

# Study of JASMONATE ZIM-Domain gene family to waterlogging stress in *Cucumis sativus* L.

Jiawei Pan<sup>1</sup>, Jingyun Tu<sup>1</sup>, Rahat Sharif<sup>1</sup>, Xiaohua Qi<sup>1,2</sup>, Xuewen Xu<sup>1,2\*</sup>, and Xuehao Chen<sup>1,2\*</sup>

<sup>1</sup> School of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, Jiangsu, China

<sup>2</sup> Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Yangzhou 225009, Jiangsu, China

\* Corresponding author, E-mail: [xxu323@yzu.edu.cn](mailto:xxu323@yzu.edu.cn); [xhchen@yzu.edu.cn](mailto:xhchen@yzu.edu.cn)

## Abstract

Jasmonate ZIM Domain proteins (JAZs) provide a response to multiple abiotic stresses by critically regulating the JA signaling pathway. In this study, a total of 11 *CsJAZ* genes were identified in the cucumber genome database. *In silico* analysis revealed the presence of different *cis*-acting regulatory elements related to growth, stress and hormonal responsiveness in the upstream region of *CsJAZ* genes. The transcriptional expression pattern analysis showed that all *CsJAZ* genes were expressed in the tissues tested and all *CsJAZ* genes responded to plant hormone GA, suggesting they may participate in complex hormone signaling networks. An expression analysis based on qRT-PCR, indicated that cucumber *CsJAZ8* was decreased significantly by waterlogging treatment but induced by exogenous methyl jasmonate application. Therefore, we speculate that *CsJAZ8* could be a potential regulator of the response of cucumber to waterlogging stress. Additionally, transient expression assay showed that the *CsJAZ8* protein is localized in the nucleus and could be involved in the regulation of various biological and cellular processes. Furthermore, Y2H assay revealed that cucumber *CsJAZ* proteins exhibit homologous and heterologous interactions. Subsequently, this research will contribute to the understanding of the *CsJAZ* gene family in *Cucumis sativus*. It also provides further insight into the role of *CsJAZ* proteins in waterlogging stress and may provide a new frontier in cucumber breeding for waterlogging tolerance.

**Citation:** Pan J, Tu J, Sharif R, Qi X, Xu X, et al. 2021. Study of JASMONATE ZIM-Domain gene family to waterlogging stress in *Cucumis sativus* L. *Vegetable Research* 1: 3 <https://doi.org/10.48130/VR-2021-0003>

## INTRODUCTION

Jasmonate (JA) and its derivatives are generally used as plant growth regulating compounds. These biomolecules have specific functions in plant responses to differing biotic and abiotic stresses<sup>[1,2]</sup>. The induction in plant immunity post JA application has been previously reported against cold stress<sup>[3]</sup>, drought stress<sup>[4]</sup>, high temperature stress<sup>[5]</sup> and salt stress<sup>[6]</sup>. Additionally, JA can regulate various aspects of growth and development, including seed germination<sup>[7]</sup>, root growth<sup>[8]</sup>, hypocotyl growth<sup>[9]</sup>, stamen development<sup>[10]</sup>, and flowering and blooming<sup>[11]</sup>. In general, JA in plants is synthesized from  $\alpha$ -Linolenic acid (ALA) via the octadecane pathway<sup>[12]</sup>. Firstly, the synthesis of unsaturated fatty acids to 12-oxo-phytyadienoic acid (12-OPDA) is completed in the chloroplast, which is oxidized to 13-hydroperoxylinolenic acid (13-HPOTE) under the action of lipoxygenase (LOX), which is then oxidized to 12-oxo-phytyadienoic acid under the catalysis of allene oxide synthase (AOS) and allene oxide cyclase (AOC). With the help of transporter proteins, 12-OPDA is transported to the peroxisome for downstream reaction, and JA is finally formed after 12-OPDA reductase and a three-step  $\beta$  oxidation reaction. After JA is transported to the cytoplasm, active JAs (+)-7-iso-JA-L-isoleucine ((+)-7 is formed under the action of JA amino acid synthetase (JAR1) -iso-JA-Ile)<sup>[13]</sup>.

The JAZ (JASMONATE ZIM domain protein) repressor protein is a key stress marker gene, specific to plants. JAZ belongs to the TIFY family, which is characterized by a

conserved TIFY motif (also known as ZIM), and there is a TIF[F/Y] XG near the N-terminus, which exhibits homo- and heteromeric interactions<sup>[14]</sup>. Its C-terminus also contains a Jas motif SLX2FX2KRX2RX5PY (also known as CCT\_2). The existence of the Jas motif makes the JAZ protein different from other members of the TIFY protein family<sup>[15]</sup>. JAZ does not contain a DNA-binding domain and is functionally dependent on protein interactions<sup>[15]</sup>. Under normal conditions, the plant endogenous JA content is low, the JAZ inhibitor binds to the transcription factors (TFs), and recruits the co-inhibitor TOPLESS (TPL) by interacting with the adaptor protein NINJA (novel interactor of JAZ, NINJA) and TPL-related protein (TPR) to inhibit the activity of TFs. The JAZ inhibitor protein tightly binds to the MYC2 transcription factor required to inhibit JA-induced gene expression. The JAZ protein targeted by the SCF<sup>COI1</sup> complex plays a negative regulatory role in the JA signaling pathway. In the process of hormone accumulation and perception, the active JA-Ile is identified by SCF<sup>COI1</sup>, which promotes the binding of JAZ protein and COI1 (CORONATINE INSENSITIVE 1)<sup>[16,17]</sup>, and facilitates the ubiquitination and degradation of the JAZ protein. Moreover, the 26S proteasome releases transcription factors from NINJA and TPL, leading to the release of MYC2 transcription factor, initiating transcriptional reprogramming of cells, and activating the JA response<sup>[18–20]</sup>.

Cucumber (*Cucumis sativus* L.) ( $2n = 2x = 14$ ) belongs to the family *Cucurbitaceae* and is an important vegetable crop. Cucumber is widely cultivated for its fruit in warm climates

around the world. Waterlogging is becoming an increasing threat causing abiotic stress owing to current climate change. Under the background of frequent rainfall and flood disasters caused by global warming, regional seasonal rainfall continues to rise which leads to the upsurge of the water table, resulting in waterlogged soil<sup>[21–23]</sup>. Cucumber is extremely sensitive to waterlogging stress due to its less developed root system along with poor water absorption capacity<sup>[24,25]</sup>. The publication of the cucumber genome sequence in 2009<sup>[26]</sup> made it feasible to carry out extensive molecular work relating to the breeding of cucumber. Further, the availability of the cucumber genome database made it significantly convenient in the identification of genes controlling various agronomic traits and regulating cucumber response to environmental stress.

Since JA plays a key role in enhancing plant tolerance to a variety of stresses, JA could be of great importance to elucidate the transcriptional regulatory mechanism of JAZ protein under waterlogging stress in cucumber. Although many members of the JAZ gene family have been extensively identified and studied in various crops<sup>[14,27–31]</sup>, the function and regulatory mechanisms regarding this gene family in cucumber waterlogging tolerance remain poorly understood. The present study comprised of bioinformatics analysis of the JAZ gene family, a key gene family in the jasmonic acid signal transduction pathway in cucumber. A complete set of 11 *CsJAZ* genes were identified and characterized. Additionally, the phylogenetic relationship, protein conserved motif, gene structure analysis, chromosomal location, gene duplication, selective evolutionary pressure, *cis*-acting regulatory analysis, and transcription expression of the cucumber JAZ gene under waterlogging stress were analysed in different tissues under hormone application. We also examined the subcellular localization of *CsJAZ8* in tobacco cells and characterized their homologous or heterologous interactions with the JAZ family. This study presents a theoretical foundation for further studies regarding the function of the *CsJAZ* gene in cucumber, and may provide a new development in cucumber breeding for waterlogging tolerance.

## RESULTS

### Genome-wide identification of *CsJAZ* genes in *Cucumis sativus*

In order to identify the *CsJAZ* genes from the cucumber genome, SMART and NCBI CD-searches were carried out to

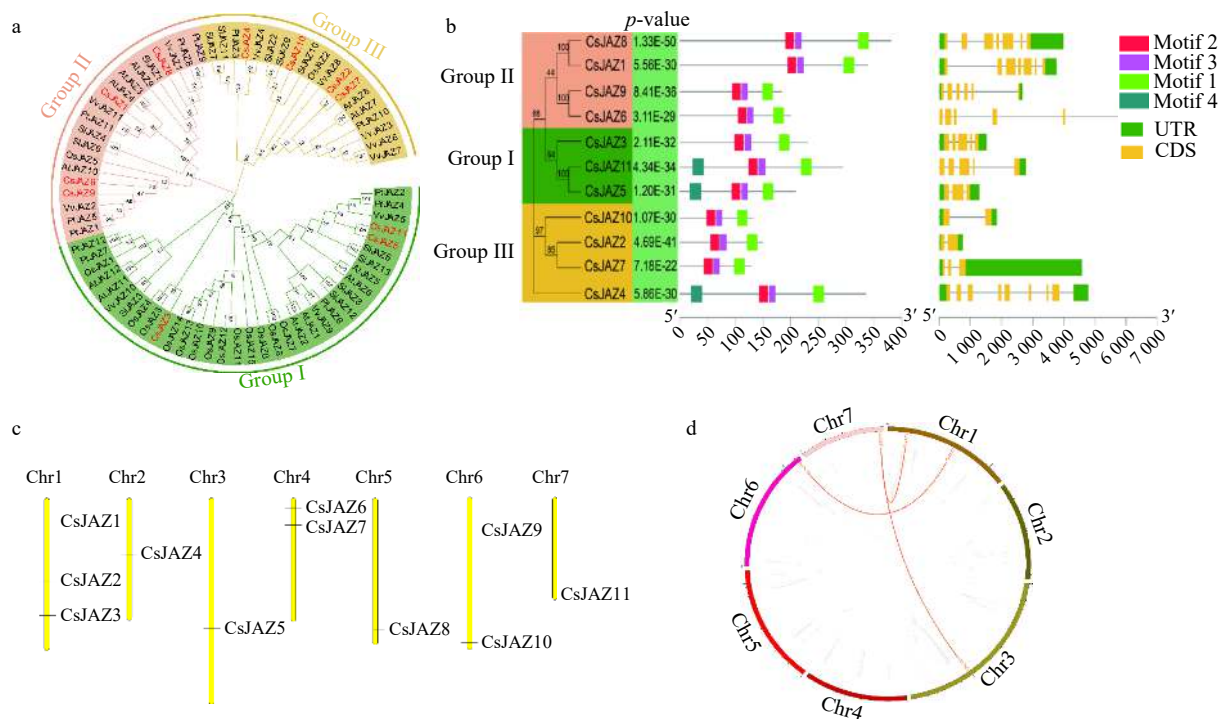
confirm the JAZ protein from their possession of conserved TIFY and Jas domains in the generated amino acid coding sequence. Next, 11 non-redundant *CsJAZ* genes were identified in the cucumber genome. According to the chromosomal location of these genes, all *CsJAZ* genes are systematically numbered, from *CsJAZ1* to *CsJAZ11*. The protein characteristics of the identified JAZ genes were studied using the protparam online server. The length of the protein sequence encoded by these *CsJAZ* genes ranged from 129aa (*CsJAZ7*) to 381aa (*CsJAZ8*). The molecular weight of the proteins were between 14,743.72 kDa (*CsJAZ10*) to 39,666.79 kDa (*CsJAZ8*). The isoelectric point ranged from 6.71 (*CsJAZ4*) to 10.42 (*CsJAZ7*), with most of them being concentrated at 9. The hydrophilicity and hydrophobicity of 11 *CsJAZ* protein sequences were predicted, and the results showed that *CsJAZ* proteins were all hydrophilic proteins (GRAVY < 0) (Table 1).

### Phylogenetic, gene structure, and conserved motifs analysis of *CsJAZ* genes in *Cucumis sativus*

To gain insightful knowledge regarding the evolutionary relationship of JAZ genes from cucumber, we utilized MEGA X software to perform ClustalW multiple sequence alignment of the JAZ protein sequences, retrieved from cucumber, *Arabidopsis thaliana*, *Populus trichocarpa*, *Vitis vinifera*, *Solanum lycopersicum*, and *Oryza sativa*. An unrooted phylogenetic neighbor-joining tree was constructed on JAZ protein using MEGA X software. It can be seen from the phylogenetic tree that these JAZs are divided into 3 independent branches (Group I – Group III) according to the homology relationship (Fig. 1a). Among these subgroups, there are three *CsJAZ* genes in Group1 (*CsJAZ3*, *CsJAZ5* and *CsJAZ11*), accounting for about 27.3% of the total. Group II has four *CsJAZ* genes (*CsJAZ1*, *CsJAZ6*, *CsJAZ8* and *CsJAZ9*). Group3 has four *CsJAZ* Genes (*CsJAZ2*, *CsJAZ4*, *CsJAZ7* and *CsJAZ10*). The results additionally showed that the evolutionary similarity of cucumber JAZ protein with *Populus trichocarpa*, *Vitis vinifera*, *Arabidopsis thaliana*, and *Solanum lycopersicum* is higher than that of *Oryza sativa*. This is consistent with the fact that the former four species and cucumber are dicotyledonous plants. The emergence time is later than the differentiation from the lineage of monocots. No species-specific population was found in the phylogenetic tree of the cucumber *CsJAZ* gene. Although there are differences between each subfamily of different species, members within the same subfamily are similar in nature, which indicates the conservative nature of JAZ genes in plantae.

**Table 1.** Summary information on the *CsJAZ* gene family in cucumber.

Gene name	Gene identifier	Chr	Start (bp)	End (bp)	ORF (aa)	MW (KDa)	PI	GRAVY
<i>CsJAZ1</i>	Csa1G042920	1	4573626	4577405	340	36 619.75	9.2	-0.25
<i>CsJAZ2</i>	Csa1G435720	1	15995968	15996717	150	16 930.92	7.21	-0.767
<i>CsJAZ3</i>	Csa1G597690	1	22692966	22694494	231	25 028.26	9.98	-0.455
<i>CsJAZ4</i>	Csa2G222060	2	10711729	10716520	336	36 967.37	6.71	-0.816
<i>CsJAZ5</i>	Csa3G645940	3	25350295	25351576	209	22 874.76	8.94	-0.631
<i>CsJAZ6</i>	Csa4G009880	4	1470022	1476285	200	22 487.17	6.98	-0.553
<i>CsJAZ7</i>	Csa4G062400	4	4986428	4991013	129	14 861.18	10.42	-0.425
<i>CsJAZ8</i>	Csa5G628650	5	25425307	25429296	381	39 666.79	9.3	-0.236
<i>CsJAZ9</i>	Csa6G091930	6	6249153	6251831	184	20 306.27	9.41	-0.317
<i>CsJAZ10</i>	Csa6G523460	6	28126619	28128465	132	14 743.72	9.5	-0.805
<i>CsJAZ11</i>	Csa7G448810	7	18408423	18411214	295	32 092.09	9.31	-0.758



**Fig. 1** (a) Phylogenetic analysis of JAZ proteins in 11 *C. sativus* (Cs), 12 *A. thaliana* (At), 13 *S. lycopersicum* (Sl), 15 *O. sativa* (Os), 11 *V. vinifera* (Vv), and 12 *P. trichocarpa* (Pt). ClustalW multi-sequence alignment was performed and an unrooted phylogenetic neighbor-joining tree was constructed from JAZ protein by using MEGA X software. Genes IDs are listed in Supplemental Table S3. (b) Phylogeny, gene structure, and motif compositions of the JAZ gene family in cucumber. (c) Chromosome distribution of *CsJAZ* in the genome of cucumber. The chromosome numbers were demonstrated at the top of each bar and the scale is in megabases (Mb). (d) Gene duplication of *CsJAZ* genes on 7 chromosomes of cucumber genome. Gray lines represent all syntenic blocks in the cucumber genome. Duplicated gene pairs are identified with red lines inside the circle. Each chromosome number is represented using different colours.

The evolution of gene families is largely manifested in the variation of conserved motifs in family members<sup>[32]</sup>. The MEME online prediction tool was used to identify the conserved motifs in the *CsJAZ* protein of cucumber (Fig. 1b and Table 2). All the 11 *CsJAZ* genes contain either motif 1 (Jas) and motif 2 (TIFY). In the three subgroups, *CsJAZ* genes contain motifs ranging from 1 to 4. It is worth noting that closely related genes in the phylogenetic tree have similar gene structural components, indicating similar functions within subgroups. The results show that all *CsJAZ* proteins contain conserved motifs. The evolution of gene families is largely reflected in the diversity of gene structures inside the family members<sup>[32]</sup>. In order to better understand the structure of the *CsJAZ* gene of cucumber, the exon-intron structure of the *CsJAZ* gene was analyzed using the website Gene Structure Display Server (GSDS) (<http://gsds.cbi.pku.edu.cn>) (Fig. 1b). The *CsJAZ* genes ranged from 2 (*CsJAZ2* and *CsJAZ10*) to 9 (*CsJAZ4*) exons and 1 to 8 introns. The clustering results with the evolutionary tree showed that genes with similar gene structures clustered together. In Group I, the two genes *CsJAZ3* and *CsJAZ11* both have 5 exons and 4 introns, whereas *CsJAZ5* has 3 exons and 2 introns. Both *CsJAZ1* and *CsJAZ8* in Group II have 7 exons and 6 introns, the *CsJAZ6* and *CsJAZ9* in Group II have 6 exons and 5 introns. In Group III, *CsJAZ2* and *CsJAZ10* both have 2 exons and 1 intron, *CsJAZ7* has 3 exons and 2 introns, *CsJAZ4* has 9 exons and 8 introns. We observed that the *CsJAZ* genes are both conservative and

variable in the Group, which may be related to the different modes of action and distribution in cucumber.

### Chromosomal location, duplication events, selective evolutionary pressure analysis of *CsJAZ* genes in *Cucumis sativus*

The distribution of cucumber *CsJAZ* genes on chromosomes were visualized using MapChart software (Fig. 1c). The results showed that 11 *CsJAZ* genes were distributed on seven chromosomes of cucumber. There were three *CsJAZ* genes on the Chr1 accounting for 27.3% of the total. Chr2, Chr3 and Chr5 have only one *CsJAZ* gene. There were two *CsJAZ* genes on Chr4, Chr6 and Chr7, accounting for 18.2% of the total. By mapping *CsJAZ* genes on chromosomes, we found that although the number of *CsJAZ* genes was small, they were distributed on each chromosome where chromosome 1 possessed the highest number of genes.

To investigate the gene duplication events, we used the Multiple Collinearity Scan toolkit (MCScanX) with default parameters to detect the duplicated blocks in the cucumber genome. The segment duplication events were characterized by copying the whole gene block from one chromosome to another, while adjacent homologous genes with no more than one intervening gene on the same chromosome were considered a tandem duplication event. We analyzed the tandem and segment duplication genes in the whole genome of cucumber. Three gene pairs were generated from five tandem duplicated genes (Fig. 1c and Supplemental

**Table 2.** Information on conserved motifs in cucumber JAZ proteins.

Motif	E-value	Sites	Width	Sequence	Logo
1	1.6e-077	11	20	DLPJARKASLQRFLEKRRKDR	
2	5.7e-074	11	15	QMTIFYNGKVCVYBD	
3	2.6e-013	11	15	PEDKAKAIMALASKG	
4	4.0e-003	3	21	EKSNSFAQTCNLLSQYLKEKRT	

Table S1). Meanwhile, five segment duplicated genes were identified and they formed three pairs (Supplemental Table S1). Combined with the phylogenetic tree (Fig. 1a), we found that the majority of duplicated pairs were clustered together in the same subgroups. The ratio Ka/Ks of non-synonymous substitution (Ka) and synonymous substitution (Ks) can reflect the selection criterion during biological evolution. As a result, the Ka/Ks ratios between *CsJAZ* genes were all greater than one, indicating that *CsJAZ* genes were mainly subjected to positive selection (Supplemental Table S2).

### Putative *cis*-acting regulatory elements in the promoter regions of *CsJAZ* genes

In order to obtain information on the *cis*-acting regulatory elements of the *CsJAZ* gene family, we analyzed the sequence of the promoter region of each *CsJAZ* gene (Supplemental Table S4). The -1500 bp promoter regions upstream of 11 *CsJAZ* genes were extracted and the *cis*-acting elements were analyzed using the Plant CARE website (Fig. 2). The results uncovered the potential regulatory role of *CsJAZ* genes as they carry hormones, growth and stress related *cis*-acting elements (Supplemental Table S5). Hormonal responsive *cis*-elements such as TCA-element and SARE (involved in salicylic acid responsiveness), ABRE (involved in abscisic acid responsiveness), CGTCA-motif and TGACG-motif (involved in MeJA-responsiveness) were found dominantly in the majority of the *CsJAZ* genes; Meanwhile, auxin responsive *cis*-acting elements known as TGA-box and TGA-element, gibberellin-responsiveness *cis*-acting elements named TATC-box and P-box were also identified in the upstream region of some genes. The presence of *cis*-acting elements involved in light responsiveness named G-box, GATA-motif, GT1-motif, and circadian which are related to circadian control, highlights the diverse roles of *CsJAZ* genes

in regulating growth and providing response to environmental stimuli. In addition, some other stress-responsive *cis*-acting regulatory elements named LTR (low temperature-responsive), ARE (anaerobic induction *cis*-elements), TC-rich repeats (involved in defense and stress) were also recognized in the promoter region of *CsJAZ* genes. Therefore, the presence of these important *cis*-acting elements in *CsJAZ* genes confirms their potential involvement in controlling multiple biological functions in cucumber.

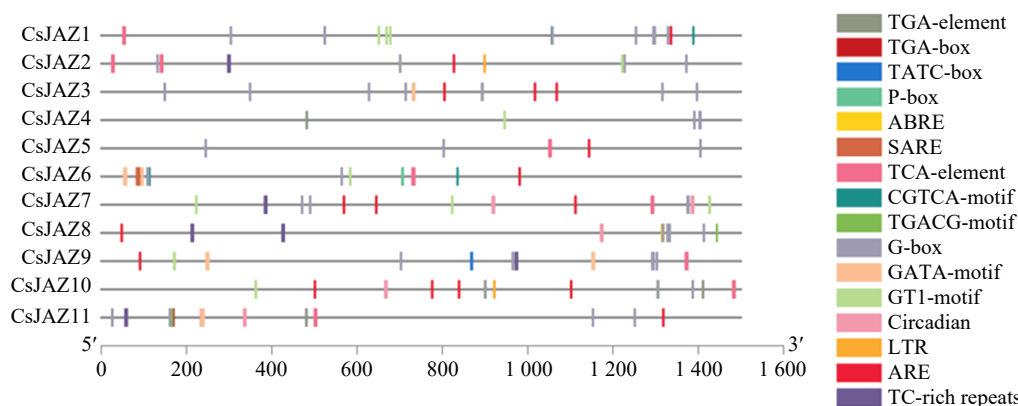
### Morphological changes in responses to waterlogging and waterlogging+MeJA

Morphological observation of the ARs developed over hypocotyl at 3, 5, and 7 d post waterlogging stress were carried out. Relatively similar phenotypes of control check (CK), Waterlogging (WL), and Waterlogging+MeJA (WL+MeJA) were observed at 3 d. The appearance of ARs on hypocotyl of WL stressed plants was clear at 5 d, whereas no ARs were found on CK and WL+MeJA treated hypocotyl. After 7 d of waterlogging stress, WL treatment produced a large number of ARs, and the number of ARs was higher than that of CK and MeJA treated plants (Fig. 3).

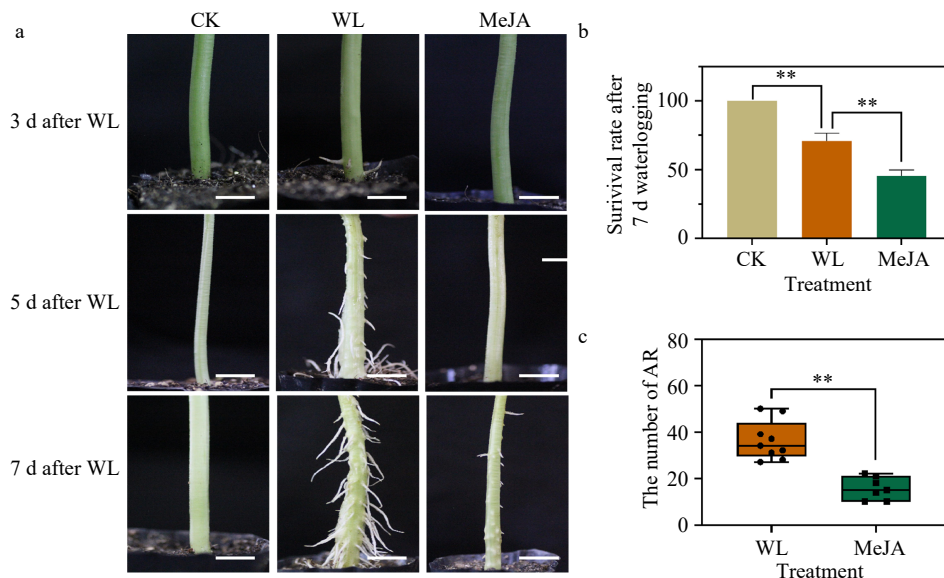
The graphical representation of the observed phenotypes revealed that the survival rate of CK treatment was 100%. In contrast, only 50% of the WL+MeJA treated plants survived. Interestingly, the survival rate of WL treated plants was about 64%. The number of ARs in WL treatment was significantly higher than that in MeJA treatment.

### Expression of *CsJAZ* genes in response to waterlogging treatment

Following the bioinformatic analysis of *CsJAZ* genes, we performed quantitative PCR analysis using the cDNA of



**Fig. 2** Prediction of *cis*-acting elements in *CsJAZ* promoters associated with plant hormones and stress are shown. The distribution of *cis*-acting elements in the 1.5 Kbp upstream promoter region of the *CsJAZ* genes associated with plant hormones, growth and stress are illustrated.



**Fig. 3** Morphological changes and statistical collation in response to waterlogging and waterlogging+MeJA. (a) The response of WL and WL+MeJA after 3, 5, 7 d waterlogging treatment. (b) The survival rate of WL and WL+MeJA after 7 d of waterlogging treatment. (c) Comparison of the adventitious root (AR) number capacity of WL and WL+MeJA after 7 d of waterlogging treatment. \*\*  $p < 0.01$ .

waterlogging stressed hypocotyl organs of the cucumber plant. The expression of the *CsJAZ* was investigated for different stress periods, 6, 12, 48 and 96 h respectively (Fig. 4). We found that between the 11 *CsJAZ* genes, the majority of the *CsJAZ* genes displayed down-regulated expression trends under waterlogging stress. Among them, *CsJAZ1*, *CsJAZ2*, *CsJAZ3*, *CsJAZ4*, *CsJAZ8*, *CsJAZ9*, *CsJAZ10*, and *CsJAZ11* decreased under waterlogging stress. Similarly, in addition to *CsJAZ4*, the expression of other *CsJAZ* were suppressed under waterlogging stress and also in the control (CK), but increased markedly under MeJA application. On the other hand, the transcript level of *CsJAZ5*, *CsJAZ6* and *CsJAZ7* induced significantly under waterlogging stress and waterlogging stress plus MeJA application. The mRNA level of *CsJAZ1* rose to a maximum of 0.2 at 6 h under MeJA but fell again at 12, 24, 48, and 96 h in CK and also under waterlogging stress plus MeJA application. Concurrently, the expression level of *CsJAZ1*, *CsJAZ2*, and *CsJAZ3* decreased at most of the timepoints in CK and waterlogging treated samples but contrastingly increased under MeJA treatment and peaked to a maximum of 0.17, 0.004 and 0.07 fold respectively. Therefore, we speculate that the JAZ genes, important regulators of the jasmonic acid signaling pathway, are involved in cucumber response to waterlogging stress.

### Transcriptional expression patterns of *CsJAZ* in different tissues and post exogenous GA treatment

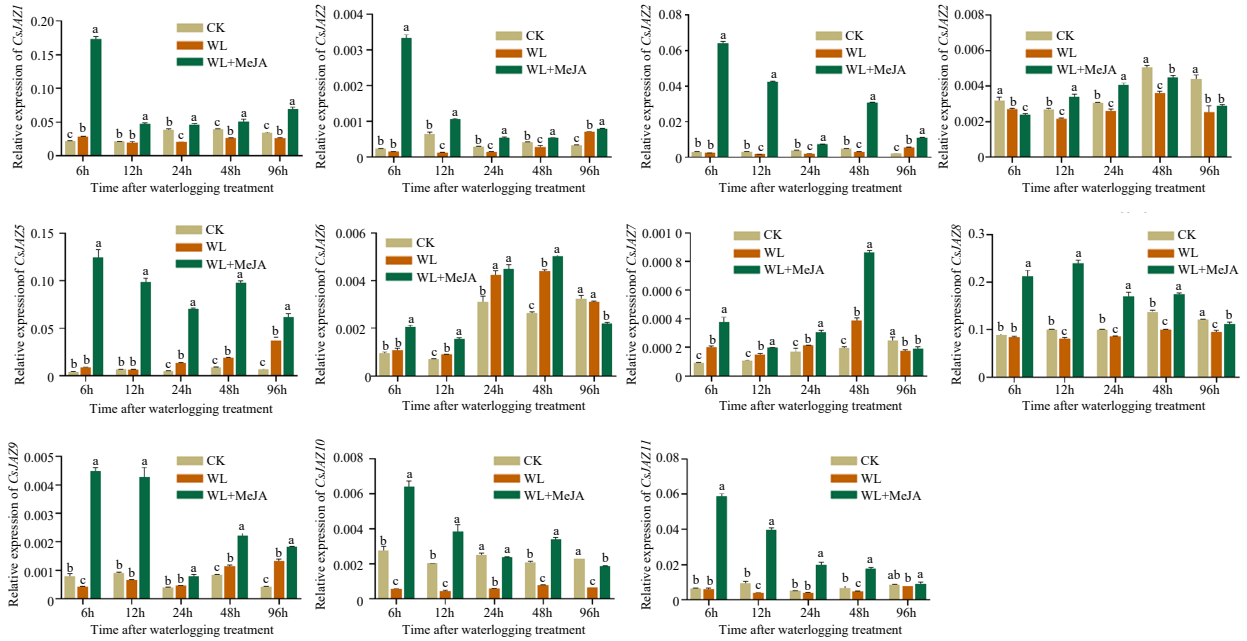
The RPKM values of *CsJAZ* in different tissues and post exogenous GA treatment were downloaded from the cucumber database (<http://cucurbitgenomics.org>) (Supplemental Table S6). Generally, all the *CsJAZ* genes were expressed in leaf, stem, ovary, tendril, fruit, root, male flower, and female flower (Fig. 5). Moreover, six genes (*CsJAZ1*, *CsJAZ2*, *CsJAZ7*, *CsJAZ8*, *CsJAZ9*, and *CsJAZ11*) were highly expressed in male flowers and three genes (*CsJAZ3*, *CsJAZ5*, and *CsJAZ10*) were highly expressed in fruit. *CsJAZ1* was highly expressed in both leaf and stem. The *CsJAZ4* and *CsJAZ6*

displayed dominant expression patterns especially in ovary tissue. Interestingly, further analysis revealed a correlation between tissue expression and evolution of the *CsJAZ* gene. For example, the *CsJAZ5* and *CsJAZ11* genes in group I, the *CsJAZ2* and *CsJAZ7* genes in group III are closely related to the phylogeny of each homologous gene pair, but the tissue expression patterns are not consistent. These results indicate that *CsJAZ* genes have different expression patterns and tissue specificity. Moreover, the 11 *CsJAZ* genes were divided into three distinct groups. According to cluster analysis, eight organ tissues were also clustered into three distinct groups on the basis of their specific expression patterns.

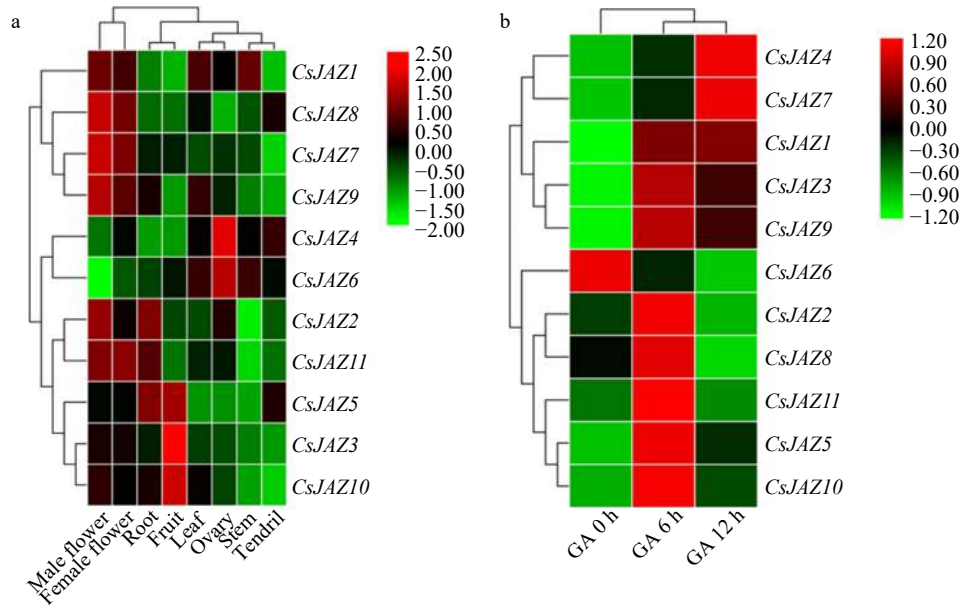
In response to GA treatment, all the *CsJAZ* genes were expressed following post exogenous GA application. In detail, the majority of *CsJAZ* genes (except *CsJAZ6*) were strongly upregulated after 6 h GA treatment. Among them, *CsJAZ2*, 5, 8, 10, 11 subsequently decreased at 12 h. On the other hand, *CsJAZ1*, 3, 9 were upregulated at 6 and 12 h after exogenous GA treatment. Noticeably, *CsJAZ6* was significantly decreased by GA treatment from 6 h to 12 h. These results indicate that all *CsJAZ* genes respond to the plant hormone GA, and they may participate in complex hormone signaling networks.

### Subcellular localization of *CsJAZ* proteins

In order to determine the subcellular localization of *CsJAZ* protein in cucumber cells, we cloned the CDS sequences of *CsJAZ8* with specific primer, and finally generated a *CsJAZ*-pCambia1301 construct. The construct encodes the *CsJAZ* protein fused with GFP. The *CsJAZ*-GFP fusion driven by the 35S promoter was introduced into the Tobacco epidermis. The GFP signal is detected in the nucleus of the transformed cell under a laser confocal microscope (Fig. 6), which is consistent with the prediction of Cell-PLoc 2.0 (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi>). Genes residing in the nucleus are generally considered as functional genes and could be involved in the regulation of various biological and cellular processes<sup>[33,34]</sup>.



**Fig. 4** Expression of *CsJAZ* genes in response to waterlogging treatment. The different lowercase letters above the bars indicate the significance ( $p < 0.05$ ) of the relative expression level between two samples. The data for quantitative RT-PCR analysis were the mean and standard deviation of three bio-duplicates in the hypocotyl and three technical duplicates in each biological sample. The Y-axis is the relative level of expression.

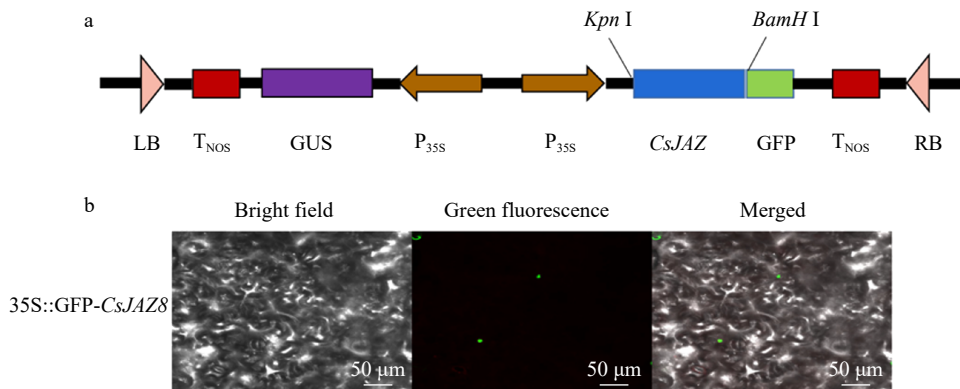


**Fig. 5** Heatmap representation of *CsJAZ* genes in different tissue and post exogenous GA treatment of cucumber. The log<sub>2</sub> transformation method was used to normalize and convert the RPKM values and displayed on the colored scale bar.

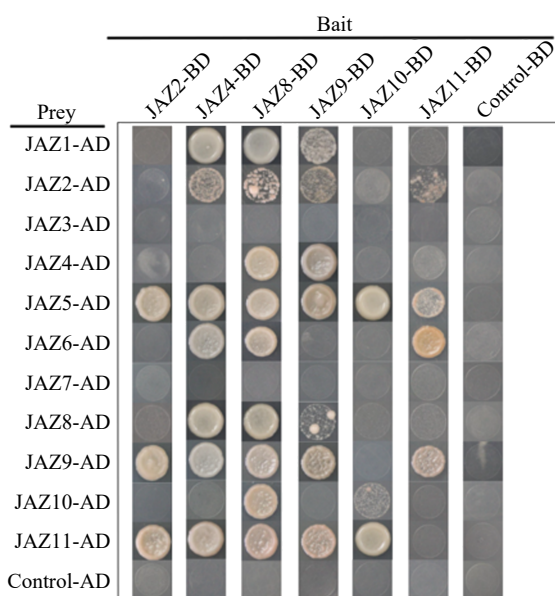
**Homo- and heterodimeric interaction of *CsJAZ* proteins**

In *Arabidopsis*, some JAZ proteins exhibit homologous and heterologous interactions mediated by the ZIM domain<sup>[14]</sup>. For this purpose, we used a yeast two-hybrid (Y2H) assay to determine whether these proteins interact to form homodimers or heterodimers. Due to the high degree of similarity in the nucleotide sequence of homologous genes,

we selected 8 *CsJAZ* genes which were suppressed under waterlogging stress (*CsJAZ1*, *CsJAZ2*, *CsJAZ3*, *CsJAZ4*, *CsJAZ8*, *CsJAZ9*, *CsJAZ10*, and *CsJAZ11*), and cloned them. Through the toxicity self-activation test, we found that *CsJAZ1* and *CsJAZ3* have a self-activation phenomenon and cannot be mutually verified. Finally, 66 protein combinations were tested (Fig. 7). As shown in Fig. 7, the cucumber *CsJAZ* protein has homologous and heterologous interactions. The experimental



**Fig. 6** Subcellular localization analysis of CsJAZ8-GFP fusion protein transiently expressed in *Nicotiana tabaccum* L. (a) Schematic representation of constructs used for subcellular localization of the CsJAZ8 protein. (b) The photographs were taken using green fluorescence (GFP), visible light, and merged light. Bar = 50 μm.



**Fig. 7** Yeast two-hybrid analysis of Homo- and heteromeric interactions in CsJAZ proteins. The full-length CsJAZ was fused with pGADT7 and pGBKT7 to generate prey and bait vectors, respectively, and then transformed into the yeast strain AH109. The interactions were determined by growth on SD-Trp/-Leu/-His/-Ade nutritional selection medium. The mating of pGBKT7-53/pGADT7-T7 was used as a positive control, and pGBKT7-Lam/pGADT7-T7 was used as a negative control.

results show that three of the six proteins (CsJAZ8, CsJAZ9, and CsJAZ10) exhibit homodimeric interactions, among which CsJAZ8 and CsJAZ9 exhibit strong homologous interactions, while CsJAZ10 homodimers interact with other members and displayed weak interaction capacity. As baits, CsJAZ4, CsJAZ8 and CsJAZ9 can interact strongly with other JAZ protein members. Meanwhile, the prey subordinate protein CsJAZ9 can interact with JAZ2, 4, 8, 9, 11, but not with CsJAZ10. On the other hand, as bait, CsJAZ9 can also interact with JAZ2, 4, 8, 9, 11, however, not interact with CsJAZ10. CsJAZ8 showed interaction with CsJAZ4, 8, and 9, both as prey and bait. Similarly, CsJAZ4 interacted with CsJAZ8 and 9 both as prey and bait. Therefore, we speculate that the cucumber JAZ gene may work in corresponding order to respond to

waterlogging stress and perhaps other environmental stimuli through intra-family interaction.

## DISCUSSION

The JAZ proteins belong to a plant-specific family known as TIFY<sup>[35]</sup>. To date, JAZ family proteins have been investigated in numerous plant species such as *Arabidopsis thaliana*<sup>[14,17,36]</sup>, *Oryza sativa*<sup>[27]</sup>, *Glycine soja*<sup>[29]</sup>, *Vitis vinifera*<sup>[28]</sup>, *Nicotiana attenuata*<sup>[37]</sup>, *Zea mays*<sup>[38]</sup>, *Saccharum spp.*<sup>[39]</sup>, *Melampsora larici-populina*<sup>[30]</sup>, *Lycoris Aurea*<sup>[40]</sup>, *Camellia Sinensis*<sup>[31]</sup>, *Brassica juncea var. tumida*<sup>[41]</sup>. However, there is currently no study available on the comprehensive understanding of JAZ proteins in cucumber. In this report, we retrieved the protein sequences of CsJAZ genes from the cucumber genome database. After that, we performed extensive bioinformatic analysis to unravel their evolutionary distribution and potential functions in the cucumber genome.

JAZ protein plays an important role in plant response to biotic and abiotic stresses as a key transcriptional inhibitor of plant jasmonic acid signaling pathway<sup>[42-44]</sup>. Analysis of CsJAZ promoters revealed a large number of hormones, growth and stress-related elements, but the types and numbers of elements contained in the different genes are not the same, indicating that the type and degree of response to hormone by cucumber CsJAZ gene is different, leading to functional differences between genes. In addition, some genes also contain multiple stress responsive cis-elements at the same time. It is speculated that CsJAZ may be involved in the regulation of different hormone signals to respond to environmental stress. In view of the presence of abiotic stresses response and hormone response cis-acting regulatory elements in the CsJAZ promoter region (Fig. 2), suggesting their potential involvement in regulating multiple abiotic stresses and could be vital in enhancing the resistance of cucumber against waterlogging stress.

JA deficient mutants *opr3/dde1* and *dde2-2* produced more ARs than the wild type<sup>[45,46]</sup>. Gutierrez et al.<sup>[47]</sup> showed that the accumulation of JA in *Arabidopsis gh3* mutants was twice that of the wild type. This higher accumulation of JA resulted in the inhibition of AR. Another study highlighted the negative role of JA in the development of ARs. The arresting of the growth of ARs was observed in the Pepino

(waterlogging sensitive line) and was found to be due to the increase in endogenous JA content. In contrast, the Zaoer N (resistant line) displayed reduced JA accumulation activity<sup>[48]</sup>. Similarly in the current study, the application of MeJA suppressed the ARs growth under waterlogging stress. The mechanism of how JA inhibits the growth of adventitious roots under waterlogging stress however needs further study.

We found that the JAZ gene, an important regulator of the jasmonic acid signaling pathway, was involved in cucumber response to waterlogging stress. Under waterlogging stress, the relative expression levels of all CsJAZ genes were up-regulated or down-regulated (Fig. 4). CsJAZ4 and CsJAZ8 genes were down-regulated at different time points after waterlogging stress. Therefore, we hypothesized that CsJAZ8 could be a potential candidate in regulating the mechanism of cucumber to waterlogging stress. In line with that, previous studies have shown that JAZ proteins are crucial in enhancing the defense mechanism of different plant species against various adverse conditions. For example, overexpression of *GsJAZ2* enhances the resistance of soybean against alkali stress by regulating the intracellular pH homeostasis, maintaining the osmotic balance and expression of stress specific genes<sup>[49]</sup>. Overexpression of Apple *MdJAZ2* also improved the tolerance of transgenic *Arabidopsis* to NaCl treatment<sup>[50]</sup>. There was no significant difference in the phenotype of cotton *GhJAZ2* RNAi seedlings under salt stress, which may possess redundant functions with other family members in *GhJAZ2* RNAi strains, but overexpression of *GhJAZ2* increased the sensitivity of transgenic cotton to salt stress<sup>[51]</sup>. These studies showed that JAZ protein is widely involved in the study of salt and alkaline tolerance of plants. In addition, the JAZ protein plays a crucial role in plant resistance to biotic stress. Transgenic wheat lines that overexpressed truncated *TaJAZ1* without Jas motif, accumulated more reactive oxygen species, which enhanced resistance to powdery mildew<sup>[52]</sup>. To date, no studies have explored the role of CsJAZ in cucumber waterlogging stress. Therefore, the specific role of the CsJAZ gene in cucumber needs further study.

In addition, exogenous MeJA treatment under waterlogging stress can significantly induce the expression of the JAZ genes. For example, the sugarcane *ScJAZ1-ScJAZ7* gene increased sharply and peaked at 3 h after MeJA treatment before gradually down-regulating at 6 h to 12 h following treatment<sup>[39]</sup>. Similarly, most *Camellia Sinensis* JAZ genes were also significantly induced by MeJA treatment, and showed induced expression at 4 and 12 hours<sup>[31]</sup>. Delay in the response of *LaJAZ5* genes to MeJA was observed, whereas *LaJAZ6* was unaffected by MeJA<sup>[40]</sup>. In our study, the expression of CsJAZ genes was largely affected by both the MeJA treatment and waterlogging stress. We observed that six of the 11 CsJAZ genes (*CsJAZ1, 2, 3, 5, 9, 10* and *11*) were up-regulated and reached their peak value at 6 h post MeJA treatment, showing consistency with previously reported studies<sup>[31]</sup> (Fig. 4).

Gene expression patterns are often related to gene function. Previous studies have shown that JAZ genes are differentially and constitutively expressed in plants. *TaJAZ1, -4, -10, -11, and -14* respectively were expressed in the root, stem, leaf, stamen, and pistil tissues of wheat<sup>[53]</sup>. In sugarcane,

seven *ScJAZ* genes were dominantly expressed in root, leaf, bud, stem pith, stem epidermis, and most of them were abundant in the bud and leaf tissues<sup>[39]</sup>. In *H. brasiliensis*, most *HbJAZ* genes such as *HbJAZ1, HbJAZ2, HbJAZ7, HbJAZ8, HbJAZ9, HbJAZ10, and HbJAZ11* were expressed at higher levels in leaves than in bark<sup>[54]</sup>. Similarly in our study, the expression activities of 11 CsJAZ genes in various tissues of cucumber were observed (Fig. 5). These different expression patterns suggested that the 11 CsJAZ genes play a crucial role in the developmental processes of cucumber plants. For example, *CsJAZ3, CsJAZ5, and CsJAZ10* displayed high expression levels in the fruit, and *CsJAZ1, CsJAZ2, CsJAZ4, CsJAZ6, CsJAZ7, CsJAZ8, CsJAZ9, and CsJAZ11* had low levels in the same tissues. Only *CsJAZ1* is highly expressed in leaves and stems, suggesting that *CsJAZ1* may play an important role in sensing jasmonic acid signals in these structures. Collectively, the tissue-specific expression of CsJAZ genes indicates their possible involvement in cucumber growth and developmental activities.

A complex signal regulation network composed of a variety of plant hormone crosstalk is ubiquitous in plant growth and development<sup>[55,56]</sup>. The Jas domain at the C-terminal of the JAZ protein is very conserved, which interacts with ethylene (ETH) and other TFs, making the JA signaling pathway cross-linked with the ethylene signaling pathway in regulating plant development<sup>[57]</sup>. The weakly conserved NT domain at the N-terminal of the JAZ protein can interact with DELLA protein, and the main role of DELLAs is to inhibit gibberellin (GA) signal transmission. The interaction between the two results in the JA and GA signal pathway being linked in regulating the plant growth defense balance<sup>[58]</sup>. For instance, the majority of the *TaJAZ* genes in *Triticum aestivum*<sup>[53]</sup> and tea plant JAZ genes<sup>[31]</sup> respond to the plant hormone GA, the relative expression levels of these genes were upregulated after GA treatment. In our study, all the CsJAZ genes were changed significantly when post exogenous GA, and showed their responsive nature. Interestingly, not each of these responding genes contained the cis-acting elements involved in gibberellin-responsiveness like P-box or TATC-box. The promoter sequences of *CsJAZ6* and *9* contained the GA responsive elements, indicating that *CsJAZ6* and *9* were directly involved in GA signaling pathway of cucumber. *CsJAZ1, 2, 3, 4, 5, 7, 8, 10, and 11* may respond to GA treatment by other unknown regulative pathways. These results indicated that CsJAZ genes may participate, directly or indirectly, in complex hormone signaling networks.

The subcellular localization experiment is generally performed to understand the behavioral nature of a certain gene. The JAZ protein of *Arabidopsis*<sup>[59]</sup> and *Camellia Sinensis*<sup>[31]</sup> plays the role of a nuclear protein, whereas the *JAZ6* of sugarcane is a protein located in the cytoplasm and cell membrane<sup>[39]</sup>. *OsJAZ1* was located in the cytoplasm, but it acted as a nuclear protein in the presence of the JA signal<sup>[60]</sup>. In addition, there may also be differences in subcellular localization of JAZ in the same species. For example, *LaJAZ3, LaJAZ4, LaJAZ6, and LaJAZ7* of *Lycoris aurea* were located in the nucleus, *LaJAZ2* and *LaJAZ5* were located in the cytoplasm, while *LaJAZ1* exists in the nucleus and cytoplasm<sup>[40]</sup>. Our study found that GFP-labeled *CsJAZ8* is specifically localized to the nucleus (Fig. 6), which highlighted its



involvement in the activation or suppression of transcription factors and determines the fate of the targeted proteins.

A TIFY/ZIM domain mediates homologous and heterologous interactions between JAZ proteins and also mediates interactions with NINJA, independent of plant hormones<sup>[14,19,61]</sup>. In previous studies, we found that all CsJAZ proteins contained a conservative TIFY motif in the ZIM domain. The yeast two-hybrid system proved that there were indeed homologous or heterologous dimer interactions between cucumber CsJAZ proteins (Fig. 7). Three of the six CsJAZ proteins (CsJAZ8, CsJAZ9, and CsJAZ10) exhibited homodimeric interactions. CsJAZ4 and CsJAZ8 can interact extensively with other JAZ protein members as baits. Homologous or heterologous interactions between JAZ proteins were also verified in *Lycoris aurea*<sup>[40]</sup> and *Gossypium hirsutum*<sup>[51]</sup>. Therefore, we hypothesized that cucumber JAZ protein may jointly respond to waterlogging stress through synergistic interaction among family members. However, the detailed network of cucumber JAZ protein regulating waterlogging stress interactions requires further verification to determine the specific role of the JAZ transcription factor in this regulation under waterlogging stress.

## CONCLUSION

In conclusion, our study is the first to integrate genome-wide identification of the JAZ genes in cucumber. Eleven CsJAZ genes in the cucumber genome were identified and characterized. The following analysis, phylogenetic relationships, chromosomal location, gene duplication, selective evolutionary pressure, conserved domains, gene structure analysis, *cis*-acting regulatory elements of the JAZ family members, revealed the distinct roles of CsJAZ genes. Moreover, a quantitative PCR analysis of 11 CsJAZ genes revealed that these genes may be crucial in regulating the response of cucumber to waterlogging. Transcriptional expression patterns of CsJAZ in different tissues and under exogenous GA treatment were visualized. The subcellular localization experiment revealed that GFP-labeled CsJAZ8 is specifically localized to the nucleus. Further, cucumber CsJAZ proteins exhibit homologous and heterologous interactions. Our study explored research on the CsJAZ family in cucumber, and provides insightful knowledge regarding the role of CsJAZ proteins in waterlogging stress and could be useful in future cucumber breeding programs.

## MATERIALS AND METHODS

### Genomic identification of JAZ genes in cucumber

The whole-genome data of cucumber was downloaded from the Cucurbit Genomics Database (CuGenDB, <http://cucurbitgenomics.org>), the plant Jasmonate ZIM-domain protein sequence and cucumber protein data were also downloaded through NCBI (<https://www.ncbi.nlm.nih.gov>) for blastall comparison. The protein sequence was obtained from Pfam (<http://pfam.xfam.org>)<sup>[62]</sup>. The NCBI Conserved Domain Database (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>) and the SMART program (<http://smart.embl-heidelberg.de>) were used to further identify the JAZ

protein structure and protein conserved domains and to remove redundancy. Finally, a total of 11 non-redundant CsJAZ genes were identified in the cucumber genome named CsJAZ1~CsJAZ11. ExPASy (<https://web.expasy.org/protparam>) and PSORT tool (<https://www.genscript.com/psort.html>)<sup>[63,64]</sup> were used to calculate protein sequence length, molecular weight, and isoelectric point (pI). The subcellular localization of all JAZ proteins of cucumber in different organisms were predicted using the online tool Cell-PLoc-2.0 (<http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2>)

### Phylogenetics, gene structure, motif composition analysis

In order to study the relationship between the JAZ gene phylogeny of cucumber, 12 *Arabidopsis thaliana*, 11 *Vitis vinifera*, 15 *Oryza sativa*, 13 *Solanum lycopersicum*, and 12 *Populus trichocarpa* JAZ protein sequences and 11 CsJAZ of cucumber were used. The amino acid sequences of conserved Jas domain were used for phylogenetic tree construction. Firstly, we performed ClustalW multiple sequence alignment of the JAZ protein sequences of six species, and set all parameters to default values<sup>[65]</sup>, we then used the Neighbor-joining method<sup>[66]</sup>, set the bootstrap replicates value to 1000 for phylogenetic tree construction using MEGA X software. According to the branches of the phylogenetic tree, it was divided into three subgroups, Group I, Group II, and Group III.

The cucumber whole-genome sequence was aligned with mRNA sequences to confirm the CsJAZ gene structures. The conserved motifs in the JAZ protein of cucumber are identified using the Motif Elicitation (MEME) online prediction tool (<http://meme-suite.org/index.html>)<sup>[67]</sup>, and the maximum number of motifs was set to 10, other parameters were set as default. In order to better understand the structure of the CsJAZ genes of cucumber, the Gene Structure Display Server program (GSDS) (<http://gsds.gao-lab.org>) was used to map the whole genome sequence and CDS sequence of the CsJAZ genes and included the CsJAZ map of gene structure, and the exon and intron structure of CsJAZ genes.

### Chromosomal location, gene duplication analysis, and selective evolutionary pressure analysis

The distribution of the cucumber JAZ protein on the particular chromosome was visualized using MapChart software<sup>[68]</sup>, according to the documents of genome annotation available on the website of the cucumber genome.

The Multiple Collinearity Scan toolkit (MCScanX) with default parameters was used to examine the gene duplication events of CsJAZ genes<sup>[69]</sup>. KaKs\_Calculator2.0 software was used to perform non-synonymous (Ka)/synonymous (Ks) analysis on the members of the cucumber CsJAZ gene family (previously described by Wang et al., 2010<sup>[69]</sup>) to further analyze segmentally and tandemly duplicated CsJAZ genes. Ka/Ks ratio < 1 means purifying selection, ratio = 1 means neutral evolution, and ratio > 1 means positive selection<sup>[70]</sup>.

### Identification of *cis*-elements of cucumber JAZ genes

The 1500 bp upstream genomic DNA sequences of 11 CsJAZ genes were extracted and the *cis*-regulatory elements were analyzed using the Plant CARE website (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html>)<sup>[71]</sup>.

### Plant growth and waterlogging treatments

The waterlogging tolerant cucumber line Zaoer-N was used as the test material. On August 18, 2019, it was sown in a cave containing vermiculite : peat : perlite (1:3:1 v/v/v). The seedlings were grown in a glass greenhouse, 14 h at 28 °C/10 h at 18 °C (day/night) photocycle, and the relative humidity was 75%. Selecting Zaoer-N seedlings with the same two leaves and one heart (seedling age is about 15–20 d) for waterlogging treatment (waterlogging to the growth place of the first cotyledon), the seedlings were transferred into plastic containers filled with water<sup>[72]</sup>. After the preliminary exploration of different concentrations of MeJA treatment, a total of three treatments were set up, namely non-flooded treatment (CK), ordinary waterlogging treatment (WL), and waterlogging plus 500 µmol/L MeJA. The cucumber hypocotyls were collected at 6, 12, 24, 48, 96 h after treatment. Under all conditions including the control, three biological replicates were collected for each sample, which consisted of nine hypocotyls of the same line. All samples were immediately frozen in liquid nitrogen and kept at –80 °C to extract total RNA.

### Total RNA Extraction, qRT-PCR analysis and transcriptomic analysis of *CsJAZ* in different tissues

The extract of cucumber hypocotyl total RNA was carried out using the TaKaRa MiniBEST Plant RNA Extraction Kit (TaKaRa, China), from 200 mg of hypocotyl samples, Nanodrop One (Thermo Fisher Scientific, USA) and 1% agarose gel electrophoresis was used to detect concentration and RNA completeness. 1 µg RNA was used in the first strand cDNA synthesis using a 20 µl reaction volume using the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, China). Quantitative RT-PCR was performed using SYBR® Premix Ex Taq™ II (TaKaRa, China). The qRT-PCR reaction conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The primers used for the qRT-PCR assay of JAZ genes are listed in Supplemental Table S7. For each sample, the internal reference gene (ACTIN) was used to normalize the abundance of mRNA, and three replicates were performed. The relative expression level of genes was calculated using the  $2^{-\Delta\Delta CT}$  method<sup>[73]</sup>, and SPSS 16.0 software (IBM Corporation, USA) data was used for analysis. The different lowercase letters above bars indicated the significance ( $p < 0.05$ ) of the relative expression level between two samples.

For measuring the relative amount of corresponding transcripts of *CsJAZ* genes in different plant tissues and post exogenous GA treatment, the transcriptomic data of *CsJAZ* genes were obtained from the cucumber genome database and visualized using TBtools<sup>[74]</sup>.

### Subcellular localization analysis

The coding region of the *CsJAZ8* gene was amplified by PCR with specific primers *CsJAZ8*-GFP-F and *CsJAZ8*-GFP-R (Supplemental Table S7). The PCR product was ligated to the BamHI-KpnI sites of GFP fusion expression vector pCAMBIA1301-GFP to generate the transient expression vector pCAMBIA1301-GFP-*CsJAZ8* by ClonExpress II One Step Cloning Kit (Vazyme Biotech, China). Next, the *CsJAZ8*-GFP fusion driven by 35S promoter was transformed into *Agrobacterium tumefaciens* GV3101 strain competent cells<sup>[75]</sup>. The cells were collected by centrifugation and suspended in a buffer solution containing 10 mM MES (pH 5.5), 10 mM MgCl<sub>2</sub>

and 200 µM acetylcholine, with a 600 nm optical density of 1.0. Using the tobacco transient transformation method, the cells were injected into one month old Ben's tobacco leaves with a needleless syringe. After 48–72 h, green fluorescence was detected in the tobacco epidermal cells under a laser confocal microscope (ZEISS LSM 980 with Airyscan2, Germany).

### Yeast two-hybrid (Y2H) assay

Yeast two-hybrid system (Clontech, USA) based on Matchmaker gal4 was used. The pGBKT7 vector contains the GAL4 DNA binding domain, and the pGADT7 vector contains the GAL4 activation domain. The CDS sequence of the *CsJAZ* gene was amplified by PCR with gene-specific primers (Supplemental Table S7), and the full-length cDNA of *CsJAZ* was cloned into pGBKT7 (bait vector) and pGADT7 (prey vector). The inactive BD-*CsJAZ2/CsJAZ4/CsJAZ8/CsJAZ9/CsJAZ10/CsJAZ11* and AD-*CsJAZ1/CsJAZ2/CsJAZ3/CsJAZ4/CsJAZ5/CsJAZ6/CsJAZ7/CsJAZ8/CsJAZ9/CsJAZ10/CsJAZ11* plasmids were mixed and co-transformed into the yeast strain AH109. Yeast cells were cultured on a medium lacking leucine and tryptophan (SD/-Leu/-Trp) at 28 °C for 3–4 d. Then, yeast colonies were screened on a medium lacking leucine, tryptophan, histidine, and adenine (SD/-Leu/-Trp/-His/-Ade) at 28 °C for 3 d to determine possible interaction.

### Statistical analysis

The statistical significance was performed using SPSS software (version 23.0, SPSS Inc., USA), using statistical analysis ANOVA and 95% and 99% confidence intervals ( $p \leq 0.05$  and  $p \leq 0.01$ ). The analyzed data were expressed as means  $\pm$  standard deviation (SD) of three replicates for all measured parameters. GraphPad Prism 8.0 (GraphPad Software, Inc., LA Jolla, CA, USA) was used to produce the graphs in accordance with the data.

### ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (grant nos. 32030093 and 31801883) and Natural Science Foundation of Jiangsu Province (BK20180913).

### Conflict of interest

The authors declare that they have no conflict of interest.

**Supplementary Information** accompanies this paper at (<http://www.maxapress.com/article/doi/10.48130/VR-2021-0003>)

### Dates

Received 21 December 2020; Accepted 15 April 2021; Published online 29 April 2021

### REFERENCES

1. Howe GA, Jander G. 2008. Plant Immunity to Insect Herbivores. *Annual Review of Plant Biology* 59:41–66
2. Raza A, Charagh S, Zahid Z, Mubarak MS, Javed R, et al. 2020. Jasmonic acid: a key frontier in conferring abiotic stress tolerance in plants. *Plant Cell Reports* 1–29

## Waterlogging responsive JAZ genes

3. Fung RWM, Wang CY, Smith DL, Gross KC, Tian M. 2004. MeSA and MeJA increase steady-state transcript levels of alternative oxidase and resistance against chilling injury in sweet peppers (*Capsicum annuum* L.). *Plant Science* 166:711–9
4. Savchenko T, Kolla VA, Wang CQ, Nasafi Z, Hicks DR, et al. 2014. Functional convergence of oxylipin and abscisic acid pathways controls stomatal closure in response to drought. *Plant Physiology* 164:1151–60
5. Yang J, Fei K, Chen J, Wang Z, Zhang W, et al. 2020. Jasmonates alleviate spikelet-opening impairment caused by high temperature stress during anthesis of photo-thermo-sensitive genic male sterile rice lines. *Food and Energy Security* 9:e233
6. Salimi F, Shekari F, Hamzei J. 2016. Methyl jasmonate improves salinity resistance in German chamomile (*Matricaria chamomilla* L.) by increasing activity of antioxidant enzymes. *Acta Physiologiae Plantarum* 38:1
7. Xu Q, Truong TT, Barrero JM, Jacobsen JV, Hocart CH, et al. 2016. A role for jasmonates in the release of dormancy by cold stratification in wheat. *Journal of Experimental Botany* 67:3497–508
8. Wang Z, Liu L, Su H, Guo L, Zhang J, et al. 2020. Jasmonate and aluminum crosstalk in tomato: Identification and expression analysis of WRKYs and ALMTs during JA/Al-regulated root growth. *Plant Physiology and Biochemistry* 154:409–18
9. Chen J, Sonobe K, Ogawa N, Masuda S, Nagatani A, et al. 2012. Inhibition of arabidopsis hypocotyl elongation by jasmonates is enhanced under red light in phytochrome B dependent manner. *Journal of Plant Research* 126:161–8
10. Qi T, Huang H, Song S, Xie D. 2015. Regulation of Jasmonate-Mediated Stamen Development and Seed Production by a bHLH-MYB Complex in Arabidopsis. *The Plant Cell* 27:1620–33
11. Cai Q, Yuan Z, Chen M, Yin C, Luo Z, et al. 2014. Jasmonic acid regulates spikelet development in rice. *Nature Communications* 5:3476
12. Gfeller A, Liechti R, Farmer EE. 2010. Arabidopsis jasmonate signaling pathway. *Science Signaling* 3:cm4
13. Wasternack C, Strnad M. 2016. Jasmonate signaling in plant stress responses and development – active and inactive compounds. *New Biotechnology* 33:604–13
14. Chini A, Fonseca S, Chico JM, Fernández-Calvo P, Solano R. 2009. The ZIM domain mediates homo- and heteromeric interactions between Arabidopsis JAZ proteins. *The Plant Journal* 59:77–87
15. Vanholme B, Grunewald W, Bateman A, Kohchi T, Gheysen G. 2007. The tify family previously known as ZIM. *Trends in Plant Science* 12:239–44
16. Chini A, Fonseca S, Fernández G, Adie B, Chico JM, et al. 2007. The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* 448:666–71
17. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, et al. 2007. JAZ repressor proteins are targets of the SCF<sup>CO11</sup> complex during jasmonate signalling. *Nature* 448:661–65
18. Browse J. 2009. Jasmonate Passes Muster: A Receptor and Targets for the Defense Hormone. *Annual Review of Plant Biology* 60:183–205
19. Pauwels L, Barbero GF, Geerinck J, Tillemans S, Grunewald W, et al. 2010. NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature* 464:788–91
20. Acosta IF, Gasperini D, Chételat A, Stolz S, Santuari L, et al. 2013. Role of NINJA in root jasmonate signaling. *Proceedings of the National Academy of Sciences of the United States of America* 110:15473–8
21. Hirabayashi Y, Mahendran R, Koirala S, Konoshima L, Yamazaki D, et al. 2013. Global flood risk under climate change. *Nature Climate Change* 3:816–21
22. Ding J, Liang P, Wu P, Zhu M, Li C, et al. 2020. Effects of waterlogging on grain yield and associated traits of historic wheat cultivars in the middle and lower reaches of the Yangtze River, China. *Field Crops Research* 246:107695
23. Mondal S, Khan MIR, Dixit S, Sta. Cruz PC, Septiningsih EM, Ismail AM. 2020. Growth, productivity and grain quality of AG1 and AG2 QTLs introgression lines under flooding in direct-seeded rice system. *Field Crops Research* 248:107713
24. Xu X, Ji J, Xu Q, Qi X, Weng Y, et al. 2018. The major-effect quantitative trait locus CsARN6.1 encodes an AAA ATPase domain-containing protein that is associated with waterlogging stress tolerance by promoting adventitious root formation. *The Plant Journal* 93:917–30
25. Qi X, Li Q, Shen J, Qian C, Xu X, et al. 2020. Sugar enhances waterlogging-induced adventitious root formation in cucumber by promoting auxin transport and signalling. *Plant, Cell & Environment* 43:1545–57
26. Huang S, Li R, Zhang Z, Li L, Gu X, et al. 2009. The genome of the cucumber, *Cucumis sativus* L. *Nature Genetics* 41:1275–81
27. Ye H, Du H, Tang N, Li X, Xiong L. 2009. Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. *Plant Molecular Biology* 71:291–305
28. Zhang Y, Gao M, Singer SD, Fei Z, Wang H, Wang X. 2012. Genome-wide identification and analysis of the TIFY gene family in grape. *Plos One* 7:e44465
29. Zhu D, Cai H, Luo X, Bai X, Deyholos MK, et al. 2012. Overexpression of a novel JAZ family gene from Glycine soja, increases salt and alkali stress tolerance. *Biochemical and Biophysical Research Communications* 426:273–9
30. Xia W, Yu H, Cao P, Luo J, Wang N. 2017. Identification of TIFY Family Genes and Analysis of Their Expression Profiles in Response to Phytohormone Treatments and *Melampsora larici-populina* Infection in Poplar. *Frontiers in Plant Science* 8:493
31. Shen J, Zou Z, Xing H, Duan Y, Zhu X, et al. 2020. Genome-Wide Analysis Reveals Stress and Hormone Responsive Patterns of JAZ Family Genes in *Camellia Sinensis*. *International Journal of Molecular Sciences* 21:2433
32. Hu R, Qi G, Kong Y, Kong D, Gao Q, et al. 2010. Comprehensive Analysis of NAC Domain Transcription Factor Gene Family in *Populus trichocarpa*. *BMC Plant Biology* 10:145
33. Baillo EH, Kimotho RN, Zhang Z, Xu P. 2019. Transcription Factors Associated with Abiotic and Biotic Stress Tolerance and Their Potential for Crops Improvement. *Genes* 10:771
34. Liberatore KL, Dukowic-Schulze S, Miller ME, Chen C, Kianian SF. 2016. The role of mitochondria in plant development and stress tolerance. *Free Radical Biology and Medicine* 100:238–56
35. Bai Y, Meng Y, Huang D, Qi Y, Chen M. 2011. Origin and evolutionary analysis of the plant-specific TIFY transcription factor family. *Genomics* 98:128–36
36. Yan Y, Stolz S, Chételat A, Reymond P, Pagni M, et al. 2007. A downstream mediator in the growth repression limb of the jasmonate pathway. *The Plant Cell* 19:2470–83
37. Oh Y, Baldwin IT, Galis I. 2013. A jasmonate ZIM-domain protein NaJAZd regulates floral jasmonic acid levels and counteracts flower abscission in *Nicotiana attenuata* plants. *PLoS One* 8:e57868
38. Zhou X, Yan S, Sun C, Li S, Li J, et al. 2015. A maize jasmonate Zim-domain protein, ZmJAZ14, associates with the JA, ABA, and GA signaling pathways in transgenic Arabidopsis. *PLoS One* 10:e121824
39. Liu F, Sun T, Wang L, Su W, Gao S, et al. 2017. Plant jasmonate ZIM domain genes: shedding light on structure and expression patterns of JAZ gene family in sugarcane. *Bmc Genomics* 18:771
40. Wang P, Yu S, Han X, Xu J, He Q, et al. 2020. Identification, molecular characterization and expression of JAZ genes in *Lycoris aurea*. *PLoS One* 15:e0230177
41. Cai Z, Chen Y, Liao J, Wang D. 2020. Genome-wide identification and expression analysis of jasmonate ZIM domain gene family in

- tuber mustard (*Brassica juncea* var. *tumida*). *PLoS One* 15:e0234738
42. Taniguchi S, Hosokawa-Shinonaga Y, Tamaoki D, Yamada S, Akimitsu K, et al. 2014. Jasmonate induction of the monoterpene linalool confers resistance to rice bacterial blight and its biosynthesis is regulated by JAZ protein in rice. *Plant, Cell & Environment* 37:451–61
  43. Yamada S, Kano A, Tamaoki D, Miyamoto A, Shishido H, et al. 2012. Involvement of OsJAZ8 in jasmonate-induced resistance to bacterial blight in rice. *Plant and Cell Physiology* 53:2060–72
  44. Peethambaran PK, Glenz R, Hönninger S, Shahinul Islam SM, Hummel S, et al. 2018. Salt-inducible expression of OsJAZ8 improves resilience against salt-stress. *BMC Plant Biology* 18:311
  45. Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, et al. 2000. The *Arabidopsis* *DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *The Plant Cell* 12:1041–61
  46. von Malek B, van der Graaff E, Schneitz K, Keller B. 2002. The *Arabidopsis* male-sterile mutant *dde2-2* is defective in the *ALLENE OXIDE SYNTHASE* gene encoding one of the key enzymes of the jasmonic acid biosynthesis pathway. *Planta* 216:187–92
  47. Gutierrez L, Mongelard G, Floková K, Păcurar DI, Novák O, et al. 2012. Auxin Controls *Arabidopsis* Adventitious Root Initiation by Regulating Jasmonic Acid Homeostasis. *The Plant Cell* 24:2515–27
  48. Xu X, Ji J, Ma X, Xu Q, Qi X, et al. 2016. Comparative Proteomic Analysis Provides Insight into the Key Proteins Involved in Cucumber (*Cucumis sativus* L.) Adventitious Root Emergence under Waterlogging Stress. *Frontiers in plant science* 7:e1515
  49. Zhao C, Pan X, Yu Y, Zhu Y, Kong F, et al. 2020. Overexpression of a TIFY family gene, *GsJAZ2*, exhibits enhanced tolerance to alkaline stress in soybean. *Molecular Breeding* 40:33
  50. An X, Hao Y, Li E, Xu K, Cheng C. 2017. Functional identification of apple *MdJAZ2* in *Arabidopsis* with reduced JA-sensitivity and increased stress tolerance. *Plant Cell Reports* 36:255–65
  51. Sun H, Chen L, Li J, Hu M, Ullah A, et al. 2017. The JASMONATE ZIM-Domain Gene Family Mediates JA Signaling and Stress Response in Cotton. *Plant and Cell Physiology* 58:2139–54
  52. Jing Y, Liu J, Liu P, Ming D, Sun J. 2019. Overexpression of *TaJAZ1* increases powdery mildew resistance through promoting reactive oxygen species accumulation in bread wheat. *Scientific Reports* 9:5691
  53. Wang Y, Qiao L, Bai J, Wang P, Duan W, et al. 2017. Genome-wide characterization of *JASMONATE-ZIM DOMAIN* transcription repressors in wheat (*Triticum aestivum* L.). *BMC Genomics* 18:152
  54. Hong H, Xiao H, Yuan H, Zhai J, Huang X. 2015. Cloning and characterisation of JAZ gene family in *Hevea brasiliensis*. *Plant Biology (Stuttgart, Germany)* 17:618–24
  55. Miransari M, Smith DL. 2014. Plant hormones and seed germination. *Environmental and Experimental Botany* 99:110–21
  56. Zou L, Pan C, Wang M, Cui L, Han B. 2020. Progress on the mechanism of hormones regulating plant flower formation. *Hereditas (Beijing)* 42:739–51
  57. Zhu Z, An F, Feng Y, Li P, Xue L, et al. 2011. Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* 108:12539–44
  58. Yang D, Yao J, Mei C, Tong X, Zeng L, et al. 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America* 109:E1192–E1200
  59. Chung HS, Howe GA. 2009. A Critical Role for the TIFY Motif in Repression of Jasmonate Signaling by a Stabilized Splice Variant of the JASMONATE ZIM-Domain Protein JAZ10 in *Arabidopsis*. *The Plant Cell* 21:131–45
  60. Fu J, Wu H, Ma S, Xiang D, Liu R, et al. 2017. OsJAZ1 Attenuates Drought Resistance by Regulating JA and ABA Signaling in Rice. *Frontiers in Plant Science* 8:2108
  61. Pauwels L, Goossens A. 2011. The JAZ proteins: a crucial interface in the jasmonate signaling cascade. *The Plant Cell* 23:3089–100
  62. Finn RD, Mistry J, Schuster-Böckler B, Griffiths-Jones S, Hollich V, et al. 2006. Pfam: clans, web tools and services. *Nucleic Acids Research* 34:D247–D251
  63. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. 2003. ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research* 31:3784–8
  64. Tamura T, Akutsu T. 2007. Subcellular location prediction of proteins using support vector machines with alignment of block sequences utilizing amino acid composition. *BMC Bioinformatics* 8:466
  65. Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–80
  66. Zhang C, Zhang H, Zhao Y, Jiang H, Zhu S, et al. 2013. Genome-wide analysis of the CCCH zinc finger gene family in *Medicago truncatula*. *Plant Cell Reports* 32:1543–55
  67. Bailey TL, Elkan C. 1994. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proceedings. International Conference on Intelligent Systems for Molecular Biology* 2:28–36
  68. Voorrips RE. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity* 93:77–8
  69. Wang Y, Li J, Paterson AH. 2013. *MCSanX-transposed*: detecting transposed gene duplications based on multiple colinearity scans. *Bioinformatics* 29:1458–60
  70. Akhunov ED, Sehgal S, Liang H, Wang S, Akhunova AR, et al. 2013. Comparative Analysis of Syntenic Genes in Grass Genomes Reveals Accelerated Rates of Gene Structure and Coding Sequence Evolution in Polyploid Wheat. *Plant Physiology* 161:252–65
  71. Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, et al. 2002. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Research* 30:325–7
  72. Xu X, Chen M, Ji J, Xu Q, Qi X, et al. 2017. Comparative RNA-seq based transcriptome profiling of waterlogging response in cucumber hypocotyls reveals novel insights into the de novo adventitious root primordia initiation. *BMC Plant Biology* 17:129
  73. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-ΔΔCT</sup> method. *Methods* 25:402–8
  74. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, et al. 2020. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* 13:1194–202
  75. Sparkes IA, Runions J, Kearns A, Hawes C. 2006. Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants. *Nature Protocols* 1:2019–25



Copyright: © 2021 by the author(s). Exclusive Licensee Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit <https://creativecommons.org/licenses/by/4.0/>.