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Effect of Overexpression of *SlbZIP39* on Plant Architecture and Fruit Size in Tomato (*Solanum lycopersicum* L.)

Yee Yee Lwin[1,2*], Min Kyaw Thu[3], Khin Hnin Yee[2], Maw Maw Khin[2]

[1]Department of Biology, School of Life Sciences, Chongqing University, Chongqing, China
[2]Department of Botany, Myingyan University, Myingyan, Republic of the Union of Myanmar
[3]Department of Botany, Yangon University, Yangon, Republic of the Union of Myanmar
Email: *yeeyeelwinbot@gmail.com

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Abstract

The transcription factors of the *bZIP* (basic leucine zipper) family are involved in the regulation of molecular analysis in fruit development, plant architecture, and response to environmental factors. Here, we performed transcription of the *SlbZIP39* gene in various tissues and fruit developmental stages. However, they revealed that the different transcription levels of tomato tissues were expressed by floral parts and immature green stages. We observed that *SlbZIP39* was induced by the treatments of tomato plants with D-mannitol, NaCl, IAA, GA3, ABA, and dehydration stress. We further revealed that Group IV *bZIP* transcription factors are expressed in fruit developmental stages in overexpressing plants. In addition, we found that overexpression of *SlbZIP39* decreased plant height and reduced fruit size. However, overexpression of *SlbZIP39* may affect early flowering and fruit development in tomato. These results suggest that the transcription factor of *SlbZIP39* is involved in abiotic stresses, regulation of fruit size, and plant architecture.

Subject Areas

Molecular Biology

Keywords

Abiotic Stresses, Hormonal Treatments, Fruit Size, Tomato (*Solanum lycopersicum*)

1. Introduction

Plant transcription factors of the *bZIP* family play a significant role in biological

advancements inclusive of morphogenesis, seed maturation, flower development, and environmental factors [1] [2]. However, *bZIP* transcription factors are involved in multiple abiotic stress tolerances. Several reports have indicated that members of the *bZIP* transcription factor family function in plant hormone signaling and environmental stresses, which include drought and salt stress [3] [4] [5] [6].

Among all the plant transcription factors, *bZIP* genes have been identified in monocotyledonous and dicotyledonous plants. Nowadays, it is reported that 75 *bZIP* genes have been classified in *Arabidopsis thaliana* [7], 89 in *Oryza sativa* [8], 125 in *Zea mays* [9], 160 in *Glycine max* [10], and 55 in *Vitis vinifera* [11]. *AREB1*, *AREB2*, and *ABF3* up-regulate ABA, drought tolerance, and water stresses in *Arabidopsis*, and they also function as master transcription factors [12] [13]. Furthermore, *AtABF3* increased tolerance to various abiotic stresses in *Alfalfa* [14]. Overexpression of *AtbZIP3* is involved in leaf development and response to the sugar signaling pathway in *Arabidopsis* [15]. However, *AtbZIP19* and *AtbZIP23* have been related to the regulation of zinc deficiency in *Arabidopsis* [16].

In plants, some researchers have confirmed that transcription factors of the *bZIP* family are induced in seed maturation [1], light response [17], sucrose signaling [18] [19], pathogen resistance [3], flower development and fertility [20], phytohormone response, development of organs and response to various stresses [2]. *AtbZIP63* may participate in the interaction of ABA and glucose in *Arabidopsis* [19]. Kang *et al*. [21], state that overexpression of *ABF3* and *ABF4* functions in ABA hypersensitivity, whereas it reduces transpiration and enhances tolerance in *Arabidopsis*. The function of *bZIP16* was developed in seed germination and seedling development of *Arabidopsis* [22]. Besides, overexpression of *GmbZIP1* affected the response of ABA in seed germination stages and abiotic stress tolerance in soybeans [23]. *AtbZIP17* might play roles as an ABA signaling pathway and is involved in seed germination and seed filling in *Arabidopsis*. Furthermore, it was genetically demonstrated that *AtbZIP17* plays a crucial role in osmotic stress and is affected as a negative regulator of storage and seed germination in *Arabidopsis* [24].

SlbZIP38, *SlbZIP1* and *LebZIP2* functions were found to be involved in tomato responses to various environmental stresses [5] [6] [25]. Furthermore, *SlAREB1* was characterized by ABA, salt, drought, and cold resistance from tomato [26]. In *Vitis vinifera*, *VIbZIP36* improved tolerance to drought treatment in seed germination [27]. Overexpression of *VIbZIP30* not only improved seed germination under dehydration stress but was also involved in drought stress in grapevine [28].

Tomato (*Solanum lycopersicum* L.) is a dicotyledonous plant that is one of 69 *SlbZIP* gene family members classified into 14 groups. In this study, we performed further experimental design by overexpressing the *SlbZIP39* gene in a tomato to investigate its expression profiles in the different tissues, abiotic

stresses, hormonal treatments, and physiological characteristics in tomato.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The WT tomato (*Solanum lycopersicum* L., cv. Micro-Tom) and transgenic seeds were sterilized before germinating, and the seedlings were then grown in 1/2 MS medium. The plants were cultivated in a greenhouse under standard conditions with 18 h light (25˚C)/6h dark (18˚C) cycles and 80% relative humidity. The different tissue samples were gathered to carry out the gene expression analysis, which included roots, stems, leaves, shoots, petioles, flowers, buds, sepals, petals, stamens, pistils, pedicles, immature, mature green, breaker, and 5-day breaker. All the tissue samples were immediately frozen in liquid nitrogen and stored at −80˚C.

2.2. Vector Construction and Transformation

The full length of *SlbZIP39* CDS without the stop codon was amplified with a primer (Table 1). The PCR fragments were cloned, then digested by *NotI* and *SbfI* and cloned into the K303 expression vector (Gateway technology) under the CaMV 35S promoter. This construction was transformed into *Solanum lycopersicum* L., cv. Micro-Tom by *Agrobacterium tumefaciens* strain GV3101 [29].

2.3. RNA Isolation and Quantitative Real-Time PCR Analysis

Total RNA from different tissues was extracted by using the E.Z.N.A.® Plant RNA Kit (Omega Bio-Tek, USA, R6827-01), according to the manufacturer's instructions. cDNAs were synthesized with the Prime-Script™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TAKARA, Japan). Quantitative real-time PCR was performed according to the instructions provided for the Bio-Rad-CFX system (Bio-Rad, USA), using SYBR Green PCR Master Mix (CWBIO, China). The tomato *UBI* gene (*Solyc07g064130*) was used as an internal control for normalization. The relative quantification of gene expression levels was calculated by the comparative $2^{-\Delta\Delta CT}$ method. All the primers used for the qRT-PCR were listed in Table 1. The experiments are repeated at least three times.

Table 1. List of the primers used in this study.

Gene Name	Forward primer	Reverse primer
SlbZIP39	ATGTCTTCGACTCCGCACT	AAACTGGAACATATCAGAAGCAGT
SlUBI-Q	GCCGACTACAACATCCAGAAGG	TGCAACACAGCGAGCTTAACC
SlbZIP39-Q	TGTCTTCGACTCCGCACTTG	GTTCTGGTTCTGCAGCTGTG
SlbZIP04-Q	ACATGGCTTCGACTCAGCAA	CACGCCAGATAGTGCTCTGA
SlbZIP07-Q	GTGCGGCACAGAACAATGTG	TAGGCTGCATTGCACAAGGA
SlbZIP10-Q	GTGAACTTAGCCGTAGGCTTGAG	CATAATGGGTTGATTGGCAGATAG
SlbZIP34-Q	*TCATGGTGGTTGATGAAAGGA*	*TGTGTTGTCATGGTGATGCTG*

2.4. Abiotic Stress and Hormone Treatments

Tomato seedlings were grown in a greenhouse under the same conditions, and all the treatments were conducted using one-month-old plants. For abiotic stress treatments, the plants were sprayed with 100 mM D-mannitol and 200 mM NaCl. For the hormone treatments, the plants were sprayed with 100 μM ABA, GA3, and IAA solutions. For the dehydration assay, leaves and fruits were left on a piece of dry filter paper at 25˚C ± 1˚C, and then all samples were collected at the designated time. All the samples were harvested at 0 h, 3 h, 6 h, 12 h, and 24 h after each treatment. The harvested samples at 0 h were used as control and stored at −80˚C. For each treatment, samples were collected from 6 plants, mixed and all the experiments were performed at least three times.

2.5. Statistical Analysis

Statistical analysis was conducted using Prism-6 (GraphPad, San Diego, CA, USA). All the experiments were repeated three times, and all the data was calculated as the expression of mean ± standard error. Comparisons between the two groups were performed using Student's t-tests. The significance values of ($*P < 0.05$; $**P < 0.01$) were considered to be compared between wild-type and transgenic plants.

3. Results

3.1. Expression Patterns of *SlbZIP39* Induced by Abiotic and Hormonal Stresses

Previous reports have indicated that *SlbZIP* transcription factors are involved in abiotic and biotic stresses [5] [6] [25]. We therefore analyzed the transcript levels of *SlbZIP39* in leaves under abiotic and hormonal stress. The transcripts of *SlbZIP39* are not only induced by NaCl and D-mannitol treatments but also affected by IAA, GA3, and ABA treatments (**Figure 1** and **Figure 2**). In NaCl and D-mannitol treatments, however, the level of *SlbZIP39* transcription was relatively down-regulated in NaCl but adequately up-regulated in D-mannitol treatment (**Figure 1(a)** and **Figure 1(b)**). During the dehydration treatments, we compared tomato leaves and fruits. The transcript of *SlbZIP39* was expressed in tomato leaves and fruits but was slightly down-regulated at 3 h and 6 h and moderately up-regulated at 12 h and 24 h in leaves (**Figure 1(c)** and **Figure 1(d)**). The level of *SlbZIP39* transcription was significantly reduced during fruit dehydration treatments (**Figure 1(d)**). In the hormone treatments, the transcript level of *SlbZIP39* was affected within 3 h to 24 h of IAA treatments, whereas it was up-regulated at 12 h and down-regulated at 3 h in tomato leaves (**Figure 2(a)**). The expression level of *SlbZIP39* was up-regulated at 24 h and moderately induced at 3 h to 12 h in GA3 treatments (**Figure 2(b)**). However, the level of *SlbZIP39* transcription was relatively responsive to ABA treatment and up-regulated at 3 h in leaves but declined at later intervals (**Figure 2(c)**). Here, we found that *SlbZIP39* responded at the same time interval after treatments

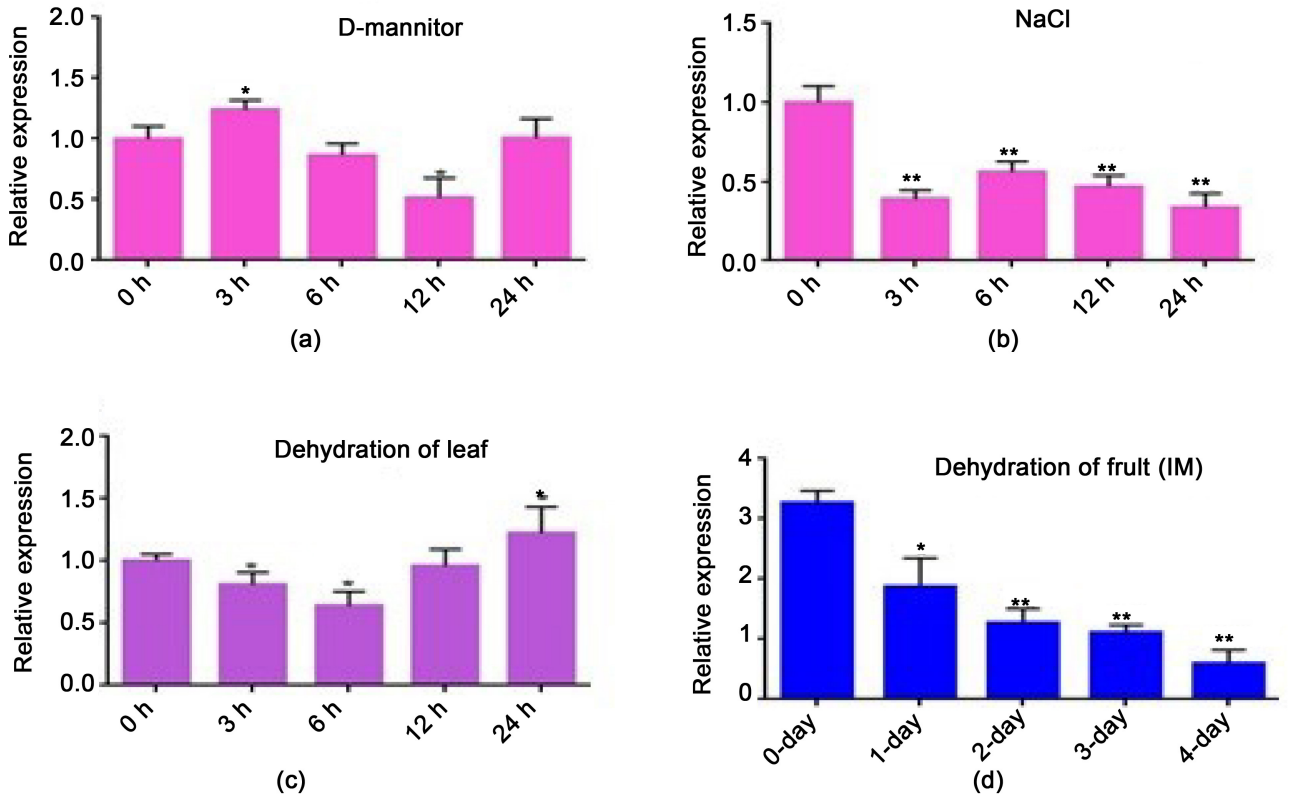

Figure 1. Expression profiles analysis of the *SlbZIP39* gene under various abiotic stresses. For control plants, the expression data were set to 0 h. (a) 100 mM D-mannitol; (b) 200 mM NaCl; (c). Dehydration of leaf; (d) Dehydration of fruit. Error bars indicate SD values from three biological replicates (n = 3). Asterisks indicate significant differences using Student's *t*-test (*$P < 0.05$; **$P < 0.01$).

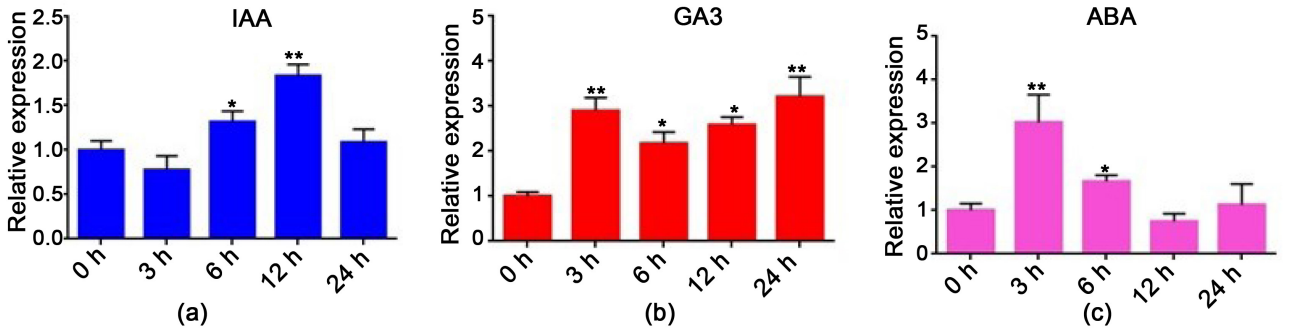

Figure 2. Expression profiles analysis of the *SlbZIP39* gene under hormonal treatments. For control plants, the expression data were set to 0 h. (a) 100 μM IAA; (b) 100 μM GA3; (c) 100 μM ABA. Error bars indicate SD values from three biological replicates (n = 3). Asterisks indicate significant differences using Student's *t*-test (*$P < 0.05$; **$P < 0.01$).

but *SlbZIP39* was more affected by NaCl, dehydration of fruits, GA3, and ABA than others. We inferred that *SlbZIP39* in tomato may respond to abiotic stresses and hormonal treatments.

3.2. Expression Patterns of *SlbZIP39* Induced by Abiotic and Hormonal Stresses

We transgenically generated overexpression of *SlbZIP39* under the 35S promoter

in tomato plants. Transgenic tomato plants were obtained from three independent lines (L-1, L-4, L-9) (Figure 3(c)). Plant heights of one-month-old WT and OE were measured for physiological processes. The *SlbZIP39* overexpressing plants showed decreased plant height compared to the wild-type (Figure 3(a), Figure 3(b)). The expression profiles of *SlbZIP39* in wild-type tomato were analyzed by qRT-PCR in various plant tissues, including roots, stems, leaves, shoots, flowers, buds, sepals, petals, stamens, and pistils, respectively (Figure 3(d)). The transcripts of *SlbZIP39* are documented as moderately high in stems, leaves, shoots, petioles, flowers, buds, and pistils; the highest transcripts of *SlbZIP39* accumulate in stamens, followed by petals and sepals. However, the lower expression level of *SlbZIP39* was expressed in roots and pedicles (Figure 3(d)). Moreover, we analyzed fruit development stages by using qRT-PCR (Figure 3(e)). The expression profiles of *SlbZIP39* in fruit developmental stages, consisting of immature green, mature green, and breaker stages, were explored. However, the transcripts of *SlbZIP39* were highly expressed at the immature green stage (IM), followed by the mature green stage (MG) and the breaker stage (Br, Br5) (Figure 3(e)). These results showed that *SlbZIP39* may affect plant architecture and fruit development processes.

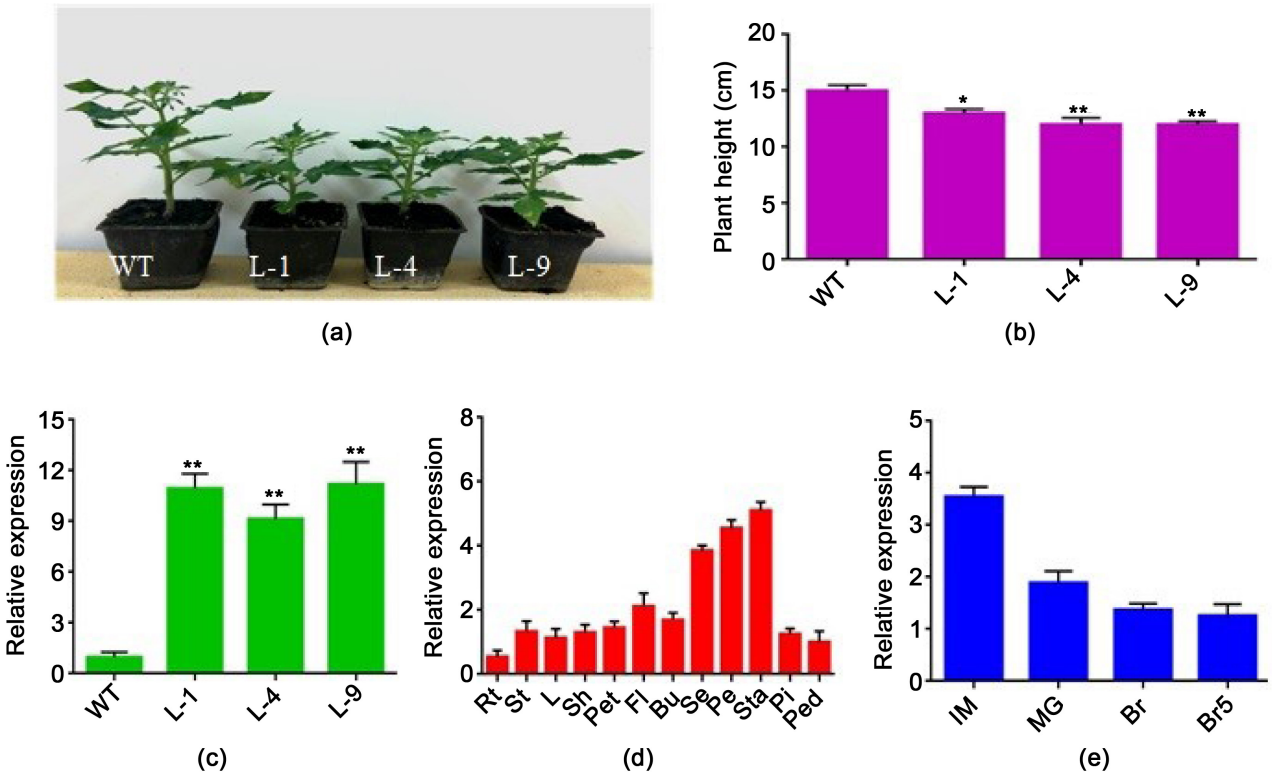

Figure 3. Phenotypical characteristics of wild-type and transgenic plants. (a) Plant architecture after 30 days of wild-type and *SlbZIP39*-OE lines; (b) Plant height; (c) *SlbZIP39* expression levels in wild-type and *SlbZIP39*-OE tomato plants; (d) and (e) *SlbZIP39* expression analysis in various tissues such as Rt, roots; St, stems; L, leaves; Sh, shoots; Pet, petioles; Fl, flowers; Bu, buds; Se, sepals; Pe, petals; Sta, stamens; Pi, pistils; Ped, pedicles; IM, immature; MG, mature green; Br, breaker; Br-5, 5 days breaker. Error bars indicate SD values from three biological replicates (n = 3). Asterisks indicate significant differences using Student's *t*-test (*$P < 0.05$; **$P < 0.01$).

3.3. Phenotypical Characters of *SlbZIP39* Overexpressing in Tomato Plants

The objectives of this study were to document and compare WT and OE plants under normal conditions. Numerous physiological parameters were measured, including plant height, flowering times, fruit weight, fruit length, and seed number, respectively. The *SlbZIP39* overexpression plant showed earlier flowering times than the WT (Figure 4(c)). In addition, the fruit size of *SlbZIP39*-OE was smaller than the WT fruits (Figure 4(d)). In tomato, *SlbZIP39* overexpression of fruit was decreased in fruit weight, fruit diameter, and the number of seeds compared to WT (Figures 4(e)-(g)). In our results, overexpression of *SlbZIP39* significantly differed in fruit weight, seed numbers, and inflorescence architecture (Figure 4). These data suggest that overexpression of *SlbZIP39* may regulate seed number and fruit development in tomato.

3.4. Tissue Specificity of *SlbZIP* Genes Expression Patterns during Fruits Development

Fruit development can be identified in several stages according to the number of days after anthesis and color. So, we investigated the expression levels of four *SlbZIP* genes in *SlbZIP39*-OE and WT at different fruit developmental stages. To verify the specific expression of *SlbZIP39*, the expression levels of *SlbZIP04* (*Solyc01g079480.2*), *SlbZIP07* (*Solyc01g100460.2*), *SlbZIP10* (*Solyc01g109880.2*)

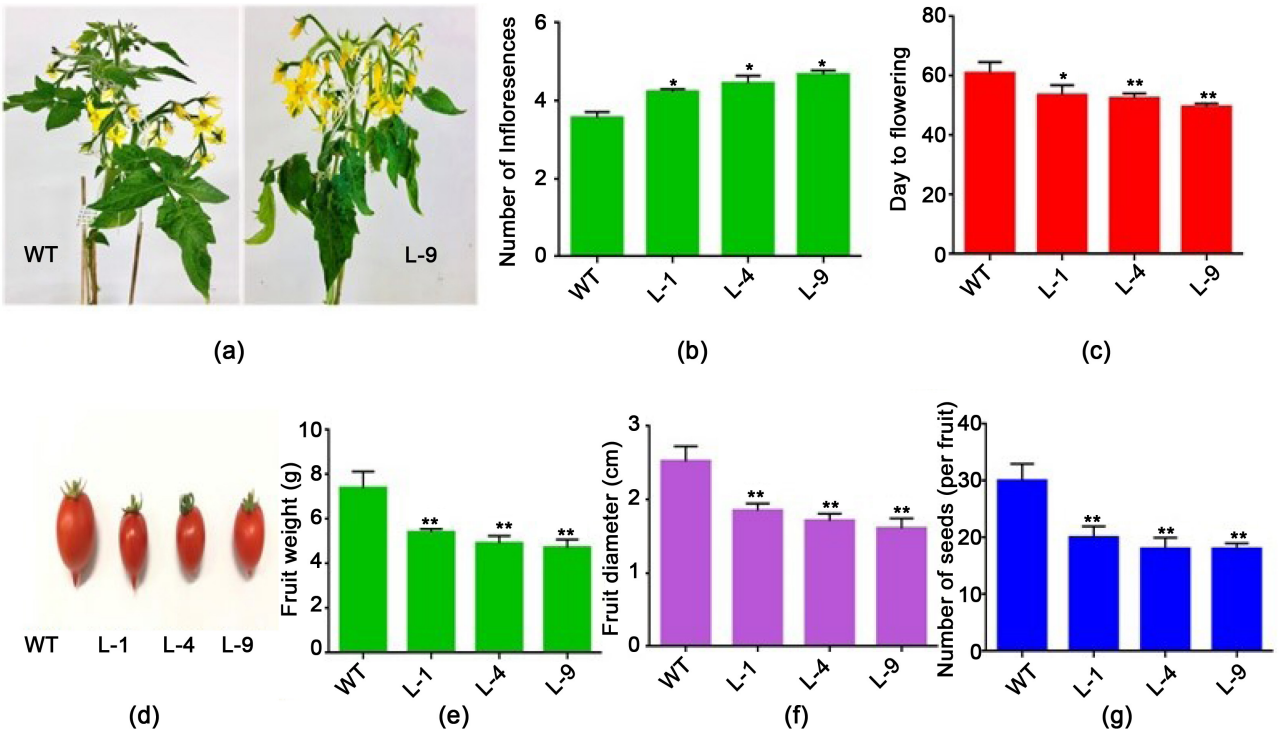

Figure 4. Phenotypical characteristics and growth parameters of *SlbZIP39* transgenic tomato plants. (a) Inflorescence architecture of the transgenic tomato plants; (b) Number of inflorescence; (c) Day to flowers; (d) Fruits characters; (e) Fruits weight; (f) Fruits diameter; (g) Number of seeds. Error bars indicate SD values from three biological replicates (n = 3) with n = 10 or 10 plants for each repeat. Asterisks indicate significant differences using Student's *t*-test (*$P < 0.05$; **$P < 0.01$).

and *SlbZIP34* (*Solyc04g080740.1*) were analyzed but they belong to group IV and contain sequences with close homology to *SlbZIP39*, as indicated in the phylogenetic tree database [30]. In our results, *SlbZIP04* and *SlbZIP07* mRNA levels were significantly up-regulated at IM and MG fruit stages, whereas they were moderately up-regulated at Br and Br-5 fruit stages than in WT (**Figure 5(a)** and **Figure 5(b)**). In addition, *SlbZIP10* transcript levels were abundantly up-regulated at MG and Br compared to WT fruit (**Figure 5(c)**). *SlbZIP10* expression was slightly up-regulated at IM and Br-5 than in WT fruit (**Figure 5(c)**). Furthermore, *SlbZIP34* transcripts were up-regulated at Br and had similar expression patterns in IM, MG, and Br-5 than in WT fruit developmental stages (**Figure 5(d)**). Significantly, *SlbZIP04* and *SlbZIP10* mRNA levels were predominantly up-regulated by *SlbZIP39*-OE and WT in fruit developmental stages (**Figure 5(a)** and **Figure 5(c)**). The transcript levels of the *SlbZIP* transcription factor

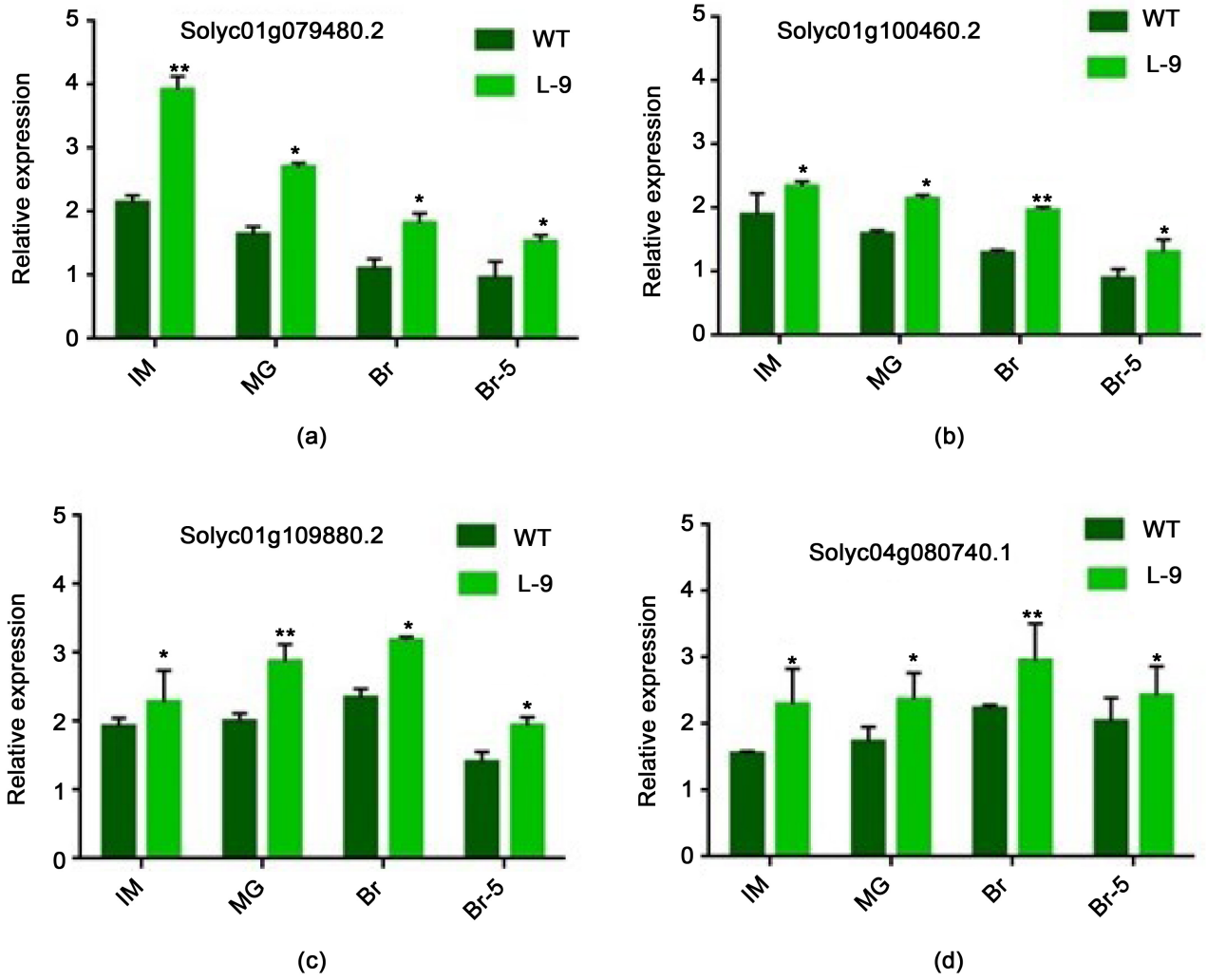

Figure 5. Expression of four *bZIP* transcription factors in WT and OE-fruits. (a) *SlbZIP04*; (b) *SlbZIP07*; (c) *SlbZIP34* and; (d) *SlbZIP10*. The tissues of fruit from different stages were used to analyze the experiment. IM, immature; MG, mature green; Br, breaker; Br-5, 5 days breaker. Error bars indicate SD values from three biological replicates (n = 3). Asterisks indicate significant differences using Student's *t*-test (*$P < 0.05$; **$P < 0.01$).

were predominantly different fruit developmental stages between *SlbZIP39*-OE and wild-type. These results assume that *SlbZIP39* may be concerned with the expression patterns of closely related *bZIP* genes.

4. Discussion

A number of *bZIP* transcription factors in plant growth and development are key processes that may participate in responses to environmental stresses. Abscisic acid (ABA) is a universal plant hormone because of its significant effects on the regulation of plant growth and development [31]. Nevertheless, the role of *bZIP* transcription factors has been involved in oxidative stress, light-dependent, and unfolded protein responses in all eukaryotes [32].

In tomato, *SlAREB1* and *SlAREB2* function in response to abiotic and biotic stresses, and may act as a link between ABA signal responses and other plant hormones; *SlAREB1* improves resistance to deficit and salt stress [3] [4]. Overexpression of *LebZIP2* might have been involved in tolerance to herbicide and oxidative stress and cell development of *Nicotiana benthamiana* [33]. However, expression of *LebZIP2* was increased by NaCl and mannitol treatments, whereas, it responded to ABA treatment [25]. The transcripts of *SlbZIP38* and *SlbZIP1* were expressed by NaCl, ABA, and GA treatments in tomato [5] [6]. Furthermore, overexpression of *MusabZIP53* was strongly increased by drought stress and ABA treatment in bananas [34]. Similarly, the expression level of *SlbZIP39* responded to NaCl treatment in tomato (**Figure 1(b)**). Besides, *SlbZIP39* was slightly induced by D-mannitol treatment in tomato leaves (**Figure 1(a)**). The present work revealed that the expression of *SlbZIP39* was also up-regulated as GA3 treatment, whereas it responded to IAA and ABA treatment (**Figures 2(a)-(c)**). Additionally, overexpression of *ABF2* has been shown to induce ABA sensitivity and dehydration tolerance in *Arabidopsis* [35]. In our research, we performed experiments to document the dehydration tolerance of fruits compared with leaves but they were affected by dehydration treatments (**Figure 1(c)** and **Figure 1(d)**). The transcript level of *SlbZIP39* was down-regulated by the dehydration of fruits (**Figure 1(d)**). This indicated that transcription factors of *SlbZIP39* may respond to abiotic stress and hormone treatments in tomato.

In expression analysis, *SlbZIP39* was detected in the different tissues of tomato (**Figure 3**). We observed that expression patterns of *SlbZIP39* were abundantly induced by sepals, petals, and stamens tissues (**Figure 3(d)**). The transcript of *SlbZIP39* is expressed in immature green fruits (**Figure 3(e)**). Previous reports documented that the expression patterns of *SlbZIP38*, *SlbZIP1* and *LebZIP2* were expressed in all tomato tissues [5] [6] [25]. Moreover, overexpression of *CabZIP1* is expressed in flowers and is involved in the plant development of *Arabidopsis* [36]. In *Arabidopsis*, the overexpression of *AtbZIP3* and *AtbZIP24* stimulated plant development [15] [37]. Furthermore, overexpression of *MubZIP53* has been linked to banana growth retardation [34]. In the present study, overexpression of *SlbZIP39* decreased plant growth in tomato (**Figure 3(a)** and

Figure 3(b)). At the mRNA level, *SlbZIP39* was expressed in all tomato tissues (Figure 3(d) and Figure 3(e)). Therefore, we suggest that it might be involved in the regulation of plant growth and development in tomato.

In our study, overexpression of *SlbZIP39* developed in the inflorescence of tomato (Figure 4(a)). In addition to the significant decrease in fruit weight, fruit diameter, and seed number, the number of inflorescence in overexpressing *SlbZIP39* was increased, whereas the flowering time was earlier, compared to the wild-type (Figures 4(b)-(g)). *SlAGO7* overexpression is involved in tomato inflorescence architecture [38]. In tomato, *SlGRAS24* and *SlGRAS40* responded to fruit sizes in the overexpression line [39] [40]. Moreover, the silencing of *SlGRAS2* is affected by smaller fruit sizes in tomato [41]. Overexpression of *SlAGL11* was dramatically influenced by tomato fruit size [42]. The transcription factor *SlAREB1* is expressed in fruit tissues and is involved in the fruit development of tomato [43]. In this study, we found that overexpression of *SlbZIP39* may be involved in fruit and seed development (Figure 4(d) and Figure 4(g)). For qPCR analysis, we performed the expression levels of *SlbZIP04*, *SlbZIP07*, *SlbZIP10*, and *SlbZIP34* (Group IV) during fruit development (Figure 5). The transcripts of *SlbZIP04* and *SlbZIP07* were up-regulated at IM and MG fruit stages (Figure 5(a) and Figure 5(b)). Furthermore, *SlbZIP10* and *SlbZIP34* were predominantly up-regulated at the Br fruit stage (Figure 5(c) and Figure 5(d)). These data suggest that *SlbZIP39* may be involved in the expression patterns of closely related *bZIP* genes.

5. Conclusion

In this study, we cloned and performed abiotic and hormonal treatments on tomato plants expressing *SlbZIP39* under greenhouse conditions. Our results have showed that evaluating the effects of overexpressing plants and wild-type fruits at developmental stages is related to *bZIP* transcription factors. We analyzed Group-IV *bZIP* transcription factors in tomato and expressed them in fruit developmental stages. However, overexpression of *SlbZIP39* was necessary to further confirm the transcription levels of the fruit size gene in tomato. Our experimental results revealed that overexpression of *SlbZIP39* might be regulated by fruit size and plant architecture.

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Authors' Contributions

Conceptualization, Y Y L, M K T and M M K; formal analysis, Y Y L and K H Y; investigation, Y Y L and M M K; data curation, Y Y L and M K T; writ-

ing-original draft preparation, Y Y L and K H Y; writing-review and editing, M K T and M M K; funding acquisition, Y Y L. All the authors have read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Alonso, R., Oñate-Sánehez, L., Weltmeier, F., *et al*. (2009) A Pivotal Role of the Basic Leucine Zipper Transcription Factor bZIP53 in the Regulation of *Arabidopsis* Seed Maturation Gene Expression Based on Heterodimerization and Protein Complex Formation. *The Plant Cell*, **21**, 1747-1761. https://doi.org/10.1105/tpc.108.062968

[2] Zhang, L., Zhang, L., Xia, C., Zhao, G., Liu, J., Jia, J. and Kong, X. (2015) A Novel Wheat bZIP Transcription Factor, TabZIP60, Confers Multiple Abiotic Stress Tolerances in Transgenic *Arabidopsis*. *Physiologia Plantarum*, **153**, 538-554. https://doi.org/10.1111/ppl.12261

[3] Orellana, S., Yañez, M., Espinoza, A., Verdugo, I., González, E., Ruiz-Lara, S. and Casaretto, J.A. (2010) The Transcription Factor SlAREB1 Confers Drought, Salt Stress Tolerance and Regulates Biotic and Abiotic Stress-Related Genes in Tomato. *Plant Cell & Environment*, **33**, 2191-2208. https://doi.org/10.1111/j.1365-3040.2010.02220.x

[4] Hsieh, T.H., Li, C.W., Su, R.C., Cheng, C.P., Sanjaya and Tsai, Y.C. (2010) A Tomato bZIP Transcription Factor, SlAREB, Is Involved in Water Deficit and Salt Stress Response. *Planta*, **231**, 1459-1473. https://doi.org/10.1007/s00425-010-1147-4

[5] Pan, Y., Hu, X., Li, C., Xu, X., Su, C., Li, J., *et al*. (2017) SlbZIP38, a Tomato bZIP Family Gene Downregulated by Abscisic Acid, Is a Negative Regulator of Drought and Salt Stress Tolerance. *Genes*, **8**, 402. https://doi.org/10.3390/genes8120402

[6] Zhu, M., Meng, X., Cai, J., Li, G., Dong, T. and Li, Z. (2018) Basic Leucine Zipper Transcription Factor SlbZIP1 Mediates Salt and Drought Stress Tolerance in Tomato. *BMC Plant Biology*, **18**, 83. https://doi.org/10.1186/s12870-018-1299-0

[7] Jakoby, M., Weisshaar, B., Droge-Laser, W., Vicente-carbajosa, J., Tiedemann, J., Kroj, T. and Parcy, F. (2002) bZIP Transcription Factors in *Arabidopsis*. *Trends in Plant Science*, **7**, 106-111. https://doi.org/10.1016/S1360-1385(01)02223-3

[8] Nijhawan, A., Jain, M., Tyagi, A.K. and Khurana, J.P. (2008) Genomic Survey and Gene Expression Analysis of the Basic Leucine Zipper Transcription Factor Family in Rice. *Plant Physiology*, **146**, 333-350. https://doi.org/10.1104/pp.107.112821

[9] Wei, K., Chen, J., Wang, Y., Chen, Y., Chen, S., Lin, Y., *et al*. (2012) Genome-Wide Analysis of bZIP-Encoding Genes in Maize. *DNA Research*, **19**, 463-476. https://doi.org/10.1093/dnares/dss026

[10] Zhang, M., Liu, Y., Shi, H., Guo, M., Chai, M., He, Q., *et al*. (2018) Evolutionary and Expression Analyses of Soybean Basic Leucine Zipper Transcription Factor Family.

BMC Genomics, **19**, 159. https://doi.org/10.1186/s12864-018-4511-6

[11] Liu, J., Chen, N., Chen, F., Cai, B., Santo, S.D., Tornielli, G.B., *et al*. (2014) Genome Wide Analysis and Expression Profile of the bZIP Transcription Factor Gene Family in Grapevine (*Vitis vinifera*). *BMC Genomics*, **15**, Article No. 281. http://www.biomedcentral.com/1471-2164/15/281 https://doi.org/10.1186/1471-2164-15-281

[12] Yoshida, T., Fujita, Y., Sayama, H., Kidokoro, S., Maruyama, K., Mizoi, J., *et al*. (2010) AREB1, AREB2, and ABF3 Are Master Transcription Factors That Cooperatively Regulate ABRE-Dependent ABA Signaling Involved in Drought Stress Tolerance and Require ABA for Full Activation. *The Plant Journal*, **61**, 672-685. https://doi.org/10.1111/j.1365-313X.2009.04092.x

[13] Barbosa, E.G.G., Leite, J.P., Marin, S.R.R., Marinho, J.P., *et al*. (2013) Overexpression of the ABA-Dependent AREB1 Transcription Factor from *Arabidopsis thaliana* Improves Soybean Tolerance to Water Deficit. *Plant Molecular Biology Reporter*, **31**, 719-730. https://doi.org/10.1007/s11105-012-0541-4

[14] Wang, Z., Su, G., Li, M., Ke, Q., Kim, S.Y., Li, H., *et al*. (2016) Overexpressing *Arabidopsis* ABF3 Increases Tolerance to Multiple Abiotic Stresses and Reduces Leaf Size in Alfalfa. *Plant Physiology & Biochemistry*, **109**, 199-208. https://doi.org/10.1016/j.plaphy.2016.09.020

[15] Sanagi, M., Lu, Y., Aoyama, S., Morita, Y., Mitsuda, N., Ikeda, M., *et al*. (2018) Sugar-Responsive Transcription Factor bZIP3 Affects Leaf Shape in *Arabidopsis* Plants. *Plant Biotechnology*, **35**, 167-170. https://doi.org/10.5511/plantbiotechnology.18.0410a

[16] Assunção, A.G.L., Herrero, E., Lin, Y.F., Huettel, B., Talukdar, S., Smaczniak, C., *et al*. (2010) *Arabidopsis thaliana*a Transcription Factors bZIP19 and bZIP23 Regulate the Adaptation to Zinc Deficiency. *The Proceedings of the National Academy of Sciences*, **107**, 10296-10301. https://doi.org/10.1073/pnas.1004788107

[17] Shen, H., Cao, K. and Wang, X. (2008) AtbZIP16 and AtbZIP68, Two New Members of GBFs, Can Interact with Other G Group bZIPs in *Arabidopsis thaliana*. *BMB Reports*, **41**, 132-138. https://doi.org/10.5483/BMBRep.2008.41.2.132

[18] Hanson, J., Hanssen, M., Wiese, A., Hendriks, M.M.W.B. and Smeekens, S. (2008) The Sucrose Regulated Transcription Factor bZIP11 Affects Amino Acid Metabolism by Regulating the Expression of Asparagine Synthetase 1 and Proline Dehydrogenase 2. *The Plant Journal*, **53**, 935-949. https://doi.org/10.1111/j.1365-313X.2007.03385.x

[19] Matiolli, C.C., Tomaz, J.P., Duarte, G.T., Prado, F.M., *et al*. (2011) The *Arabidopsis* bZIP Gene AtbZIP63 Is a Sensitive Integrator of Transient Abscisic Acid and Glucose Signals. *Plant Physiology*, **157**, 692-705. https://doi.org/10.1104/pp.111.181743

[20] Zou, M., Guan, Y., Ren, H., Zhang, F. and Chen, F. (2008) A bZIP Transcription Factor, OsABI5, Is Involved in Rice Fertility and Stress Tolerance. *Plant Molecular Biology*, **66**, 675-683. https://doi.org/10.1007/s11103-008-9298-4

[21] Kang, J.Y., Choi, H.I., Im, M.Y. and Kim, S.Y. (2002) *Arabidopsis* Basic Leucine Zipper Proteins That Mediate Stress-Responsive Abscisic Acid Signaling. *The Plant Cell*, **14**, 343-357. https://doi.org/10.1105/tpc.010362

[22] Hsieh, W.P., Hsieh, H.L. and Wu, S.H. (2012) *Arabidopsis* bZIP16 Transcription Factor Integrates Light and Hormone Signaling Pathways to Regulate Early Seedling Development. *The Plant Cell*, **24**, 3997-4011. https://doi.org/10.1105/tpc.112.105478

[23] Gao, S.Q., Chen, M., Xu, Z.S., Zhao, C.P., Li, L., Xu, H.J., *et al*. (2011) The Soybean GmbZIP1 Transcription Factor Enhances Multiple Abiotic Stress Tolerances in

Transgenic Plants. *Plant Molecular Biology*, **75**, 537-553. https://doi.org/10.1007/s11103-011-9738-4

[24] Cifuentes-esquivel, N., Celiz-Balboa, J., Henriquez-Valencia, C., Mitina, I., Arrano-Salinas, P., Moreno, A.A., *et al*. (2018) bZIP17 Regulates the Expression of Genes Related to Seed Storage and Germination, Reducing Seed Susceptibility to Osmotic Stress. *Journal of Cellular Biochemistry*, **119**, 6857-6868. https://doi.org/10.1002/jcb.26882

[25] Seong, E.S., Kwon, S.S., Ghimire, B.K., Yu, C.Y., Cho, D.H., Lim, J.D., *et al*. (2008) LebZIP2 Induced by Salt and Drought Stress and Transient Overexpression by *Agrobacterium*. *BMB Reports*, **41**, 693-698. https://doi.org/10.5483/BMBRep.2008.41.10.693

[26] Yanez, M., Caceres, S., Orellana, S., Bastias, A., Verdugo, I., Ruiz-Lara, S. and Casaretto, J.A. (2009) An Abiotic Stress-Responsive bZIP Transcription Factor from Wild and Cultivated Tomatoes Regulates Stress-Related Genes. *Plant Cell Reports*, **28**, 1497-1507. https://doi.org/10.1007/s00299-009-0749-4

[27] Tu, M., Wang, X., Feng, T., Sun, X., Wang, Y., Huang, L., *et al*. (2016) Plant Science Expression of a Grape (*Vitis vinifera*) bZIP Transcription Factor, VlbZIP36, in *Arabidopsis thaliana* Confers Tolerance of Drought Stress during Seed Germination and Seedling Establishment. *Plant Science*, **252**, 311-323. https://doi.org/10.1016/j.plantsci.2016.08.011

[28] Tu, M., Wang, X., Zhu, Y., Wang, D., Zhang, X., Cui, Y., *et al*. (2017) VlbZIP30 of Grapevine Functions in Drought Tolerance via the Abscisic Acid Core Signaling Pathway. 1-56. https://doi.org/10.1101/231811

[29] Fillatti, J.J., Kiser, J., Rose, R. and Comai, L. (1987) Efficient Transfer of a Glyphosate Tolerance Gene into Tomato Using a Binary *Agrobacterium tumefaciens* Vector. *Biotechnology*, **5**, 726-730. https://doi.org/10.1038/nbt0787-726

[30] Li, D., Fu, F., Zhang, H. and Song, F. (2015) Genome-Wide Systematic Characterization of the bZIP Transcriptional Factor Family in Tomato (*Solanum lycopersicum* L.). *BMC Genomics*, **16**, Article No. 771. https://doi.org/10.1186/s12864-015-1990-6

[31] Banerjee, A. and Roychoudhury, A. (2017) Abscisic-Acid-Dependent Basic Leucine Zipper bZIP Transcription Factors in Plant Abiotic Stress. *Protoplasma*, **254**, 3-16. https://doi.org/10.1007/s00709-015-0920-4

[32] Guedes Correa, L.G., Riano-Pachon, D.M., Guerra Schrago, C., Viicentini dos Santos, R., Mueller-Roeber, B., *et al*. (2008) The Role of bZIP Transcription Factors in Green Plant Evolution: Adaptive Features Emerging from Four Founder Genes. *PLOS ONE*, **3**, e2944. https://doi.org/10.1371/journal.pone.0002944

[33] Seong, E.S., Yoo, J.H., Kim, N.J., Choi, J.H., Lee, J.G., Ghimire, B.K., Chung, I.M. and Yu, C.Y. (2016) Morphological Changes and Increase of Resistance to Oxidative Stress by Overexpression of the LebZIP2 Gene in *Nicotiana benthamiana*. *Russian Journal of Plant Physiolology*, **63**, 124-131. https://doi.org/10.1134/S1021443716010143

[34] Shekhawat, U.K.S. and Ganapathi, T.R. (2014) Transgenic Banana Plants Overexpressing MusabZIP53 Display Severe Growth Retardation with Enhanced Sucrose and Polyphenol Oxidase Activity. *Plant Cell, Tissue and Organ Culture*, **116**, 387-402. https://doi.org/10.1007/s11240-013-0414-z

[35] Kim, S., Kang, J.Y., Cho, D.I., Park, J.H. and Kim, S.Y. (2004) ABF2, an ABRE-Binding bZIP Factor, Is an Essential Component of Glucose Signaling and Its Overexpression Affects Multiple Stress Tolerance. *The Plant Journal*, **40**, 75-87. https://doi.org/10.1111/j.1365-313X.2004.02192.x

[36] Lee, S.C., Choi, H.W., Hwang, I.S., Choi, D.S. and Hwang, B.K. (2006) Functional Roles of the Pepper Pathogen-Induced bZIP Transcription Factor, CAbZIP1, in Enhanced Resistance to Pathogen Infection and Environmental Stresses. *Planta*, **224**, 1209-1225. https://doi.org/10.1007/s00425-006-0302-4

[37] Yang, O., Popova, O.V., Süthoff, U., Lüking, I., Dietz, K. and Golldack, D. (2009) The *Arabidopsis* Basic Leucine Zipper Transcription Factor AtbZIP24 Regulates Complex Transcriptional Networks Involved in Abiotic Stress Resistance. *Gene*, **436**, 45-55. https://doi.org/10.1016/j.gene.2009.02.010

[38] Lin, D., Xiang, Y., Xian, Z. and Li, Z. (2016) Ectopic Expression of SlAGO7 Alters Leaf Pattern and Inflorescence Architecture and Increases Fruit Yield in Tomato. *Physiologia Plantarum*, **157**, 490-506. https://doi.org/10.1111/ppl.12425

[39] Huang, W., Peng, S., Xian, Z., Lin, D., Hu, G., Yang, L., Ren, M. and Li, Z. (2017) Overexpression of a Tomato miR171 Target Gene SlGRAS24 Impacts Multiple Agronomical Traits via Regulating Gibberellin and Auxin Homeostasis. *Plant Biotechnology Journal*, **15**, 472-488. https://doi.org/10.1111/pbi.12646

[40] Liu, Y., Huang, W., Xian, Z., Hu, N., Lin, D., Ren, H., Chen, J., Su, D. and Li, Z. (2017) Overexpression of SlGRAS40 in Tomato Enhances Tolerance to Abiotic Stresses and Influences Auxin and Gibberellin Signaling. *Frontiers in Plant Science*, **8**, 1659. https://doi.org/10.3389/fpls.2017.01659

[41] Li, M., Wang, X., Li, C., Li, H., Zhang, J. and Ye, Z. (2018) Silencing GRAS2 Reduces Fruit Weight in Tomato. *Journal of Integrative Plant Biology*, **60**, 498-513. https://doi.org/10.1111/jipb.12636

[42] Huang, B., Routaboul, J.M., Liu, M., Deng, W., Maza, E., Mila, I., *et al*. (2017) Overexpression of the Class D MADS-Box Gene Sl-AGL11 Impacts Fleshy Tissue Differentiation and Structure in Tomato Fruits. *Journal of Experimental Botany*, **68**, 4869-4884. https://doi.org/10.1093/jxb/erx303

[43] Bastias, A., Yanez, M., Osorio, S., Arabona, V., Gomez-Cadenas, A., Fernie, A.R. and Casaretto, J.A. (2014) The Transcription Factor AREB1 Regulates Primary Metabolic Pathways in Tomato Fruits. *Journal of Experimental Botany*, **65**, 2351-2363. https://doi.org/10.1093/jxb/eru114

Abbreviations

ABA: Abscisic acid

bZIP: Basic leucine zipper

GA: Gibberellic acid

IAA: Indole-3-acetic acid

NaOCl: Sodium hypochlorite

MS: Murashige and Skoog

TF: Transcription factor

WT: Wild-type

OE: Overexpression

qRT-PCR: Quantitative Real-time PCR