Full Length Research Paper

Cloning and expression analysis of a partial LEAFY homologue from pineapple (Ananas comosus (L.) Merr.)

Lv Lingling¹,²,³, Sun Guangming¹,⁴, Xie Jianghui², Zhang Jianxia¹, Liu Shenghui², Liu Yuge², Wei Changbin², Zeng Songjun¹ and Duan Jun¹*

¹South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong 510650, China.
²South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Science, Zhanjiang, Guangdong 524091, China.
³Graduate University of Chinese Academy of Sciences, Beijing 100049, China.
⁴National Center of Important Tropical Crops Engineering and Technology Research, Haikou, Hainan 571101, China.

Accepted 29 April, 2011

LEAFY-like genes are crucial regulators of flowering in angiosperms. A partial homologue of LEAFY, designated as AcLFY (Genebank accession no HQ433335), was isolated from pineapple (Ananas comosus L. cv. Comte de Paris) by PCR. The conserved cDNA fragment of AcLFY is 256 bp in length and contained an open reading frame of 248 bp, which encodes 82 amino acids protein. The amino acid alignment showed that AcLFY had a high identity with the related fragment of PhalLFY in Phalaenopsis hybrid cultivar (91%), LFY in Oncidium (90%) and OrcLFY in Orchis italica (88%). RT-PCR analysis showed that the AcLFY could be expressed constitutionally. Moreover, it was expressed in the flesh of young fruit and bract at low levels, and was highly expressed in the fruit, stem, petal and sepal.

Key words: Pineapple, cloning, expression, RT-PCR, LEAFY.

INTRODUCTION

Pineapple (Ananas comosus (L.) Merr.) was domesticated long before its first historic mention by Christopherus Columbus in 1493 (Morrison et al., 1973). It is a biennial tropical monocot herb of Bromeliaceae and is one of the commercially important tropical fruits constituting a major export item for some countries (Duval et al., 2001). It is the third most important fruit in the tropics and subtropics, after banana and citrus (Rohrbach et al., 2002), and fruits can be harvested in most months of the year. In 2009, pineapple world production reached 18.4 million tons and the production was 1.45 million tons in China (FAOSTAT, 2011).

Nutritionally, freshly harvested pineapple is an inexpensive source of vitamins A, B and C, calcium, phosphorus and iron. The fruit is used for canning and in the preparation of juice, jam, jelly and crystallized glaze fruit. The leaves, stem and fruits of the pineapple plant contain bromelain which is a mixture of industrially important proteolytic enzymes. That is the reason why several research groups are developing fundamental and applied studies to create new cultivars with better agronomic performance.

Environmental induction of inflorescence development that occurs prior to the scheduled forcing is a sporadic but widespread and important problem in pineapple-producing regions because the precocious flowering results in fruits that are either too small to be marketable or too few in number, which makes harvesting unprofitable (Bartholomew and Mal6zieux, 1994; Scott et al., 1993). Flower induction is a good way to make pineapple flower at the same time, however, some species are difficult to be used for force flowering.

Conventional breeding to develop improved pineapple varieties has proved to be difficult due to the high level of genomic heterozygosity and apparent genome instability (Kato et al., 2004). Moreover, it requires several generations of backcrossing which is too cumbersome and time consuming. Genetic engineering has a great potential to improve the horticultural characteristics of pineapple varieties by introducing very specific traits without altering other agronomic attributes. One of the...
most important traits of interest is the control of flowering. LEAFY (LFY) plays an important role in the reproductive transition and controls flower spatial patterning by inducing the expression of the ABC floral organ identity genes (Liu et al., 2009). The conserved helix-turn-helix structure of LFY presumes that this gene is a universal integrator of the external and internal signals that promote the induction of flowering and maintain the identity of floral meristems (Blázquez et al., 2000; Araki et al., 2001; Boss et al., 2004; Hames et al., 2008). Expression of Arabidopsis LFY gene in Citrus induces flowering within the first year. This shortening of the juvenile period is stable in seedlings (Pena et al., 2001).

Expression of loquat LFY gene (ejLFY) in Arabidopsis resulted in early-flowering too (Liu et al., 2008). So far, LFY family genes have been isolated in many plant species, including snapdragon, rice, wheat, pea and so on (Coen et al., 1990; Dornelas and Rodriguez, 2006; Hofer et al., 1997; Kelly et al., 1995; Kyozuka et al., 1998; Shitsukawa et al., 2006). The identified LFY family genes are highly conserved in both their structures and functions (Ma et al., 2005). In this study, a partial LFY-like gene (AcLFY) was cloned and its expression in different tissues was analyzed too.

MATERIALS AND METHODS

Plants of pineapple cultivar ‘Comte de Paris’ were planted in the greenhouse at natural temperature and light. When the young panicle of the plant was 4 to 5 cm in height, the apical meristems, flesh of young fruit, fruit stem, bract, leaf, petal and sepal were cut and frozen in liquid nitrogen immediately and stored at -80°C until use.

Cloning of the partial AcLFY cDNA

Total RNA was extracted from apical meristems using Column Plant RNAout 2.0 kit (Tianz, Inc, China) following the manufacturer’s instructions. First-strand cDNA was synthesized with M-MLV-reverse transcriptase from TAKARA according to the manufacturer’s instructions.

To clone the conserved region of AcLFY, a pair of degenerate primers LFY-partial-F (5’CGGAYATIAAYAARCCIAARATGMCIGAYTA3’) and LFY-partial-R (5’GCACGTGIC IKIARYKGTIGGIACRTACCA3’) was designed according to the conserved regions of LFY homologues using the Primer Premier 5 software. The PCR amplification was carried out under the following condition: 1 cycle at 94°C for 3 min; 32 cycles at 94°C for 0.5 min, 52°C for 1 min and 72°C for 1 min and 1 cycle at 72°C for 10min. PCR products were isolated and cloned into pMD18-T vector (TAKARA, Japan) for sequence.

Sequence and phylogenetic analysis

Sequence chromatograms were examined and edited using Chromas version 2.22. The deduced AcLFY amino acid sequence was aligned with homologous sequences retrieved from GenBank (http://www.ncbi.nlm.nih.gov/protein) using DNAMAN 6.0 program. Phylogenetic tree based on the amino acid sequences was constructed using DNAMAN 6.0. Gaps appearing in one sequence only were treated as nonconstant characters.

RT-PCR expression analysis

Total RNA was extracted from apical meristems, flesh of young fruit, fruit stem, bract, leaf, petal and sepal, and were reverse-transcribed. The resulting cDNAs from these tissues were then individually amplified using the primers LFydi-F (5’-TGGTACCATGACCCCTGTACTC-3’) and LFydi-R (5’-GTGGATCGAAACAAACTACCCACAGG3’). The cycle condition was: 1 cycle at 94°C for 3 min; 32 cycles at 94°C for 0.5 min, 55°C for 0.5 min and 72°C for 1 min; a final extension at 72°C for 10 min. Ac18S rRNA was used as the housekeeping gene. Ac18S rRNA-up was: ATGTTGTGACCGGTGAC and Ac18S rRNA-dn was: AGACACTAAAGCAGCCCG GTA. The PCR products were separated on a 1.0% agarose gel.

RESULTS AND DISCUSSION

Cloning and sequence analysis of AcLFY

The conserved region of LFY-like gene was isolated from pineapple cDNA and designated as AcLFY (Genbank accession no. HQ433335). AcLFY is 256 bp in length and contained an open reading frame (ORF) of 248 bp, which encodes 82 amino acids protein. An alignment of the deduced amino acid sequence of AcLFY (Ananas comosus), OrcLFY (Orchis italic) and BAC54955, PhaLFY (Phalaenopsis hybrid cultivar, AAY 40170) and LFY in Oncidium (Oncidium hybrid cultivar, ADL57240) was performed using the DNAMAN 6.0 program (Figure 1). The alignment showed that AcLFY had a high identity with the related fragment of PhaLFY (91%), LFY in Oncidium (90%) and OrcLFY (88%).

Phylogenetic analysis was conducted using the deduced pineapple LFY protein and the other amino acid sequences, and a rooted tree was generated by the maximum likelihood method with DNAMAN 6.0 program (Figure 2). The phylogenetic tree showed that AcLFY protein is more closely related to some monocot LFY proteins than to their dicot counterparts. AcLFY protein was clustered into the same subgroup ‘Poales’ with ZIZFL1 in Zea luxurians (AAX35296), SbFL in Sorghum bicolor (AAX35325) and RFL33 in Oryza rufipogon (AAY33631). The result suggests that AcLFY may potentially accelerate flowering.

RT-PCR analysis of AcLFY in pineapple tissues

Organ-specific expression patterns of AcLFY in pineapple were investigated by RT-PCR. Total RNA was extracted from the apical meristems, flesh of young fruit, fruit stem, bract, leaf, petal and sepal, and was reverse-transcribed. The resulting cDNAs were used as templates for analysis. The results showed that AcLFY was expressed in the flesh of the young fruit and bract at low levels, in fruit stem, petal and sepal at high levels, and in the apical
Figure 1. Alignment of the deduced AcLFY (Genebank accession no. HQ433335) protein sequence from Ananas comosus with other angiosperm LFY-like proteins. Identical amino acid residues in this alignment are shaded in black, 75% or more similar amino acid residues are shaded in red and black dots denote gaps. The alignment was performed with the DNAMAN 6.0 program.

Figure 2. Phylogenetic analysis of the LFY homologous genes from different plant species. Protein sequences of the entire coding region were obtained from the NCBI database: AAX35296 (Zea luxurians), AAX35325 (Sorghum bicolor), AAY33631 (Oryza rufipogon), AAY40170 (Phalaenopsis hybrid cultivar), ABR13015 (Lilium longiflorum), ADL57240 (Oncidium hybrid cultivar), BAC54955 (Orchis italica), AAS00458 (Hyacinthus orientalis), AAM27942 (Arabidopsis lyrata), AAT84086 (Chrysanthemum lavandulifolium), AAC64705 (Cucumis sativus), ABR13015 (Lilium longiflorum), ADL57240 (Oncidium hybrid cultivar), BAC54955 (Orchis italica), AAS00458 (Hyacinthus orientalis), AAM27942 (Arabidopsis lyrata), AAT84086 (Chrysanthemum lavandulifolium), AAC64705 (Cucumis sativus), ABY78032 (Prunus persica) and ABY77762 (Solanum tuberosum). The numbers next to the nodes give bootstrap values from 1000 replicates and the branch lengths are proportional to the distance.
meristems and leaf at moderate levels (Figure 3). So, it was inferred that AcLFY gene could be expressed constitutionally.

The expression patterns of LFY homologues are diverse in different plants. In Arabidopsis plants, LFY expression is observed in lateral meristem initials through the whole vegetative phase and the inflorescence meristem (Weigel et al., 1992). LILFY1 in Lilium longiflorum Thunb is expressed in young flower buds and shoot apical meristem but not in the roots, shoots, mature leaves and mature floral organs (Wang et al., 2008). Ginlfy gene in Ginkgo biloba was expressed in different organs such as the root, stem, leaf, young fruit and in the male flower bud and female flower bud in different stages. However, GinnDry in Ginkgo biloba was only expressed in the leaf, male flower bud and female flower bud in different stages (Guo et al., 2008). Analysis of StLfY expression at the mRNA level by RT-PCR showed that it was slightly expressed in the apical buds, floral buds and initial stolons (Guo et al., 2008). EcFLO mRNA was expressed during the vegetative phase of the shoot apical meristem and in developing dissected leaves (Andrea et al., 2003). The expression difference of LFY homologues is probably because of the change of the promoter sequence during evolution.

ACKNOWLEDGMENTS

This study was funded by ‘948’ program from Chinese Ministry of Agriculture (2006- G34 (A) and National standardization plan of pineapple (nyhyxz07-030). The authors are grateful to Professor Lei Jianjun and Associate Professor Chen Guoju for their helpful suggestions, and to Dr Chen Changming, Li Weiming, Dr Zhang Wei, Dr Zhang Lubin, Tian Ruixue, Zhang Shengpeng, Huang Weiting and Lv Wenying for their technical assistance.

REFERENCES


