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Genome-wide analysis of OVATE family proteins in cucumber (*Cucumis sativus* L.)

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Abstract

**Background:** OVATE family proteins (OFPs) are plant-specific proteins with the conserved OVATE domain that regulating plant growth and development. Although these OFPs have been studied in several species, the biological functions of this OFP gene family remain largely unknown in cucumber (*Cucumis sativus* L.).

**Results:** In this study, we identified 19 *CsOFPs* in cucumber. This *CsOFPs* are distributed on seven chromosomes and can be divided into four subgroups. Most *CsOFP* genes are expressed in reproductive organs although have different expression patterns. *Cis*-elements analysis showed that there are six kinds of hormone response elements in *CsOFPs* and exogenous gibberellin treatment leads to a ‘first increase then decrease’ expression pattern of *CsOFP7*, *CsOFP11* and *CsOFP12*. Ectopic expression of *CsOFP11* in *Arabidopsis* resulted in shorter and blunt siliques.

**Conclusions:** Together, these results indicated that CsOFPs may play important roles in cucumber fruit development.

**Keywords:** cucumber, *CsOFPs*, expression pattern, fruit development
**Background**

*OVATE* gene was originally identified in tomato, in which a natural mutation in *OVATE* resulted in fruit from round to pear-shaped [1]. One century ago, pear-shaped fruit form in tomato was proposed to be controlled by a single recessive quantitative trait locus (QTL), which was named *pyriform* (*pr*) [2-3]. Later, additional studies showed that *pr* was co-segregate with the locus determining oblate- to oval-shaped fruit, *pr* was renamed *ovate*. Until 2002, the *ovate* gene was cloned in tomato. A single base substitution (G-T) in *OVATE* caused a premature stop codon and the pear-shaped fruit in tomato [1]. *OVATE* gene encodes a protein with a conserved 70 amino acid C-terminal domain, which was designated as OVATE domain, and proteins containing this domain were named OVATE family proteins (OFPs) [4-6]. OFPs are widely distributed in the plants. There are 19 *AtOFPs* in Arabidopsis, 26 *SlOFPs* in tomato, 45 *ZmOFPs* in maize and 33 *OsOFPs* in rice [4-5, 7-8]. While only 31 *OsOFPs* encode full-length OFP proteins in rice [8].

So far, the biological functions of OFPs in plants remain largely unknown, although OFPs widely exist in plants. Limited studies in several different plant species including Arabidopsis, tomato, pepper, banana and rice demonstrated that OFPs play important roles in plant growth and development. Consistent with *OVATE*’s negative role in tomato fruit elongation, *CaOVATE*, an *OVATE*-like gene of *Capsicum annuum*, also plays a negative role in determining fruit shape. Knockdown of *CaOVATE* through virus-induced gene silencing in cv. “Mytilini Round” caused its fruit to a more oblong shape [9]. Some studies suggested that GS9 could interact with OsOFP14 and OsOFP8 to regulate rice grain shape and knockout of GS9 resulted in slender grains [10]. Moreover, the interaction pair MuMADS1 and MaOFP1 are antagonistically regulated by ethylene and might participated in the process of fruit ripening in banana [11]. In Arabidopsis, most *AtOFPs* seem to act as transcriptional repressors. It has been shown that *AtOFP1* acts as a transcriptional repressor of *AtGA20ox1* to suppress cell elongation [12]. *AtOFP5* was reported to act as negative regulator of the BELL–KNOX TALE during early embryo sac development [13]. Furthermore, *AtOFP1* and *AtOFP4* interact with KNAT7 to regulate secondary cell wall formation and works together with *AtKu70* involving in the repair of DNA double-strand breaks (DSBs) [14]. In addition to transcription factors, *OVATE* protein also interacts with TONNEAU1-recruiting motif family of proteins (TRMs) that can bind microtubules regulating multicellular growth in plants [15]. Together, OFP proteins can act as transcription repressors by inhibiting their target genes, or interact with transcription factors and TRMs to regulate plant growth and development.

Cucumber (*Cucumis sativus* L.) is an important horticultural crop and the fruit shape indexes are important quantitative characters in the process of breeding [16-17].
Recently, a few of genes are shown to regulate cucumber fruit length. For example, the RING-type E3 ligase short fruit 1 (SF1) promotes fruit length by regulating cell division in cucumber via degradation of 1-aminocyclopropane-1-carboxylate synthase 2 (ACS2), a rate-limiting enzyme for ethylene biosynthesis [18]. Short Fruit 2 (SF2), a Histone Deacetylase Complex 1 (HDC1) homologue, also positively regulates fruit length by promoting cell proliferation [19]. Moreover, a MADS-box transcription factor CsFUL1 negatively regulate fruit length via inhibits the expression of Superman and auxin transporters PIN1/7 [20]. Although these OFPs have been studied several plant species, only CsOFP15a was reported as a candidate gene regulating the fruit length in cucumber [21]. But whether CsOFP members regulate cucumber fruit development remains largely unknown.

Here, we identified and characterized CsOFP genes in the cucumber. Most of these CsOFPs were expressed in reproductive organs. When transformed CsOFP11 driven by the 35S promoter into the Arabidopsis, the transgenic lines exhibited shorter and blunt siliques, consistent with the OVATE’s negative role in tomato fruit elongation. These results suggested that CsOFP genes may negatively regulate the fruit length in cucumber.
Results

Identification and distribution of CsOFP genes in cucumber

To identify OVATE domain-containing proteins in cucumber, the amino acid sequences of 19 Arabidopsis AtOFP members were used as queries for BLASTp searches. There are no homologs for AtOFP2, AtOFP3, AtOFP4, AtOFP5, AtOFP10 and AtOFP18, but there are three homologs for AtOFP1, AtOFP6, AtOFP15 and two homologs for AtOFP8, respectively. In total, 21 putative CsOFP genes were identified from the cucumber genome. By checking in the NCBI’s Conserved Domain Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/docs/cdd_search.html), 19 OVATE domain-containing members were identified and named as CsOFP1a-c, CsOFP5, CsOFP6a-c, CsOFP7, CsOFP8a-b, CsOFP11, CsOFP12, CsOFP13, CsOFP14, CsOFP15a-c, CsOFP16, and CsOFP19 (Table 1 and Additional file 3: Table S1). These genes were mapped on the chromosomes according to the cucumber genome version 2 in Cucurbit Genomics Database and drew chromosomal distribution of these CsOFP genes by the online tools (http://mg2c.iask.in/mg2c_v2.0/). As shown in Additional file 1: Figure S1, these CsOFP genes are distributed over the seven cucumber chromosomes. There are five genes distributed on chromosomes 3, four genes on chromosomes 6, three genes on chromosomes 7, two genes on chromosomes 1, 2, 4 and only one gene distributed on chromosomes 5.

Gene structures and phylogenetic analysis of CsOFP genes of cucumber

To explore the evolutionary relationships between cucumber and Arabidopsis, the OVATE domain sequences of AtOFP and CsOFP members were used to generate the phylogenetic tree using MEGA5.0. Both AtOFP and CsOFP members can be divided into four subgroups, named OFP I to OFP IV, except for CsOFP1c, which failed to fall into any subgroup (Fig. 1a). In addition, gene structure analysis showed that most of AtOFPs and CsOFPs have no intron, except for CsOFP19, CsOFP14, AtOFP17 and AtOFP20, which have one intron (Fig. 1b). However, these intron-containing CsOFP and AtOFP genes are distributed in different subgroups, except that the most closely related AtOFP17 and AtOFP20 genes fall into the same subgroup.

To further investigate whether the OVATE domain of CsOFPs are highly conserved in cucumber, we aligned the tomato OVATE and CsOFPs in cucumber by Genedoc program and identified the conserved motifs by MEME (http://meme-suite.org/). The results showed that the OVATE domain contains two conserved motifs, in which they shared some conserved amino residues (Fig. 2a-b). These conserved amino residues may be critical for protein-protein interactions between OFPs and other proteins.
Expression pattern of CsOFPs in cucumber

To further study the temporal and spatial transcription patterns of the CsOFP genes in cucumber development, the expression patterns of all 19 CsOFP genes were examined by semi-quantitative RT-PCR (semi-qRT-PCR) in different tissues, including stem, leaves, tendrils, male floral buds, female floral buds, opened male flowers, opened female flowers, ovary at 4 days before anthesis, ovary at 2 days before anthesis and ovary at anthesis (Fig. 3). The results showed that the organ-specific expression of CsOFP1a, CsOFP6a, CsOFP8a, CsOFP15a and CsOFP15b was not obvious. While CsOFP1b, CsOFP5, CsOFP11 and CsOFP15C were specifically expressed in female floral buds and ovary; CsOFP1c and CsOFP6b were mainly expressed in male and female flowers; CsOFP6c was mainly expressed in opened female flowers; CsOFP16 was specifically enriched in male and female flower and tendrils; and CsOFP7 was highly accumulated in previous period of fruit development. These results suggested that OFP members may function in cucumber fruit and other organ development.

Promoter cis-element analysis

To determine whether CsOFP gene expression is respond to hormone related signaling, the promoter sequences (2000 bp upstream of the start codon) of CsOFPs were analyzed by PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). In total, 11 hormone response cis-elements were identified in CsOFP promoter regions. The number of hormone response cis-elements was variable in each CsOFP promoter region (Fig. 4a). There are ten cis-elements in CsOFP1b promoter region, but only one for CsOFP8a and CsOFP16 (Fig. 4a). These cis-elements are related to responses of auxin, gibberellin (GA), abscisic acid (ABA), salicylic acid (SA), flavonoid and jasmonic acid (JA) (Fig. 4b). For example, TATC-box, GARE-motif and P-box elements are relevant to GA. Moreover, the promoters of most CsOFP genes contain GA and ABA response Elements, rather than auxin and flavonoid elements, indicating that GA and ABA may regulate the expression of CsOFP genes in cucumber.

CsOFP gene expression in response to GA treatment

To further investigate the CsOFP gene expression in response to GA treatment, the transcript abundance of CsOFP7, CsOFP11 and CsOFP12 genes, which have cis-elements related with GA in the promotors, was measured by qRT-PCR following treatment with GA. CsOFP15b with no GA response elements was used as a control. As expected, the expression of CsOFP15b remained unchanged, while the transcript abundance of CsOFP7, CsOFP11 and CsOFP12 genes was up-regulated after spraying GA3 on female flower buds (Fig. 5a-d). The expression level reached the peak after 4h
of treatment and decreased after 12h of treatment. Together, these results confirmed that the expression of *CsOFP7, CsOFP11* and *CsOFP12* is regulated by GA.

**Overexpression CsOFP11 in Arabidopsis**

To investigate the roles of *CsOFP* in fruit development, we selected female floral buds- and ovary-expressed *CsOFP11* for further study. We introduced the *CsOFP11* under the control of 35S promoter into wild-type (WT) Arabidopsis. Three T2 transgenic lines carrying *CsOFP11* were identified. These transgenic lines displayed shorter siliques, although had no significant morphological phenotypes compared to WT (Fig. 6a-b). The transgenic line #34 exhibited shorter silique lengths than other transgenic lines, consistent with their highest expression of *CsOFP11* (Fig. 6c and g). And then the transgenic line #34 was used for further studies. An examination of the width of 15 siliques also showed that the transgenic line #34 had wider siliques compared to WT (Fig. 6d and Additional file 2: Figure S2), consistent with the founding member OVATE’s negative role in tomato fruit elongation. Interestingly, the transgenic line #34 also exhibited blunt siliques (Fig. 6f). Previous study showed the mutation of *STYLISH1(STY1)* and *NGATHA(NGA)*, both participate in the development of gynoecium by activating the expression of *YUC*, resulted in fruit with long and narrow styles in Arabidopsis [22-23]. To better understand of the *CsOFP11* gene function, we detected the expression level of *AtSTY1*, *AtNGA*, and *AtYUC4*. As expected, we found that the expression level of *AtSTY1*, *AtNGA* and *AtYUC4* was moderately decreased at three transgenic lines, especially in transgenic line #34 (Fig. 6h-j). These results indicated that *CsOFP11* may regulate fruit development.

**Discussion**

OVATE domain-containing proteins widely exist in plants. In this study, we identified 19 CsOFPs by BLASTp searches using the amino acid sequences of 19 Arabidopsis AtOFP members. These 19 CsOFP members are distributed on seven chromosomes and most of them have no intron except CsOFP19 and CsOFP14 (Additional file 1: Figure S1 and Fig. 2b). Based the sequences of the conserved OVATE domain of AtOFP and CsOFP, they are clustered into four subgroups except *CsOFP1c*, although the AtOFPs and CsOFPs show low similarity in sequence (Additional file 3: Table S1), indicating that the sequences of OVATE domain are highly conserved in Arabidopsis and cucumber.

Expression patterns analysis of CsOFPs showed that most of them were expressed in reproductive organs, consistent with the previous studies. For example, the tomato *OVATE* gene that is specially expressed in the reproductive organs at the early stages of fruit development [1]; *SIOFP7* also showed high expression in fruit at 20 days post
anthesis [25], indicating that CsOFPs may also regulate fruit development. Otherwise, CsOFP1a, CsOFP6a, CsOFP8a, CsOFP15a and CsOFP15b are ubiquitously expressed in all tissues, which are similar to AtOFP6, AtOFP12, and AtOFP16 in Arabidopsis [5]. In addition to male and female flower, CsOFP16 was also specifically enriched in tendrils. These results suggested that CsOFP members may play important roles in all aspects of development in cucumber.

Recent studies revealed that several OFPs negatively control the fruit length. For example, both OVATE and SIOPFP20 regulate fruit length in tomato [1, 21]. In melon (Cucumis melo), six candidate genes in a QTL associated with shape on chromosome 8 include CmOFP13. In cucumber, the fruit shape QTL fs3.2 interval also contains an OFP member CsOFP15a [21]. In this study, the transgenic plants also exhibited shorter and wider siliques compared to WT plants when overexpression of CsOFP11 in Arabidopsis (Fig. 6b-c). Although genetic evidence is limited, it seems likely that these OFP genes regulate fruit length in several plant species. In addition, the transgenic Arabidopsis overexpressing CsOFP11 also showed blunt siliques (Fig. 6f), a same phenotype also observed in transgenic plants carrying Class III AtOFP genes [5]. Interestingly, the mutation of heterotrimeric G-protein β subunit AGB1 and a putative receptor protein kinase ERECTA (ER) also lead to blunt siliques [26-27]. Whether OFPs regulate silique morphology downstream of ER and AGB1 proteins remains to be examined in the future.

Conclusions

In this study, we identified 19 CsOFPs in cucumber. 14 of these CsOFP members were expressed in reproductive organs. When CsOFP11 transformed into the Arabidopsis, the transgenic lines exhibited shorter but blunt siliques, indicating that these CsOFP members may be involved to fruit development. In the future, it will be worth to investigate the biological function of the CsOFPs in cucumber.

Methods

Plant materials
Cucumber (Cucumis stativus L.) inbred R461, identified by Professor Huazhong Ren (Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, China Agricultural University), were grown in a standard glass greenhouse of China Agricultural University under standard growing conditions. Water and fertilization management were performed according to standard protocol. The Arabidopsis thaliana (Columbia-0), obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio State University, Columbus, OH, USA), was used as wild-type (WT) and grown in greenhouse at 22 °C with a 16-h-light/8-h-dark
photoperiod.

**CsOFPs identification and chromosomal location in cucumber**

The sequences of 19 AtOFPs were collected from the TAIR (https://www.arabidopsis.org/) and were used as queries for BLASTp searches to identify OFP genes in the cucumber genome (http://cucurbitgenomics.org) [28]. These genes were mapped on the chromosomes according to the cucumber genome version 2 in Cucurbit Genomics Database and drew chromosomal distribution of these CsOFP genes by the online tools (http://mg2c.iask.in/mg2c_v2.0/).

**Gene structure, phylogenetic analysis, multiple sequence alignments and promoter sequence analysis.**

The amino acid sequences of OFPs in cucumber and Arabidopsis were obtained from the Cucumber Genome Database (http://cucurbitgenomics.org/) and the TAIR (https://www.arabidopsis.org/). The gene structure analysis was performed by the program GSDS2.0 (http://gsds.cbi.pku.edu.cn/). The multiple sequences were aligned using the program Genedoc and the conserved motifs were identified on line tools MEME (http://meme.sdsc.edu/). And this alignment was used to generate a phylogenetic tree with the Neighbor-Joining method using MEGA5.0 software (bootstrap=1000) [29]. The promoter sequences were obtained from the Cucumber Genome Database and then analyzed by the online tool PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

**GA treatments**

The female flower buds of 40-day-old cucumber were sprayed with 50uM GA3, and they were harvested at 0h, 0.5h, 2h, 4h, 12h, and 24h, respectively. For each time point, 2 female flower buds were harvested as one biological repeat for RNA isolation.

**RNA isolation and gene expression analysis**

Total RNA was isolated using Trizol (Sangon Biotech, Shanghai, China) and reverse-transcribed using Fast King RT Enzyme (Tiangen, Beijing, China). Subsequently, RT-qPCR was performed using a SYBR Premix Ex TaqII Kit (Takara), and cucumber Ubiquitin (Csa000874) and Arabidopsis ACTIN2 are used as the internal references, respectively. Three biological and three technical replicates were performed in each qRT-PCR experiment. Primers used are listed in Additional file 4: Table S2.
Plant transformation

The coding sequence of CsOFP11 was cloned into PBI121 binary vector to generate 35S::CsOFP11 construct by homologous recombination. This construct was transformed into plants by Agrobacterium tumefaciens-mediated floral dip transformation method [30]. Primary transformants were isolated on Murashige and Skoog (MS) medium containing 45mg/L kanamycin. The primers used are listed in Additional file 4: Table S2.

Supplementary Information

Additional file 1: Figure S1. Genome distribution of CsOFP genes in cucumber.
Additional file 2: Figure S2. The width of 15 siliques of WT and CsOFP11 transgenic lines #34. Scale bar, 0.5 cm.
Additional file 3: Table S1. The similarity of OFP members between cucumber and Arabidopsis.
Additional file 4: Table S2. List of primers used in this paper

Abbreviations

BLASTp: Basic Local Alignment Search Tool protein;
qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction;
WT: Wild-type

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests
The authors declare that they have no conflict of interest.
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Authors' contributions
L. H and X. Z conceived and designed the experiments. L. H, X. S, Z. W and X. L performed the experiments. L. H, L. Y, Z. Z, and X. Z analyzed the data. L. H and Z. Z wrote the paper. All authors read and approved the final manuscript.

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References
1. Liu JP, Van Eck J, Cong B, Tanksley SD. A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA. 2002; 99:13302-06.
2. Hedrick UP, Booth NO. Mendelian characters in tomato. Proc. Am. Soc. Hort. Sci. 1907;5: 19-24.
3. Price HC, Drinkard AW. Inheritance in tomato hybrids. Va Agric Exp Stn Bull. 1908;177:17-53.
4. Hackbusch J, Richter K, Muller J, Salamini F, Uhrig JF. A central role of Arabidopsis thaliana ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins. Proc Natl Acad Sci USA. 2005;102:4908-12.
5. Wang SC, Chang Y, Guo JJ, Zeng QN, Ellis BE, Chen JG. Arabidopsis ovate family proteins, a novel transcriptional repressor family, control multiple aspects of plant growth and development. PLoS One. 2011;6:e23896.
6. Liu D, Sun W, Yuan YW, Zhang N, Hayward A, Liu YL, et al. Phylogenetic analyses provide the first insights into the evolution of OVATE family proteins in land plants. Ann Bot. 2014;113:1219-33.
7. Rodriguez GR, Munos S, Anderson S, Sim SC, Michel A, Causse M, et al. Distribution of SUN, OVATE, LC, and FAS in the tomato germplasm and the relationship to fruit shape diversity. Plant Physiol. 2011;156:275-85.
8. Yu H, Jiang WZ, Liu Q, Zhang H, Piao MX, Chen ZD, et al. Expression pattern and subcellular
9. Tsaballa A, Pasentsis K, Darzentas N, Tsafarlis AS. Multiple evidence for the role of an ovate-like gene in determining fruit shape in pepper. BMC Plant Biol. 2015;11:46 (2011).

10. Zhao DS, Li QF, Zhang CQ, Zhang C, Yang QQ, Pan LX, et al. GS9 acts as a transcriptional activator to regulate rice grain shape and appearance quality. Nat Commun. 2018;9:1240.

11. Liu JH, Zhang J, Hu W, Miao HX, Zhang JB, Jia CH, et al. Banana ovate family protein MaOFP1 and MADS-box protein MuMADS1 antagonistically regulated banana fruit ripening. PLoS One. 2015;10:e0123870.

12. Wang SC, Chang Y, Guo JJ, Chen JG. Arabidopsis ovate family protein 1 is a transcriptional repressor that suppresses cell elongation. Plant J. 2007;50:858-72.

13. Pagnussat GC, Yu HJ, Sundaresan V. Cell-fate switch of synergid to egg cell in Arabidopsis eostre mutant embryo sacs arises from misexpression of the BEL1-like homeodomain gene BLH1. Plant Cell. 2007;19:3578-92.

14. Zhao DS, Li QF, Zhang CQ, Zhang C, Yang QQ, Pan LX, et al. GS9 acts as a transcriptional activator to regulate rice grain shape and appearance quality. Nat Commun. 2018;9:1240.

15. Liu YY, Douglas CJ. A role for OVATE FAMILY PROTEIN1 (OFP1) and OFP4 in a BLH6-KNAT7 multi-protein complex regulating secondary cell wall formation in Arabidopsis thaliana. Plant Signal Behav. 2015;10:e1033126.

16. Lazzaro MD, Wu S, Snouffer A, Wang YP, van der Knaap E. Plant organ shapes are regulated by protein interactions and associations with microtubules. Front Plant Sci. 2018;9:1766.

17. Che G, Zhang XL. Molecular basis of cucumber fruit domestication. Curr Opin Plant Biol. 2019;47:38-46.

18. Weng YQ, Colle M, Wang YH, Yang LM, Rubinstein M, Sherman A, et al. QTL mapping in multiple populations and development stages reveals dynamic quantitative trait loci for fruit size in cucumbers of different market classes. Theor Appl Genet. 2015;128;1747-63.

19. Xin TX, Zhang Z, Li S, Zhang S, Li Q, Zhang ZH, et al. Genetic regulation of ethylene dosage for cucumber fruit elongation. Plant Cell. 2019;31:1063-76.

20. Zhang Z, Wang BW, Wang SH, Lin T, Yang L, Zhao ZL, et al. Genome-wide target mapping shows histone deacetylase complex 1 regulates cell proliferation in cucumber fruit. Plant Physiol. 2019; pii: pp.00532.

21. Zhao JY, Jiang L, Che G, Pan YP, Li YQ, Hou Y, et al. A functional allele of CsFUL1 regulates fruit length through inhibiting CsSUP and auxin transport in cucumber. Plant Cell. 2019;31:1289-307.

22. Wu S, Zhang BY, Keyhaninejad N, Rodriguez GR, Kim HJ, Chakrabarti M, et al. A common genetic mechanism underlies morphological diversity in fruits and other plant organs. Nat Commun. 2018;9:4734.

23. Sohlberg JJ, Myrenas M, Kuusk S, Lagercrantz U, Kowalczyk M, Sandberg G, et al. STY1 regulates auxin homeostasis and affects apical-basal patterning of the Arabidopsis gynoecium. Plant J. 2006;47:112-23.
24. Trigueros M, Navarrete-Gomez M, Sato S, Christensen SK, Pelaz S, Weigel D, et al. The NGATHA genes direct style development in the Arabidopsis gynoecium. Plant Cell. 2009;21:1394-409.

25. Wang SC, Chang Y, Ellis B. Overview of OVATE FAMILY PROTEINS, A novel class of plant-specific growth regulators. Front Plant Sci. 2016;7:417.

26. Huang ZJ, Van Houten J, Gonzalez G, Xiao H, van der Knaap E. Genome-wide identification, phylogeny and expression analysis of SUN, OFP and YABBY gene family in tomato. Mol Genet Genomics. 2013;288:111-29.

27. Lease KA, Wen JQ, Li J, Doke JT, Liscum E, Walke JC. A mutant Arabidopsis heterotrimeric G-protein β subunit affects leaf, flower, and fruit development. Plant Cell. 2001;13:2631-42.

28. Torii KU, Mitsukawa N, Oosumi T, Matsuura Y, Yokoyama R, Whittier RF, et al. The Arabidopsis ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. Plant Cell. 1996;8:735-46.

29. Huang SW, Li RQ, Zhang ZH, Li L, Gu XF, Fan W, et al. The genome of the cucumber, Cucumis sativus L. Nat Genet. 2009;41:1275-81.

30. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731-39.

31. Clough SJ, Bent AF. Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 1999;16:735-43.

Figure legends

Fig. 1 Gene structure and phylogenetic analysis of OFPs in Arabidopsis and cucumber.
(a) Phylogenetic tree of OFPs in Arabidopsis and cucumber. The OVATE sequences of OFP members in cucumber (Cucumis sativus) (red triangle) and Arabidopsis thaliana (green circle) were used to generate the phylogenetic tree using MEGA. (b) Gene structures of OFPs. Gene structures were drawn using GSDS (http://gsds.cbi.pku.edu.cn/). Green boxes and black lines indicate exons and introns, respectively. Upstream and downstream sequences are indicated by red boxes.

Fig. 2 The amino acid sequence alignment of OVATE domains of CsOFPs.
(a) Amino acid sequence alignment of OVATE domains in CsOFP proteins. Identical amino acids are shaded in red, and similar amino acids are shaded in orange. (b-c) Two conserved motifs in OVATE protein domain. The overall height of each stack represents the conservation of the sequence at that position, and the height of letters within each stack indicates the relative frequency of the respective amino acid.
Fig. 3 Expression pattern of CsOFPs in different tissues of cucumber.
Semi-quantitative RT-PCR analysis of CsOFPs in different tissues of cucumber. The cucumber UBIQUITIN gene was used as an internal standard.

Fig. 4 Cis-elements in the promoters of CsOFPs responding to plant hormones.
(a) Cis-elements in the promoters of CsOFPs. Promoter sequences were analyzed by PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). (b) The percentages of Cis-elements in the promoters of CsOFPs that are related to hormone response.

Fig. 5 CsOFP expression triggered by GA in female flower buds.
(a-d) The cucumber female flower buds of 40-day-old cucumber were sprayed with 50uM GA3 and the expression level of CsOFP15b (a), CsOFP7 (b), CsOFP11 (c), and CsOFP12 (d) were examined at 0h, 0.5h, 2h, 4h, 12h, and 24h after treatment. The cucumber UBIQUITIN gene was used as an internal standard. Values are means ± sd of three biological replicates, and significant differences are indicated by asterisks (*, P < 0.05 and **, P < 0.01, Student’s t test).

Fig. 6 Ectopic expression of CsOFP11 in Arabidopsis.
(a) Morphology of WT and CsOFP11 transgenic plants. (b) Siliques from the primary inflorescence of WT and CsOFP11 transgenic plants. Scale bar, 0.5 cm. (c-d) Statistical analyses of silique length (c) and width (d) in WT and CsOFP11 transgenic plants. (e-f) Blunt-end siliques in CsOFP11 transgenic plants. (h-j) The expression of AtSTY1 (h), AtNGA3 (i) and AtYUC4 (j) in the inflorescence apex of WT and three CsOFP11 transgenic lines. Actin2 was used as an internal standard. Values are means ± sd of three biological replicates, and significant differences are indicated by asterisks (*, P < 0.05 and **, P < 0.01, Student’s t test).
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Fig. 2 The amino acid sequence alignment of OVATE domains of CsOFPs

The amino acid sequence alignment of OVATE domains of CsOFPs. (a) Amino acid sequence alignment of OVATE domains in CsOFP proteins. Identical amino acids are shaded in red, and similar amino acids.
are shaded in orange. (b-c) Two conserved motifs in OVATE protein domain. The overall height of each stack represents the conservation of the sequence at that position, and the height of letters within each stack indicates the relative frequency of the respective amino acid.

**Figure 3**

Expression pattern of *CsOFPs* in different tissues of cucumber. Semi-quantitative RT-PCR analysis of *CsOFPs* in different tissues of cucumber. The cucumber UBIQUITIN gene was used as an internal
Figure 4

Cis-elements in the promoters of CsOFPs responding to plant hormones. (a) Cis-elements in the promoters of CsOFPs. Promoter sequences were analyzed by PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). (b) The percentages of Cis-elements in the promoters of CsOFPs that are related to hormone response.
CsOFP expression triggered by GA in female flower buds. (a-d) The cucumber female flower buds of 40-day-old cucumber were sprayed with with 50uM GA3 and the expression level of CsOFP15b (a), CsOFP7 (b), CsOFP11 (c), and CsOFP12 (d) were examined at 0h, 0.5h, 2h, 4h, 12h, and 24h after treatment. The cucumber UBIQUITIN gene was used as an internal standard. Values are means ± sd of three biological
replicates, and significant differences are indicated by asterisks (*, P < 0.05 and **, P < 0.01, Student's t test).

Figure 6

Ectopic expression of CsOFP11 in Arabidopsis. (a) Morphology of WT and CsOFP11 transgenic plants. (b) Siliques from the primary inflorescence of WT and CsOFP11 transgenic plants. Scale bar, 0.5 cm. (c-d) Statistical analyses of silique length (c) and width (d) in WT and CsOFP11 transgenic plants. (e-f) Blunt-
end siliques in CsOFP11 transgenic plants. (h-j) The expresion of AtSTY1 (h), AtNGA3 (i) and AtYUC4 (j) in the inflorescence apex of WT and three CsOFP11 transgenic lines. Actin2 was used as an internal standard. Values are means ± sd of three biological replicates, and significant differences are indicated by asterisks (*, P < 0.05 and **, P < 0.01, Student's t test).

Supplementary Files

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- Supplementarymaterial.pdf