Fu L *et al.* (2025)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 53, Issue 4, Article number 14590
DOI:10.15835/nbha53414590
Research Article

Pan-genome wide identification and expression analysis of the *OFP* family genes in response to abiotic and biotic stresses in cucumber

Lili FU[1a], Wenhan YIN[2b], Jingyu CHEN[1], Jia'ni WANG[1], Yuanxiu WANG[1], Xinghui ZHOU[3*], Haoju LIU[1*]

[1]*Jiangxi Agricultural University, College of Bioscience and Bioengineering, Nanchang, 330045, China; 15970686602@163.com; 3197513044@qq.com; wjn20050520@qq.com; wangyuanxiu2002@jxau.edu.cn; liuhaoju@mail.jxau.edu.cn (*corresponding author)*
[2]*South China Agricultural University, College of Life Sciences, Guangzhou, 510642, China; 1437474518@qq.com*
[3]*Guilin Tourism University, Guilin, 541000, China; zxh4541311@126.com (*corresponding author)*
[a,b]*These authors contributed equally to the work*

Abstract

Ovate family proteins (OFPs), comprising a recently characterized family of transcriptional repressors, contribute to various plant growth and developmental processes. However, systematic research on the *OFP* gene family in cucumber (*Cucumis sativus* L.) is still lacking. In this work, we conducted a pan-genome-wide identification of the *OFP* gene family in cucumber, and the chromosomal locations, gene structure and protein properties, *cis*-elements, collinearity and phylogenetic relationship of the *CsOFP* family members were analyzed. Phylogenetic analysis classified the cucumber OFP proteins into nine distinct subgroups, with members of the same clade sharing similar motif arrangements. Notably, all *CsOFP* genes lack introns except for *CsOFP15* and *CsOFP20*. Expression analysis identified distinct transcriptional patterns for *CsOFP* genes, including tissue-specific accumulation (notably in roots, ovaries, and flowers) and differential expression under diverse abiotic (cold, heat, salt) and biotic (powdery mildew, root-knot nematode, downy mildew) stresses. These findings presented here will help to understand the functions of *CsOFP* genes in cucumber development, growth, and stress response.

Keywords: abiotic stress; biotic stress; cucumber (*Cucumis sativus* L.); expression profile; ovate family protein (OFP)

Introduction

OVATE family proteins (OFPs) are plant-specific transcriptional repressors and are acknowledged for their roles in important growth and developmental processes in land plants (Dangwal and Das, 2018). The *OFP* gene was first identified in tomato, where its mutation was shown to promote fruit elongation,

Received: 19 May 2025. Received in revised form: 25 Nov 2025. Accepted: 25 Nov 2025. Published online: 10 Dec 2025.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

transforming round fruits into pear-shaped (Liu *et al*., 2002). Structurally, OFP proteins contain a conserved 70 amino acid C-terminal domain, which was designated as OVATE domain and can specifically interact with other transcription factors, forming complex protein interaction networks that participate in organ morphogenesis and stress responses (Liu and Douglas, 2015; Wang *et al*., 2016; Tan *et al*., 2021).

Over the years, the *OFP* genes have been identified in many plants, prompting extensive research into their biological functions (Snouffer *et al*., 2020). Many studies have revealed that *OFP* genes widely participate in plant growth and developmental processes. For example, AtOFP1 interacts with AtBLH3 to form a functional protein complex that modulates the timing of vegetative-to-reproductive phase transition in *Arabidopsis* (Zhang *et al*., 2016). In addition, AtOFP1 modulates gibberellin homeostasis through transcriptional repression of *AtGA20ox1*, consequently restricting cellular elongation (Wang *et al*., 2007). AtOFP4 regulates secondary cell wall formation through its interaction with KNAX7 protein (Li *et al*., 2011). Two tomato OFP proteins, OVATE and SlOFP20, were found to genetically and physically interact with specific members of the TONNEAU1 RECRUITMENT MOTIF (TRM) proteins, forming a regulatory network that coordinates cell division and organ shape (Wu *et al*., 2018; Zhang *et al*., 2023). Importantly, the OFP-TRM interaction module appears to be evolutionarily conserved across plant species as a fundamental mechanism governing organ shape determination. OsOFP9 directly interacts with the brassinosteroid (BR) signaling modulators GS9 and DLT, coordinately regulating leaf angle and grain size in rice by opposing their transcriptional activities (Lu *et al*., 2025). These systematic investigations collectively establish OFPs as master regulators that orchestrate developmental programs through transcriptional networks and protein-protein interactions in higher plants. Moreover, OFPs are particularly interesting as some have been verified to play critical roles in regulating plant responses to diverse stresses. For example, it has been established in rice that OsOFP6 significantly contributes to tolerance against both cold and drought (Ma *et al*., 2017). Overexpression of *Populus trichocarpa PtOFP1* in *Arabidopsis* enhanced drought tolerance across developmental stages, improving both seedling survival and mature plant viability (Wang *et al*., 2021a). Overexpression of *TaOFP29a-A* in transgenic wheat improved drought tolerance, resulting in enhanced root growth and biomass accumulation under drought stress (Wang *et al*., 2020). These findings suggested that plant OFPs serve as key regulators in regulating plant growth and development, as well as various stresses.

Cucumber (*Cucumis sativus* L.) has gained widespread popularity as an important cash crop due to its refreshing taste and unique flavor characteristics. However, during cultivation, cucumber varieties are susceptible to various biotic and abiotic stresses that can significantly compromise yield. Building on the prior identification of 20 *CsOFP* genes (Han *et al*., 2022), this study systematically characterized their tissue-specific and stress-responsive expression patterns and also uncovered prevalent amino acid variations across different accessions using the cucumber pan-genome. We comprehensively characterized the *CsOFP* genes through phylogenetic, structural, and promoter analyses, and utilized RNA-seq data to determine their expression patterns across tissues and under various stresses. These findings establish a valuable foundation for elucidating the biological functions and regulatory mechanisms of *CsOFP* genes in cucumber growth, development, and stress responses.

Materials and Methods

Pan-genome-wide identification of OFP genes from Cucumis sativus

The cucumber genome sequence data were downloaded from the Cucurbit Genomics Data website (http://cucurbitgenomics.org/organism/20), including three East Asian cultivated accessions (9930, XTMC, and Cu2), one Xishuangbanna cultivated accession (Cuc80), three Indian wild accessions (Cuc64, W4, and W8), three Eurasian cultivated accessions (Cuc37, Gy14, and 9110gt), and two Indian cultivated accessions

(Hx14 and Hx117). The Hidden Markov model (HMM) profile of the OVATE domain (PF04844) was retrieved from the Pfam database (http://pfam.xfam.org/) and employed as a query to systematically screen the cucumber genome sequence data using HMMER3.0 with a stringent e-value cutoff of $1 \times e^{-5}$. The OFP proteins were individually verified using PFAM and the Simple Modular Architecture Research Tool (SMART, http://smart.embl-heidelberg.de/) to confirm the presence of conserved OFP domains. Proteins lacking the OVATE domain were removed, and sequences without complete reading frames were manually deleted to finalize the set of identified cucumber *OFP* gene family members.

Chromosome distribution and sequence analysis

The chromosomal positions and lengths of *OFP* gene family members in the cucumber genome were retrieved from the Cucumber Genome Database, and their chromosomal locations were visualized using the MG2C website (http://mg2c.iask.in/mg2c_v2.1/index.html). Protein sequences, coding sequence (CDS) lengths, and genomic DNA (gDNA) lengths of each member were also obtained from the cucumber database. Molecular weights (MWs), isoelectric points (pIs), and total average hydrophobicity (GRAVY) values of cucumber OFP proteins were predicted via the ExPASy tool (http://web.expasy.org/protparam/). Subcellular localization of OFP proteins was further analyzed using the Plant-mPLoc server (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/).

Phylogenetic tree construction, gene structure analysis and conserved motif identification

The gene IDs of the *OFP* family members in *Arabidopsis* were retrieved from a previous study (Wang *et al*., 2011), and their corresponding protein sequences were downloaded from TAIR (www.arabidopsis.org/). Subsequently, multiple sequence alignment of all OFP protein sequences from cucumber and *Arabidopsis* was performed, and a phylogenetic tree was constructed with MEGA11 based on the alignment results. The phylogenetic tree was created using the neighbor-joining (NJ) method. Similarly, a separate phylogenetic tree for the CsOFP gene family was constructed using MEGA11. Gene sequence information including mRNA and corresponding genomic DNA (gDNA) sequences was downloaded from the cucumber genome database, and gene structure visualization was conducted using TBtools software (Chen *et al*., 2023). Motif composition analysis of cucumber OFP proteins was predicted via the online tool Multiple Expectation Maximization for Motif Elicitation (MEME, http://meme-suite.org/tools/meme).

Cis-element and synteny analysis

For *cis*-element analysis, genomic DNA sequences spanning 2.0 kb upstream of the translation initiation codon for cucumber *OFP* genes were extracted using TBtools and submitted to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to identify *cis*-regulatory elements associated with developmental growth, hormone and stress responsiveness. To analyze the replication events involving the *OFP* genes across species, genome data for cucumber, *Arabidopsis*, and tomato were downloaded, and synteny analysis of *OFP* genes in cucumber, *Arabidopsis*, and tomato was performed using the One Step MCScanX module in TBtools employing the standard settings based on the previous description (Zhao *et al*., 2024).

Expression analysis of the CsOFP genes according to RNA-seq data

For analyze the expression patterns of cucumber *CsOFP* genes across different tissues, RNA-seq data related to different tissues including root, stem, leaf, male flower, female flower, ovary, and tendril were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) under BioProject ID PRJNA80169. We determined the expression patterns of *CsOFP* genes under various abiotic stresses by analyzing RNA-seq data from the following published datasets: cold stress (0, 2, 6, and 12 h at 6 °C; BioProject ID PRJNA438923), heat stress

(0, 3, and 6 h at 42 °C; BioProject ID PRJNA634519), and salt stress (leaves and roots of seedlings after salt treatment; BioProject IDs PRJNA477930 and PRJNA511946). Gene expression profile data under various biotic stresses were obtained in public available transcriptome data from NCBI including powdery mildew (PM, *Sphaerotheca fuliginea* infection, PRJNA321023), downy mildew (DM, *Pseudoperonospora cubensis* infection, PRJNA285071), and (RKN, *Meloidogyne incognita* infection, PRJNA419665) as previously described (Yang *et al*., 2022; Wang *et al*., 2024; Xu *et al*., 2025). The expression of the *CsOFP* genes was analyzed based on these data, and the expression values are indicated as the transcripts per kilobase million (TPM) values. The expression values were $\log_2$-transformed using TBtools software to generate heat maps (Chen *et al*., 2023), and differentially expressed genes (DEGs) were defined based on a criterion of $\log_2$(fold change) ≤ -1 or $\log_2$(fold change) ≥ 1 versus the control.

Results

Pan-genome-wide identification of CsOFP genes from C. sativus

To study the variation of *CsOFP* genes, we initially quantified the number of *CsOFP* genes in 12 different cucumber varieties, and their accession genomes were obtained based on available genomic data from earlier studies (Li *et al*., 2022; Wang *et al*., 2024). Through HMMER searches and online database comparisons, we determined the composition of the *OFP* gene family in each cucumber accession. As shown in Table A1, a total of 20 *CsOFP* genes were identified and ordered based on their chromosomal positions. Amongst them, all 20 genes were present in 12 selected cucumber accessions, while the lines "XTMC", "Cu2", and "HX14" contained only 19 *CsOFP* genes (Table A1).

To comprehensively characterize the *CsOFP* genes across different cucumber accessions, we first performed statistical analyses of the identified CsOFP protein sequence lengths. As shown in Table A2, only two CsOFPs (CsOFP4 and CsOFP7) exhibited completely identical protein sequence lengths across all 12 studied cucumber accessions. In addition, among the 12 accessions, CsOFP3, CsOFP8, and CsOFP13 each exhibited two protein length variants, one of which was unique to a single accession ("CUC64", "W8", and "CUC37", respectively) (Table A2). In contrast, CsOFP10, CsOFP17, and CsOFP19 also had two variants, but both were distributed across several accessions. The remaining CsOFPs exhibited quite a few variations in protein sequence lengths across different cucumber accessions (Table A2), suggesting potential functional diversification of them among these cultivars.

Characterization of CsOFP family genes from v2 version of Chinese Long 9930

Subsequent analyses primarily focused on the genes identified in the Chinese Long '9930' v2 genome, as it encompassed all 20 CsOFPs whose protein lengths were representative of diverse cucumber varieties. Among the 20 *CsOFPs*, *CsOFP5* had the longest gDNA (1588 bp), while *CsOFP6* had the shortest gDNA (225 bp). In contrast, *CsOFP17* possessed the longest CDS (1407 bp), and *CsOFP6* had the shortest CDS (225 bp) (Table 1). The encoded proteins ranged from 74 to 468 amino acids in length, with molecular weights spanning 8.57-54.43 kDa (CsOFP6 being the smallest and CsOFP17 the largest). Their isoelectric points (pI) ranged from 4.34 to 10.37, with an equal distribution of acidic (50%, pI < 7) and basic (50%, pI > 7) (Table 1). All CsOFP proteins exhibited negative GRAVY values (-0.419 to -0.38), confirming that they are hydrophilic. Subcellular localization predictions using Plant-mPLoc revealed that most CsOFP proteins localized to the nucleus, except for CsOFP8 (extracellular), CsOFP9 (chloroplast), CsOFP13 (cytoplasm), and CsOFP16 (mitochondria) (Table 1).

Table 1. Basic information of the *OFP* gene family in cucumber

Name	Gene ID	Chromosome	Location	gDNA (bp)	CDS (bp)	Protein				
						Length (aa)	pI	MW (kDa)	GRAVY	Subcellular prediction
CsOFP1	Csa1G168910.1	1	10494259-10494916	658	516	171	6.08	19.04	-0.646	Nucleus
CsOFP2	Csa1G246610.1	1	12389294-12390133	840	741	246	10.37	28.24	-0.9	Nucleus
CsOFP3	Csa2G004680.1	2	751409-752247	839	720	239	10.17	27.8	-0.82	Nucleus
CsOFP4	Csa2G361530.1	2	17235991-17236828	838	813	270	9.56	31.05	-0.738	Nucleus
CsOFP5	Csa3G146670.1	3	9886324-9887911	1588	618	205	5.87	23.13	-0.419	Nucleus
CsOFP6	Csa3G203770.1	3	13934510-13934734	225	225	74	10.36	8.57	-0.255	Nucleus
CsOFP7	Csa3G730160.1	3	27275911-27277195	1285	1032	343	9.72	38.81	-0.752	Nucleus
CsOFP8	Csa3G778360.1	3	30063619-30064457	839	705	234	5.47	25.89	-0.361	Extracellular
CsOFP9	Csa3G778370.1	3	30074776-30075486	711	504	167	9.47	18.65	-0.237	Chloroplast
CsOFP10	Csa4G038760.1	4	3296230-3297352	1123	906	301	9.06	34.47	-0.842	Nucleus
CsOFP11	Csa4G332100.1	4	13407761-13408980	1220	1008	335	9.81	37.65	-0.827	Nucleus
CsOFP12	Csa5G613560.1	5	24153619-24154666	1048	834	277	5.2	30.99	-0.591	Nucleus
CsOFP13	Csa6G046300.1	6	3736954-3737822	869	684	227	4.7	25.96	-0.424	Cytoplasm
CsOFP14	Csa6G212870.1	6	12904894-12905448	555	555	184	9.85	21.96	-0.55	Mitochondria
CsOFP15	Csa6G454380.1	6	21768374-21769438	1065	858	285	4.34	31.99	-0.535	Nucleus
CsOFP16	Csa6G512880.1	6	26488823-26489433	611	507	168	6.1	19.57	-0.827	Nucleus
CsOFP17	Csa6G520290.1	6	27741376-27742885	1510	1407	468	9.56	54.43	-1.103	Nucleus
CsOFP18	Csa7G234150.1	7	8301163-8301890	728	594	197	6.9	22.25	-0.401	Nucleus
CsOFP19	Csa7G388340.1	7	14375342-14376328	987	837	278	5.03	30.86	-0.545	Nucleus
CsOFP20	Csa7G446730.1	7	17708434-17709411	978	750	249	5.35	27.52	-0.886	Nucleus

Phylogenetic analysis of the OFP gene family between cucumber and Arabidopsis

To analyze the evolutionary relationships among cucumber OFP proteins, we first performed a multiple sequence alignment of all 19 Arabidopsis and 20 cucumber OFP proteins using MAFFT and then constructed a neighbor-joining (NJ) phylogenetic tree with MEGA11. As shown in Figure 1, these OFP proteins were classified into nine subgroups (designated Ia to Ij, with the exception of Ig), which is consistent with previous studies (Li *et al*., 2019b). The cucumber OFP proteins were distributed across all nine subgroups: Ih contained three members (CsOFP5, CsOFP18, and CsOFP19); Ii had one (CsOFP20); Ij comprised four (CsOFP13, CsOFP8, CsOFP12, and CsOFP15); Ib included two (CsOFP3 and CsOFP2); Ic contained one (CsOFP10); Ia had four (CsOFP6, CsOFP4, CsOFP1, and CsOFP9); If consisted of two (CsOFP16 and CsOFP17); Id

contained one (CsOFP14); and Ie included two members (CsOFP7 and CsOFP11). The phylogenetic analysis showed that each cucumber OFP clusters with at least one *Arabidopsis* homolog, providing evidence for a shared evolutionary origin (Figure 1).

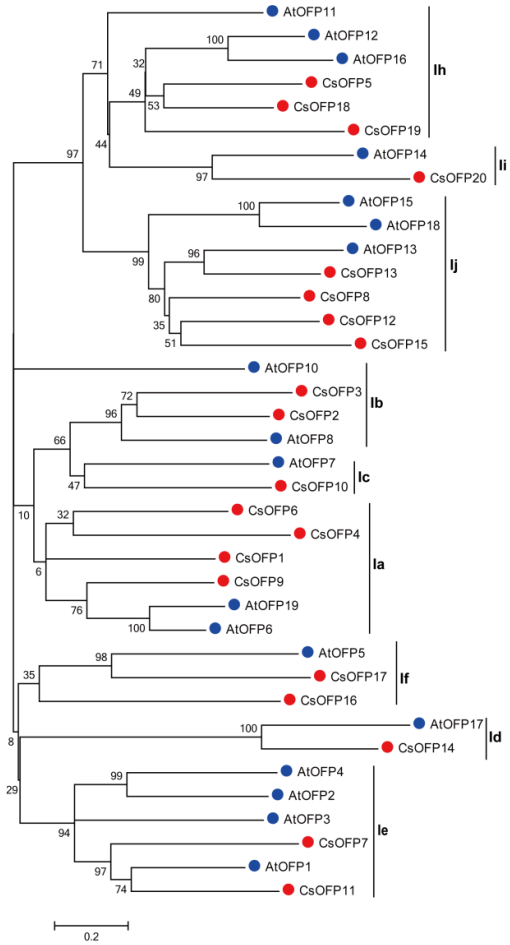

Figure 1. Evolutionary relationship analysis of *OFP* family proteins between cucumber and *Arabidopsis*. The NJ tree was constructed using MEGA11 with 1000 bootstrap replicates

Conserved domain analysis of CsOFP proteins

To analyze the conservation of cucumber OFP proteins, we identified their conserved motifs using the MEME tool, revealing 10 distinct motifs (Motif 1-10; Figure 2). Nearly all cucumber OFP proteins contained Motif 1 and Motif 2, except for CsOFP2/CsOFP20 (lacking Motif 1) and CsOFP6 (lacking Motif 2) (Figure 2). In addition, phylogenetically closely related cucumber OFP proteins exhibited similar conserved motif compositions. For instance, CsOFP5 and CsOFP18 (both in subgroup Ih) shared an identical motif order: Motif 5, Motif 2, and Motif 1. Notably, some CsOFPs possessed unique compositions: CsOFP8/CsOFP16 contained Motif 9; CsOFP1/CsOFP17 featured Motif 10; and CsOFP19 harbored three copies of Motif 8 (Figure 2). These substantial motif variations among the 20 CsOFPs suggest potential functional divergence of them.

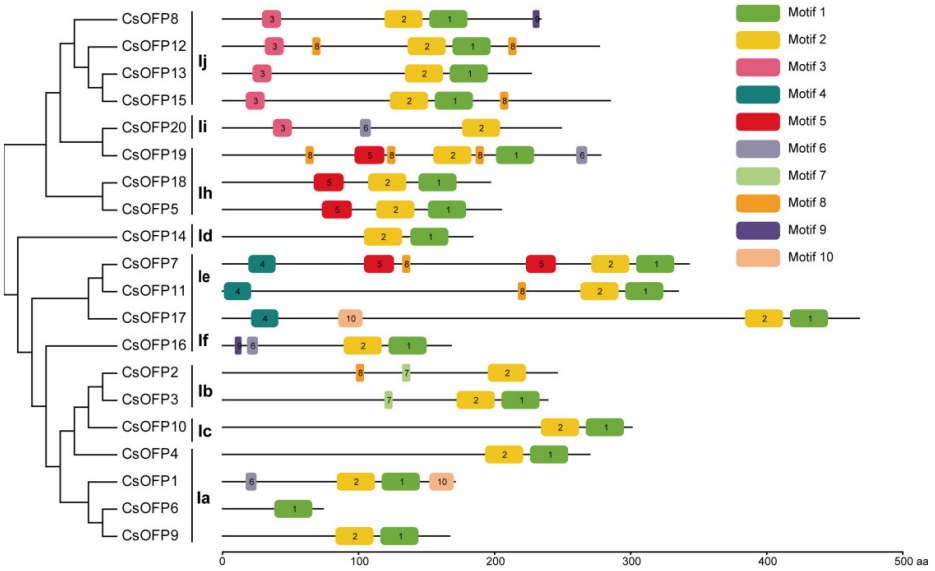

Figure 2. Conserved motif analysis of cucumber *OFP* family proteins based on the phylogenetic relationship. Different motifs are represented by different colored boxes with numbers 1-10

Gene structure analysis of CsOFP genes

To determine the structural characteristics of cucumber *OFP* genes, we analyzed the exon-intron distribution patterns of the *OFP* gene family using the online tool GSDS. As a result, a relatively simple gene structure was found among cucumber *OFPs*. Only *CsOFP15* and *CsOFP20* contained introns, while all other members consisted solely of exons and have no intron (Figure 3). This structural simplicity may reflect evolutionary conservation within the cucumber *OFP* gene family.

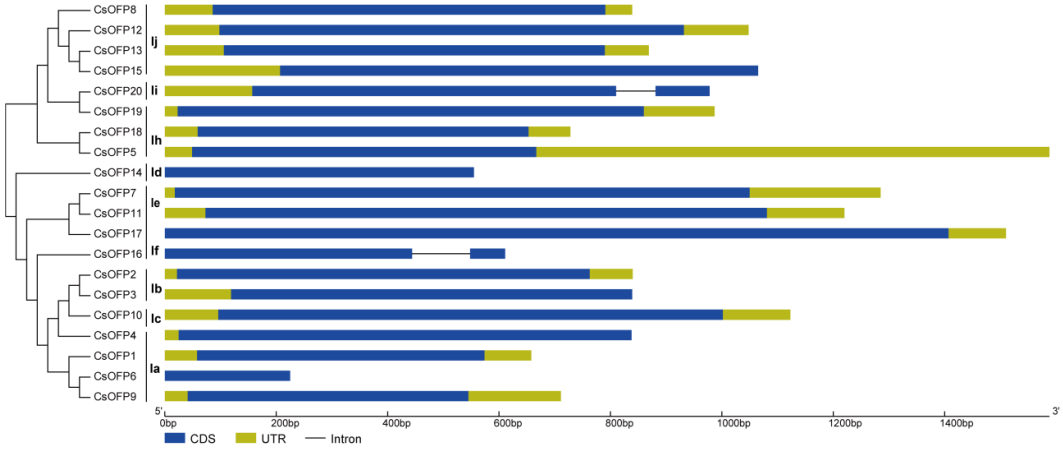

Figure 3. Gene structure analysis of *CsOFP* genes. The yellow and blue boxes indicate UTRs and CDSs, respectively

Chromosomal location and synteny analysis of CsOFP genes

We extracted the chromosomal locations of *CsOFP* genes from the genome annotation files, and the chromosomal location of *CsOFP* genes were figured using the MG2C online tool for visualization (Figure 4). All of the *CsOFP* genes were distributed across all seven cucumber chromosomes, with the highest density

observed on chromosome 3 (4 genes) and chromosome 6 (5 genes). Notably, chromosome 5 contained only a single gene (*CsOFP17*) (Figure 4).

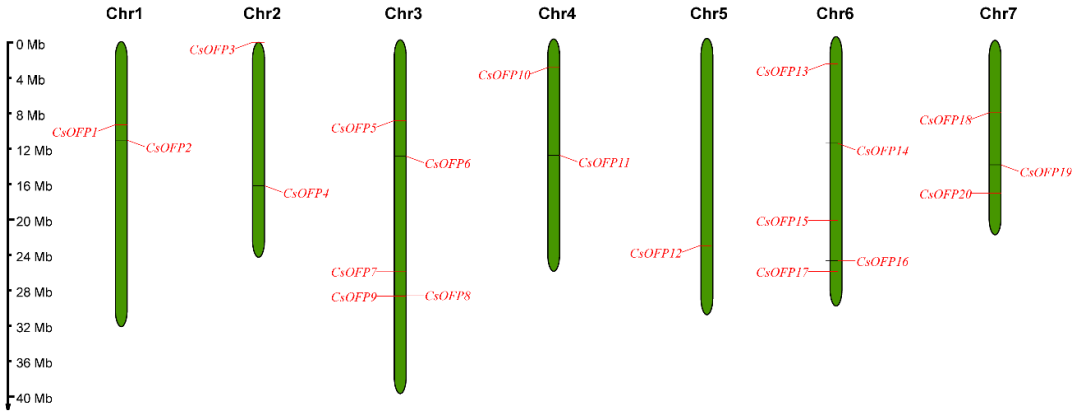

Figure 4. Chromosome distribution of *CsOFP* genes. The scale is in megabases (Mb). The scale bar on the left indicates the length of the chromosome

We further performed collinearity analysis of *OFP* gene family members among cucumber, *Arabidopsis*, and tomato (Figure 5). The results demonstrated that 14 orthologous gene pairs were identified between cucumber and *Arabidopsis*, and cucumber *OFP* genes also shared 14 gene pairs with tomato, suggesting conserved evolutionary relationships among these species (Figure 5).

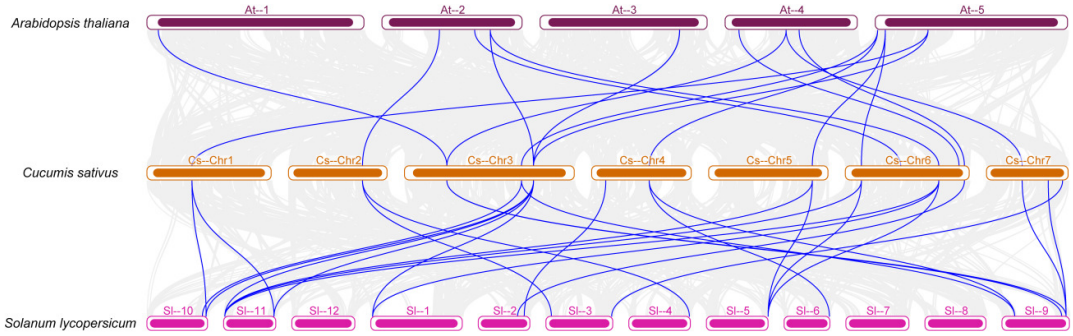

Figure 5. Collinearity analysis of *OFP* genes among cucumber, *Arabidopsis*, and tomato. The syntenic *OFP* gene pairs were highlighted with red lines

Notably, all *OFP* members showed syntenic relationships between cucumber and tomato, indicating high genomic conservation during their long-term divergence as closely related species (Figure 5).

Cis-elements analysis of putative CsOFP promoters

To elucidate the transcriptional regulation and potential functions of cucumber *CsOFP* genes, 2.0-kb sequence upstream of the translation initiation codon of each *CsOFP* gene was analyzed by PlantCARE for *cis*-acting element prediction. In total, 30 *cis*-elements were identified in the putative promoters of the *CsOFP* genes, including 6, 11, and 13 kinds of development-, hormone-, and stress-responsive *cis*-elements (Figure 6). Amongst these *cis*-elements, MYB (drought-responsive element) and ARE (anaerobic induction element), were most abundant and present in nearly all 20 *CsOFP* genes. In addition, a series of *cis*-elements related to growth and stress responsiveness, such as as-1, CAT-box, MBS, STRE, and others, were identified in the promoter regions of *CsOFP* genes. Furthermore, ethylene-responsive elements (ERE) were identified in 18

CsOFP genes, abscisic acid-responsive elements (ABRE) in 13 genes, gibberellin-responsive elements (GARE/P-box/TATC-box) in 12 genes, salicylic acid-responsive elements (TCA-element) in 12 genes, methyl jasmonate-responsive motifs (TGACG/CGTCA) in 8 genes, and auxin-responsive elements (AuxRR-core/TGA-element) in 8 genes (Figure 6). These findings suggest that *CsOFP* genes play crucial roles in various cucumber growth and developmental processes by participating in hormone metabolism and signal transduction networks.

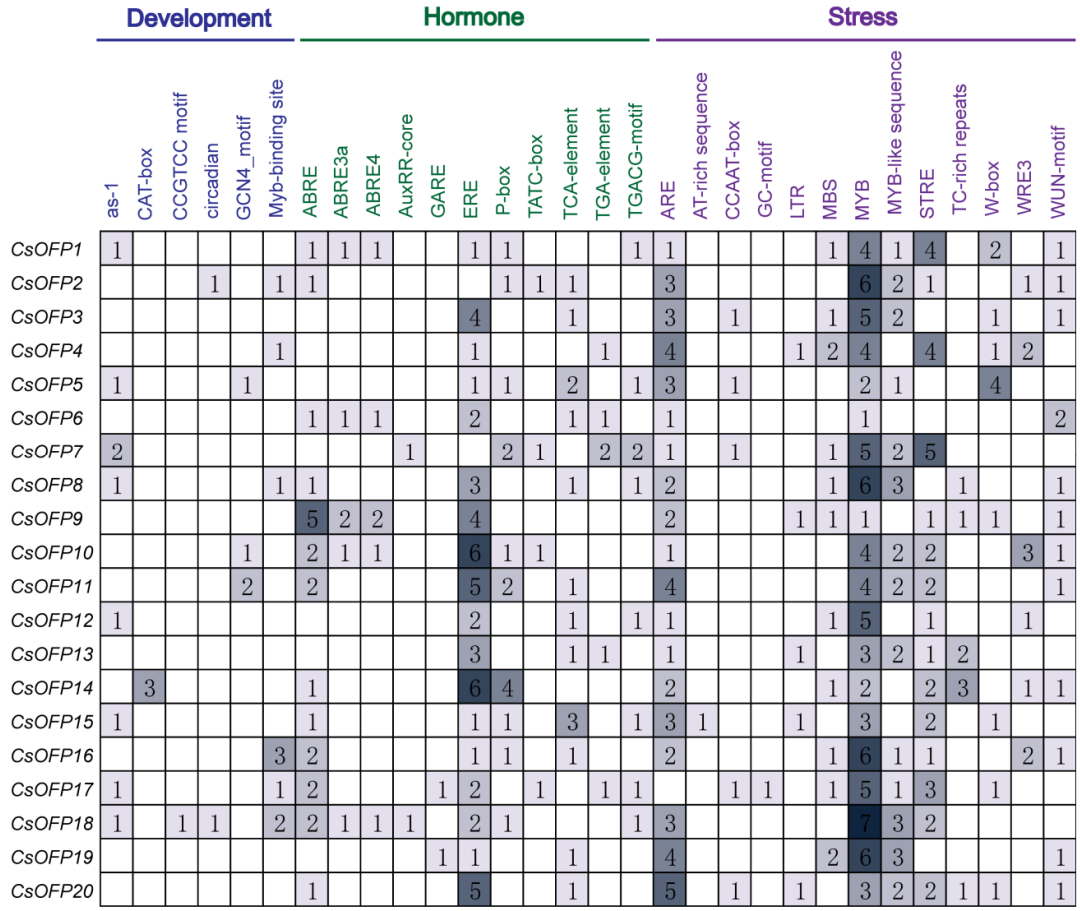

Figure 6. The *cis*-elements analysis of promoter regions in *CsOFP* genes

Expression of CsOFP genes in various tissues

To further investigate the expression patterns of cucumber *OFP* genes across different tissues, we analyzed publicly available RNA-seq data (Figure 7). The results revealed that four genes (*CsOFP2*, *CsOFP5*, *CsOFP12*, and *CsOFP20*) exhibited relatively high expression levels in all examined plant tissues, suggesting they may function as constitutively expressed genes closely associated with growth and developmental regulation. Notably, *CsOFP7*, *CsOFP11*, *CsOFP14*, and *CsOFP20* showed significantly higher expression in roots compared to other tissues, indicating their potential roles in root growth. *CsOFP5* displayed particularly strong expression in tendrils, implying its specific involvement in tendril development (Figure 7). Furthermore, elevated expression of *CsOFP7*, *CsOFP12*, *CsOFP15*, and *CsOFP20* in both female and male flowers suggests their functional importance in floral development (Figure 7).

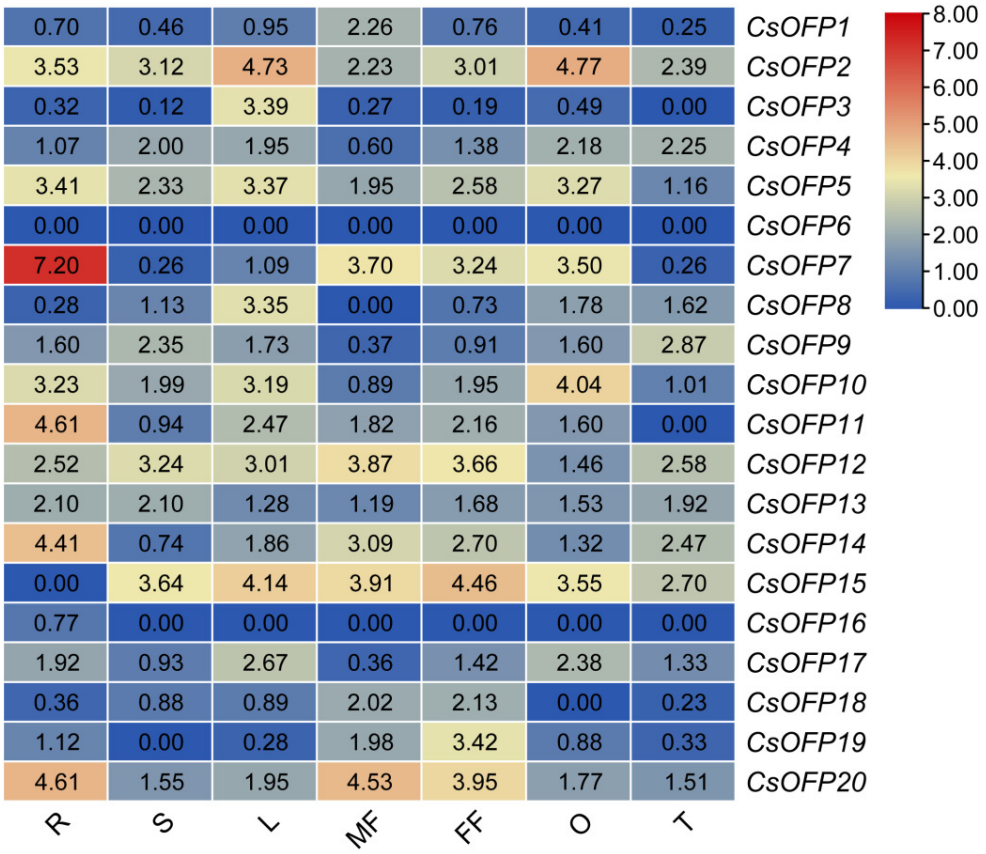

Figure 7. Expression of *CsOFP* genes in various cucumber tissues

The color scale from red to blue represent the log2(TPM+1) values of each *CsOFP* gene. R, root; S, stem; L, leaf; MF, male flower; FF, female flower; O, ovary; T, tendril

Expression patterns of CsOFP genes under diverse abiotic stresses

To investigate the effects of abiotic stress on the expression of cucumber *CsOFP* genes, this study analyzed existing RNA-seq data to examine their expression patterns under various abiotic stress conditions. Under cold stress, only 7 *CsOFP* genes exhibited significant expression changes. Notably, *CsOFP12* and *CsOFP14* showed marked upregulation, while *CsOFP1*, *CsOFP8*, *CsOFP9*, *CsOFP16*, and *CsOFP20* were significantly downregulated under cold stress (Figure 8A). Under heat treatment, a total of 10 *CsOFP* genes showed significant upregulation, while 2 *CsOFP* genes were downregulated. Notably, *CsOFP7* and *CsOFP19* exhibited the most pronounced upregulation at 6 h under heat treatment (Figure 8B). Under salt stress conditions, only three *CsOFP* genes exhibited significant differential expression. Compared to the control, *CsOFP16* showed marked upregulation in leaves but no significant change in roots under salt treatment. Additionally, *CsOFP16* expression was significantly downregulated in salt-stressed roots, while *CsOFP15* displayed notable downregulation in leaves under salt stress (Figure 8C).

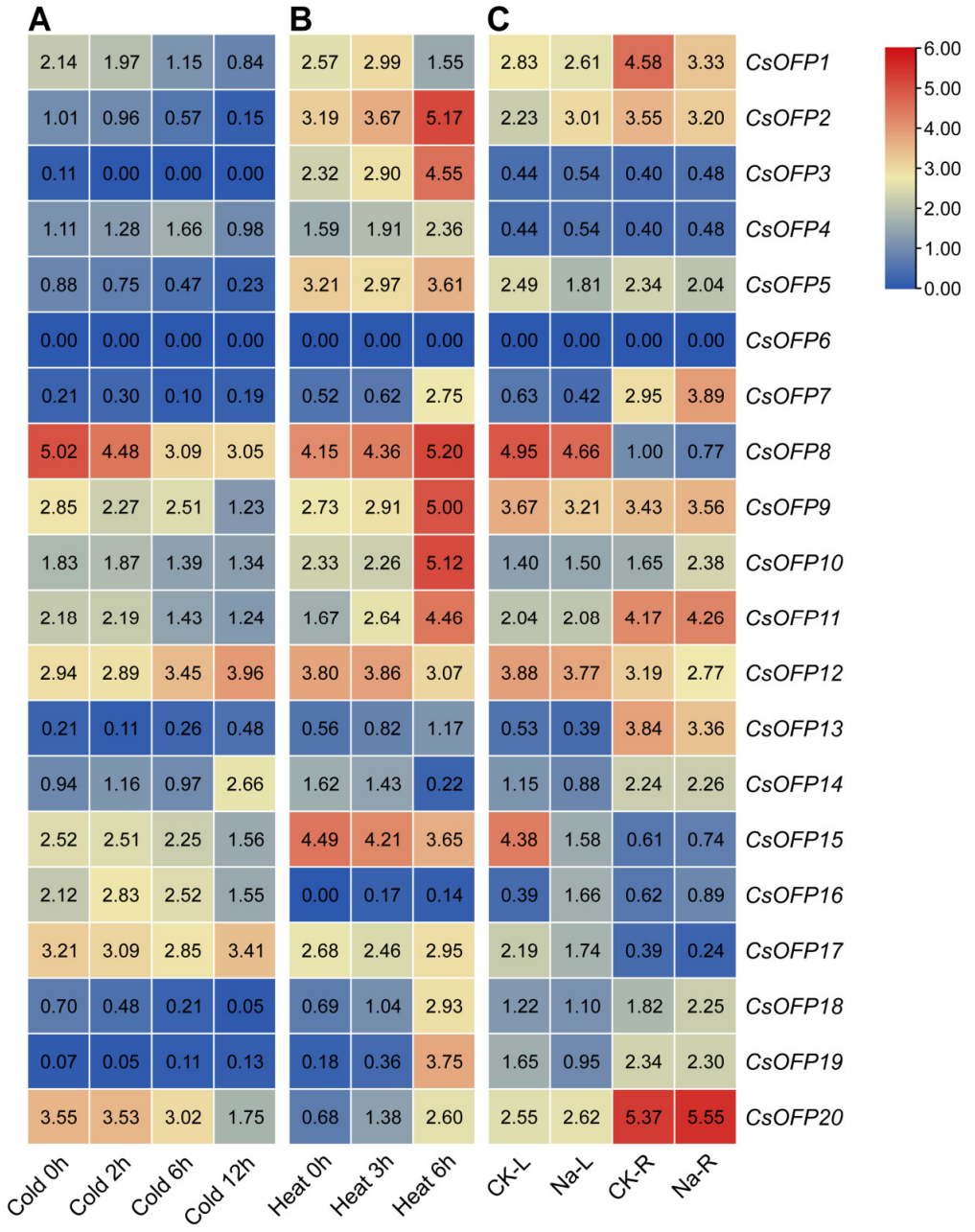

Figure 8. Expression analysis of *CsOFP* genes in response to various abiotic stresses including cold (A), heat (B), and salt (C)

CK-L and Na-L denote leaf samples from control and salt-stressed plants, respectively; CK-R and Na-R represent root samples from control and salt-stressed plants, respectively. The data shown in the boxes represent log2(TPM+1) values. Compared to the control, *CsOFP* genes with a threshold of |log2 (fold change)| ≥ 1 were defined as differentially expressed

Gene expression patterns of CsOFPs in response to various biotic stresses

To identify *CsOFP* genes involved in biotic stress responses, we analyzed RNA-seq data from cucumber plants infected with *Sphaerotheca fuliginea* (PM treatment), *Meloidogyne incognita* infection (RKN treatment), and *Pseudoperonospora cubensis* (DM treatment), calculating TPM values and generating expression heatmaps.

The results revealed differential expression patterns of *CsOFP* genes under the three biotic stress conditions using both susceptible and resistant cucumber lines (Figure 9). Under PM treatment, three and 6 *CsOFP* genes showed upregulated expression in susceptible line (D8) and resistant line (SSL508-28), respectively. Notably, the expression of *CsOFP4* was upregulated in the susceptible line, while the transcripts of *CsOFP11*, *CsOFP13*, *CsOFP15*, and *CsOFP20* were exclusively upregulated in resistant line. Both *CsOFP3* and *CsOFP16* exhibited upregulated expression in both types of lines (Figure 9A), suggesting their crucial roles in PM resistance. Under root-knot nematode (RKN) treatment, a total of 9 *CsOFP* genes exhibited differential expression. Among them, *CsOFP2* and *CsOFP7* showed significant upregulation in both susceptible (CC3) and resistant (IL10-1) cultivars in response to RKN stress, while *CsOFP14* was downregulated in both types of cultivars under RKN treatment (Figure 9B). Additionally, *CsOFP9*, *CsOFP10*, *CsOFP11*, *CsOFP12*, and *CsOFP18* were specifically upregulated only under RKN treatment in susceptible cultivar, whereas *CsOFP19* displayed downregulated expression exclusively in susceptible cultivar (Figure 9B). Under DM treatment, *CsOFP20* exhibited significant up-regulated expression in both DM-susceptible (Vlaspik) and DM-resistant (PI197088) cultivars, whereas *CsOFP8*, *CsOFP10*, *CsOFP12*, *CsOFP15*, and *CsOFP17* showed marked decreased transcriptions in both types of cultivars (Figure 9C). Notably, *CsOFP11* and *CsOFP3* displayed cultivar-specific expression patterns, with *CsOFP11* being upregulated exclusively and *CsOFP3* downregulated only in resistant cultivar (Figure 9C).

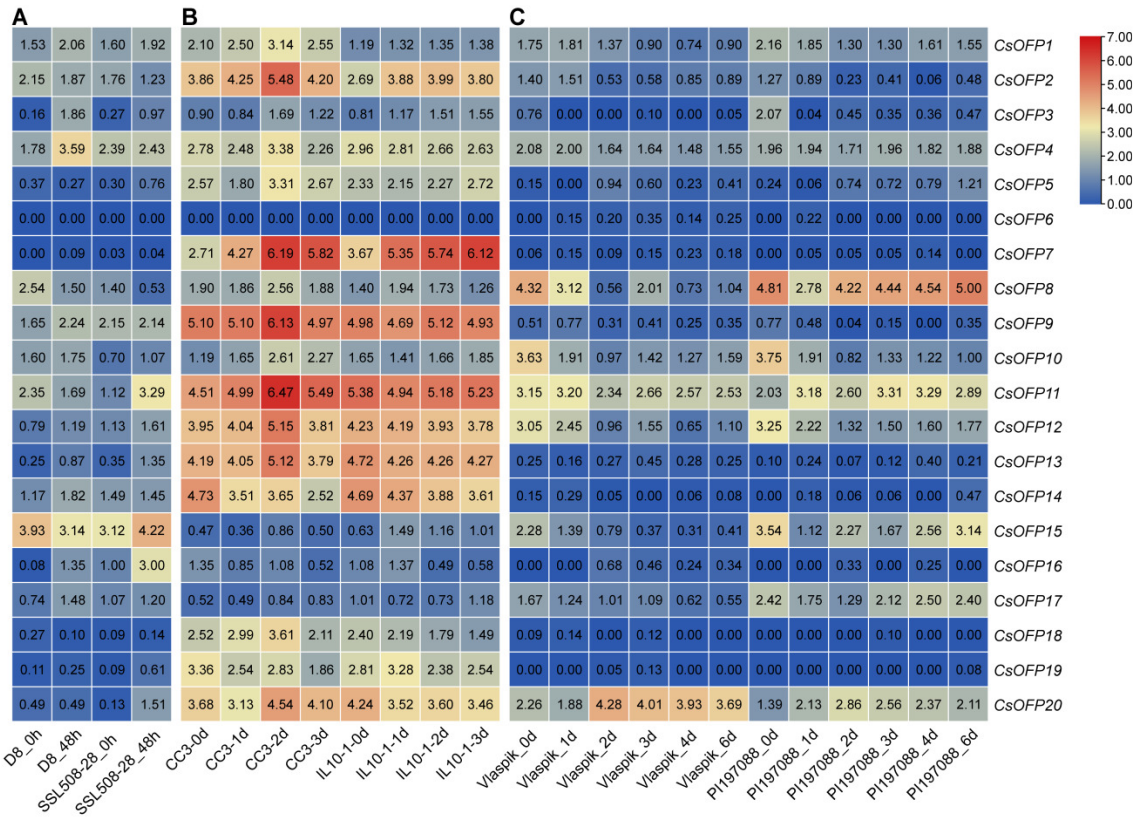

Figure 9. Expression analysis of *CsOFP* genes in response to various biotic stresses including PM treatment (A), RKN treatment (B), and DM treatment (C)

The data shown in the boxes represent log2 transformed (TPM+1) values. Compared to the control, *CsOFP* genes with a threshold of |log2 (fold change)| ≥ 1 were defined as differentially expressed

Discussion

The establishment of the cucumber pan-genome has enabled researchers to conduct more comprehensive analyses of genetic diversity, genome structure, and their associations with key traits such as disease resistance and stress tolerance, providing valuable resources for gene function exploration and molecular breeding (Xu *et al*., 2025). This study represents the first systematic identification of 20 *CsOFP* genes based on the cucumber pan-genome (Table A1), elucidating their genomic distribution patterns, evolutionary characteristics, and functional diversity. In the previous reports, genomic analyses have characterized the *OFP* gene family in multiple plant species, such as pummelo (*Citrus maxima*, 16 *CitOFP* genes) (Wu *et al*., 2022), grape (*Vitis vinifera*, 16 *VvOFP* genes) (Dong *et al*., 2024), *Arabidopsis* (*Arabidopsis thaliana*, 18 *AtOFP* genes) (Wang *et al*., 2011), mango (*Mangifera indica*, 25 *MiOFP* genes) (Wu *et al*., 2024), apple (*Malus domestica*, 26 *MdOFP* genes) (Li *et al*., 2019a), Chinese cabbage (*Brassica rapa*, 29 *BraOFP* genes) (Wang *et al*., 2021b), rice (*Oryza sativa*, 31 *OsOFP* genes) (Yu *et al*., 2015; Ahmad *et al*., 2023), and banana (*Musa acuminate*, 49 *MaOFP* genes) (Zhang *et al*., 2020). Comparative analysis revealed that the number of cucumber *OFP* genes is relatively lower, and no direct relationship was found between the number of *OFP* family genes and the size of above plant genomes. In addition, comparative genomic analysis of synteny among cucumber, tomato, and *Arabidopsis* identified diverse gene correspondence patterns, ranging from one-to-one to one-to-multiple orthologous relationships (Figure 5), indicating stronger genetic affinities and potential co-evolutionary trajectories between these species.

Notably, CsOFP proteins exhibit sequence length polymorphism across different cucumber accessions (Table A2). Among the 20 CsOFPs, only two (CsOFP4 and CsOFP7) were entirely conserved in protein length across all 12 accessions, suggesting their functions may be critical and invariant. In addition, minor variations of 1-2 amino acids were observed in CsOFP8, CsOFP10, and CsOFP19 across accessions, which may due to insertions or deletions and could lead to functional divergence among the variants. In contrast, the other 15 CsOFPs exhibited length variations, with CsOFP2 and CsOFP11 showing particularly pronounced differences, implying potential functional diversification. Furthermore, specific gene absence was linked to cultivar groups: *CsOFP6* was missing in East Asian cultivated accessions (XTMC and Cu2), while *CsOFP18* was absent from the Indian cultivated accession 'Hx14' (Table A1). Analysis of variation patterns also revealed that CsOFP1 variations were primarily confined to Indian wild and East Asian cultivated accessions, whereas CsOFP17 was completely conserved in both East Asian and Xishuangbanna cultivated accessions (Table A2). In summary, the pervasive length variation in CsOFP protein sequences across accessions may be associated with natural selection from environmental stresses and artificial selection during domestication, thereby potentially leading to the functional diversification of this gene family.

The phylogenetic analysis showed a close relationship between cucumber and *Arabidopsis* OFP proteins (Figure 1), while the combination of phylogenetic and motif analysis revealed that CsOFPs within the same clade share motifs and likely have similar functions (Figure 2). For example, ectopic expression of *CsOFP19* (previously named as *CsOFP12-16c*) in *Arabidopsis* produced shorter and blunt siliques (Han *et al*., 2022). Similarly, overexpression of its *Arabidopsis* homolog *AtOFP16* also induces blunt-end silique phenotypes (Wang *et al*., 2011). In addition, gene structure analysis revealed that the vast majority of the *CsOFP* genes were intron-free, and only two *CsOFPs* harbors one intron (Figure 3), which was consistent with the previous results in cotton (Zhang *et al*., 2022), sorghum (An *et al*., 2024), grape (Dong *et al*., 2024). This phenomenon likely results from an evolutionarily conserved pattern associated with the need for rapid environmental signal transduction. Previous research has shown that intronless genes improve stress response efficiency by minimizing transcriptional processing time (Lai *et al*., 2022).

Gene expression patterns can provide important clues for gene function, and tissue-specific expression patterns further elucidate functional diversification of *CsOFP* genes. For instance, *CsOFP2*, *CsOFP10*, and

CsOFP17 shows the highest expression in ovary (Figure 7), suggesting their possible roles in ovary development. In previous studies, many *OFP* genes were found to acts as a key regulator of fruit development in different plants, such as melon *CmOFP13* (Ma *et al*., 2022), pepper *CaPCR1* (Liu *et al*., 2024), grape *VvOFP4* (Dong *et al*., 2024). It should be noted that *CsOFP5*, *CsOFP7*, *CsOFP11*, *CsOFP14*, and *CsOFP20* exhibited higher transcription levels in root as compared to other tissues, while *CsOFP3*, *CsOFP8*, and *CsOFP17* were expressed at the highest levels in leaf (Figure 7). Correspondingly, *TaOFP* genes showed different expression patterns in wheat, with *TaOFP21-A* and *TaOFP22-D* exhibited the highest transcripts in leaves at the seedling stage, while *TaOFP19-A/B/D*, *TaOFP29a-A*, and *TaOFP3-B* were highly expressed in roots at the flag leaf visible stage (Wang *et al*., 2020). Relevant reports have demonstrated that *OFP* genes serve as key regulatory nodes integrating phytohormone signaling pathways and environmental stimuli to modulate various aspects of plant growth and development. For example, rice *OsOFP1* modulate brassinosteroid (BR) responses to control grain morphology and plant structure (Xiao *et al*., 2017), *OsOFP6* mediates auxin (IAA)-dependent lateral root development (Ma *et al*., 2017), while *OsOFP3* and *OsOFP8* can regulate leaf angle through BR signaling (Yang *et al*., 2016; Xiao *et al*., 2020). A recent study showed that OsOFP9 plays a pivotal role in regulating key agronomic traits including seed germination, grain development, and leaf angle establishment through its coordination of multiple phytohormone signaling pathways (Lu *et al*., 2025). LsOFP6 interacts with LsBLH2 and enhances LsBLH2-mediated transcriptional active of *LsGA20ox1* and *LsGA2ox8*, thereby modulating bioactive GA levels to control bolting transition in lettuce during the rosette stage (Chen *et al*., 2025). In this study, a series of *cis*-elements related to hormone responsiveness, were identified in the promoter regions of *CsOFP* genes (Figure 6), implying that *CsOFPs* may also differentially regulate plant architecture through distinct hormonal pathways.

Plant OFPs were also found to play key roles in regulating plant response to various stresses. For example, overexpressing *OsOFP8* enhanced disease resistance, drought tolerance, and cold stress tolerance in rice (Yang *et al*., 2016). Drought-induced *TaOPF29a-A* confers wheat drought tolerance by enhancing root growth and biomass under drought treatment (Wang *et al*., 2020). Heterologous overexpression of *PpOFP1* in both yeast and tomato plants significantly improved their salt stress resistance (Tan *et al*., 2021). In this study, a total of 7, 10, and 3 CsOFP genes exhibited significant differential expression under cold, heat, and salt stress, respectively, and their promoter regions were found to be enriched with a series of stress-related cis-acting elements (Figures 6 and 8). Moreover, RNA-seq analysis revealed that 7, 9, and 8 *CsOFP* genes exhibited significant differential expression in response to powdery mildew (PM), root-knot nematode (RKN), and downy mildew (DM) infections, respectively (Figure 9), and many stress-responsive *cis*-elements were observed in the promoters of *CsOFP* genes (Figure 6). In pepper, *CazOFP15*, *CazOFP16* and *CazOFP17* exhibit significant salt-responsive expression patterns, suggesting their functional involvement in salinity stress adaptation (Luo *et al*., 2022). In rice, five genes (*OsOFP11*, *OsOFP25*, *OsOFP29*, *OsOFP30*, and *OsOFP31*) displayed varied expression patterns in brown planthopper, striped rice stemborer, and rice leaf folder infestations (Ahmad *et al*., 2023). Distinct stress-responsive *PbrOFP* genes were also reported in Chinese pear, with two genes (*Pbr010426.1* and *Pbr010427.1*) showing significant upregulation during *Venturia nashicola* infection and five members exhibiting induced expression under PEG-induced drought stress (Ding *et al*., 2020). Therefore, the differential expression patterns of *CsOFP* genes under multiple stresses suggest their role as master regulators orchestrating cucumber's defense signaling networks.

Conclusions

In this study, a total of 20 *OFP* family genes were identified through pan-genome analysis. Among 12 cucumber accessions, only two CsOFP members maintained consistent protein lengths. Phylogenetic analysis with *Arabidopsis* OFPs classified these 20 CsOFPs into nine distinct subgroups. Gene structure and conserved motif analyses demonstrated high divergence during evolution. Promoter and tissue-specific expression analyses indicated that *CsOFP* genes play extensive regulatory roles in cucumber growth and development. Expression analysis via RNA-seq data showed that some *CsOFP* genes may respond to different abiotic stresses (cold, heat, and salt) and biotic stresses (powdery mildew, root-knot nematode, and downy mildew). These findings provide novel insights into the functional diversity of the *OFP* gene family and serve as a valuable resource for further investigation of *CsOFP* gene functions in cucumber.

Authors' Contributions

Conceptualization: XZ and HL; Data curation: LF and WY; Formal analysis: LF, JC and JW; Funding acquisition: HL; Investigation: XZ and HL; Methodology: LF and HL; Resources: YW; Software: WY and YW; Writing - original draft: FL, XZ and HL; Writing - review and editing: WY, XZ and HL.

All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the Science and Technology Research Project of Jiangxi Provincial Department of Education, grant number GJJ200436.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ahmad S, Ahmad N, Bayar J (2023). Genome-wide identification, characterization, and expression analysis of the Ovate family protein in *Oryza sativa* under biotic and abiotic stresses. Plant Stress 10:100228. *https://doi.org/10.1016/j.stress.2023.100228*

An Y, Xia X, Zhang X, Liu L, Jiang S, Jing T, Zhang F (2024). Genome-wide identification of the sorghum OVATE gene family and revelation of its expression characteristics in sorghum seeds and leaves. Scientific Reports 14(1):15123. *https://doi.org/10.1038/s41598-024-66103-z*

Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, ... Xia R (2023). TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. Molecular Plant 16(11):1733-1742. *https://doi.org/10.1016/j.molp.2023.09.010*

Chen H, Qi Y, Wang Y, Liu J, Lu R, Zhao X, ... An G (2025). LsBLH2-LsOFP6-LsKANT3 module regulates bolting by orchestrating the gibberellin biosynthesis and metabolism in lettuce. Plant Biotechnology Journal 23(5):1668-1682. *https://doi.org/10.1111/pbi.14614*

Dangwal M, Das S (2018). Identification and analysis of OVATE family members from genome of the early land plants provide insights into evolutionary history of OFP family and function. Journal of Molecular Evolution 86(8):511-530. *https://doi.org/10.1007/s00239-018-9863-7*

Ding B, Hu C, Feng X, Cui T, Liu Y, Li L (2020). Systematic analysis of the *OFP* genes in six Rosaceae genomes and their roles in stress response in Chinese pear (*Pyrus bretschneideri*). Physiology and Molecular Biology of Plants 26(10):2085-2094. *https://doi.org/10.1007/s12298-020-00866-3*

Dong Y, Huang L, Liu J, Nong H, Li H, Zhang W, ... Tao J (2024). Genome-wide identified *VvOFP* genes family and *VvOFP4* functional characterization provide insight into fruit shape in grape. International Journal of Biological Macromolecules 276(Pt 1):133880. *https://doi.org/10.1016/j.ijbiomac.2024.133880*

Han L, Song X, Wang Z, Liu X, Yan L, Han D, ... Zhang X (2022). Genome-wide analysis of OVATE family proteins in cucumber (*Cucumis sativus* L.). Journal of Integrative Agriculture 21(5):1321-1331. *https://doi.org/10.1016/S2095-3119(21)63788-7*

Lai W, Zhu C, Yang S, Hu Z, Liu S, Zhou Y (2022). Comprehensive identification of the VQ family genes in cucumber and their roles in response to abiotic and biotic stresses. Scientia Horticulturae 295:110874. *https://doi.org/10.1016/j.scienta.2021.110874*

Li E, Wang S, Liu Y, Chen JG, Douglas CJ (2011). OVATE FAMILY PROTEIN4 (OFP4) interaction with KNAT7 regulates secondary cell wall formation in *Arabidopsis thaliana*. Plant Journal 67(2):328-341. *https://doi.org/10.1111/j.1365-313X.2011.04595.x*

Li H, Dong Q, Zhao Q, Ran K (2019a). Genome-wide identification, expression profiling, and protein-protein interaction properties of ovate family proteins in apple. Tree Genetics & Genomes 15(3):45. *https://doi.org/10.1007/s11295-019-1354-5*

Li H, Dong Q, Zhu X, Zhao Q, Ran K (2019b). Genome-wide identification, expression, and interaction analysis for ovate family proteins in peach. Molecular Biology Reports 46(4):3755-3764. *https://doi.org/10.1007/s11033-019-04817-4*

Li H, Wang S, Chai S, Yang Z, Zhang Q, Xin H, ... Zhang Z (2022). Graph-based pan-genome reveals structural and sequence variations related to agronomic traits and domestication in cucumber. Nature Communications 13(1):682. *https://doi.org/10.1038/s41467-022-28362-0*

Liu J, Van Eck J, Cong B, Tanksley SD (2002). A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proceedings of the National Academy of Sciences of the United States of America 99(20):13302-13306. *https://doi.org/10.1073/pnas.162485999*

Liu T, Dong Y, Gao S, Zhou Y, Liu D, Wang J, ... Li F (2024). Identification of *CaPCR1*, an *OFP* gene likely involved in pointed versus concave fruit tip regulation in pepper (*Capsicum annuum* L.) using recombinant inbred lines. Theoretical and Applied Genetics 137(7):161. *https://doi.org/10.1007/s00122-024-04675-0*

Liu Y, Douglas CJ (2015). 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 Signaling & Behavior 10(7):e1033126. *https://doi.org/10.1080/15592324.2015.1033126*

Lu CY, Ren XY, Zhou Y, Jia SS, Bai H, Zhao DS, ... Li QF (2025). OsOFP9 regulates diverse key traits of rice by integrating multiple plant hormones. Plant Journal 121(5):e70044. *https://doi.org/10.1111/tpj.70044*

Luo Y, Yang S, Luo X, Li J, Li T, Tang X, ... Qin C (2022). Genome-wide analysis of *OFP* gene family in pepper (*Capsicum annuum* L.). Frontiers in Genetics 13:941954. *https://doi.org/10.3389/fgene.2022.941954*

Ma J, Li C, Zong M, Qiu Y, Liu Y, Huang Y, ... Wang J (2022). *CmFSI8*/*CmOFP13* encoding an OVATE family protein controls fruit shape in melon. Journal of Experimental Botany 73(5):1370-1384. *https://doi.org/10.1093/jxb/erab510*

Ma Y, Yang C, He Y, Tian Z, Li J (2017). Rice OVATE family protein 6 regulates plant development and confers resistance to drought and cold stresses. Journal of Experimental Botany 68(17):4885-4898. *https://doi.org/10.1093/jxb/erx309*

Snouffer A, Kraus C, van der Knaap E (2020). The shape of things to come: ovate family proteins regulate plant organ shape. Current Opinion in Plant Biology 53:98-105. *https://doi.org/10.1016/j.pbi.2019.10.005*

Tan Q, Jiang S, Wang N, Liu X, Zhang X, Wen B, ... Li L (2021). OVATE family protein PpOFP1 physically interacts with PpZFHD1 and confers salt tolerance to tomato and yeast. Frontiers in Plant Science 12:759955. *https://doi.org/10.3389/fpls.2021.759955*

Wang D, Cao Z, Wang W, Zhu W, Hao X, Fang Z, ... Tang Y (2020). Genome-wide characterization of *OFP* family genes in wheat (*Triticum aestivum* L.) reveals that *TaOFP29a-A* promotes drought tolerance. Biomed Research International 2020:9708324. *https://doi.org/10.1155/2020/9708324*

Wang H, Chen JG, Chang Y (2021a). Identification, expression, and interaction analysis of Ovate family proteins in *Populus trichocarpa* reveals a role of PtOFP1 regulating drought stress response. Frontiers in Plant Science 12:650109. *https://doi.org/10.3389/fpls.2021.650109*

Wang R, Han T, Sun J, Xu L, Fan J, Cao H, Liu C (2021b). Genome-wide identification and characterization of the *OFP* gene family in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). PeerJ 9:e10934. *https://doi.org/10.7717/peerj.10934*

Wang S, Chang Y, Ellis B (2016). Overview of OVATE family proteins, a novel class of plant-specific growth regulators. Frontiers in Plant Science 7:417. *https://doi.org/10.3389/fpls.2016.00417*

Wang S, Chang Y, Guo J, Chen JG (2007). Arabidopsis Ovate Family Protein 1 is a transcriptional repressor that suppresses cell elongation. Plant Journal 50(5):858-872. *https://doi.org/10.1111/j.1365-313X.2007.03096.x*

Wang S, Chang Y, Guo J, Zeng Q, Ellis BE, Chen JG (2011). *Arabidopsis* ovate family proteins, a novel transcriptional repressor family, control multiple aspects of plant growth and development. PLoS One 6(8):e23896. *https://doi.org/10.1371/journal.pone.0023896*

Wang Z, Yin M, Han J, Wang X, Chang J, Ren Z, Wang L (2024). Pan-genome-wide identification and transcriptome-wide analysis of *ZIP* genes in cucumber. Agriculture 14(1):133. *https://doi.org/10.3390/agriculture14010133*

Wu Q, Sun J, Fu J, Yu H, Wang X, Wang S, ... Xu Q (2022). Genome-wide identification of ovate family in Citrus and functional characterization of *CitOFP19*. Plant Science 321:111328. *https://doi.org/10.1016/j.plantsci.2022.111328*

Wu Q, Xia R, Yang J, Chen R, Zeng Z, Fan C (2024). Identification and comprehensive analysis of *OFP* genes for fruit shape influence in mango. Genes 15(7):823. *https://doi.org/10.3390/genes15070823*

Wu S, Zhang B, Keyhaninejad N, Rodríguez GR, Kim HJ, Chakrabarti M, ... van der Knaap E (2018). A common genetic mechanism underlies morphological diversity in fruits and other plant organs. Nature Communications 9(1):4734. *https://doi.org/10.1038/s41467-018-07216-8*

Xiao Y, Liu D, Zhang G, Tong H, Chu C (2017). Brassinosteroids regulate OFP1, a DLT interacting protein, to modulate plant architecture and grain morphology in rice. Frontiers in Plant Science 8:1698. *https://doi.org/10.3389/fpls.2017.01698*

Xiao Y, Zhang G, Liu D, Niu M, Tong H, Chu C (2020). GSK2 stabilizes OFP3 to suppress brassinosteroid responses in rice. Plant Journal 102(6):1187-1201. *https://doi.org/10.1111/tpj.14692*

Xu H, Liu K, Zhao L, Chen C, Wang L, Ren Z (2025). Pan-genome identification and expression analysis of lipoxygenase genes in cucumber. Agriculture 15(3):245. *https://doi.org/10.3390/agriculture15030245*

Yang C, Shen W, He Y, Tian Z, Li J (2016). OVATE family protein 8 positively mediates brassinosteroid signaling through interacting with the GSK3-like kinase in rice. PLoS Genetics 12(6):e1006118. *https://doi.org/10.1371/journal.pgen.1006118*

Yang S, Zhu C, Chen J, Zhao J, Hu Z, Liu S, Zhou Y (2022). Identification and expression profile analysis of the *OSCA* gene family related to abiotic and biotic stress response in cucumber. Biology 11(8):1134. *https://doi.org/10.3390/biology11081134*

Yu H, Jiang W, Liu Q, Zhang H, Piao M, Chen Z, Bian M (2015). Expression pattern and subcellular localization of the ovate protein family in rice. PLoS One 10(3):e0118966. *https://doi.org/10.1371/journal.pone.0118966*

Zhang B, Li Q, Keyhaninejad N, Taitano N, Sapkota M, Snouffer A, van der Knaap E (2023). A combinatorial TRM-OFP module bilaterally fine-tunes tomato fruit shape. New Phytologist 238(6):2393-2409. *https://doi.org/10.1111/nph.18855*

Zhang J, Miao H, Xie B, Wang J, Jia C, Zhang J, ... Liu J (2020). Genomic and transcriptional analysis of banana ovate family proteins reveals their relationship with fruit development and ripening. Biochemical Genetics 58(3):412-429. *https://doi.org/10.1007/s10528-020-09951-4*

Zhang L, Tehseen Azhar M, Che J, Shang H (2022). Genome-wide identification, expression and evolution analysis of OVATE family proteins in cotton (*Gossypium* spp.). Gene 834:146653. *https://doi.org/10.1016/j.gene.2022.146653*

Zhang L, Zhang X, Ju H, Chen J, Wang S, Wang H, ... Chang Y (2016). Ovate family protein1 interaction with BLH3 regulates transition timing from vegetative to reproductive phase in *Arabidopsis*. Biochemical and Biophysical Research Communications 470(3):492-497. *https://doi.org/10.1016/j.bbrc.2016.01.135*

Zhao L, Wang K, Wang Z, Chu S, Chen C, Wang L, Ren Z (2024). Pan-genome analysis of *TRM* gene family and their expression pattern under abiotic and biotic stresses in cucumber. Horticulturae 10(9):908. *https://doi.org/10.3390/horticulturae10090908*

Appendix

Table A1. Identification of *OFP* family genes in different cucumber accessions

Gene name	Accession name												
	9930-v2	9930-v3	XTMC	Cu2	Cuc80	Cuc64	Cuc37	Gy14	9110gt	W4	W8	Hx14	Hx117
CsOFP1	1G168910	1G015160	1G017580	1G015660	1G016210	1G016480	1G017550	1G024920	1G016730	1G017430	1G016590	1G027530	1G030840
CsOFP2	1G246610	1G024220	1G021760	1G019200	1G034240	1G034430	1G025620	1G028320	1G021280	1G022070	1G022450	1G033190	1G035280
CsOFP3	2G004680	2G002260	2G001210	2G001170	2G002210	2G001190	2G001160	2G001230	2G002240	2G001190	2G001170	2G002170	2G002190
CsOFP4	2G361530	2G028590	2G030050	2G028790	2G101760	2G068930	2G098630	2G036900	2G030300	2G033950	2G041070	2G037050	2G040020
CsOFP5	3G146670	3G013290	3G019640	3G015790	3G013740	3G015790	3G013780	3G016660	3G015660	3G015880	3G014060	3G019470	3G023950
CsOFP6	3G203770	6G040960	/	/	6G079940	6G035650	6G037340	6G046200	6G036510	6G033240	6G033610	6G052960	6G046130
CsOFP7	3G730160	3G033200	3G051720	3G039240	3G045380	3G054680	3G049110	3G051930	3G040660	3G038910	3G038390	3G056510	3G059320
CsOFP8	3G778360	3G037710	3G058280	3G044740	3G049850	3G059120	3G053520	3G058480	3G045240	3G044470	3G042870	3G062100	3G063810
CsOFP9	3G778370	3G037720	3G058290	3G044750	3G049860	3G059130	3G053530	3G058490	3G045250	3G044480	3G042880	3G062110	3G063820
CsOFP10	4G038760	4G005140	4G006090	4G008160	4G005140	4G013370	4G005110	4G008060	4G006110	4G006140	4G006070	4G005090	4G005060
CsOFP11	4G332100	4G027080	4G033530	4G027380	4G085200	4G030140	4G087250	4G031470	4G028450	4G021260	4G024230	4G031300	4G032450
CsOFP12	5G613560	5G035260	5G055460	5G051390	5G059850	5G040610	5G052640	5G058220	5G043500	5G037570	5G041990	5G055960	5G064340
CsOFP13	6G046300	6G004380	6G004370	6G005260	6G006490	6G004480	6G004380	6G007410	6G004370	6G004380	6G004370	6G006410	6G004450
CsOFP14	6G212870	6G019090	6G028710	6G022420	6G046070	6G019690	6G018100	6G026480	6G021480	6G018550	6G021630	6G027960	6G025570
CsOFP15	6G454380	6G040950	6G052560	6G035960	6G079930	6G035640	6G037330	6G046190	6G036500	6G033230	6G033600	6G052950	6G046120
CsOFP16	6G512880	6G048660	6G060360	6G044500	6G098830	6G043200	6G045060	6G054860	6G045250	6G042040	6G042310	6G060730	6G053910
CsOFP17	6G520290	6G051110	6G063790	6G046990	6G101140	6G045610	6G047540	6G057310	6G047710	6G044450	6G044730	6G064090	6G056380
CsOFP18	7G234150	7G022480	7G023260	7G020860	7G032140	7G022650	7G033600	7G020930	7G019990	7G018510	7G028430	/	7G024000
CsOFP19	7G388340	7G027990	7G036490	7G026840	7G038180	7G028490	7G039840	7G035090	7G026170	7G024620	7G037850	7G036280	7G040200
CsOFP20	7G446730	7G033180	7G041800	7G032130	7G044450	7G036800	7G045010	7G040380	7G033500	7G030910	7G044090	7G042480	7G048490

Notes: The gene IDs in the table omit the abbreviations representing different cucumber accessions. "/" indicates genes that were not identified in the cucumber accession

Table A2. Length of OFP proteins sequences in different cucumber varieties

Protein name	East Asian cultivated accession				Xishuang-banna cultivated accession	Indian wild accession			Eurasian cultivated accession			Indian cultivated accession	
	9930-v2	9930-v3	XTMC	Cu2	Cuc80	Cuc64	W4	W8	Cuc37	Gy14	9110gt	Hx14	Hx117
CsOFP1	171	289	180	180	171	180	180	189	171	170	171	171	171
CsOFP2	246	246	246	195	265	265	268	205	265	195	246	133	265
CsOFP3	239	239	239	239	239	238	239	239	239	239	239	239	239
CsOFP4	270	270	270	270	270	270	270	270	270	270	270	270	270
CsOFP5	205	300	403	205	264	205	300	205	205	205	205	205	205
CsOFP6	74	156	/	/	126	156	156	214	156	156	126	88	156
CsOFP7	343	343	343	343	343	343	343	343	343	343	343	343	343
CsOFP8	234	234	234	234	234	234	234	236	234	234	234	234	234
CsOFP9	167	174	180	174	174	180	162	174	178	161	174	167	180
CsOFP10	301	301	300	300	300	301	300	300	301	300	300	300	300
CsOFP11	335	335	178	181	216	335	325	325	269	262	335	262	335
CsOFP12	277	277	277	277	186	275	186	186	186	186	186	186	186
CsOFP13	227	227	227	227	227	227	227	227	537	227	227	227	227
CsOFP14	184	219	184	184	184	209	184	181	184	219	219	184	184
CsOFP15	285	294	294	294	290	290	245	123	290	290	290	294	294
CsOFP16	168	182	175	168	168	168	168	168	175	175	175	175	168
CsOFP17	468	468	468	468	468	468	441	441	468	468	441	441	441
CsOFP18	197	197	203	197	203	197	203	177	197	203	203	/	203
CsOFP19	278	278	278	278	277	277	278	277	278	278	278	278	278
CsOFP20	249	268	268	268	268	268	268	268	268	233	268	268	268

Notes: "/" indicates genes that were not identified

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.