Genetics and Molecular Research

Genomic identification of group A bZIP transcription factors and their responses to abiotic stress in carrot

F. Que, G.L. Wang, Y. Huang, Z.S. Xu, F. Wang and A.S. Xiong

State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Horticulture, Nanjing Agricultural University, Nanjing, China

Corresponding author: A.S. Xiong
E-mail: xiongaisheng@njau.edu.cn

Genet. Mol. Res. 14 (4): 13274-13288 (2015)
Received May 12, 2015
Accepted August 13, 2015
Published October 26, 2015
DOI http://dx.doi.org/10.4238/2015.October.26.24

ABSTRACT. The basic-region/leucine-zipper (bZIP) family is one of the major transcription factor (TF) families associated with responses to abiotic stresses. Many members of group A in this family have been extensively examined and are reported to perform significant functions in ABA signaling as well as in responses to abiotic stresses. In this study, 10 bZIP factors in carrot were classified into group A based on their DNA-binding domains. The *cis*-acting regulatory elements and folding states of these 10 factors were analyzed. Evolutionary analysis of the group A members suggested their importance during the course of evolution in plants. In addition, *cis*-acting elements and the folding state of proteins were important for DNA binding and could affect gene expression. Quantitative RT-PCR was conducted to investigate the stress response of 10 genes encoding the group A factors. Six genes showed responses to abiotic stresses, while four genes showed other special phenomenon. The current analysis on group A bZIP family TFs in carrot is the first to investigate the TFs of Apiaceae *via* genome analysis. These results provide new information for future studies on carrot.

Key words: bZIP; Group A; Transcription factor; Evolution; Abiotic stress; Carrot

INTRODUCTION

Abiotic stress has emerged as an important factor affecting the growth and development of vegetable crops and limiting their yield (Vinocur and Altman, 2005). Among the various environmental factors, soil salinization, low and high temperatures, and drought are the main stressors for plant growth. For their survival, plants have evolved several mechanisms to resist these stresses (Vinocur and Altman, 2005). Previous studies have revealed that transcription factors (TFs) participate in plant's responses to abiotic stress (Chen et al., 2002).

Of the numerous TFs present in plants, seven major TF families have been reported to participate in such responses (Finkelstein and Lynch, 2000). The basic-region/leucine-zipper (bZIP) TF family, for example, performs significant functions in resisting abiotic stresses (Yáñez et al., 2009). The bZIP TFs are one of the most conserved proteins present in all eukaryotes (Riechmann et al., 2000). The bZIP domain of bZIP TFs is highly conserved and harbors a DNA-binding basic region as well as a leucine (Leu) zipper dimerization motif (Wang et al., 2011). The DNA-binding basic region is highly conserved and is used for binding DNA in a sequence-specific manner. The Leu zipper dimerization motif, which is not conserved, has the property to dimerize (Ellenberger et al., 1992). Based on their DNA-binding domains, 75 bZIP TFs in *Arabidopsis* have been classified into 10 groups of which 13 have been assigned to group A (Jakoby et al., 2002). A number of bZIP family TFs have been reported to be involved in abscisic acid (ABA)-mediated stress signaling (Fujita et al., 2005). ABA performs an important function in the response of plants to abiotic stress (Fujita et al., 2011).

In *Arabidopsis*, many members of the group A bZIP family TFs have been designated as ABA-responsive element (ABRE) -binding-factors (ABFs) or ABRE-binding-proteins (AREBs) (Finkelstein and Lynch, 2000; Jakoby et al., 2002). A number of studies have evaluated the function of group A TFs in rice, their results demonstrating that the overexpression of *OsABI5* lead to a higher sensitivity to salt stress (Zou et al., 2008), and that of *OsABF1* and *OsABF2* enhanced the abiotic stress signaling (Hossain et al., 2010a,b). In *Arabidopsis*, the roles of AREB1 and AREB2 in response to ABA, drought, and high salt stresses have been well-examined (Hsieh et al., 2010). Although several reports on bZIP family TFs in *Arabidopsis* and rice are available, similar studies in carrot are lacking. Transcriptional regulation of group A TFs under abiotic stresses has not been sufficiently investigated in carrot.

Carrot (*Daucus carota* L.) is a biennial plant with the highest cultivation area among Apiaceae species. Molecular studies on this significant vegetable, best known for its rich nutrient content, are limited. In the present study, 75 bZIP family TFs were identified in carrot; ten members were classified under group A, based on carrot genome data (Xu et al., 2014). The bZIP motifs of the 10 genes, their upstream *cis*-regulatory elements, and their folding states were also analyzed. The expression profiles of the 10 genes, under 4 different environment stimuli (i.e., drought, heat, cold, and, salt) were analyzed to evaluate the responses of *DcbZIPA* genes to abiotic stresses. The results could help in elucidating the function of *DcbZIPA* genes in stress response as well as the structure of *bZIP* genes in carrot.

MATERIAL AND METHODS

Plant materials, growth conditions, and treatments

The carrot cultivar 'Kurodagosun' was used as the experimental material. This cultivar originated in Japan. It has cylindrical roots and shows high tolerance to heat stress. The plantlets

were grown in an artificial climate chamber at Nanjing Agricultural University (32°02′N,118°50′E). Two-month-old plantlets were subjected to high (38°C) and low (4°C) temperature, high salt (0.2 M NaCl), and dehydration (200 g/L PEG) conditions. The plant samples were collected at 0, 1, 2, 4, 8, and 12 h, after the treatment. The whole treated plants were harvested, frozen in liquid nitrogen, and stored at -70°C for further study.

Sequence database searches

Sequences of the *Arabidopsis* bZIP TF family were obtained from The *Arabidopsis* Information Resource (http://www.arabidopsis.org/) while those of the carrot were downloaded from CarrotDB, which is a genomic and transcriptomic database of *D. carota* (http://apiaceae.njau.edu.cn/carrotdb/index.php; Xu et al., 2014).

Motif recognition and phylogenetic analysis

Motifs of the selected genes were analyzed using the MEME (Version 4.9.1) suite (Bailey et al., 2006). Multiple alignments of bZIP protein sequences were performed by Clustal X 1.83 software (Thompson et al., 1997) and the phylogenetic tree was constructed with MEGA 5.0 (Tamura et al., 2011).

cis-Regulatory element analysis and prediction of folding state

Putative *cis*-acting regulatory elements were discovered using the plant database PlantCARE (Lescot et al., 2002). The lengths of the upstream sequence for study ranged from 900 to 1500 bp. Predictions were performed employing the FoldIndex program (http://bioportal.weizmann.ac.il/fldbin/findex) (Prilusky et al., 2005).

RNA isolation and relative quantitative real-time (qRT)-PCR analysis

Total RNA was extracted using an RNA kit (Tiangen, Beijing, China) according to the manufacturer instructions. RNA from each of the treated samples was reverse transcribed into cDNA using the Prime ScriptRT reagent kit (TaKaRa, Dalian, China).

The primers for the selected *DCbZIPA* genes were designed using Primer Premier 5.0 software. *Tubulin* gene of carrot was selected as the internal control. Sequences of all the primers used in this study are provided in Table1. The primers were synthesized by Genscript Nanjing Inc. (Nanjing, China). qRT-PCR was performed in an ABI7300 qRT-PCR system (Applied Biosystems, Foster City, CA, USA) using a SYBR Premix *Ex Taq* kit (TaKaRa, Dalian, China) with the following reaction conditions: 95°C for 30 s followed by 40 cycles of 95°C for 5 s and 60°C for 30 s for annealing and extension. Melting curve analysis was performed at 65°C for 10 s (61 cycles) to test the specificity of amplification. The experiments were repeated thrice using independent RNA samples.

RESULTS

Phylogenetic analysis of bZIP TF family in carrot

To analyze the bZIP TF family in carrot, a phylogenetic tree was constructed using the

amino acid sequences of 68 proteins from *Arabidopsis* and 75 proteins from carrot. In the tree, the 75 DcbZIP TFs were arranged in 10 subfamilies (A-I, and S), according to the classification of the bZIP family in *Arabidopsis* (Figure 1). We analyzed the constituents of each subfamily (group) of the bZIP in carrot, based on this phylogenetic tree. This included Group A, the third-largest group in the family (13%) with 10 members and Group S, which included 31% of all bZIP family members and was the largest (Figure 2).

Table 1. Primer sequences for qRT-PCR amplification of Tubulin and 10 genes from group A bZIP TFs in carrot.

Gene number	Gene name	Oligonucleotide sequences
DcbZIPA1	Dck13424-Forward primer	5'-AATTCTAGTTTTGGAATTGGAT-3'
	Dck13424-Reverse primer	5'-GGTCTCTGTTGTAGTTGTGGCT-3'
DcbZIPA2	Dck24387-Forward primer	5'-CAATGGTGGATATTATGGTGAG-3'
	Dck24387-Reverse primer	5'-TACAGTTGCTTGTTTGGGGAAA-3'
DcbZIPA3	Dck21551-Forward primer	5'-ATTCTTGGTTAAGGCGGGTGTG-3'
	Dck21551-Reverse primer	5'-GCATTATCTGTTGCTGAGTTGG-3'
DcbZIPA4	Dck19713-Forward primer	5'-TTTGGTGAGATGACTGTGGAGG-3'
	Dck19713-Reverse primer	5'-TGTGGGGAACTTTGTTGTGGGA-3'
DcbZIPA5	Dck70030-Forward primer	5'-TTGACGAGGTGTGGCAGGATAT-3'
	Dck70030-Reverse primer	5'-TGCGGAGCACTTTGTTGAGGTA-3'
DcbZIPA6	Dck73230-Forward primer	5'-CCGTGCCTACTGTGCCACACAT-3'
	Dck73230-Reverse primer	5'-GCTGGAACACTCAGCGATCCTT-3'
DcbZIPA7	Dck03542-Forward primer	5'-CATGAATCCACAATATCACTAT-3'
	Dck03542-Reverse primer	5'-TACCCCTGCCTTGACCAAAAAA-3'
DcbZIPA8	Dck02510-Forward primer	5'-ACAGATTTTCAGGGGAGGGTTA-3'
	Dck02510-Reverse primer	5'-TCAAGAGTGAGGGAATGGATTG-3'
DcbZIPA9	Dck48330-Forward primer	5'-CCTTTGGGGAAATATAATAAGC-3'
	Dck48330-Reverse primer	5'-GGGCGGCAAGAGGAGAGTTGGG-3'
DcbZIPA10	Dck27084-Forward primer	5'-GAATCTCCAAGAATCTCTAGGC-3'
	Dck27084-Reverse primer	5'-TAATCATTTTCACGGGAACACC-3'
Tubulin	Tubulin-Forward primer	5'-GAGTGGAGTTACCTGCTGCCTTC-3'
	Tubulin-Reverse primer	5'-ATGTAGACGAGGGAACGGAATCAAG-3'

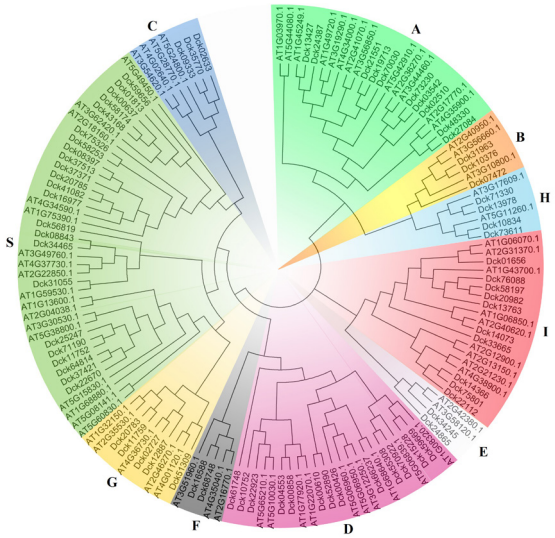


Figure 1. Phylogenetic tree of carrot and *Arabidopsis* bZIP family TFs. A total of 75 carrot and 68 *Arabidopsis* bZIP domain protein sequences were aligned by Clustal X 1.83, and the phylogenetic tree was constructed via MEGA5.0. Backgrounds with different colors represent different subfamilies.

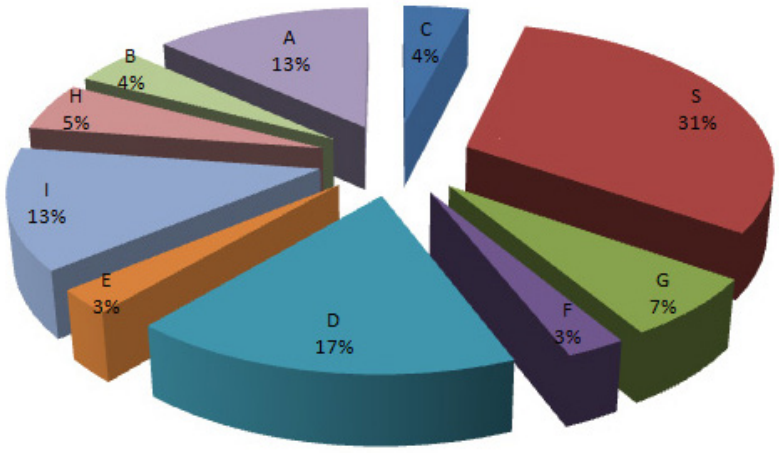


Figure 2. Classification of bZIP family TFs in carrot and the percentage of every subfamily.

Motif analysis of the carrot bZIP TF family

We deciphered the conserved motifs in the 75 bZIP TFs in carrot using MEME (Version 4.9.1). The logos of these motifs are listed in Figure 3. The constituent motifs of each TF in the bZIP family are shown in Figure 4. Motif 1 appeared in all the members, suggesting that it is a basic motif with a significant function in bZIP TFs. Motif 2 was found only in the members of group D and in a single member of group S (Dck22667). Motifs 4, 6, and 7 were exclusive to the group D while Motifs 5, 8, and 9 appeared only in the group A. The constituents of groups A and D were abundant compared to the remaining subfamilies, which implied that the groups A and D were special among the subfamilies of bZIP family TFs in carrot. The factors Dck48330 and Dck27084, unlike other members of group A, exhibited only Motif 1, indicating that these two TF might have fewer functions than the other members of the bZIP family in carrot.

Evolution of bZIP TFs among different plant species

We analyzed the bZIP TF family and group A members of the bZIP family among different plants. Among 42 selected plant species, the gene sequences of group A bZIP family members of only *Ricinus communis* (Jin et al., 2014), *Vitis vinifera* (Liu et al., 2014), *Solanum lycopersicum* (Xu et al., 2013), and *Hordeum vulgare* (Pourabed et al., 2015) have been published. In addition, there are few reports on genome-wide studies on the bZIP TF family in other selected plants. These studies used the same process adopted for carrot in the current study to examine the number of bZIP TFs and group A members (Figure 5). The number of bZIP TFs in algae and land plants differed remarkably. The number of TFs in algae was less than 20, while that in the land plants was greater than 25. The number of group A members in algae, bryophyte, pteridophyta, and gymnosperms was less than 5. No group A member was found in *Ostreococcus lucimarinus*. On the contrary, the number of group A members in land plants, except *Picea abies*, *Selaginella moellendorffii*, and *Physcomitrella patens* was greater than 5. The average number of bZIP family TFs per Mb in *P. abies*, *Arabidopsis thaliana*, and carrot was 0.002, 1.016, and 0.156, respectively. Among the selected plant species, the average number of bZIP family TFs per Mb was the highest in *A. thaliana.*

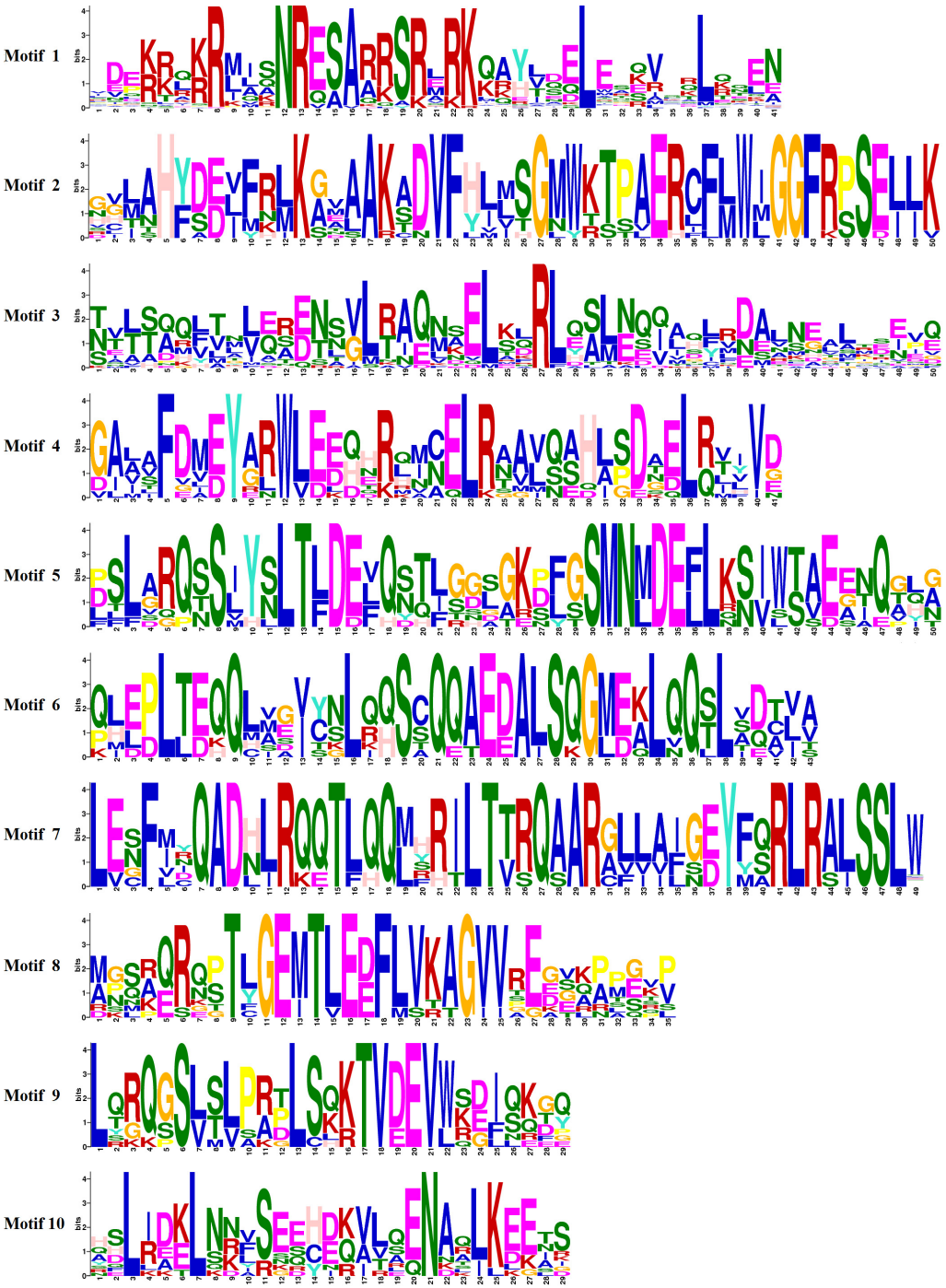


Figure 3. Sequence logos of bZIP TF domains in carrot.

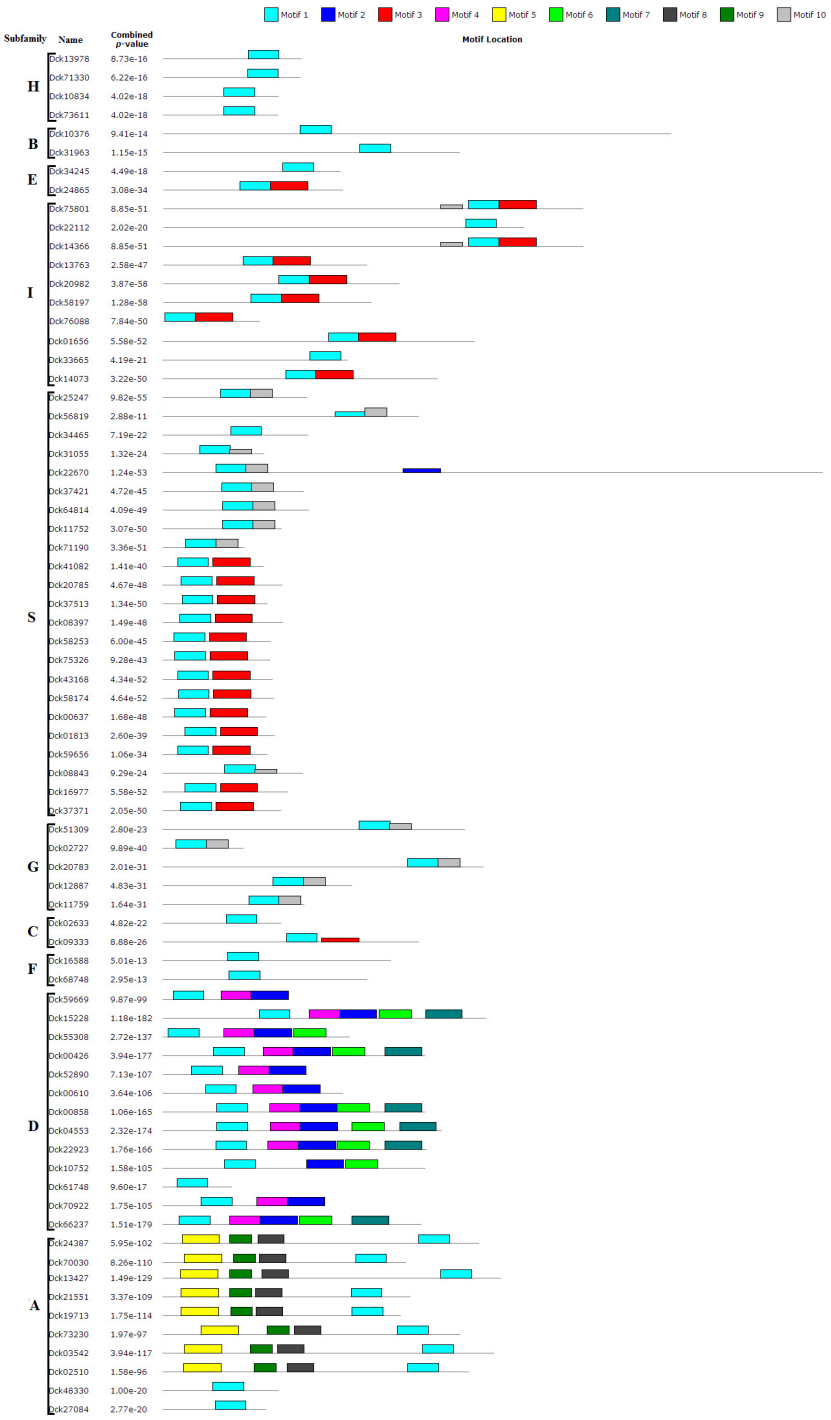
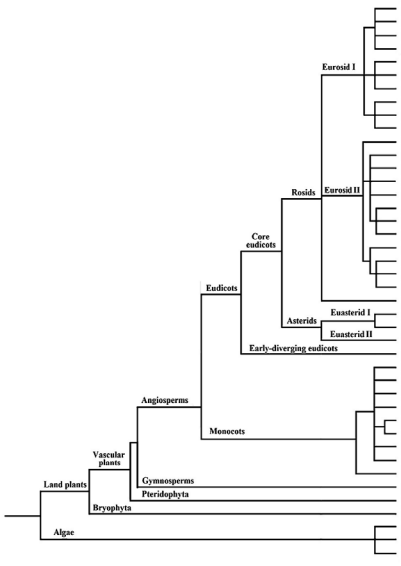


Figure 4. Motifs of bZIP TFs from different subfamilies in carrot.



Plants	Group A	Total bZIP family factors	Percentage of Group A in the family/%	Genomic Size(Mb)	Average number of bZIP family factor per Mb
Pyrus bretschneideri	14	89	15.7	527	0.169
Malus domestica	16	114	14.0	742	0.154
Fragaria vesca	11	52	21.1	240	0.217
Prunus persica	10	50	20.0	265	0.189
Cucumis melo	12	79	15.2	450	0.176
Cucumis sativus	15	118	12.7	350	0.337
Citrullus lanatus	9	61	14.8	425	0.144
Glycine max	42	266	15.8	1100	0.242
Cicer arietinum	21	97	21.6	738	0.131
Medicago truncatula	13	66	19.7	500	0.132
Citrus sinensis	28	105	26.7	367	0.286
Theobroma cacao	28	107	26.2	430	0.249
Populus trichocarpa	39	215	18.1	480	0.448
Gossypium raimondii	64	272	23.5	775.2	0.351
Linum usitatissimum	22	107	20.6	373	0.287
Jatropha curcas	13	54	24.1	410	0.132
Manihot esculenta	18	89	20.2	760	0.117
Ricinus communis	6	50	12.0	350	0.143
Carica papaya	7	46	15.2	370	0.124
Arabidopsis lyrata	15	80	18.8	206.7	0.387
Arabidopsis thaliana	23	127	18.1	125	1.016
Brassica rapa	23	133	17.3	485	0.274
Vitis vinifera	13	47	27.7	490	0.096
Solanum lycopersicum	12	70	17.1	900	0.078
Solanum tuberosum	19	95	20.0	840	0.113
Daucus carota	10	75	13.3	480	0.156
Nelumbo nucifera	13	62	20.1	929	0.067
Zea mays	29	218	13.3	2300	0.095
Sorghum bicolor	18	123	14.6	730	0.168
Brachypodium distachyon	21	95	22.1	260	0.365
Aegilops tauschii	14	65	21.5	4000	0.016
Oryza sativa subsp. indica	13	94	13.8	430	0.219
Oryza sativa subsp. japonica	19	140	13.6	430	0.325
Hordeum vulgare	18	157	11.5	5000	0.031
Setaria italica	16	104	15.4	490	0.212
Phoenix dactylifera	10	68	14.7	685	0.099
Picea abies	4	44	9.1	19600	0.002
Selaginella moellendorffii	5	25	20.0	212	0.118
Physcomitrella patens	1	67	1.5	480	0.140
Ostreococcus lucimarinus	0	7	0	13.25	0.519
Chlorella sp. NC64	3	13	23.1	46	0.283
Chlamydomonas reinhardtii	1	19	5.3	130	0.146

Figure 5. Evolution of bZIP family TFs among plants.

Analysis of *cis*-regulatory elements

We examined the upstream sequences of genes from the group A, in carrot, based on their genome sequences. The region 900-1500 bp upstream of the gene was investigated in all the cases except for *DcbZIPA3*, for which only the region 473 bp upstream was considered. A number of *cis*-regulatory elements were identified when a search was made for *bZIP* genes in the PlantCARE database. We selected 11 elements related to abiotic stress and hormone responsiveness for further investigations (Figure 6). Almost all members of group A exhibited the *cis*-regulatory elements involved in abiotic stress (i.e., drought, heat, low temperature) or defense and stress responsiveness. The *cis*-regulatory elements involved in hormone (i.e., abscisic acid, gibberellins, ethylene, MeJA, salicylicacid, and auxin) responsiveness were identified in *DcbZIPA1* and *DcbZIPA4-10*. Among the 10 genes studied, seven had drought responsive elements, two had heat responsive elements, and only *DcbZIPA10* showed the low-temperature responsive element. The numbers of *cis*-acting elements in *DcbZIPA2*, *3,* and *9* were low, contrary to the large numbers observed in *DcbZIPA5* and *6*. Furthermore, three genes (*DcbZIPA1*, *DcbZIPA5* and *7*) had one ABRE element, while *DcbZIPA6* had four.

Prediction of bZIP protein (group A) folding state

The folding state of the group A bZIP proteins was predicted via the FoldIndex program. The prediction results revealed disordered regions in the selected proteins gathered at the end of the ORF domain (Figure 7). Table 2 shows the specific data for the proteins. The percentages of disordered residues in DcbZIPA1, 2, and 5 were less than 50%, while those of the remaining members of group A bZIP proteins were more than 50%. The charge values of these proteins

ranged from 0.000 to 0.071. The phobic values of the proteins were uniform and ranged from 0.373 to 0.429, with little difference between them.

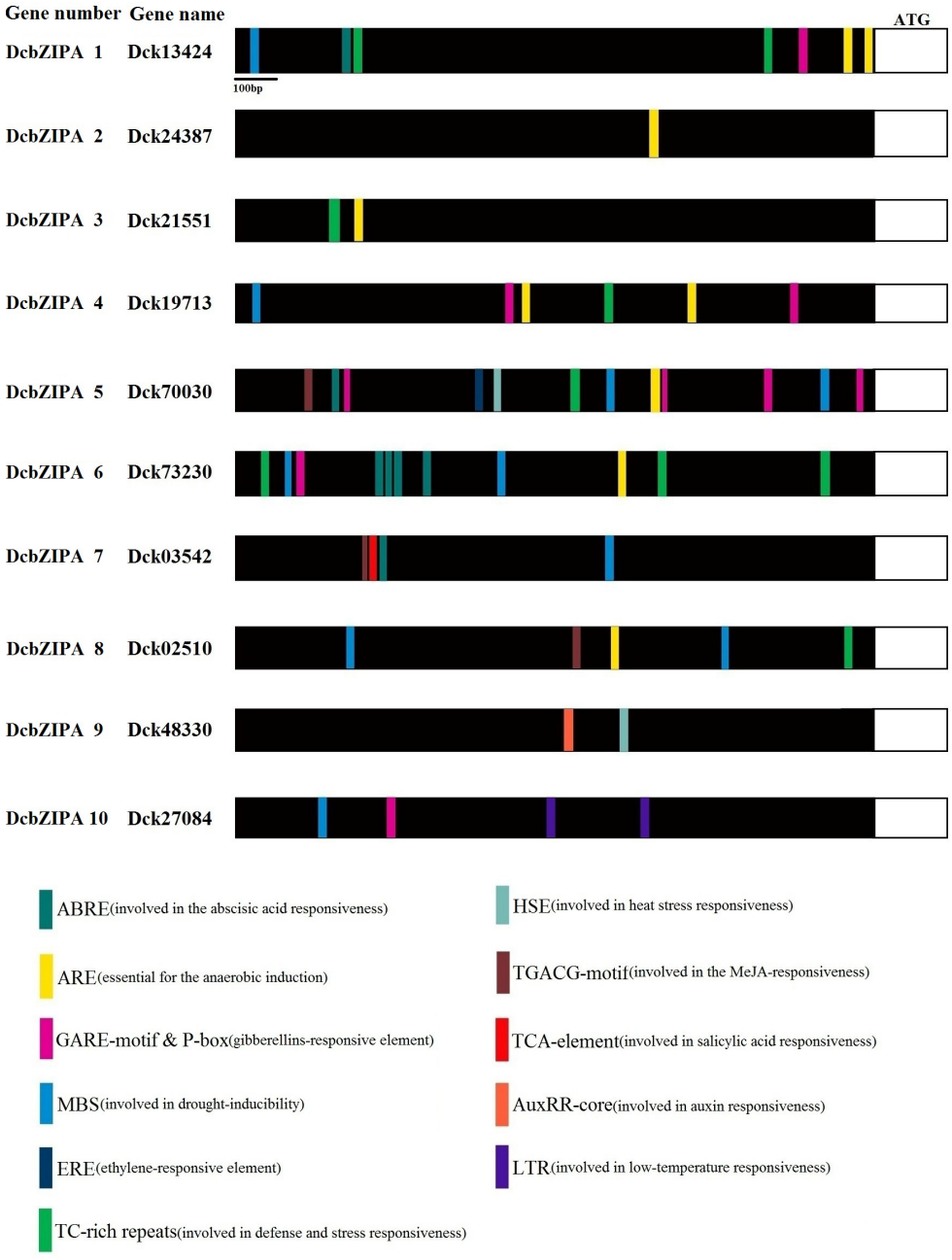


Figure 6. Regulatory regions of carrot bZIP genes. The 5' upstream regions of the bZIP genes are displayed. Elements involved in the different physiological processes are marked in different colors.

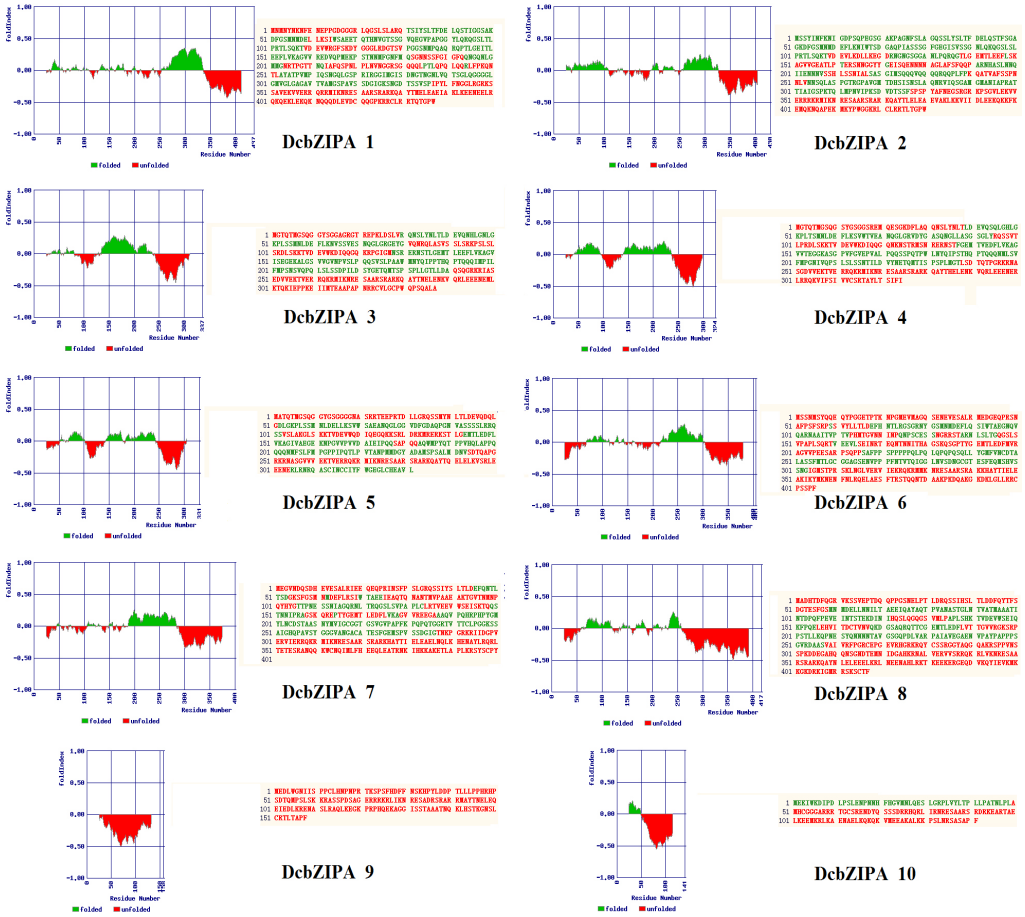


Figure 7. Prediction of the folding state of group A members of bZIP TFs. Ordered regions of the protein are marked in green, and disordered regions are in red. Relative amino acid residue sequences are also marked in green (ordered) and red (disordered).

Table 2. Prediction of the folding states of amino acid sequences of group A members of bZIP factors from *D. carota*.

Factor number	No. of amino acid	No. of disordered regions	Longest disordered region	No. of disordered residues	Percentage of disordered residues (%)	Charge value	Phobic value
DcbZIPA 1	460	9	101	213	46.3	0.021	0.420
DcbZIPA 2	430	8	104	191	44.4	0.030	0.429
DcbZIPA 3	337	3	97	173	51.3	0.015	0.427
DcbZIPA 4	324	3	87	167	51.5	0.015	0.428
DcbZIPA 5	331	4	61	157	47.4	0.000	0.426
DcbZIPA 6	405	7	102	250	61.7	0.000	0.417
DcbZIPA 7	451	9	114	244	54.1	0.003	0.418
DcbZIPA 8	417	5	160	256	61.3	0.007	0.404
DcbZIPA 9	158	1	158	158	100.0	0.063	0.373
DcbZIPA10	141	1	92	92	65.2	0.071	0.386

qRT-PCR analysis of the expression of selected *bZIP* genes under abiotic stress

The expression genes encoding the group A members of bZIP family in response to various abiotic stresses (i.e., low temperature, salt, drought, and heat) were assessed by qRT-PCR. Among the 10 selected genes, the expression of *DcbZIPA 1*, *3*, *4*, *5*, *9*, and *10* was clearly detected, that of *DcbZIPA2*, *6*, and *8* was only partly detected, whereas for *DcbZIPA7* it was undetectable (Figure 8).

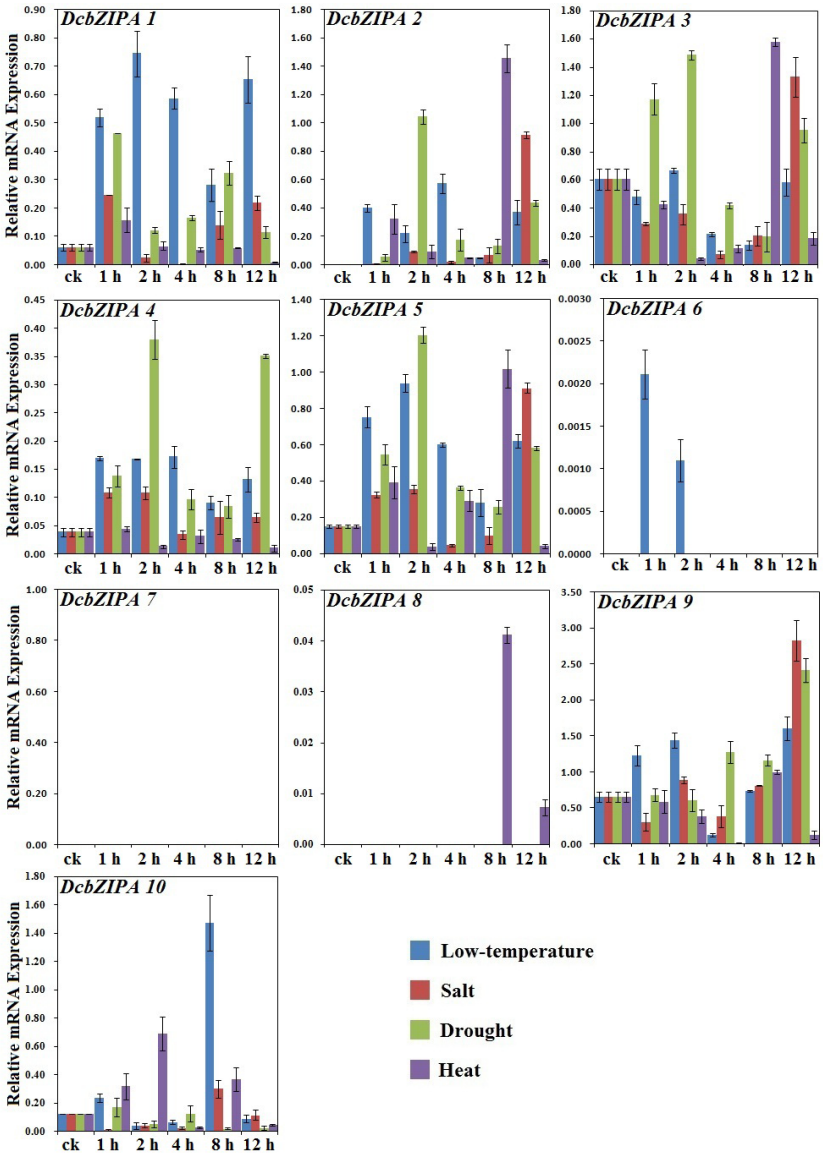


Figure 8. Expression profiling of the 10 bZIP genes of group A under abiotic stress. The mRNA level of Tubulin was defined as 1. Experiments were repeated thrice using independent RNA samples. Error bars were calculated based on three replicates.

Low-temperature treatment

Eight genes (*DcbZIPA1-6*, *9*, and *10*) responded to the cold stress. *DcbZIPA1*, *2*, *4*, and *5* were upregulated after cold treatment. The expression of *DcbZIPA2* was not detected in control check (CK). It was only detected after the cold treatment. The expression of *DcbZIPA6* was detected only 1 and 2 h after the treatment while that of *DcbZIPA7* and *8* were not detected.

Salt treatment

Seven genes (*DcbZIPA1-5*, *9*, and *10*) responded to the salt stress while the expressions of 3 genes (*DcbZIPA6-8*) were not detected. The expression of *DcbZIPA2* was not observed in CK but it peaked 12 h after salt treatment.

Drought treatment

In response to drought treatment, seven genes (*DcbZIPA1-5*, *9*, and *10*) exhibited varied expressions. *DcbZIPA1*, *4*, and *5* were upregulated after drought treatment. The expression of *DcbZIPA2* was not detected in CK but was detected after PEG treatment (200 g/L) and peaked at 2 h after the treatment. The expression of *DcbZIPA6-8* was not observed under the drought stress.

Heat treatment

In response to heat treatment, eight genes (*DcbZIPA1-5*, *7*, *9*, and *10*) exhibited varying expression. The expression of *DcbZIPA8* was detected only after 8 and 24 h of the heat treatment. The expression of *DcbZIPA2* was not detected in CK but was detected after treatment and peaked at 8 h.

DISCUSSION

Group A members of bZIP TFs in carrot

bZIP TFs are important in the response of plants to abiotic stress (Yáñez et al., 2009). In *Arabidopsis*, this TF family has been divided into ten (A-I and S) groups according to their DNA-binding domain (Jakoby et al., 2002). The bZIP family of *D. carota* has also been classified into 10 subfamilies; 10 out of 75 members of this family belong to the group A. Analysis of the motifs of this family supports this classification.

Evolution of bZIP TFs among plant species

During evolution, the plant genome must change to adapt to environmental variations and become more complex. Plant development and growth depends on the proper regulation of several genes (Cheon et al., 2011). bZIP TFs have been reported to play vital roles in these regulation processes (Wray et al., 2003). Comparison of the genome size of algae and land plants, in this study, showed that their genome sizes are drastically different. The number of bZIP factors was also considerably different between algae and land plants. The number of group A bZIP factors in land plants was obviously larger than that in algae, except for *P. abies*, *S. moellendorffii*, and *P. patens*. These results indicate that the group A bZIP TFs are associated with plant evolution and

perform key roles in the process. Compared to those of other plants, the genome size of carrot (Apiaceae, ~480 Mb; Xu et al., 2014) is about 4 times that of the model plant, *A. thaliana* (125 Mb) (*Arabidopsis* Genome Initiative, 2000) and similar to those of rice (*Oryza sativa* ssp. *indica*, 430 Mb;Yu et al., 2002) and grapevine (*Vitis vinifera*,487 Mb;Jaillon et al., 2007). The average number of bZIP family TFs per Mb in *A. thaliana*, rice (ssp. *indica*), grapevine, and carrot was 1.016, 0.219, 0.096, and 0.156, respectively. In plants, bZIP TFs appeared before the origin of algae and have been duplicated many times during plant evolution. bZIP TFs might thus have expanded during plant evolution.

Group A members of bZIP TFs among plants

In plants, ABA is an important factor mediating their response to abiotic stresses (Gorji et al., 2013).The phytohormone ABA takes part in the regulation of many stress-inducing genes, including some bZIP TFs (Mehrotra et al., 2014). A number of group A bZIP TFs have been reported to function in ABA signaling (Fujita et al., 2011). In *Arabidopsis, AtbZIP39*, *36*, and *38* are reported to function in ABA signaling (Choi et al., 2000; Finkelstein and Lynch, 2000; Uno et al., 2000; Lopez-Molina et al., 2001). In *O. sativa,* similar reports on *OsABF1* (Hossain et al., 2010b), *OsABI5* (Zou et al., 2008), and *OsABF2* are available (Hossain et al., 2010a). ABRE is reported to be the main *cis*-acting regulatory element in ABA-responsive gene expression (Hossain et al., 2010a). Analysis of the *cis*-regulatory elements of the 10 genes encoding DcbZIPA in carrot revealed that four (*DcbZIPA1*, *DcbZIPA5-7*) had ABRE elements. These genes might perform important functions in ABA signaling in carrot.

Response of group A members to abiotic stress

Abiotic stress is a serious limiting factor during carrot production. Drought, high salt, heat, and low temperature are the main abiotic stresses severely affecting carrot production (Shinozaki et al., 2003; Chauhan et al., 2013). In this study, 2-month-old carrot plants were subjected to high (38°C) and low temperature (4°C), high salt (0.2 M NaCl), and dehydration (200 g/L PEG) stress. The expression of the 10 selected genes was analyzed by qRT-PCR. Expression of seven of these genes was detected after the treatment. The expression of other genes (*DcbZIPA6-8*) was either not detected or only partly detected. The expression of *DcbZIPA2* was unique because it could not be detected in CK but was detected after the treatment.

The study of protein intrinsic disorder (ID) is an emerging field in basic plant science (Dunker et al., 2002). ID is reported to appear in many highly regulated pathways that can influence the DNA binding (Kragelund et al., 2012). According to a previous study, ID is a feature of whole proteins resulting from a high number of charged amino acid residues and low hydrophobicity (Dunker et al., 2001). To examine the group A members of the bZIP family in carrot further, we predicted the folding state of all members of the subfamily. We found three genes, i.e., *DcbZIPA6-8*, the expressions of which were either not detected or only partially detected and differed from the others in terms of several indices. The percentages of disordered residues in these genes were higher than those of the other genes, except for *DcbZIPA9* and *10*; their charge values were also lower. During analysis of *cis*-elements, these three genes revealed several elements upstream of their ORFs that are associated with abiotic stresses. However, the expression of the three genes was not detected after the treatments. The folding state of the proteins expressed by these genes might probably be associated with this phenomenon.

CONCLUSIONS

The group A of bZIP family TFs is important in the evolution of plants. Carrot genome harbors 75 genes encoding the bZIP family TFs; 10 of these are classified into group A. The modulation of expression of these genes under abiotic stresses (salt, drought, cold, and heat) suggests that this group is widely involved in the response to abiotic stress in carrot. The behavior of four 'special' members as discussed above needs further study to find a plausible explanation. The *D. carota* genome sequence offers a significant resource for analyzing the bZIP TFs. The results presented in this study will facilitate further study on the group A bZIP TFs regarding their functions in response to abiotic stresses.

ACKNOWLEDGMENTS

Research supported by the New Century Excellent Talents in University (#NCET-11-0670), Jiangsu Natural Science Foundation (#BK20130027), and Priority Academic Program Development of Jiangsu Higher Education Institutions Project (PAPD).

REFERENCES

Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796-815.

Bailey TL, Williams N, Misleh C and Li WW (2006). MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic. Acids Res.* 34: W369-W373.

Chauhan H, Khurana N, Agarwal P, Khurana JP, et al. (2013). A seed preferential heat shock transcription factor from wheat provides abiotic stress tolerance and yield enhancement in transgenic *Arabidopsis* under heat stress environment. *PloS One* 8: e79577.

Chen W, Provart NJ, Glazebrook J, Katagiri F, et al. (2002). Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. *Plant Cell* 14: 559-574.

Cheon SA, Jung KW, Chen YL, Heitman J, et al. (2011). Unique evolution of the UPR pathway with a novel bZIP transcription factor, Hxl1, for controlling pathogenicity of *Cryptococcus neoformans*. *PLoS Pathogens* 7: e1002177. doi:10.1371/journal.ppat.1002177.

Choi H, Hong J, Ha J, Kang J, et al. (2000). ABFs, a family of ABA-responsive element binding factors. *J. Biol. Chem.* 275: 1723-1730.

Dunker AK, Brown CJ, Lawson JD, Iakoucheva LM, et al. (2002). Intrinsic disorder and protein function. *Biochemistry* 41: 6573-6582.

Dunker AK, Lawson JD, Brown CJ, Williams RM, et al. (2001). Intrinsically disordered protein. *J. Mol. Graph. Model.* 19: 26-59.

Ellenberger TE, Brandl CJ, Struhl K and Harrison SC (1992). The GCN4 basic region leucine zipper binds DNA as a dimer of uninterrupted α helices: crystal structure of the protein-DNA complex. *Cell* 71: 1223-1237.

Finkelstein RR and Lynch TJ (2000). The *Arabidopsis* abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. *Plant Cell* 12: 599-609.

Fujita Y, Fujita M, Satoh R, Maruyama K, et al. (2005). AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. *Plant Cell* 17: 3470-3488.

Fujita Y, Fujita M, Shinozaki K and Yamaguchi-Shinozaki K (2011). ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J. Plant Res.* 124: 509-525.

Gorji A, Hajianfar R and Rostamforody B (2013). Effects of cold and other abiotic stress on plants. *Intl. J. Agron. Plant Prod.* 4: 3597-3604.

Hossain MA, Cho JI, Han M, Ahn CH, et al. (2010a). The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice. *J. Plant Physiol.* 167: 1512-1520.

Hossain MA, Lee Y, Cho J-I, Ahn C-H, et al. (2010b). The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. *Plant Mol. Biol.* 72: 557-566.

Hsieh TH, Li CW, Su RC, Cheng CP, et al. (2010). A tomato bZIP transcription factor, SlAREB, is involved in water deficit and salt stress response. *Planta* 231: 1459-1473.

Jaillon O, Aury JM, Noel B, Policriti A, et al. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449: 463-467.

Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, et al. (2002). bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci.* 7: 106-111.

Jin Z, Xu W and Liu A (2014). Genomic surveys and expression analysis of bZIP gene family in castor bean (*Ricinus communis* L.). *Planta* 239: 299-312.

Kragelund BB, Jensen MK and Skriver K (2012). Order by disorder in plant signaling. *Trends Plant Sci.* 17: 625-632.

Lescot M, Déhais P, Thijs G, Marchal K, 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 Res.* 30: 325-327.

Liu J, Chen N, Chen F, Cai B, et al. (2014). Genome-wide analysis and expression profile of the bZIP transcription factor gene family in grapevine (*Vitis vinifera*). *BMC Genomics* 15: 281. doi:10.1186/1471-2164-15-281.

Lopez-Molina L, Mongrand S and Chua N-H (2001). A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 98:4782-4787.

Mehrotra R, Bhalothia P, Bansal P, Basantani MK, et al. (2014). Abscisic acid and abiotic stress tolerance-different tiers of regulation. *J. Plant Physiol.* 171: 486-496.

Pourabed E, Ghane Golmohamadi F, Soleymani Monfared P, Razavi SM, et al. (2015). Basic leucine zipper family in barley: genome-wide characterization of members and expression analysis. *Mol. Biotechnol.* 57: 12-26.

Prilusky J, Felder CE, Zeev-Ben-Mordehai T, Rydberg EH, et al. (2005). FoldIndex: a simple tool to predict whether a given protein sequence is intrinsically unfolded. *Bioinformatic*s 21: 3435-3438.

Riechmann JL, Heard J, Martin G, Reuber L, et al. (2000). *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290: 2105-2110.

Shinozaki K, Yamaguchi-Shinozaki K and Seki M (2003). Regulatory network of gene expression in the drought and cold stress responses. *Curr. Opin. Plant Biol.* 6: 410-417.

Tamura K, Peterson D, Peterson N, Stecher G, et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731-2739.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, et al. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876-4882.

Uno Y, Furihata T, Abe H, Yoshida R, et al. (2000). Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. *Proc. Natl. Acad. Sci. USA*97: 11632-11637.

Vinocur B and Altman A (2005). Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr. Opin. Biotechnol.* 16: 123-132.

Wang J, Zhou J, Zhang B, Vanitha J, et al. (2011). Genome-wide expansion and expression divergence of the basic leucine zipper transcription factors in higher plants with an emphasis on sorghum. *J. Integr Plant Biol.* 53: 212-231.

Wray GA, Hahn MW, Abouheif E, Balhoff JP, et al. (2003). The evolution of transcriptional regulation in eukaryotes. *Mol. Biol. Evol.* 20: 1377-1419.

Xu R, Zhang S, Lu L, Cao H, et al. (2013). A genome-wide analysis of the RNA helicase gene family in *Solanum lycopersicum*. *Gene* 513: 128-140.

Xu ZS, Tan HW, Wang F, Hou XL, et al. (2014). CarrotDB: a genomic and transcriptomic database for carrot. Database (Oxford) 2014: bau096.

Yáñez M, Cáceres S, Orellana S, Bastías A, et al. (2009). An abiotic stress-responsive bZIP transcription factor from wild and cultivated tomatoes regulates stress-related genes. *Plant Cell Rep.* 28: 1497-1507.

Yu J, Hu S, Wang J, Wong GK, et al. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* 296: 79-92.

Zou M, Guan Y, Ren H, Zhang F, et al. (2008). A bZIP transcription factor, OsABI5, is involved in rice fertility and stress tolerance. *Plant Mol. Biol.* 66: 675-683.