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MOLECULAR CHARACTERISATION AND EXPRESSION ANALYSIS OF ACC OXIDASE GENE FROM *GUZMANIA RUIZ & PAV.*

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Abstract

ACC oxidase is the last key enzyme of ethylene synthesis pathway, while ethylene is a key factor affecting flowering in ornamental bromeliad. To understand ACC oxidase gene's characteristics and its effect on ornamental bromeliad flowering, we cloned 1504bp full-length cDNA sequence (GenBank: JX972145) and 2546bp corresponding genomic sequence (GenBank: JX972146)of *GoACO1* (ACC oxidase gene) from *Guzmania* variety: *Ostara*. Prokaryotic expression study showed that expression of *GoACO1* can produced a 41 KD protein precipitation in *Escherichia coli* DE3(BL-21); Real-time quantitative analysis showed that *GoACO1* can express in all tested tissues including floral organ, bract, leaf and scape, and expression quantity in bract was the highest. Through constructing plant overexpression vector, transforming into *Arabidopsis thaliana*, and investigating blossom character of T2 generation seeds, we found that first flowering time of the goal *Arabidopsis thaliana* was 1.5 days earlier, and their peak flowering time(the number of flowering more than 50%) was 1.8 days earlier, compared with wild type one. Taken together, our results suggested that *GoACO1*can express in all kinds of tissues and seems to promote *Arabidopsis thaliana* flowering earlier.

Key words: ACC oxidase gene, *Guzmania*, cDNA sequence, *Ostara*.

Introduction

Guzmania is a kind of very popular high-grade cut flower and potted flower, and it is also main ornamental bromeliad variety in flower market. As well as edible bromeliad-pineapple, they both are *Bromeliaceae* plants. Guzmania grow slowly and have a long vegetative period, so a long growth cycle is necessary for flowering. In commercialized production, artificial forcing flowering methods often being used for sale earlier, and it also can reduce production cost and improve production efficiency. The medicament used was mainly ethylene (such as ethephon) (Manica *et al*., 1994; Danijela *et al*., 2006; Yuri & Jose, 2006). All of those treatments that can increase ethylene content can promote guzmania flowering, including exogenous application of ethephon, shaking and ACC treatment (Danijela *et al*., 2006). Ethylene can cause all tried bromeliads flowering rapidly, it is a key factor to induce ornamental bromeliads flowering (Kuan *et al*., 2005; Danijela *et al*., 2006; Yuri & Jose, 2006).Though mechanisms about how exogenous application of ethylene promote bromeliad flowering remain unknown, it is generally believed that there is a close connection with endogenous ethylene biosynthesis and transmission mechanism. Ethylene seems to be the only control factor to floral formation in bromeliad (Danijela *et al*., 2006).

As a kind of plant hormone, ethylene as a wide-range influence on seed germination, plant growth, senescence of leaf and floral organ, fruit ripening and sex differentiation. ACC oxidase catalyze ACC forming ethylene under aerobic conditions (Yang & Bradford, 2008) which is the last key enzyme in ethylene biosynthesis pathway, so it is also named as ethylene-forming enzyme (EFE) (Yang & Bradford, 2008). The ACC oxidase gene can be induced to express by plant itself development situation, external environment change and hormone signal. Flowering, pollination, senescence of petal and leaf, seeds germination and fruit maturity can all cause ACC oxidase gene express (Llop-Tous *et al*., 2000; Hiroko & Kazuo, 2012). The ACC oxidase gene has been cloned from many plant species such as *Paeonia suffruticosa Andr* (Fan *et al*., 2012), *Dendrobium officinale* (Xu *et al*., 2013), *Paeonia lactiflora* (Zhao *et al*., 2014), *Tulip* (Kazumi *et al*., 2007), *Petunia* (Huang *et al*., 2007). And many existing researches showed that inhibition of ethylene biosynthesis can make florescence longer, anti-aging or delay fruit ripening, which is helpful to preservation and storage longer (Michael & Wu, 1992; Silva *et al*., 2015). Most of these studies were executed by using antisense RNA technology or RNAi technology to reduce endogenous ethylene content. For example, *Dianthus caryophyllus* (Yu & Bao, 2004), *Tulipagesneriana*, *Paeonia suffruticosa Andr*, *Musa nana Lour* (Huang *et al*., 2005), *Cucumismelo*, *Brassica oleracea L* (Chen *et al*., 2005), *Brassica juncea*, *Pear* (Qiao, 2008).

Many studies on ethylene synthesis genes have been performed in bromeliad, but most of them focused on pineapple. Some studies about the cloning of ethylene biosynthesis pathway gene and control of flowering duration time had made some progress. For example, Wang *et al.* (2007) launched a research on delaying pineapple natural blossom. Yuri & Jose (2006) had cloned a pineapple ACC synthase gene, and then executed gene silence experiment, finally 2 strains whose ACC synthase gene were inhibited were obtained. By investigating flowering trait, they found that the initial blooming time of the two strains was delayed significantly. However, similar research was rarely in ornamental bromeliad, there is still no report in literature. In GenBank, there were only two ethylene response genes (AY263359, AY294285, 1), while no ethylene biosynthetic genes(including ACC synthase gene).

In order to investigate biological function of ACC synthase gene in *Guzmania* (*GoACO1*) on flowering and lay a foundation for research on promoting precocious flowering or extension florescence in ornamental bromeliads, we isolated full-length cDNA and genomic DNA sequences of *GoACO1*, then carried out bioinformatics analysis and expression research in this study.

Materials and Methods

The Guzmania'*Ostara*', *Guzmania wittmackii* × *Guzmania lingulata* cultivar, was used as an experimental plant. The plants were grown in a greenhouse of Flower Research and Development Center, Zhejiang Academy of Agricultural Science, China. In order to obtain full length cDNA sequence of the goal gene, full-length cDNA library was constructed by extract RNA from floral organ and bract of flowering *Ostara* plants; In order to obtain corresponding DNA sequence, DNA from leaves of *Ostara* plants were also extracted.

Acquisition of *GoACO1* full length cDNA sequence: Since 2008, we had constructed successfully a full length cDNA library of *Ostara*'s floral organ and bract, and obtained 1758 high quality sequences through 5'EST sequencing to 2004 positive clones (Liu *et al*., 2009). After all these sequences were analyzed by blast alignment, 3 ESTs belonged to the same contig of ACC oxidase gene were obtained, included |ppfca0_001923. z1. scf|, |ppfca0_0002_G12. ab1|, and |ppfca0_001745. z1. scf|. Then, through primer walking sequencing to |ppfca0_001923. z1. scf| monoclone, goal cDNA sequence of *GoACO1* was obtained.

Acquisition of *GoACO1* DNA sequence: In order to obtain *GoACO1's*corresponding DNA sequence, two PCR special primers were designed on the basis of *GoACO1* cDNA sequence as follows: FP: GGGGATTGTAGATT AGAGGCAATCG; RP: GCATAAAATCTGCTTCACAA TAGATTACAC. DNA was extracted from leaves and used as template in PCR reaction. PCR reaction system (25ul) followed to Long PCR Enzyme Mix protocol (MBI, K0181). The reaction program was as follow: Preliminary denaturalization 94ºC 2 minutes, Denaturalization 95ºC 20 S (seconds), Anneal 68ºC 30 S, Extending 68ºC 3 M, Cycles 5C; Denaturalization 95ºC 20 S, Anneal 66ºC 30S, Extending 68ºC 3M, Cycles 5C; Denaturalization 95ºC 20 S, Anneal 64ºC 30 S, Extending 68ºC 3.5 M, Cycles 5C; Denaturalization 95ºC 20 S, Anneal 62ºC 30 S, Extending 68ºC 4 M, Cycles 25C; Final Extending 68ºC 15 M, End 4ºC +∝. After PCR reaction finishing, desired PCR product performed electrophoresis on agarose gel. Through TA cloning and sequencing, DNA sequence of ACC oxidase gene was obtained.

Bioinformatics analyses of *GoACO1*: *GoACO1's* structure characteristic, putative amino acid sequence were analyzed by bioinformatics methods such as BlastN, BlastP, ExPASy, ProtParam, SPOMA, CDART(NCBI), Clustal X(1. 81), and molecular phylogenetic tree was constructed using MEGA 4.1 software.

Prokaryotic expression of *GoACO1* in *Escherichia coli*: To verify whether *GoACO1* can be expressed for a complete and effective protein, prokaryotic expression experiment was performed. According to ends of *GoACO1* full length cDNA sequence, *MCS* region (Multiple Clone Site) of *pET-28* vector, and introduction of enzyme cutting sites: *NdeI* and *SalI*, Primers were designed as follows:

Forward primer: GGAATTCCATATGGAGAGTAAA TTCCCAATCATC

Reverse primer: ACGCGTCGACTTA CTAGGTTGC AATTGGCG

Amplification of purpose fragment: Purpose gene was obtained by PCR reaction from DH10B which containing the target gene-PDNR-LIB carrier. Then PCR product contained purpose gene was recycled and purified. After digesting PCR product and *pET-28* carrier with *Nde I* and *SalI* (NEB) double restriction enzyme, and then connect them together. Finally, these products were transformed into DH5a competent cells.

Protein expressionof*GoACO1*: pET-28 and aco-pET-28 vector were transformed into competent cells of expression strain DE3 (BL-21).Then the bacteria liquid and IPTG (the final concentration was 0.3 mM) were mixed and incubated for 3h's in 37ºC,180 r/min condition. Expressed protein was detected by SDS-PAGE electrophoresis. Finally, three different IPTG concentrations induced experiments in room temperature and three different temperature conditions induced experiments with 0.3mM IPTG were carried out respectively, and their expressed protein was also detected.

Tissues expression of *GoACO1*: Design of primers as follows: Actin, a housekeeping gene, was used as inner reference to rectify expression amount of target gene. In the paper, *Goactin1* (GenBank: HQ184438) was obtained by author from *Guzmania ostara* formerly. Primers of *GoACO1* and *Goactin1* were designed as follows:

***Goactin1*-F *(*forward primer):** 5'-GCTTGCCTACATT GCCCT-3';

***Goactin1*-R *(*reverse primer):** 5'-ATTGTTGAACCCCC GCTT-3';
GoACO1-F: 5'-GCGGGAGCAGATGGACGAG-3';
GoACO1-R: 5'-GGAGGTGGCGGAGGAAGAA-3'.

Extraction and inverse transcription of total RNA: Total RNA was extracted from scape, leaf, bract and floral organ, respectively. Then their concentration, purity and integrality characteristics were detected by ultraviolet spectrometry and agarose gel electrophoresis. Finally, superior quality total RNA were reverse transcription into cDNA.

Preparation of standard curve: Firstly, common PCR multiplication of *GoACO1* and *Goactin1* were proceeded separately (*System 9600*, Perkin Elmer). Their PCR products were identified by agarose gel electrophoresis, and were diluted by tenfold gradient for 5 times. Finally, their 4 consecutive concentration were picked out as standard sample for preparing standard curves.

Real-time quantitative PCR analysis: Real-time quantitative PCR reaction system (25ul) included 1ul cDNA, 0. 5ul primer-F (20pmol/ul), 0. 5ul primer-R (20pmol/ul), 12. 5ul 2×mix, 1ul Sybr Green I(10×) and 9.5ul ddH_2O. PCR programs were showed in Table 1 (*PRISM 7700*, Sequence Detector ABI).

Table 1. Quantitative PCR programs.

Genes	Preliminarydenaturalization	Denaturalization	Anneal	Extending	Cycles
GoACO1	94ºC, 2min	94ºC, 30sec	58ºC,30sec	72ºC, 30sec	35
Goactin1	94ºC, 2min	94ºC, 30sec	60ºC, 30sec	72ºC, 30sec	35

Overexpression of *GoACO1* in *Arabidopsis thaliana*: In order to verify the function of *GoACO1* in plant, we transformed its plant overexpression vector into *Arabidopsis thaliana*, then investigated flowering habit of the transformed *Arabidopsis thaliana* plants.

The steps were as follows:

Construction of plant overexpression vector: Primer were designed as follows: Sac-acc-f: CTAGAGCTCGGGGATTGTAGATTAGAGG; Sal-acc-r: CTAGTCGACCCAATAGTAGCCCACATAGA. *GoACO1* was amplified from T vector containing the goal gene. Then both the amplified PCR product and plant expression vector *Pcambia 2300* were digestion by *SAC1* and *SAL1* enzyme (*fermentas*) and connected by the T4 ligase. Finally, the connected product was transformed into E. coli DH5a through heat shock method.

Transforming into *Arabidopsis thaliana*: Plasmid of the above positive clone was extracted and transformed into *agrobacterium* strain EHA105 by electroporation method. Then, these strains were transformed into *Arabidopsis thaliana* by floral dip method (Chen *et al*., 2013). T1 generation seeds were collected, and transformants were selected on plates containing Kan antibiotics. Transformant plants were transplanted into soil and cultivate to maturity. Finally, T2 generation seeds were harvested.

Investigation of flowering habit: T2 generation seeds were sowed in the soil. Until maturity, their flowering habit were investigated and analyzed.

Results

Cloning of *GoACO1*: Based on full-length cDNA library and a lot of EST sequences information being obtained previously, three EST monoclones were classified into ACC oxidase gene by BLAST alignment. Through sequencing to |ppfca0_001923.z1.scf|, which is one of 3 EST monoclones, full length cDNA sequence of ACC oxidase gene was obtained. The gene has 1504 bp bases and can encode a sequence of 317 amino acids. We named it *GoACO1* (GenBank accession number: JX972145). According to the full length cDNA sequence, two specific primers were designed for amplify its DNA sequence. DNA was extracted from leaf of *Guzmania Ostara* as a template. A band about 2500bp with expectations was obtained by PCR amplification. Through sequencing to the band, a2546bp sequence was obtained. By BLAST comparison analysis, the 2546bp sequence was determined to be corresponding DNA sequence of*GoACO1* (GenBank:JX972146). Furthermore, we can find out that there were three introns in the DNA sequence, and cDNA sequence was divided into four parts by these introns (Fig. 1).

Bioinformatics analyses of *GoACO1*: The 1504 bp cDNA sequence of *GoACO1*contains a 954 bp ORF (open reading frame) from the start of the 79th base to the end of the 1032th base that encodes 317 amino acid residuals. ProtParam analysis indicated that the theoretical molecular weight and isoelectric point were36.1 kD and 5.28, respectively, and there were 50 negatively charged amino acids (Asp+Glu) as well as 40 positively charged amino acids (Arg+Lys). While secondary structure analysis with the SPOMA program revealed that the protein consisted of alpha helices (44.48%), random coils (25.55%), extended strands (21.14%) and beta turns (8.83%) (Combet *et al*., 2000). Conservative domain analysis by using the CDART program(NCBI) showed that there were two typical conserved domains, DIOX_N superfamily and 20G-FeII_Oxy superfamily domains in the gene (Fig. 2) (Marchler-Bauer *et al*., 2015).

Nucleotide-nucleotide blast analysis showed that full length cDNA sequence of *GoACO1*washighly homologue to ACC oxidase genes of *Ananas comosus, Oryza sativa, Phyllostachys edulis, Musa acuminata, Musa abb, Saccharum officinarum, and Hordeum vulgare*. Homologue to that of *Ananas comosus* (gb|AY049052. 1|) was 94 percent, and was the highest. Moreover, through comparing the deduced amino acid sequences (BlastP) with protein data bank, we found that *GoACO1* also had very high homologue to ACC oxidase of many other species in the data bank. The maximum value in amino acid homologue was 76 percent, which came from *Musa acuminate* (emb|CAA64856. 1|), *Actinidia chinensis* (gb|AEM62885. 1|), *Phyllostachys pubescens* (dbj|BAB32502. 1|), and *Dendrobium hybrid* (gb|ADN65042. 1|).

Based on multi-sequencing comparison between GoACO1 and amino acid sequence of ACC oxidase genes in other species by Clustal X (1.81) program, circular molecular phylogenetic tree was established using MEGA 4.1 program (Neighbor Joining method) (Fig. 3). Together with ACC oxidase genes of those Poaceae plants including *Oryza sativa* (gi|755773|, gi|2952328|), *Hordeum vulgare* (gi|397740900|, gi|397740892|, gi|397740898|, gi|397740894|), *Saccharum officinarum* (gi|41615359|), *Saccharum arundinaceum* (gi|123255925|), *Phyllostachys pubescens* (gi|12862572|), and *Brachypodium distachyon* (gi|357137917|), *GoACO1* were clustered to the same group.

Prokaryotic expression of *GoACO1*: The pET-28 and aco-pET-28 vector were transformed into E. coli DE3 competent cells (BL-21), respectively. The bacterium which was transformed successfully was induced to express by three different concentrations IPTG solution, and expressed protein was detected by SDS-PAGE electrophoresis (Fig. 4). Under different concentration IPTG solution induction (0.1mM, 0.3mM and 0.5mM), there was no obvious difference among them, except for 0mM IPTG solution (Fig. 4-A). Furthermore, the bacterium also was induced to express under three different temperature conditions with 0.3mM IPTG. The result showed that*GoACO1*can express under 18ºC, 24ºC and 37ºC (Fig. 4-B). All of the three temperature conditions can induce *GoACO1* expression, but content of produced protein increased with temperature increasing, and that of 37ºC was the highest. Finally, 0.3mM IPTG, 37ºC, 180 r/min for 3h was used as optimal induction condition to performed induction expression experiment. The result showed that a 41 KD protein precipitation was generated (Fig. 4-C), but supernatant had no target band, which indicated that there was no soluble protein produced.

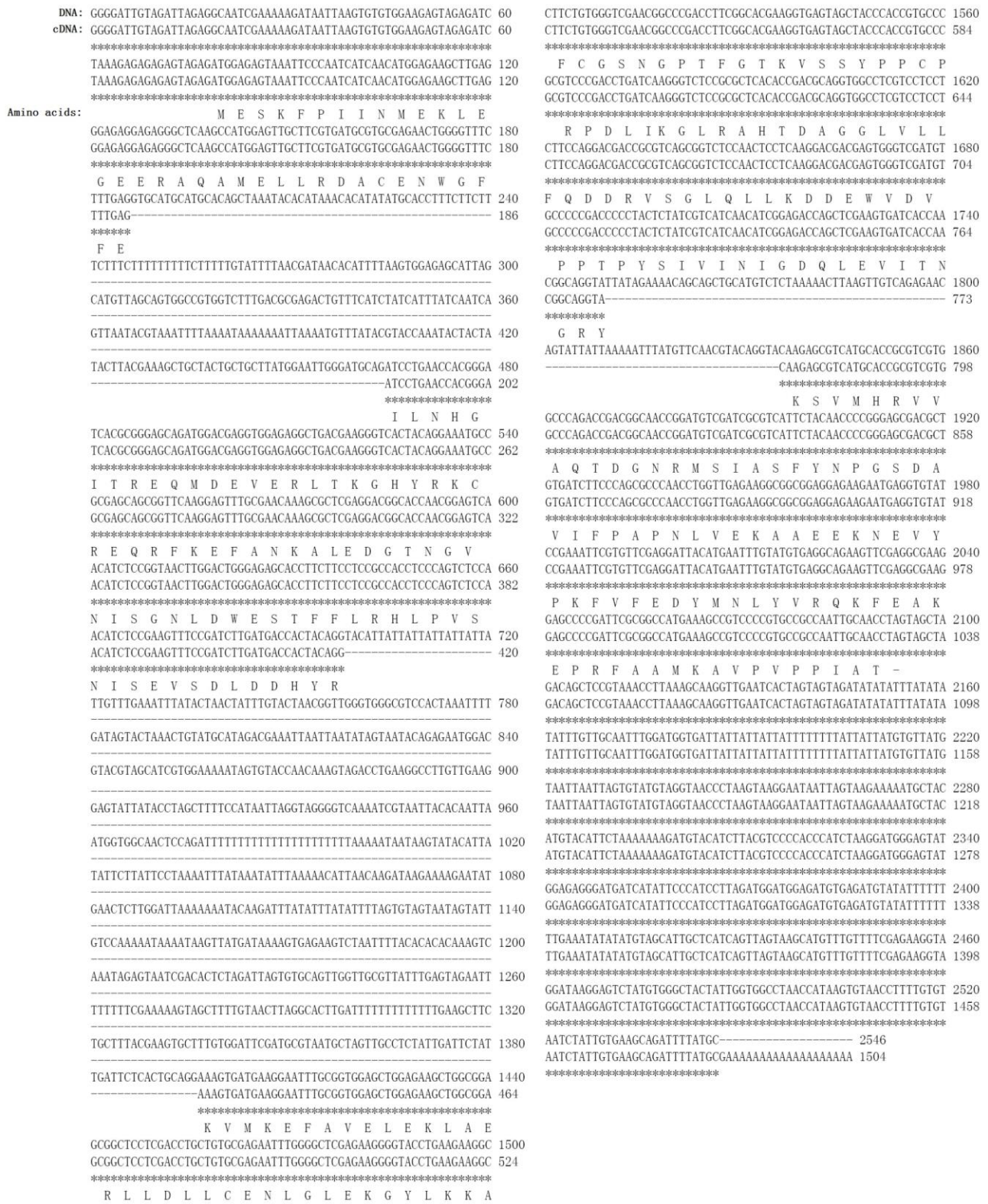

Fig. 1. cDNA, DNA and putative amino acids sequence of *GoACO1*.

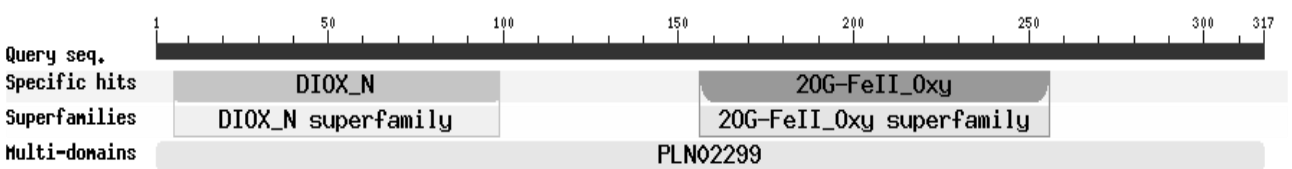

Fig. 2. Conserved domainsof*GoACO1*putative amino acids sequence.

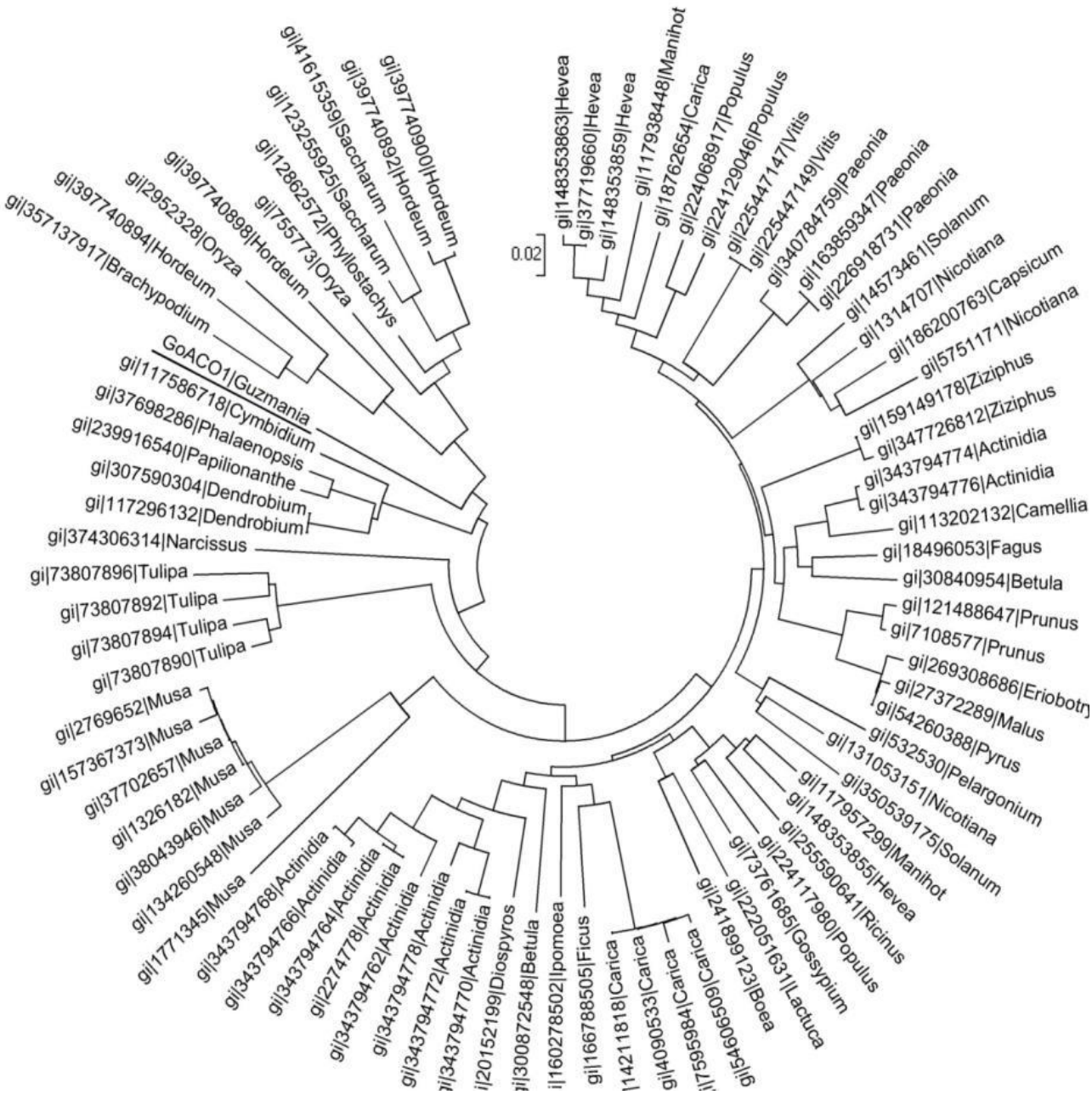

Fig. 3. Molecular evolution analyses in putative amino acid sequence of *GoACO1*.

Tissues expression of *GoACO1*: After experiment on which making amplification curve and dissociation curve of *GoACO1* and *Goactin1*were finished by three parallel quantitative PCR reactions, their Ct values were obtained. Through calculating expression quantity of *GoACO1* based on *Goactin1* (inner reference gene), relative expression quantity of *GoACO1* in different tissues were obtained (Fig. 5-B). *GoACO1* can express in all tested tissues. Its expression quantity in bract more than other three tissues, and that of floral organ was similar to scape. However, *GoACO1's* expression quantity in leaf was the least. The fact showed that *GoACO1's*expression probably have a close relationship with transition from vegetative to reproductive growth.

Overexpression of *GoACO1* in *Arabidopsis thaliana*: *GoACO1's* plant overexpression vector was transformed into *Arabidopsis thaliana* by floral dip method (Chen *et al*., 2013), seeds of T1 generation were collected and planted. Until T1 generation plant were cultivated to maturity, T2 generation seeds were harvested and were sowed in the soil once again. Flowering traits of T2 generation adult plant were observed and analyzed. The result showed that the average first flowering date of goal *Arabidopsis thaliana* containing *GoACO1* gene was 1.5 days earlier, and their peak-flowering period (flowering more than 50%) was 1.8 days earlier, compared with wild type *Arabidopsis thaliana* (Fig. 6). Student's t test showed that Sig. (2-tailed) of first flowering and peak flowering were 0.022 and 0.024 respectively, which were less than 0.05. There was a significant difference between the transgenic and wild-type plants (Fig. 6). Therefore, we thought that overexpression of *GoACO1* seems to promote plant blossom earlier.

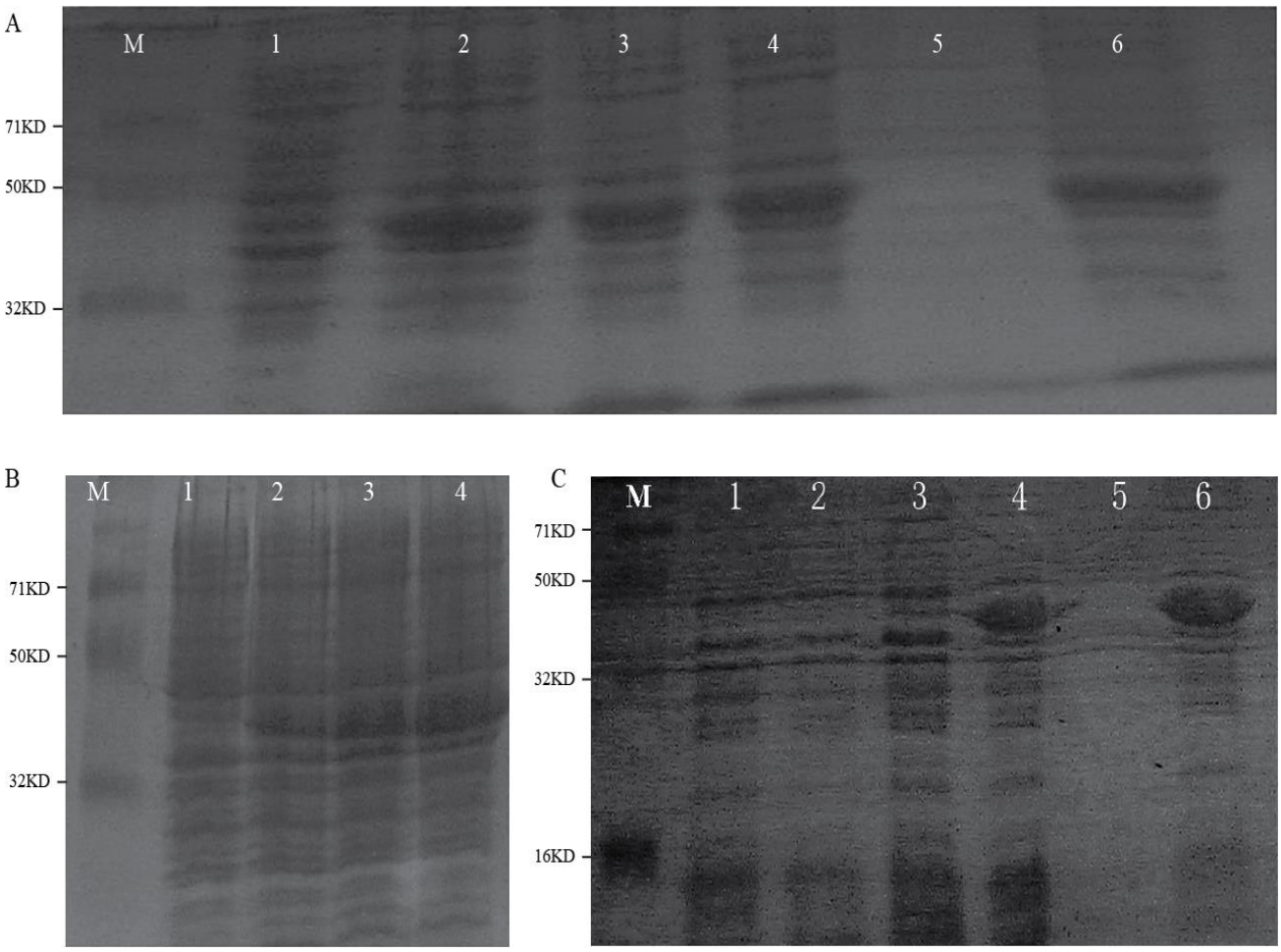

Fig. 4. SDS-PAGE analysis of recombinant GoACO1 protein inducement

A. Inducement under different IPTG concentration: lane 1, 0mMIPTG; lane 2, 0.1mMIPTG; lane 3, 0.3mMIPTG; lane 4, 0.5mMIPTG; lane 5, Supernatant of 0.3mMIPTG; lane 6, Deposition of 0.3mMIPTG

B. Inducement under different temperature: lane 1,24ºC 0mMIPTG; lane 2, 18ºC 0.3mMIPTG; lane 3,24ºC 0.3mMIPTG; lane 4, 37ºC 0.3mMIPTG;

C. Inducement under 0.3mM IPTG, 37ºC condition: lane 1, the whole lysate of E. coli cells containing the pET-28 vector without 0. 3mM IPTG induction; lane 2 the whole lysate of E. coli cells containing the pET-28 vector with 0. 3mM IPTG induction;lane 3, the whole lysate of E. coli cells containing the aco-pET-28 vector without 0. 3mM IPTG induction;lane 4, the whole lysate of E. coli cells containing the aco-pET-28 vector with 0. 3mM IPTG induction;lane 5, the supernatant of E. coli cells containing the aco-pET-28 vector with 0.3mM IPTG induction;lane 6, the deposition of E. coli cells containing the aco-pET-28 vector with 0. 3mM IPTG induction.

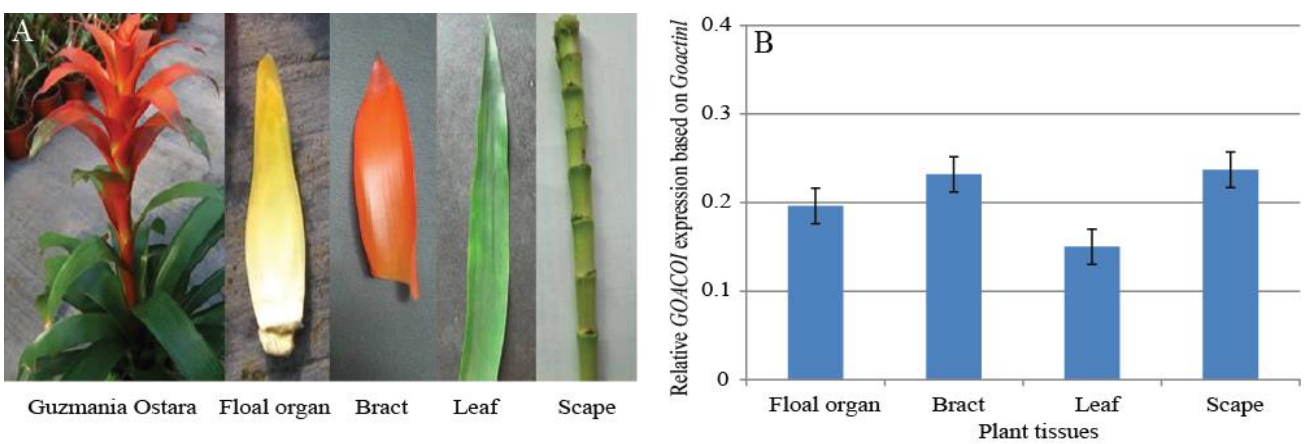

Fig. 5. Expression of*GoACO1*in various tissues in Real-time quantitative PCR experiment.

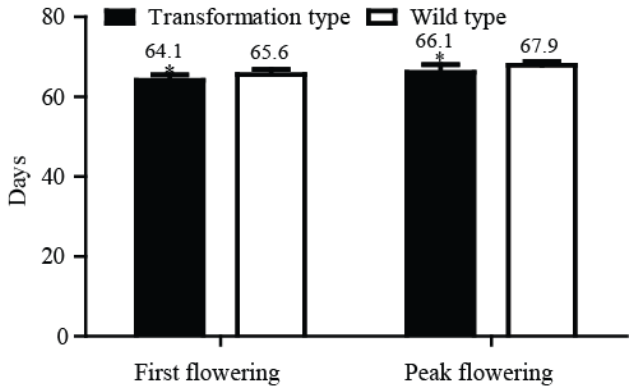

Fig. 6. Comparison of flowering time(calculate from seeding) between transformation and wild type *Arabidopsis thaliana*.
Note: The asterisk(*) represent significant difference at the 0.05 level (2-tailed)

Discussion

In this study, through comparing full-length cDNA and DNA sequence of *GoACO1*, we found that there exist three introns in the DNA, which divided cDNA sequence into four parts. This result was in agreement with *TgACO1*-4 of tulip (Kazumi *et al*., 2007). Intron was inserted sequence in eukaryotic cell DNA. These sequences were transcribed into RNA, but were removed immediately without editing and translation. Intron in higher plant also play an important role in gene expression, such as starting gene expression, affecting gene expression pattern, and enhancing gene expression level (Alan & Jason, 2000; Xie & Wu, 2002; Patricia *et al*., 2014). For example, if *adhl's* (alcohol dehydrogenase gene*)* intron in maize was removed, expression level of *adhl* reduced obviously. Furthermore, introns of *adhl* and *shl* gene in maize can make expression level of report gene increase 100 times in transgenic maize (Callis *et al*., 1987). At present, the phenomenon that intron promoted gene expression was found mainly in monocotyledon include those from the maize *Adh1, Sh1, Bz1, Hsp82, actin*, and *GapA1* genes and the rice *salT, Act1,* and *tpi* genes. (Alan & Jason,2000; Xie & Wu, 2002; Furger, 2002; Patricia *et al*., 2014). Though the phenomenon was also observed in dicotyledon include those from the petunia rbcS gene *SSU301*, the potato *ST-LS1* gene , and the Arabidopsis *UBQ3,UBQ10, PAT1, atpk1, A1 EF-1a*, and *At eEF-1b* genes (Alan & Jason,2000), their increasing extent was only 2-5 times (Alan & Jason, 2000; Xie & Wu, 2002). Guzmania was monocotyledon, multiple introns of *GoACO1* may play an important role in regulation of gene expression, but still need further study to determine the specific role.

To date, *Escherichia coli* was a biology which was studied most and clearest in genetic background, gene expression and regulation. As a host of gene expression, it has advantages such as cultivating easily, growing fast, genetic operation expediently, many alternative expression vectors, different genotype strains, and high expression level for most exogenous gene in *Escherichia coli*. Recombinant protein produced by overexpression often became inclusion body in *Escherichia coli* cytoplasm or clearance between membrane and wall. In this study, *GoACO1*'s expression protein was41 KD in *Escherichia coli* DE3 (BL-21). However, its predicted molecular weight was 36.1 KD. Owing to pET-28a contained His tags, its actual protein molecular weight was about39 KD to 43 KD. Therefore, the 41 KD protein obtained was accord with expectation. In addition, the recombinant protein was existed as form of inclusion body. Miscellaneous protein in inclusion body was low, so it only was need be broke by ultrasonic and centrifugation to separate and purify goal protein.

ACC oxidase gene was a key enzyme gene in ethylene biosynthesis pathway. Numerous studies have found that ethylene associate closely with development of flower organs (O'Neill *et al*., 1993). For example, after *caulis dendrobii* pollinating, ethylene content of flower organ increased significantly within 9 hours (Ketsa & Rugkong, 2000; Kanjana *et al*., 2011). Previous studies also showed that general nutritive or reproduction organs and organizations existed ACC oxidase activity in plant (Hoffman *et al*., 1982). For example, in the process of fruit ripening (Moon & Callahan, 2004), flower organ and seeds forming (Calvo *et al*., 2004). This was consistent with our results. Namely, floral organ, bract, leaf and stem all existed *GoACO1's* expression. This result was in agreement with ACC oxidase gene of most other plants, including *PlACO* of *Paeonia lactiflora*, *TgACO1, TgACO3* and *TgACO5* of *tulip* and so on (Kazumi *et al*., 2007; Zhao *et al*., 2014). But, different from the high expression level of *PlACO* in leaf (Zhao *et al*., 2014), *GoACO1* expression level in leaf was the lowest.

Conclusions

In this paper, we obtained full-length cDNA and corresponding genomic sequence of *GoACO1* (ACC oxidase gene) from Guzmania variety: *Ostara*. The *GoACO1*produced a 41 KD protein precipitation in *Escherichia coli*. Furthermore, it can express in all kinds of tested tissues and seems to promote *Arabidopsis thaliana* flowering earlier.

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