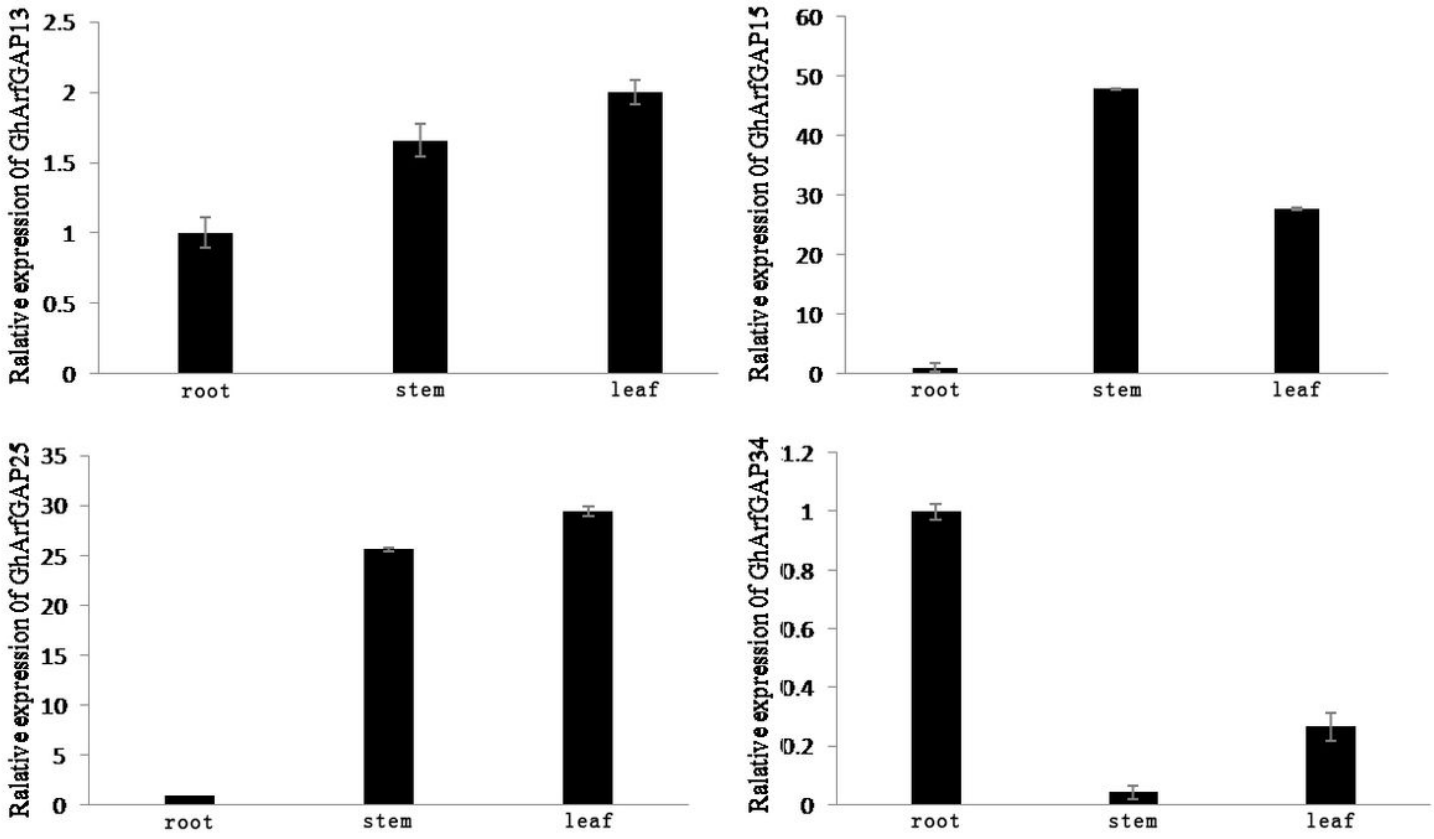

Figure 7

Tissue-specific expression analysis of GhArfGAP13、GhArfGAP15、GhArfGAP25、GhArfGAP34 in upland cotton

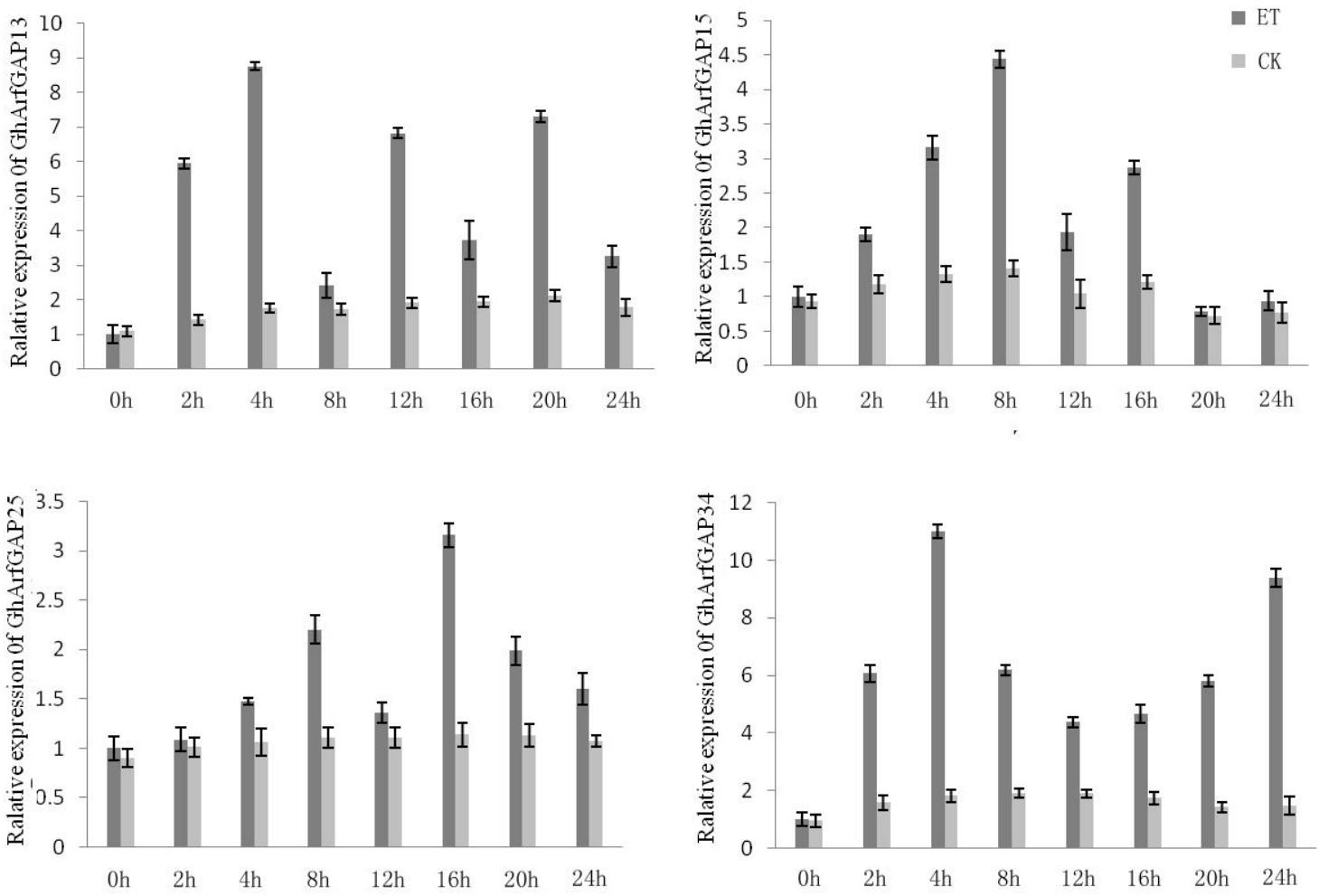

Figure 8

Different time expression analysis of GhArfGAP13、GhArfGAP15、GhArfGAP25、GhArfGAP34 in upland cotton

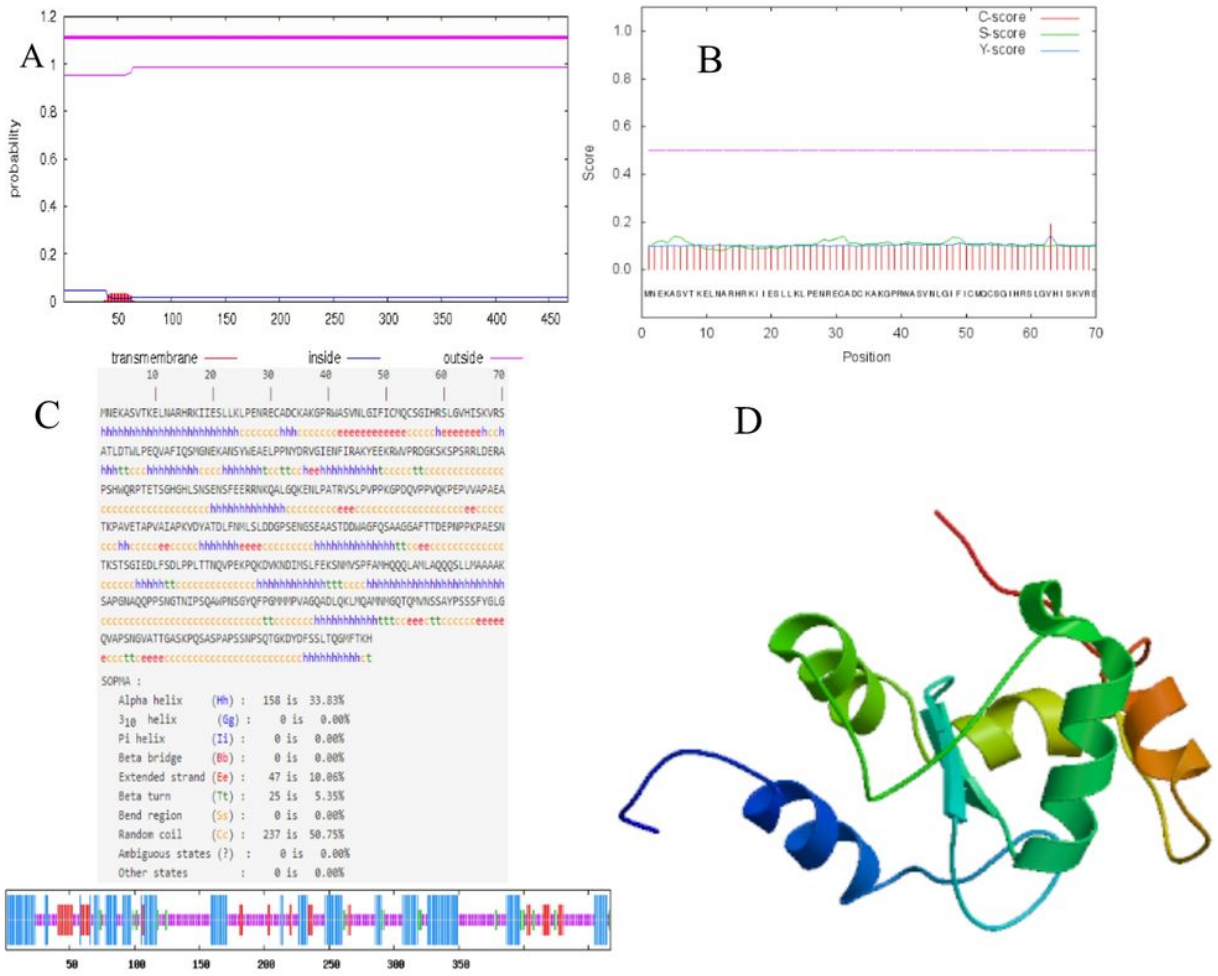

Figure 9

Structural analysis of gharfgap25 protein. A:transmembrane domain prediction of GhArfGAP25 protein. B:predicted signal peptide of GhArfGAP25 (C score: The score for origihal cleavage site;Y score: The score for general cleavage site;S score: The score for signal peptide).C:secondary structure prediction of GhArfGAP25 protein (Hh: Alpha helix; Ee: Extended strand; Tt: Beta turn; Cc: Random coil).D:predicted tertiary structure of the GhArfGAP25 protein.

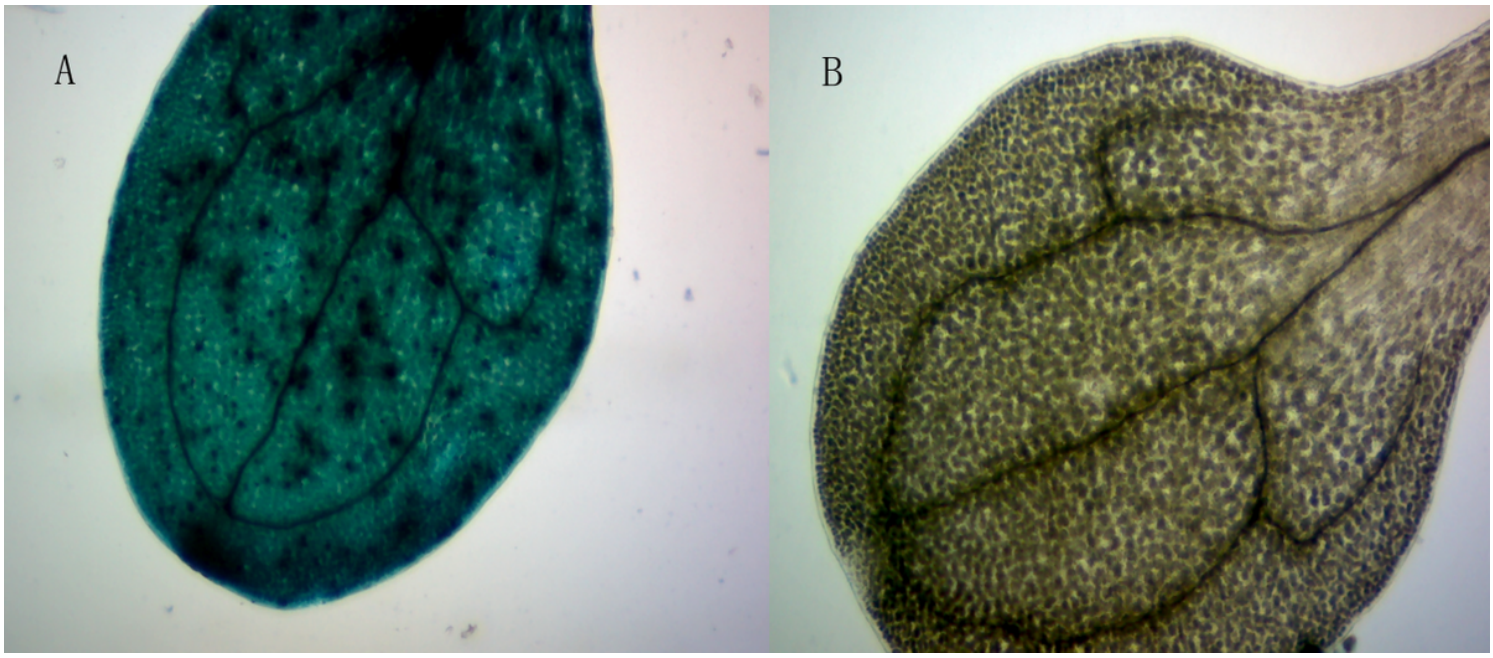

Figure 10

GUS staining of positive plants A:transgenic Arabidopsis; B: wild type arabidopsis thaliana

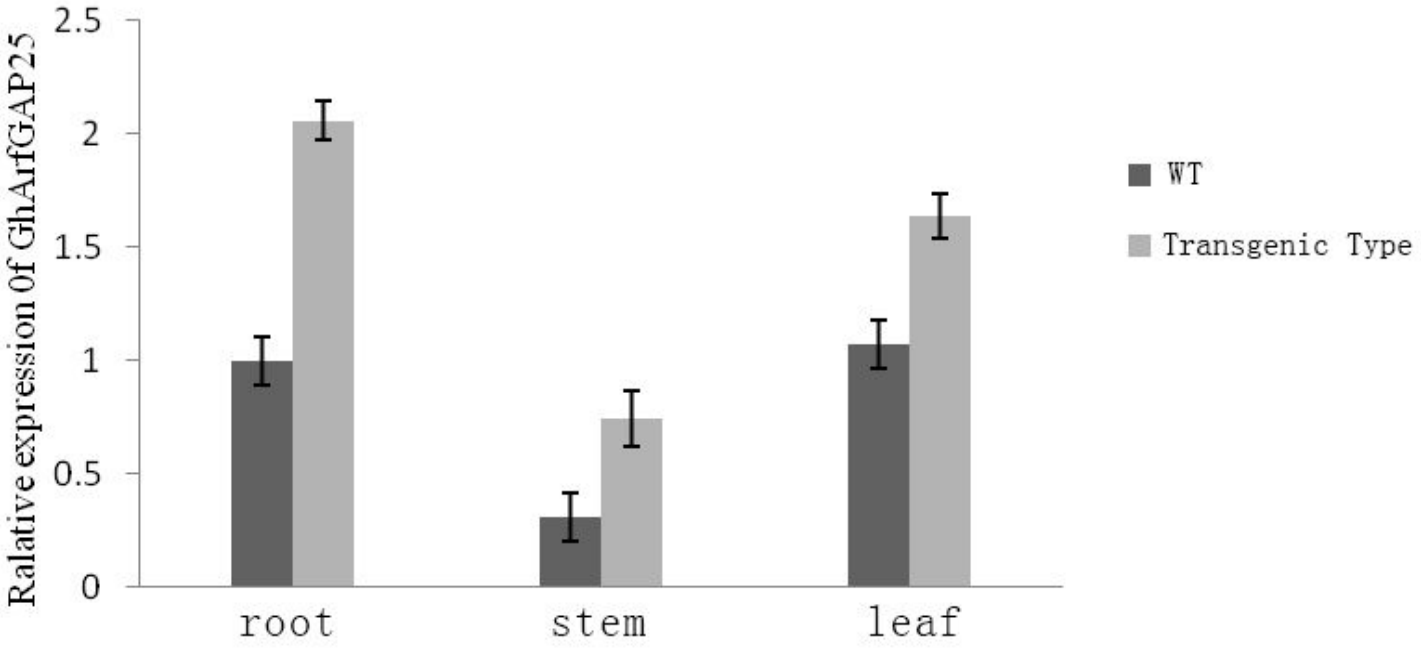

Figure 11

The expression analysis of GhArfGAP25 in different tissues of transgenic Arabidopsis thaliana plants

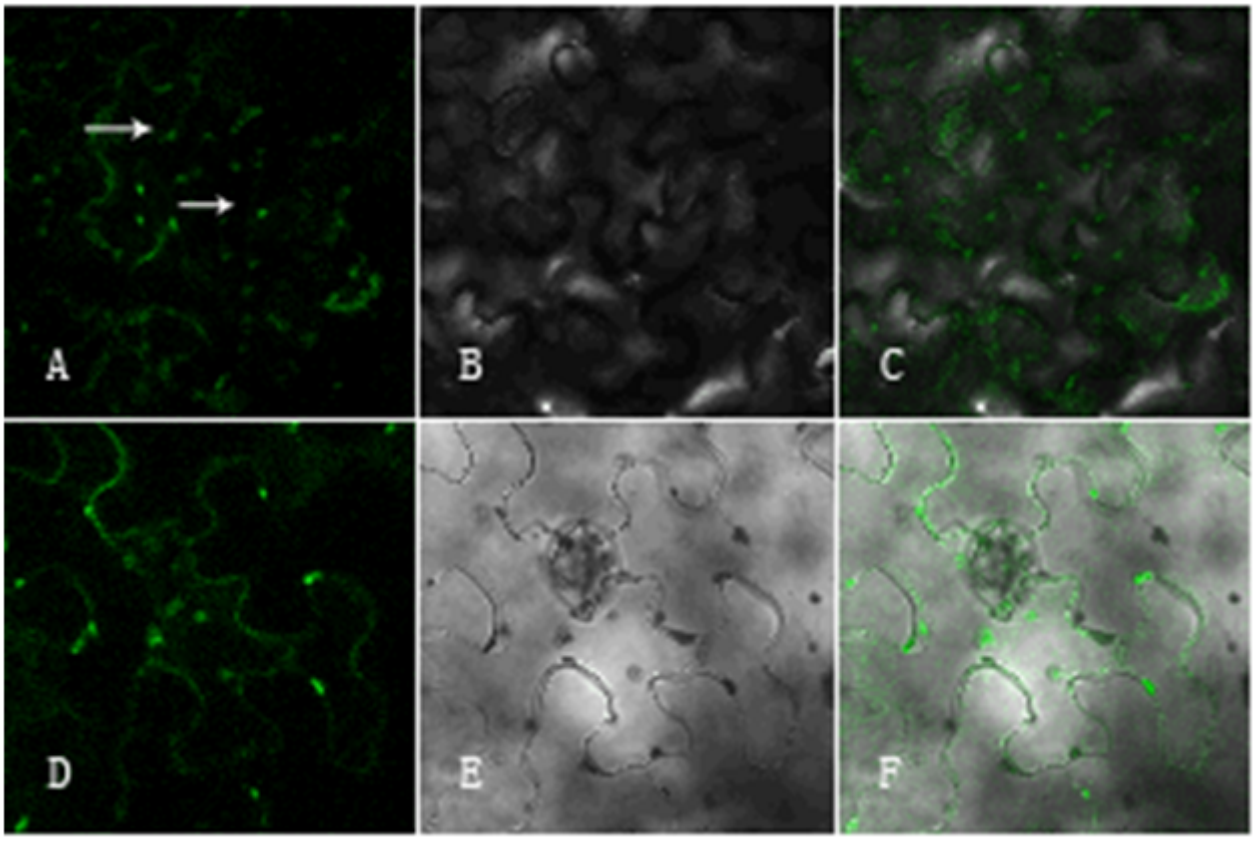

Figure 12

subcellular localization of pCambia1304-GhArfGAP25 and pCambia1304 A-C:subcellular localization of pCambia1304-GhArfGAP25; D-F：subcellular localization of pCambia1304

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