Plant Science 254 (2017) 82–94

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Curd development associated gene (*CDAG1*) in cauliflower (*Brassica oleracea* L. var. *botrytis*) could result in enlarged organ size and increased biomass

Hui Li[b,1], Qian Liu[a,1], Qingli Zhang[a], Erjun Qin[a], Chuan Jin[a], Yu Wang[a], Mei Wu[a], Guangshuang Shen[a], Chengbin Chen[a], Wenqin Song[a], Chunguo Wang[a,*]

[a] College of Life Sciences, Nankai University, Tianjin, 300071, China
[b] College of Horticulture and Landscape, Tianjin Agricultural University, Tianjin, 300384, China

ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 24 October 2016
Accepted 25 October 2016
Available online 7 November 2016

Keywords:
Cauliflower (*Brassica oleracea* L. var. *botrytis*)
Organ size
OSR gene
CDAG1
High-yielding crops

ABSTRACT

The curd is a specialized organ and the most important product organ of cauliflower (*Brassica oleracea* L. var. *botrytis*). However, the mechanism underlying the regulation of curd formation and development remains largely unknown. In the present study, a novel homologous gene containing the Organ Size Related (OSR) domain, namely, *CDAG1* (*Curd Development Associated Gene 1*) was identified in cauliflower. Quantitative analysis indicated that *CDAG1* showed significantly higher transcript levels in young tissues. Functional analysis demonstrated that the ectopic overexpression of *CDAG1 in Arabidopsis* and cauliflower could significantly promote organ growth and result in larger organ size and increased biomass. Organ enlargement was predominantly due to increased cell number. In addition, 228 genes involved in the *CDAG1*-mediated regulatory network were discovered by transcriptome analysis. Among these genes, *CDAG1* was confirmed to inhibit the transcriptional expression of the endogenous *OSR* genes, *ARGOS* and *ARL*, while a series of ethylene-responsive transcription factors (ERFs) were found to increased expression in 35S:*CDAG1* transgenic *Arabidopsis* plants. This implies that *CDAG1* may function in the ethylene-mediated signal pathway. These findings provide new insight into the function of *OSR* genes, and suggest potential applications of *CDAG1 in breeding high-yielding crops.*

1. Introduction

Organ size is one of the most important morphological features of plants. Different plant species even those closely related, usually exhibit obvious organ size differences [1]. For example, *Arabidopsis thaliana* and *Brassica oleracea* L. var. *botrytis* both belong to the same genus, and show close evolutionary relationship, but display obvious differences in the sizes of their leaves, flowers, seeds, and stems. However, in the same plant species, the organ size of each individual plant shows almost no difference under the same growth conditions [2]. It is the clear evidence that organ size of plants is under strict genetic control, although it is often influenced by various environmental signals [2]. In addition, organ size is also a very valuable agronomic trait. The sizes of seeds, fruits, leaves or flowers directly affect the yields or qualities of some crops and other economic plants [3]. Previous reports have indicated that the final organ size in plants is controlled by the number and size of cells [2,4]. In the early phase of organ development, cell division is active, and leads to an overall increase in cell number, which is the main factor maintaining organ growth. Subsequently,

Abbreviations: OSR domain, organ size related domain; *CDAG1*, *Curd Development Associated Gene 1*; *ANT*, *AINTEGUMENTA*; *AN3*, *ANGUSTIFOLIA 3*; *AtGRF5*, *GROWTH-REGULATING FACTOR5*; *GIF*, *GRF-INTERACTING FACTOR*; *JAG*, *JAGGED*; *SWP*, *STRUWWELPETER*; *SMP1*, *SWELLMAP1*; *KLU*, *KLUH*; *ARGOS*, *Auxin-Regulated Gene Involved in Organ Size*; *ARF2*, *AUXIN RESPONSE FACTOR2*; *BOP1*, *BLADE ON PETIOLE1*; *PPDs*, *PEAPOD1 2* ; *BB*, *BIG BROTHER*; *CNR1*, *Cell Number Regulator 1*; *ARL*, *ARGOS-LIKE*; *ROT3*, *ROTUNDIFOLIA3*; *BoREM1*, *Brassica oleracea Reproductive Meristem Gene 1*; *CCE1*, *Cauliflower Curd Expression 1*; MS, Murashige & Skoog; CTAB, hexadecyl trimethyl ammonium bromide; RACE, Rapid Amplification of cDNA Ends; UTR, Untranslated Region; GSPs, gene-specific primers; RPKM, the number of reads per kilobase of exon region per million mapped reads; NJ, neighbor-joining; *ERFs*, *ethylene-responsive transcription factors*; *ZAR1*, *Zea mays ARGOS1*; GO, Gene Ontology.

* Corresponding author.
E-mail addresses:
lihui@tjau.edu.cn (H. Li), 15822076271@163.com (Q. Liu), 13553162779@163.com (Q. Zhang), erjunqin@126.com (E. Qin), emojinchuan@163.com (C. Jin), 1581257798@qq.com (Y. Wang), alexmaymolecular@126.com (M. Wu), 13752041387@163.com (G. Shen), htg1979@163.com (C. Chen), songwenqin53@gmail.com (W. Song), wangcg@nankai.edu.cn (C. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plantsci.2016.10.009
0168-9452/

the rate of cell division decreased and eventually ceased. Further organ growth then mainly results from cell expansion [1]. A series of genes and transcription factors have been identified to function in determining the final organ size by regulating cell division and/or proliferation in plants. For instance, *AINTEGUMENTA* (*ANT*) [5,6], *ANGUSTIFOLIA 3* (*AN3*) [7], *GROWTH-REGULATING FACTOR5* (*AtGRF5*) [7], *GRF-INTERACTING FACTOR* (*GIF*) [8], *JAGGED* (*JAG*) [9], *STRUWWELPETER* (*SWP*) [10], *SWELLMAP1* (*SMP1*) [11], *KLUH* (*KLU*) [12], *EBP1* [13], *AtMRB1* [14] and *Auxin-Regulated Gene involved in Organ Size* (*ARGOS*) [15] in *Arabidopsis* and *LaAP2L1* [16] in *Larix* positively regulate cell proliferation. The ectopic overexpression of these genes or transcription factors could prolong cell proliferation, thereby increasing organ size. Mutants with a loss of function of these genes usually have a smaller organ size than that of wild-type plants [5,13,15]. Several other genes that also affect the final organ size by regulating cell proliferation have been confirmed, for example *AUXIN RESPONSE FACTOR2* (*ARF2*) [1,17], *BIG BROTHER* (*BB*) [18] and *DA1* [19,20] in *Arabidopsis*; *CNR1* (*Cell Number Regulator* 1) in maize [21] and *Physalis floridana* [22]; and *FORMOSA* in *Antirrhinum majus* [23]. However, unlike *ANT* or *ARGOS*, which act as positive cell proliferation regulators, these genes negatively regulate organ size by inhibiting cell proliferation and division. Cell size is another important factor that determines organ size in plants. A few cell size-associated genes also function in final organ size control, such as *ARGOS-LIKE* (*ARL*) [24], *ROTUNDIFOLIA3* (*ROT3*) [25], *AtGRF1/2* [26], *BIGPETAL* [27], *AtTOR* [28], *ORGAN SIZE RELATED1* (*OSR1*) [29] and *OSR2* [30] in *Arabidopsis*. In addition, genes such as *OSR1* and *EBP1 in Arabidopsis* as well as *OsARGOS* [31] in *Oryza sativa* were showed to function in both cell proliferation and cell expansion. However, as a complicated morphological feature and an important agronomic trait in some crops, our knowledge about organ size regulation in plants, especially in crops and economic plants remains poor.

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is one of the most important *B. oleracea* varieties, and thanks to its high nutrition content is planted worldwide. The most significant characteristic of cauliflower is that, unlike other *B. oleracea* varieties, a specialized organ called the curd forms during floral development. The curd is composed of many indeterminate inflorescences and shortened inflorescence branches [32,33]. In addition to being a specialized organ, the curd directly determines the economic value of cauliflower. *BobCAL* and *BobAP1*, homologous with *Arabidopsis CAL* and *AP1*, play important roles in the formation of cauliflower curd [34–37]. *BoREM1* (*Brassica oleracea reproductive meristem gene 1*) [38] and *CCE1* (*Cauliflower curd expression 1*) [39] are also specifically expressed in cauliflower curd. However, the underlying basis of curd formation in cauliflower is still not well understood. In our previous study, to uncover the regulation of curd development in cauliflower, a comparative transcriptome analysis of curds was conducted during different developmental phases. A series of genes showing significant differential expression was identified. Among these genes, a gene containing the OSR domain, named *CDAG1* (*Curd Development Associated Gene 1*), was identified. *OSR* genes, such as *ARGOS*, *ARL*, *OSR1* and *OSR2* play crucial roles in regulating organ size in *Arabidopsis* [15,24,29,30]. Comparative transcriptome data indicated that *CDAG1* showed significant differential expression during curd development in cauliflower. *CDAG1* showed markedly high expression during early curd development, whereas expression levels dramatically decreased in mature curds with decreased final size. Consequently, it was speculated that *CDAG1* containing the OSR domain may function in regulating cauliflower curd development. Nevertheless, the roles of *CDAG1* in cauliflower still require further elucidation.

2. Materials and methods

2.1. Plant materials

Arabidopsis thaliana (Columbia ecotype) was used in the present study. Seeds were surface sterilized in 30% bleach for 12 min and washed five times with sterilized water. Then, the seeds were planted on Murashige & Skoog (MS) medium and vernalized in darkness at 4 °C for 2 days before the plates were transferred to a culture room at 23 °C with a 16 h/8 h light/dark photoperiod. For genetic transformation and morphological examination, seedlings were transferred to soil 10 days after germination and planted in a growth chamber at 23 °C with 40–65% relative humidity and a 16 h/8 h light/dark photoperiod. The homogeneous cauliflower seeds were first treated with 75% ethanol for 5 min and 2% NaClO for 10 min, and then rinsed thrice with sterile distilled water. The sterilized seeds were also planted on MS medium under controlled conditions with a 16 h/8 h light/dark cycle at 25 °C and 22 °C, respectively. The explants coming from the cultured hypocotyl segments were used for genetic transformation by *Agrobacterium*-mediated transformation method. Positive transgenic plants were selected by kanamycin and further identified by molecular methods. All of the identified transgenic lines were transplanted to soil, and grew in a growth chamber at 25 °C with a 16 h/8 h light/dark cycle.

2.2. CDAG1 cloning and sequence analysis

To clone the full-length cDNA of *CDAG1*, 5′- and 3′-RACE (Rapid Amplification of cDNA Ends) were conducted using a SMART™ RACE cDNA Amplification Kit (Clontech, USA) according to the manufacturer's instructions. The gene-specific primers (GSPs) for 5′- and 3′-RACE were designed based on the corresponding unigene sequences (Supplementary Table S1). Primers for amplifying the full-length cDNA of *CDAG1* including the 5′UTR (Untranslated Region) and 3′URT regions were designed based on the sequences obtained by RACE (Supplementary Table S1). In addition, Total DNA was isolated from 15-day-old cauliflower seedlings by the hexadecyl trimethyl ammonium bromide (CTAB) method. Total RNA was also isolated from 15-day-old cauliflower seedlings using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. Then, the first-strand cDNA synthesis was conducted with M-MLV reverse transcriptase (Promega, USA), and RT-PCR analysis of *CDAG1* was performed. The PCR and RT-PCR products were cloned using the T-A cloning method. DNA sequencing was performed by an ABI3770 sequencer (Applied Biosystems, USA). Sequence analysis was carried out using the Blast and Clustal W software [40].

2.3. qRT-PCR transcript expression assay

Total RNAs from the cotyledons and true leaves of cauliflower seedlings, transgenic cauliflower leaves and transgenic *Arabidopsis* leaves were extracted using TRIzol reagent (Invitrogen, USA). The first-strand cDNAs of these RNAs were used as the templates to conduct qRT-PCR analysis using specific primer pairs (Supplementary Table S1). The *Actin* gene was selected as an internal control. FastStart Universal SYBR Green Master (Roche, Germany) was used for qRT-PCR. The relative expression levels of each gene were calculated by the comparative $2^{-\Delta\Delta CT}$ method. Three biological replicates and three technological replicates were performed to ensure the reliability of quantitative analysis.