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

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

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29 **Abstract**

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

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

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

43

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

45

46 **Background**

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

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

82 Cucumber (*Cucumis sativus* L.) is an important horticultural crop and the fruit shape
83 indexes are important quantitative characters in the process of breeding [16-17].

84 Recently, a few of genes are shown to regulate cucumber fruit length. For example, the
85 RING-type E3 ligase short fruit 1 (SF1) promotes fruit length by regulating cell division
86 in cucumber via degradation of 1-aminocyclopropane-1-carboxylate synthase 2 (ACS2),
87 a rate-limiting enzyme for ethylene biosynthesis [18]. Short Fruit 2 (SF2), a Histone
88 Deacetylase Complex 1 (HDC1) homologue, also positively regulates fruit length by
89 promoting cell proliferation [19]. Moreover, a MADS-box transcription factor CsFUL1
90 negatively regulate fruit length via inhibits the expression of *SUPERMAN* and auxin
91 transporters *PIN1/7* [20]. Although these OFPs have been studied several plant species,
92 only *CsOFP15a* was reported as a candidate gene regulating the fruit length in
93 cucumber [21]. But whether CsOFP members regulate cucumber fruit development
94 remains largely unknown.

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

101

102

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, leave, 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 promoters, 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 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 plants²⁴. 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]; *SLOFP7* 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 *SIOFP20* 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 sativus* 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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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 $\pm$ 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 $\pm$ sd of three biological replicates, and significant differences are indicated by asterisks (*, $P < 0.05$ and **, $P < 0.01$, Student's *t* test).

Figures

Fig. 1 Gene structure and phylogenetic analysis of OFPs in Arabidopsis and cucumber

Figure 1

Gene structure and phylogenetic analysis of OFPs in Arabidopsis and cucumber. (a)Phylogenetic tree of OFPs in Arabidopsis and cucumber. The OVATE sequences ofOFP 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

Figure 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

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

standard.

Fig. 4 *Cis*-elements in the promoters of *CsOFPs* responding to plant hormones

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.

Fig. 5 *CsOFP* expression triggered by GA in female flower buds

Figure 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 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 $\pm$ 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

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 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 $\pm$ 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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