Genome-wide analysis of OFP gene family in pepper (Capsicum annuum L.)

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Ovate family proteins (OFPs) are transcriptional inhibitors that regulate plant growth and development and play important roles in the synthesis of secondary cell walls during pollen development. This study identified the pepper OFP gene family based on the genome-wide analysis and used bioinformatics methods to provide a fundamental profile of the gene family. 74 OFP genes with typical Ovate domain were identified in cultivated pepper Zunla-1, wild pepper Chiltepin and CM334. Chromosome mapping revealed that CazOFP genes were unevenly distributed on 11 chromosomes and Chr00 in Zunla-1, CacOFP genes on 12 chromosomes in Chiltepin, and CamOFP genes on 12 chromosomes and two Scaffolds in CM334. Gene structure analysis revealed that CaOFP genes possessed 1-3 exons, and the analysis of physicochemical properties suggested that CaOFPs were hydrophilic. Many cis-acting elements were identified in the promoter region of CaOFP genes, including ABRE, ARE, Box 4, G-box, TC-rich, and TCT-motif. The expression patterns of pepper at different growth stages showed that CaOFP genes were actively involved in the growth and fruit development of pepper, and CazOFP16 and CazOFP17 were actively involved in response to multiple hormones and stress events. qRT-PCR was also used to verify the expression of CazOFP gene in two developmental stages of seven pepper varieties with different fruit shapes, and it was found that CaOFP genes may be involved in the formation of fruit type in pepper. This study provides theoretical and practical evidence for future research on the OFP gene family.

KEYWORDS
pepper, OFP genes, phylogenetic tree, expression characteristics analysis, qRT-PCR
Introduction

Transcription factors (TFs) play fundamental roles in the growth and development of higher plants, as well as in their responses to the external environment. Typical TFs in higher plants possess DNA-binding domains, transcription regulation domains, oligomerization sites, and nuclear localization signals; the interaction between these domains and cis-acting elements enables TFs to regulate gene expression (Jiang et al., 2011). Ovate family proteins (OFPs) are plant-specific, multigene family members usually with a conserved Ovate domain. Homologs of OFPs have been reported in higher plants, mosses, and lycophytes (Wang et al., 2016).

Pepper is an important vegetable with high standards for its quality, spiciness, and shape, but less research has been done to study its fruit shape. Fruit shape is one of the most important quality traits of pepper, and is also one of the main attributes for the classification of pepper fruit types (Paran and Van Der Knaap, 2007). In crops, yield is regulated by environmental factors and fruit morphology. Genes regulating fruit morphology were first identified in tomatoes, and OFP genes are highly involved in this process (Liu et al., 2002; Snouffer et al., 2020). According to previous studies, OFP genes were found to be involved in the regulation of shape in different varieties of crops, such as rice, tomato, and melon. Moreover, overexpression of OFP destroyed plant organ shape in the process of phytohormone regulation, which suggested that OFP genes are involved in regulating hormones and signaling pathways within developing organs (Snouffer et al., 2020). OFP genes related to pepper fruit shape were cloned from different pepper varieties, and there were significant differences among the different fruit shapes (Tsaballa et al., 2011). A recent study revealed that virus-induced gene silencing of the pepper ortholog \textit{CaOFP20} resulted in increased fruit elongation on two independent backgrounds (Borovsky et al., 2021).

OFP genes play important roles in the growth and development of plants, including flowering (Yuan et al., 2016), growth of roots and stems (Xu et al., 2018), and formation of secondary cell walls (Li et al., 2011). Yuan et al. (2016) detected 12 \textit{VvOFP} genes in different tissues, and the expression levels of \textit{VvOFP1}, \textit{VvOFP2}, \textit{VvOFP4}, \textit{VvOFP5}, \textit{VvOFP6}, \textit{VvOFP7}, \textit{VvOFP12}, and \textit{VvOFP17} were relatively high in the week before flowering, suggesting that OFP TFs regulate fruit development during anthesis and the week before anthesis, whereas \textit{VvOFP5} and \textit{VvOFP2} are involved in the regulation of flowering and reproductive growth, respectively (Yuan et al., 2016). Li et al. (2011) also found that \textit{AtOFP4} regulates the formation of secondary cell walls via its interaction with the KNAX7 protein.

In this study, we systematically characterized the \textit{CaOFP} transcription factors at the genome-wide level, analyzed its phylogenetic tree, physicochemical properties, conserved structure, chromosomal localization, response to biotic and abiotic stresses, and provided a theoretical basis for studying the function of OFP genes in pepper.
Gene structure, conserved motifs, and phylogenetic analysis of OFP genes in pepper

The phylogenetic tree was constructed based on the CaOFPs protein sequences in pepper and AtOFPs protein sequences in Arabidopsis thaliana. MEGA X was used to construct a phylogenetic tree of CaOFP genes according to the neighbor-joining method with 1000 bootstrap reiterations (Kumar et al., 2016). The structures of CaOFP genes were analyzed using the online tool GSDS 2.0 (http://gsds.gao-lab.org/) (Hu et al., 2015), and the analysis of conserved motifs was conducted using Meme (https://meme-suite.org/meme/) (Bailey et al., 2015) and visualized using TBtools (Chen et al., 2020). Physicochemical properties of the OFP gene family were analyzed using the online tool Expasy (Artimo et al., 2012).

Chromosome mapping and collinearity analysis of OFP genes in pepper

The structure file of OFP genes in pepper was extracted by TBtools to determine their starting and ending position in the chromosomes (Chen et al., 2020). The protein database was constructed using the Zunla-1 genomic protein file, Chiltepin genomic protein file and CM334 genomic protein file for comparison. The collinearity analysis was performed using MCScanX software (Wang et al., 2013). The chromosome mapping and collinearity results of OFP genes were visualized using TBtools (Chen et al., 2020).

Selection pressure and cis-acting element analysis of OFP genes in pepper

The online tool Pal2nal (http://www.bork.embl.de/pal2nal/) (Suyama et al., 2006) was used for the analysis of selection pressure of nine pairs of paralogous genes. The 2000-bp upstream sequences of the OFP genes were extracted using Bedtools (Quinlan, 2014), analyzed using the online tool Plantcare (Lescot et al., 2002), and visualized using TBtools.

Characterization of OFP-gene expression in pepper

Taking zunla-1 as the reference genome, the gene expression data of pepper were obtained from the NCBI (Accession No. GSE45037) and Pepper Information Data Center (Qin et al., 2014; Liu et al., 2017) (http://pepperhub.hzau.edu.cn). Experimental treatment and data analysis were conducted as described by (Liu et al., 2017), and the gene expression data were visualized using TBtools (Chen et al., 2020).

cDNA synthesis and qRT-PCR analysis

Expression pattern analysis was investigated using qRT-PCR. Its amplification was carried out using SYBR Green Pro Taq HS (Takara, Dalian, China) according to the manufacturer’s instructions. qRT-PCR was performed on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, United States) using the following program: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. The CaActin (GenBank No. DQ832719) and CaUbiquitin (GenBank No. AY496112) pepper genes were amplified as two control genes. Three biological replicates and three measurements for each replicate were performed under identical conditions. Analysis of the relative mRNA expression data was performed using 2^–ΔΔCt (Livak and Schmittgen, 2001). All primers, designed by Primer3plus (http://www.primer3plus.com/cgi-bin/dev/primer3plus.cgi) and used in this study, were listed in Supplementary Table S1.

Results

Identification, phylogenetic analysis, and physicochemical properties of pepper OFP genes

After aligning to the pepper proteomic database, 74 CaOFP genes were identified in Zunla-1, Chiltepin and CM334 (Table 1; Supplementary Table S2), and the conserved structures were found in all CaOFP genes. Phylogenetic data showed that the pepper OFP genes were divided into two subfamilies, including Class I and II. The number of CaOFP genes of Zunla-1, Chiltepin and CM334 in two subfamilies was 12, 12, and 13, respectively (Figure 1). All OFP genes were named according to their positions on the chromosomes. CaOFPs consisted of 103–436 amino acids; their molecular mass ranged from 11.82 to 49.45 (kDa), and the isoelectric point (pI) ranged from 4.33 to 9.94. The results indicated that CaOFPs are amphoteric proteins, with the instability index ranging from 31.34 to 76.51. The total average hydrophilicity was between -1.030–0.072, and almost all CaOFPs were hydrophilic proteins except for CamOFP25, which was 0.072 hydrophobic protein.

Structure characterization and motif analysis of CaOFP genes

The motif analysis of OFP genes based on 10 motifs showed that the highest number of motifs was recorded for CamOFP17, CamOFP21, CamOFP17, CamOFP18, CamOFP20, CamOFP21, CamOFP10 and CamOFP20, containing 9 motifs (Figure 2C). According to their motifs, Class I can be divided into motif 1, motif 2, motif 4, motif 6 and motif 9 as Class I-A subclade and
| Gene_id          | Rename | Number of amino acids | Molecular weight | pI   | Instability index | GRAVY |
|------------------|--------|-----------------------|------------------|------|-------------------|-------|
| Capana01g003669  | CazOFP1| 190                   | 21,309.00        | 9.54 | 75.18             | -0.502|
| Capana01g003670  | CazOFP2| 241                   | 27,214.99        | 4.33 | 46.62             | -0.509|
| Capana02g001663  | CazOFP3| 381                   | 44,895.92        | 8.40 | 66.03             | -1.020|
| Capana02g002672  | CazOFP4| 308                   | 35,371.74        | 9.64 | 59.98             | -1.009|
| Capana03g000334  | CazOFP5| 281                   | 31,625.45        | 8.66 | 47.31             | -0.691|
| Capana03g003702  | CazOFP6| 231                   | 26,239.81        | 4.67 | 52.50             | -0.630|
| Capana04g000381  | CazOFP7| 274                   | 31,578.51        | 7.09 | 65.11             | -0.717|
| Capana05g002442  | CazOFP8| 228                   | 25,629.91        | 4.68 | 53.89             | -0.358|
| Capana06g000283  | CazOFP9| 339                   | 38,208.89        | 9.66 | 63.37             | -0.797|
| Capana06g000645  | CazOFP10| 155                  | 17,809.74        | 5.38 | 44.69             | -0.048|
| Capana07g001811  | CazOFP11| 164                 | 18,936.63        | 8.99 | 66.82             | -0.678|
| Capana07g003310  | CazOFP12| 282                 | 32,488.54        | 9.14 | 43.56             | -0.873|
| Capana08g002905  | CazOFP13| 239                 | 26,380.44        | 5.37 | 38.63             | -0.308|
| Capana08g002909  | CazOFP14| 176                 | 20,071.91        | 9.11 | 54.07             | -0.520|
| Capana08g002909  | CazOFP15| 145                 | 16,616.02        | 8.67 | 51.31             | -0.385|
| Capana08g002909  | CazOFP16| 145                 | 16,616.02        | 8.67 | 51.31             | -0.385|
| Capana08g002909  | CazOFP17| 320                 | 36,192.28        | 4.55 | 46.98             | -0.351|
| Capana08g002909  | CazOFP18| 168                 | 19,101.53        | 4.42 | 38.06             | -0.330|
| Capana08g002909  | CazOFP19| 165                 | 18,469.72        | 5.20 | 52.40             | -0.420|
| Capana08g002909  | CazOFP20| 111                 | 12,768.27        | 4.66 | 35.64             | -0.372|
| Capana09g002104  | CazOFP21| 320                 | 36,161.30        | 4.83 | 46.56             | -0.529|
| Capana10g000348  | CazOFP22| 135                 | 15,068.29        | 7.64 | 48.28             | -0.591|
| Capana10g002165  | CazOFP23| 250                 | 28,182.00        | 4.35 | 55.62             | -0.657|
| Capana10g002905  | CazOFP24| 406                 | 46,225.56        | 9.72 | 51.58             | -0.823|
| Capan10g001844   | CacOFP1| 231                 | 26,215.81        | 4.62 | 51.89             | -0.594|
| Capan10g003395   | CacOFP2| 406                 | 46,197.50        | 9.68 | 51.17             | -0.821|
| Capan10g001492   | CacOFP3| 381                 | 44,981.97        | 8.21 | 67.09             | -1.030|
| Capan10g002400   | CacOFP4| 335                 | 38,506.07        | 8.92 | 67.33             | -0.986|
| Capan11g000309   | CacOFP5| 281                 | 31,548.33        | 8.67 | 47.72             | -0.707|
| Capan11g000354   | CacOFP6| 264                 | 30,636.70        | 8.75 | 68.19             | -0.672|
| Capan11g000990   | CacOFP7| 227                 | 25,532.79        | 4.68 | 53.23             | -0.353|
| Capan11g000272   | CacOFP8| 339                 | 38,208.89        | 9.66 | 63.37             | -0.797|
| Capan11g000600   | CacOFP9| 155                 | 17,809.74        | 5.38 | 44.69             | -0.048|
| Capan11g000809   | CacOFP10| 164                | 18,936.63        | 8.99 | 66.82             | -0.678|
| Capan11g000595   | CacOFP11| 192               | 21,483.16        | 9.54 | 76.51             | -0.505|
| Capan11g000596   | CacOFP12| 274               | 30,519.62        | 4.33 | 43.22             | -0.407|
| Capan11g000292   | CacOFP13| 282               | 32,520.60        | 9.14 | 43.56             | -0.881|
| Capan11g000909   | CacOFP14| 239               | 26,394.46        | 5.37 | 37.50             | -0.308|
| Capan11g001043   | CacOFP15| 340               | 38,301.09        | 9.75 | 68.07             | -0.807|
| Capan11g001855   | CacOFP16| 147              | 16,850.28        | 8.68 | 50.75             | -0.366|
| Capan11g001856   | CacOFP17| 320              | 36,274.43        | 4.55 | 44.01             | -0.515|
| Capan11g001857   | CacOFP18| 315              | 35,270.45        | 4.94 | 33.27             | -0.520|
| Capan11g001859   | CacOFP19| 238              | 27,195.28        | 4.77 | 44.44             | -0.541|
| Capan11g001861   | CacOFP20| 313              | 35,222.48        | 5.25 | 43.47             | -0.406|
| Capan11g001863   | CacOFP21| 320              | 36,140.41        | 4.97 | 46.63             | -0.509|
| Capan11g000323   | CacOFP22| 128              | 14,703.09        | 8.77 | 45.06             | -0.669|
| Capan11g000324   | CacOFP23| 120              | 13,854.05        | 8.25 | 45.97             | -0.594|

(Continued on following page)
motif 1, motif 2, motif 4, motif 6, motif 7, motif 9 as Class I-B subclade, and OFP genes in Group I only contained 4–5 motifs. The 16 OFP genes in Class I-A subclade did not contain introns, while the Class I-B subclade had 1-2 exons. The largest number of exons was presented by CacOFP18, which contained five exons, and the number of exons in Class II was higher than that in Class I. Sequence comparison of 74 pepper OFP genes showed that all OFP genes contained motif1 and 69 OFP genes contained motif2 with the typical conserved structure of OVATE domain except for CazOFP24, CacOFP2, CamOFP4, CamOFP8, and CamOFP19 (Figure 3). Gene structure analysis showed that the OFP genes possessed 1-5 exons (Figure 2D).

Chromosome mapping and collinearity analysis of CaOFP genes

Chromosome mapping analysis showed that 24 CaOFP genes were unevenly distributed on 11 chromosomes and Chr00 in Zunla-1, and 24 CaOFP genes unevenly on 12 chromosomes in Chiltepin (Figure 4A). Among them, ZChr10 and CChr10 had the highest number of OFP genes (8 and 7 genes, respectively), while Chr04, Chr05, Chr07, and Chr12 in Zunla-1 and Chiltepin had only one OFP gene each. Interestingly, only ZChr00 contained one OFP gene.

Six genes (CaOFP15–CaOFP21) on ZChr10 and CChr10 were tandem repeats with colinear regions as well as other chromosomes in Zunla-1 and Chiltepin, but ZChr08 vs. CChr08 and CChr00 vs. ZChr00 did not have colinear regions (Figure 4A). In another comparison (Figure 4B), most chromosomes had colinear regions except for ZChr00, ZChr08, MChr08, and scaffold2890 in Zunla-1 or CM334.

Analysis of evolutionary selection pressure of CaOFPs

Gene synonymous substitution rate ($K_s$), nonsynonymous substitution rate ($K_a$), and their ratio ($K_a/K_s$)
can reflect the selection pressure in the evolutionary process. The evolutionary selection pressure ($K_a/K_s$) of the seven pairs of paralogous genes identified among 74 CaOFP genes were greater than one, suggesting that OFP genes were subjected to positive selection. Out of these, nine pairs were under evolutionary selection pressure. The $K_a/K_s$ of two pairs (CacOFP6-CacOFP4 and CamOFP14-CamOFP18) were less than one, indicating that they were under purifying selection, while their $K_a/K_s$ of the other seven pairs were more than one, while the other seven pairs were all above 1, indicating that these genes were under positive selection during the evolution of the CaOFP genes in pepper (Table 2; Supplementary Table S3).

### TABLE 2 Analysis of the evolutionary pressure of selection on the OFP genes in pepper.

| Collateral homologous | Synonymous substitution rate ($K_s$) | Non-synonymous substitution rate ($K_a$) | Selective pressure ratio ($K_a/K_s$) |
|----------------------|-----------------------------------|--------------------------------------|-----------------------------------|
| CazOFP4-CazOFP8      | 2.3995                            | 4.2491                               | 1.7709                            |
| CacOFP6-CacOFP4      | 59.1311                           | 3.2503                               | 0.055                             |
| CacOFP16-CacOFP11    | 2.1397                            | 2.8974                               | 1.3541                            |
| CacOFP23-CacOFP22    | 0.409                             | 0.977                                | 2.389                             |
| CacOFP8-CacOFP23     | 2.4928                            | 16.504                               | 6.6207                            |
| CacOFP17-CacOFP21    | 1.2441                            | 1.3557                               | 1.0897                            |
| CamOFP11_CamOFP24    | 1.1054                            | 6.2469                               | 5.6512                            |
| CamOFP10_CamOFP20    | 1.2441                            | 1.3557                               | 1.0897                            |
| CamOFP14_CamOFP18    | 2.3596                            | 2.1842                               | 0.9256                            |

FIGURE 1
Phylogenetic analysis of OFP genes in pepper. Caz: Zunla-1; Cac: Chiltepin; Cam: CM334. The solid diamond with red color represented CazOFP gene of Zunla-1; the solid star with green color represented CacOFP gene of Chiltepin; and the solid circle with blue color represents CamOFP gene of CM334.
Cis-acting element analysis of CaOFP genes

The analysis of the 2000-bp upstream sequence of CaOFP genes showed that the OFP gene family contained numerous basic elements, such as the CAAT box and TATA box (Figure 5), as well as the ABA-related cis-acting element ABRE, anaerobic induction-required cis-acting element ARE, photoresponse-related conserved element Box 4, photoresponse-related cis-acting element G-box, defense and stress response-related cis-acting element TC-rich repeats, photoresponse element TCT-motif, cis-acting element involved in salicylic acid responsiveness TCA-element, MYB binding site involved in drought-inducibility MBS and cis-acting element involved in low-temperature responsiveness LTR (Supplementary Table S4). It was
FIGURE 3
Sequence alignment of OFP transcription factor motifs in pepper.
speculated that these genes may respond to biological stress and be involved in plant growth.

Expression patterns of OFP genes in different tissues and development stages of pepper

The expression levels of 24 CazOFP genes in five tissues including developing roots, developing stems, mature leaves, closed flower buds and open flowers from Zunla-1 pepper data were analyzed. It showed that CazOFP17–CazOFP21 were not expressed, and other 19 CazOFP were expressed in five different tissues on different levels (Figure 6A). During the nine stages of fruit development in Zunla-1, 22 CazOFP genes except for CazOFP18 and CazOFP20 were expressed in the first eight stages, showing that these CazOFP genes were mainly involved in early fruit development. One gene (CazOFP10), by contrast, was mainly involved in late fruit development (Figure 6B).

Characterization of CazOFP genes expression patterns under different stresses in pepper

As showed in Figure 7, the expression of CazOFP16, CazOFP17, and CazOFP18 in pepper leaves was up-regulated upon salicylic acid (SA, 2 mM) treatment. Jasmonic acid (JA, 10 μM) treatment similarly up-regulated the expression of CazOFP16 and CazOFP17. Pepper OFP genes expression was mainly up-regulated in roots after indole acetic acid (IAA, 2 μM) treatment; the expression of CazOFP24 in roots was higher than in leaves at a late expression stage. When treated with gibberellic acid (GA3, 2 μM), the expression of CazOFP16, CazOFP17, and CazOFP21 was higher than that of other OFP genes, and the level of up-regulation was greater in roots than in leaves. Abscisic acid (ABA, 30 μM) treatment up-regulated the expression of CazOFP11 and CazOFP12 in roots at stage AR4, while CazOFP17 had the highest expression level in leaves at stage AL5. The expression of CazOFP17 was significantly up-regulated upon salicylic acid (SA, 2 mM), JA, IAA, GA3, and ABA treatments, which suggested that CazOFP17 participates in plant response to the hormone, whereas CazOFP18, CazOFP19, and CazOFP20 were not expressed.

The expression of CazOFP17 was up-regulated in roots under cold stress at stage FR1 (Root/Freezing 1), and the expression of CazOFP16 was up-regulated in leaves at stage RL3 when treated with H2O2 (Figure 7). Heat stress up-regulated the expression of CazOFP16 in roots at stage HR3 and that of CazOFP17 in leaves at stage HL5 (Figure 7). When treated with D-mannitol, the expression of CazOFP15 was up-regulated in leaves; the expression of CazOFPs was higher in roots than in leaves. Salt stress up-regulated the expression of CazOFP16 in leaves at stage NL4 (Figure 7). In summary, CazOFP18-21 were not expressed, and the number of genes expressed in roots was higher than that in leaves.
Expression patterns of **OFP** genes in seven pepper varieties with different fruit shapes by qRT-PCR experiments

In order to verify whether CaOFP genes involve in pepper development and fruit shape formation (Figure 8A), eight CazOFP genes were randomly selected and qRT-PCR performed. These genes except for CazOFP12 were mainly expressed in developmental stage 1 (fruit with mature green) of seven pepper varieties with different fruit shapes (Figure 8B), indicating that these genes were mainly involved in early fruit development, which were consistent with transcriptome results mentioned above. In seven pepper varieties with different fruit shapes, the expression of CazOFP3 and CazOFP12 at developmental stage 2 (fruit at breaker plus 5 days) in T803 was higher than that at developmental stage 1 (fruit with mature green), as well as CazOFP14 in Zunla-1. Moreover, CazOFP3, CazOFP9 and CazOFP14 were expressed in two developmental stages of seven pepper varieties, showing that the three genes may be involved in the formation of pepper fruit shape.

**Discussion**

**Characteristics of OFP gene family in pepper**

We identified 24 CaOFP genes in cultivated pepper Zunla-1, 24 in wild pepper Chiltepin, and 26 in another cultivated pepper...
CM334. So far, OFP genes have been identified in many species with the development of genome sequencing, such as 18 AtOFP genes in *A. thaliana* (Wang et al., 2011), 35 RsOFP genes in *Raphanus sativus*, 31 SlOFP genes in *Solanum lycopersicum* (Huang et al., 2013), 29 BrOFP genes in *Brassica rapa* (Wang et al., 2021), and 8 VvOFP genes in *Vitis vinifera* (Wang et al., 2018).

In reported studies, the tomato SlOFP genes can be divided into three subfamilies (Huang et al., 2013), and the apple OFP genes can be divided into 15 subfamilies by carrying out evolutionary analysis of 727 protein sequences from 32 species (Li et al., 2019), while Liu et al. found that OFP genes in angiosperms were divided into 11 subfamilies by phylogenetic tree analysis (Liu et al., 2014). In the study, the CaOFP genes were divided into two subfamilies, namely Class I and Class II by identifying and classifying three varieties of pepper (Zunla-1, Chiltepin and CM334). However, these CaOFP genes can be subdivided according to their conserved domains and motifs. For example, Class I contains only six motifs, while Class II contains motif1, motif5, motif8, and motif10. These results indicated that the subfamily classification of OFP genes was quite different according to their plant properties, conserved domains and exon size. In a previous study, OFP genes of *Selaginella moellendorfii*, an early terrestrial plant, were found to have more than dozens of exons (Dangwal and Das, 2018), while there were 1-5 exons in the CaOFP gene family of pepper, and Class I-A subclade did not contain introns according to its subgroup classification.

Some studies have found that OFP was not only involved in the regulation of plant growth and development, but also in plant stress response and fruit morphology formation. Liu et al. cloned OVATE in tomato for the first time and found that it could control the shape of tomato fruit. A point mutation of OVATE gene led to premature termination of translation, which increased the longitudinal diameter of wild tomato fruit and limited the growth of its neck, thus developing from round fruit to pear fruit (Liu et al., 2002). In the yeast two-hybrid assay, nine OVATE proteins were found to interact with TALE homeobox proteins, and AtOFP1 and AtOFP5 co-regulated the subcellular localization of a TALE homeobox protein BLH1. When these two genes were co-expressed in tobacco leaves, the BLH1 protein was transported from the nucleus to the cytoplasm (Hackbusch et al., 2005). Numerous studies have shown that OFP played a role by interacting with KNOX and BELL transcription factors. For example, Schmitz et al. found that OsOFP2 could regulate KNOX-BELL function to participate in the development process of rice (Schmitz et al., 2015). Another study found that the CmOFP13 gene *faqs8.1* could alter the recessive structure of the...
gene in melon, which could lead to structural variation and affect the melon fruit morphology (Martínez et al., 2022). Current biochemical evidence suggests that OFPs may function in this feedback through interactions with three amino acid loop extension (TALE) proteins, as well as through interactions with additional proteins within signaling pathways (Snouffer et al., 2020). Expression patterns of OFP genes in different tissues and development stages of pepper and seven pepper varieties with different fruit shapes indicated that some CaOFP genes were actively involved in the growth and development of pepper, and participated in plant stress response process and fruit morphological development through mediating mediates.

Expression profiles of CaOFP genes in pepper

Recent studies have found that OFP gene was responsive to salt stress in plants. Tang et al. found that PpOFP1 had salt tolerance through yeast experiments in vitro (Tan et al., 2021). Ma et al. found that the OsOFP6 overexpression lines had slower water loss and less H$_2$O$_2$ accumulation under drought condition, while the RNAi lines had faster water loss and higher H$_2$O$_2$ content, which indicated that OsOFP6 may have drought resistance in rice (Ma et al., 2017). Characterization of CaOFP genes expression patterns under different stresses in pepper showed that CazOFP15, CazOFP16 and CazOFP17 can respond to regulation and participate in the salt stress process. Aabscisic acid responsiveness, salicylic acid responsiveness, defense and stress responsiveness, drought-inducibility, low-temperature responsiveness were also found in the upstream 2000 bp of pepper CaOFP genes. Defense and stress responsiveness, drought-inducibility, low-temperature responsiveness, etc. Now studies on OFP mainly focus on growth and development, including defense and stress response, low temperature response and drought induction, while no other studies under SA, JA, IAA, GA$_3$, ABA, H$_2$O$_2$, Mannitol stress have been reported. According to the analysis of cis-acting elements in pepper, It is speculated that CazOFP plays a functional role in responding to relevant elements, which provides new insights for subsequent studies on pepper’s response to biotic and abiotic stresses. By studying the expression profile of CazOFP genes under different tissues.
and stress conditions, this study provides theoretical support for studying the stress response process of OFP and its participation in the regulation of growth and development in pepper.

OFP genes have been found to reduce the length of hypocotyls, rosette leaves, stem leaves, inflorescence stems and floral organs in Arabidopsis mutants, while AtOFP1 regulates the target gene AtGA20ox1, resulting in insufficient gibberellin synthesis. These results indicated that OFP transcription factors negatively regulated plant growth and development (Wang et al., 2007). OFP can also promote chlorophyll accumulation and delay leaf senescence. Zhou et al. overexpressed SlOFP20 in tomato and found that the expressions of genes encoding transcription factors SlGLK1, SlGLK2 and HY5 related to chloroplast development and chlorophyll level were significantly up-regulated, which indicated that OFP had a positive regulatory effect on chlorophyll accumulation and retarding leaf senescence (Zhou et al., 2019). At present, it had been reported that OFP was involved in the fruit shape development process of pepper, and CaOVATE was found to be involved in the fruit shape process of tomato through the study of pepper varieties with different fruit shapes (Tsaballa et al., 2011). Expression pattern analysis of Citrus OFPs (CitOFPs) showed that CitOFP19 had significantly higher expression level in the ovaries of round pummelo than in those of pear-shaped pummelo (Wu et al., 2022). We also found that the CazOFP genes were up-regulated in the early stages of pepper fruit development (Dev1–Dev5) in the study. However, CazOFPs expression tended to be down-regulated in the late fruit stages. The expression levels of CazOFP9 and CazOFP13 in thin pepper varieties were higher than those in large pepper varieties, suggesting that CazOFP9 and CazOFP13 were involved in the fruit shape development of thin pepper varieties. In brief, CazOFP genes with different expression levels in pepper may be involved in plant growth and development, thus playing both positive and negative regulatory roles.

Fruit shape is an important quality and yield trait of pepper. High quality pepper and excellent fruit shape help to improve its market competitiveness and increase economic output value. The mechanism and functional verification of OFP transcription factors regulating the growth and development of pepper will become the focus of future research. With the development of plant genome field, it is helpful to explore more OFP gene

FIGURE 8
Expression of eight CazOFP genes in two developmental stages of seven pepper varieties with different fruit shapes. (A) Fruit diagrams of seven varieties with different fruit shapes. These varieties (M1-M7) are zunla-1, ZJ-5, 11C255-1, ZJ11, HYL, ZJ-1 and T803. Above is the fruit development stage 1 (fruit with mature green). Below is the fruit development stage 2 (fruit at breaker plus 5 days). (B) The levels of these eight CazOFP genes detected by qRT-PCR.
functions and provide theoretical and practical support for the study of plant shape and morphological development.

Data availability statement

Publicly available datasets were analyzed in this study. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

YL, SY, XL, XZ, and CQ conceived and designed the experiments and drafted the manuscript. JL, TL, XT, and FL performed the experiments. YL, SY, and CQ analyzed the data. All authors read and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.941954/full#supplementary-material

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