Genome-wide characterization of NtHD-ZIP IV: different roles in abiotic stress response and glandular Trichome induction

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Abstract

Background: The plant-specific homeodomain-leucine zipper class IV (HD-ZIP IV) gene family has been involved in the regulation of epidermal development.

Results: Fifteen genes coding for HD-ZIP IV proteins were identified (NtHD-ZIP-IV-1 to NtHD-ZIP-IV-15) based on the genome of N. tabacum. Four major domains (HD, ZIP, SAD and START) were present in these proteins. Tissue expression pattern analysis indicated that NtHD-ZIP-IV-1, −2, −3, −10, and −12 may be associated with trichome development; NtHD-ZIP-IV-8 was expressed only in cotyledons; NtHD-ZIP-IV-9 only in the leaf and stem epidermis; NtHD-ZIP-IV-11 only in leaves; and NtHD-ZIP-IV-15 only in the root and stem epidermis. We found that jasmonates may induce the generation of glandular trichomes, and that NtHD-ZIP-IV-1, −2, −5, and −7 were response to MeJA treatment. Dynamic expression under abiotic stress and after application of phytohormones indicated that most NtHD-ZIP IV genes were induced by heat, cold, salt and drought. Furthermore, most of these genes were induced by gibberellic acid, 6-benzylaminopurine, and salicylic acid, but were inhibited by abscisic acid. NtHD-ZIP IV genes were sensitive to heat, but insensitive to osmotic stress.

Conclusion: NtHD-ZIP IV genes are implicated in a complex regulatory gene network controlling epidermal development and abiotic stress responses. The present study provides evidence to elucidate the gene functions of NtHD-ZIP IVs during epidermal development and stress response.

Keywords: Nicotiana tabacum, Expression pattern, MeJA, ABA, GA, SA

Background

Plants have developed a complex regulatory network to adapt to extreme environmental stresses, in which jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) act as pivotal defense signal molecules [1–3]. Plant trichomes are involved in defense responses towards insect predation, UV damage, toxin sequestration, and excess transpiration. Trichomes are grouped into two types, glandular and non-glandular. Glandular trichomes can synthesize and secrete large numbers of specialized metabolites, including terpenes, phenylpropanoids, sucrose esters, and flavonoids [4, 5]. These natural plant compounds not only protect plants against insect pests, but also contribute to the production of industrial chemicals for use in flavors, aromas, and pharmaceuticals [6–8]. In Arabidopsis, it was reported that exogenous application of JA and GA induced the occurrence of non-glandular trichomes [9]. In tomato, exogenous application of JA resulted in a dramatic increase in glandular trichome density [10].

The plant-specific homeodomain-leucine zipper (HD-Zip) gene family plays a crucial role in abiotic stress response and plant development [11–13]. These proteins can be further grouped into 4 subfamilies according to their structural features, conserved domains, and physiological functions [14–17]. The Class IV HD-Zip (hereafter “HD-Zip IV”) gene family...
is associated with lipid transport, epidermal development, cuticle biosynthesis, and anthocyanin deposition [18–20]. HD-ZIP IVs are also implicated in mediating plant defense to osmotic stress [21, 22]. In Arabidopsis, the HD-ZIP IV family comprises 16 genes; the first identified HD-ZIP IV gene (GL2) was implicated in root hair differentiation and trichome development [23, 24]. Two AtHD-ZIP IVs, ML1 and PDF2, have been involved in regulating epidermis and embryo development and determining floral organ identity [25, 26]. One AtHD-ZIP IV gene, AtANL2, controls epidermal cell proliferation, root development, and anthocyanin accumulation [27]. Two closely-related and functionally-redundant AtHD-ZIP IVs, HDG11 and HDG12, regulate branching of the trichome [19]. HD-ZIP IVs has been characterized in various groups other than Arabidopsis, namely maize, rice, soybean, and cucumber [18, 19, 28–30]. It was found that HD-ZIP IVs are primarily expressed in the epidermal tissue. Moreover, Arabidopsis, maize, rice, soybean, and cucumber possess only non-glandular trichomes. The recently published expression profile of HD ZIP IVs in tomatoes suggests that each member may fulfill distinct functions in plant development [31]. Up to now, the specific roles of HD-ZIP IVs in the induction of glandular trichomes has remained enigmatic.

The common tobacco (Nicotiana tabacum), a broadleaf crop with large yields and planting areas, has glandular trichomes on the surface of its leaves. These trichomes produce various terpenoids, alkaloids and defensive proteins, together representing up to 30% dry weight of the leaf [32–34]. Diterpenoids, including labdanoids and cembranoids, are more abundant in Pinus and Nicotiana than in other genera [35, 36]. In addition, cembranoids have neuroprotective, anti-microbial, and anti-tumor properties, and can help in the treatment of human immunodeficiency virus [37–40]. However, knowledge concerning the occurrence of glandular trichomes is fragmentary.

N. tabacum is an excellent model to clarify the gene functions of HD-ZIP IVs in dicotyledons. To elucidate the potential functions of NtHD-ZIP IVs in abiotic stress response and plant development, N. tabacum HD-ZIP IV genes were identified by the computational analysis of N. tabacum genome resource. We analyze gene structure, synteny, phylogeny, tissue expression pattern, and the expression profile under various exogenous hormones and abiotic stresses. In particular, we compare the transcript level of HD-ZIP IVs in the sub-epidermal and epidermal layers. Our study lays the foundation for characterization of HD-ZIP IVs in epidermis-related functions.

Results
Identification and analysis of HD-ZIP IV genes in N. tabacum
Based on the latest genome data of tobacco, 32 HD-ZIP IV genes were identified in N. tabacum genomes. These HD-ZIP IV proteins had conserved domains namely HD, LZ, SAD and START. The positions of the HD-ZIP IV genes showed a scattered distribution pattern in the tobacco chromosome (Table 1). Chromosome 4 had three HD-ZIP IV gene copies, chromosomes 1, 11, 13, and 23 contained two copies, and chromosomes 2, 6, 8, 10, 12, 14, 17, and 22 individually had one copy. Moreover, nine pairs of HD-ZIP IVs were duplicated in tobacco genome (Fig. 1). The molecular weight of HD-ZIP IV proteins ranged from 49.66 to 91.77 kDa, the predicted full-length amino acid sequences ranged from 359 to 828, and the number of exons ranged from 4 to 11.

Evolutionary analysis showed that 74 HD-ZIP IV proteins (32 from tobacco, 13 from tomato, 16 from Arabidopsis, and 13 from rice) were clustered into 5 groups (Fig. 2). Each group contained HD-ZIP IVs from the four species. The result of the phylogenetic analysis was consistent with the taxonomic classification: the HD-ZIP IV genes from the solanaceous plants (tobacco and tomato) had highly homologous sequences; and the HD-ZIP IVs of eudicots (tobacco and Arabidopsis) were more closely clustered than were those of the monocot (rice).

Gene structure analysis can give insights into the origin and evolution of the HD-ZIP IV gene family in tobacco. A phylogenetic tree was constructed to verify the consistency of the exon-intron pattern and the phylogenetic classification. The tobacco HD-ZIP IV genes were divided into 15 categories, which we designate with the prefix “Nt”: NtHD-ZIP-IV-1 to NtHD-ZIP-IV-15 (Fig. 3a). The closely related NtHD-ZIP IV genes had a similar gene structure. Similarly to the situation found in other plants, the features of the NtHD-ZIP IV gene family varied substantially, and the exon number varied from 4 to 11 (Fig. 3b). It is noteworthy that non intron sequence was inserted in the conserved domain. Analysis of conserved motifs found that 20 motifs were present in the 15 NtHD-ZIP IV proteins (Fig. 3c, Additional file 1: Figure S1). There were usually similar motif patterns in closely-related proteins in the phylogenetic tree, thus indicating evolutionary and functional conservation within a clade.

Spatial gene expression of HD-ZIP IVs
The expression pattern of 15 NtHD-ZIP IVs in five tobacco tissues was investigated to assess their role in the epidermal development. There were no trichomes on the cotyledons, but many glandular and non-glandular trichomes occurred on the outer surface of leaves and stems (Fig. 4a). As shown in Fig. 4b, NtHD-ZIP-IV-1 and NtHD-ZIP-IV-2 were specifically expressed in the leaf, root, and stem epidermis. No expression of these genes was detected in cotyledons and in stems without epidermis. This indicates that NtHD-ZIP-IV-1 and NtHD-ZIP-
are trichome-specific genes. The expression of NtHD-ZIP-IV-3, NtHD-ZIP-IV-10, and NtHD-ZIP-IV-12 was weak in stems without epidermis; this suggests that these three genes may relate to trichome development. Five NtHD-ZIP IV genes (NtHD-ZIP-IV-4, −5, −6, −13, and −14), had similar expression patterns: not expressed in cotyledons, but expressed in leaves, roots, stem epidermis, and stems without epidermis. NtHD-ZIP-IV-7 showed a consistent transcript level in five tissues. These genes may have complex roles in tobacco development. Notably, NtHD-ZIP-IV-8 was expressed only in the cotyledons, NtHD-ZIP-IV-9 only in the leaf and stem epidermis, NtHD-ZIP-IV-11 only in the leaf, and NtHD-ZIP-IV-15 only in the root and stem epidermis. These results indicated that each NtHD-ZIP IV gene may be associated with the development of different plant organs.

MeJA application induced the initiation of long-stalk glandular trichomes
Only non-glandular and short-stalked glandular trichomes are present on the surface of tobacco T.I.1112 plants. After MeJA application, long-stalk glandular trichomes were observed, and the density was significantly

| Table 1 HD ZIP IV gene family in N. tabacum |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gene locus      | Location        | Gene length (bp)| Exon Number | CDS (bp) | Length AA | Mw (KDa) | pI |
| Nitab4.5_0003055g0010 | Nt01 | 5126 | 11 | 2481 | 827 | 91.61 | 5.13 |
| Nitab4.5_0002229g0180 | Nt01 | 5450 | 9 | 1482 | 494 | 55.37 | 4.49 |
| Nitab4.5_0001176g0050 | Nt02 | 4565 | 10 | 2103 | 701 | 77.47 | 5.37 |
| Nitab4.5_0001315g0160 | Nt04 | 7912 | 11 | 2184 | 728 | 81.22 | 6.11 |
| Nitab4.5_0001180g0060 | Nt04 | 3246 | 9 | 2235 | 745 | 82.23 | 7.11 |
| Nitab4.5_0002107g0010 | Nt04 | 5492 | 9 | 2274 | 758 | 82.43 | 6.36 |
| Nitab4.5_0004843g0050 | Nt06 | 5529 | 9 | 2442 | 814 | 88.52 | 6.41 |
| Nitab4.5_0002442g0010 | Nt08 | 3724 | 10 | 2154 | 718 | 79.14 | 6.71 |
| Nitab4.5_0002083g0010 | Nt10 | 6035 | 10 | 2007 | 669 | 73.61 | 4.71 |
| Nitab4.5_0000143g0600 | Nt11 | 5690 | 5 | 1513 | 371 | 70.03 | 4.74 |
| Nitab4.5_0002888g0060 | Nt11 | 6556 | 9 | 2289 | 763 | 84.35 | 5.63 |
| Nitab4.5_0002342g0030 | Nt12 | 6223 | 8 | 1692 | 564 | 63.32 | 4.65 |
| Nitab4.5_0000080g0020 | Nt13 | 6576 | 9 | 2460 | 820 | 90.43 | 5.11 |
| Nitab4.5_0001198g0350 | Nt13 | 5884 | 8 | 1077 | 359 | 49.66 | 4.99 |
| Nitab4.5_0000091g0520 | Nt14 | 4639 | 10 | 2154 | 718 | 78.73 | 6.15 |
| Nitab4.5_0002347g0050 | Nt17 | 3697 | 10 | 2121 | 707 | 77.45 | 5.83 |
| Nitab4.5_0000351g0080 | Nt22 | 5405 | 11 | 2016 | 672 | 75.28 | 6.71 |
| Nitab4.5_0000411g0020 | Nt23 | 5533 | 11 | 2484 | 828 | 91.77 | 4.97 |
| Nitab4.5_0002155g0030 | Nt23 | 4841 | 10 | 2055 | 685 | 75.43 | 5.99 |
| Nitab4.5_0004973g0030 | Nt23 | 4848 | 8 | 2277 | 759 | 83.18 | 6.08 |
| Nitab4.5_0003059g0020 | Nt23 | 6627 | 11 | 1971 | 657 | 71.85 | 5.31 |
| Nitab4.5_0011619g0010 | Nt23 | 4670 | 9 | 1989 | 663 | 72.52 | 6.27 |
| Nitab4.5_0009124g0030 | Nt23 | 5337 | 10 | 2121 | 707 | 77.47 | 5.78 |
| Nitab4.5_0004851g0070 | Nt23 | 7277 | 11 | 1912 | 404 | 73.91 | 6.66 |
| Nitab4.5_0003066g0010 | Nt23 | 3284 | 10 | 2154 | 718 | 79.04 | 6.72 |
| Nitab4.5_0000631g0010 | Nt23 | 6547 | 11 | 2046 | 682 | 74.52 | 6.17 |
| Nitab4.5_0008369g0020 | Nt23 | 4116 | 10 | 2052 | 684 | 74.94 | 5.34 |
| Nitab4.5_0007217g0030 | Nt23 | 8378 | 10 | 2058 | 686 | 74.96 | 5.67 |
| Nitab4.5_0002094g0010 | Nt23 | 3562 | 10 | 2052 | 684 | 74.96 | 5.67 |
| Nitab4.5_0002214g0010 | Nt23 | 2466 | 9 | 1077 | 359 | 66.77 | 8.35 |
| Nitab4.5_0005109g0040 | Nt23 | 5319 | 9 | 2466 | 822 | 89.82 | 5.98 |
| Nitab4.5_00019165g0010 | Nt23 | 4817 | 4 | 1843 | 381 | 52.13 | 5.31 |
increased with the increase of the MeJA concentration; this was not the case for the non-glandular and short-stalked glandular trichomes (Fig. 5a, b). These results indicated that the morphogenesis of different trichome types was regulated by different networks.

Detecting the transcript level of 15 NtHD-ZIP-IVs in the epidermis found that most NtHD-ZIP IV genes were not response to MeJA treatment except NtHD-ZIP-IV-1, -2, -5, and -7. NtHD-ZIP-IV-1, -2, and -7 were inhibited under MeJA application, whereas the transcription level of NtHD-ZIP-IV-5 increased after MeJA treatment (Fig. 5c).

Expression pattern of NtHD-ZIP IV genes under abiotic stress and hormone treatments

Plant hormones are key regulators in plant growth and development, as are various environmental stimuli. The NtHD-ZIP IV genes had diverse responses to the various hormone treatments (Fig. 6). Following ABA treatment, NtHD-ZIP-IV-1, 2, -3, -5, -7, -9, -10, and -13 were inhibited, NtHD-ZIP-IV-6, -11, and -12 were slightly induced, whereas NtHD-ZIP-IV-4, -8, -13, and -14 showed no response. GA treatment induced expression of NtHD-ZIP-IV-4, -5, -6, -9, -10, -12, and -13, whereas the remaining NtHD-ZIP IV genes showed no response. Similarly, most NtHD-ZIP IV genes were activated by 6-BA treatment (but not NtHD-ZIP-IV-8, -10, and -15). Following SA treatment, NtHD-ZIP-IV-1, -2, and -3 were inhibited, NtHD-ZIP-IV-6, -8, and -15 did not respond, and the transcript level of the remaining NtHD-ZIP IV genes increased. Compared with other NtHD-ZIP IV genes, NtHD-ZIP-IV-9 and -14 could be upregulated at a constant rate by...
The findings suggested that NtHD-ZIP IVs might be implicated in complex networks, with each member having distinct functions. We found that most NtHD-ZIP IVs could be activated by abiotic stress, to varying degrees. NtHD-ZIP IV genes were more sensitive to heat stress than to salt, drought, and cold stress. Following high salinity treatment, the expression of NtHD-ZIP-IV-1, -2, -3, -9, -11, -12, and -13 was upregulated, whereas the remaining genes showed no clear changes. Under drought stress, most of NtHD-ZIP IV genes were upregulated, except for NtHD-ZIP-IV-8 and -15. Conversely, most NtHD-ZIP IV genes were not obviously activated by cold, except for NtHD-ZIP-IV-6, -7, -10, and -11. Among the four genes, NtHD-ZIP-IV-7 and -11 showed the strongest response to cold stress. Under heat stress, most NtHD-ZIP IV genes were significantly activated, except for NtHD-ZIP-IV-5, -8, -9, -13, and -15; in those that were activated, the expression levels were high. Moreover, the transcription levels of NtHD-ZIP-IV-1, -2, -4, -6, -7, and -11 were high at each sampling occasion after application of heat stress.

**Discussion**

**HD-ZIP IV genes are conserved during evolution**

Plant-specific HD-ZIP IVs have been involved in the regulation of epidermal development, including root hairs, trichomes, cuticles and stomates [17]. Sequence
analysis indicates that HD-ZIP IVs are highly conserved during the evolution or separation processes of various plant species. However, there were different epidermal characteristics in different plant species. Here, we characterized the 15 NtHD-ZIP IV genes in the tobacco genome.

There are mainly three principal evolutionary mechanisms of gene duplications: tandem duplication, segmental duplication, and transposition events [41]. In plants, segmental duplication is the most frequent mechanism due to the property of diploidized polyploid [42]. In the present study, some NtHD-ZIP IV genes were distributed in duplicated blocks, indicating that segmental duplications have contributed to the gene duplication of NtHD-ZIP IVs. The phylogenetic analysis of the HD-ZIP IVs from different species suggested that the HD-ZIP IV duplications within species were first clustered into the same clade, and then grouped together with other species. This finding indicates that HD-ZIP IVs diversified and expanded after the radiation of species.

Some HD-ZIP IVs may play crucial roles in the trichome formation

The regulatory network that controls unicellular trichome formation has been well studied in Arabidopsis [43, 44]. Similarly to the situation found in the tomato and potato, trichomes in tobacco are typically multicellular structures. Prior
to now, there has been a fragmented understanding of the molecular mechanisms underlying multicellular trichome formation. Two paralogous HD-ZIP IVs (HDG11 and HDG12) were involved in the trichome branching. Specifically, hdg11 mutants had more branched trichomes in the leaves, and hdg12 mutants had more normal trichomes than occurred in the wild type. The excessive-branching morphology of the trichome in hdg11 mutants was enhanced by hdg12, revealing a synergistic effect on the trichome development. In our study, the HDG11 and HDG12 homologous genes were HD-ZIP-IV-11 and HD-ZIP-IV-12. NtHD-ZIP-IV-11 was expressed only in the leaf. NtHD-ZIP-IV-12 was strongly expressed in the cotyledons and stem epidermis, whereas weak expression was detected in stems without epidermis. From this, we deduce that NtHD-ZIP-IV-12 may be related to epidermal development. In tomato, an HD-ZIP IV gene (Wo) is involved in the initiation of multicellular trichomes [45, 46]. Suppression of Wo expression by RNA interference decreases the density of type I trichomes. The homologous gene of Wo in Arabidopsis, PDF2, may regulate shoot epidermal cell differentiation [47]. These results indicate that the formation of multicellular trichomes might be regulated by a distinct network unlike the unicellular trichomes. Further, these HD-ZIP IVs may act different roles in the initiation of the unicellular and multicellular trichomes. Here, the predicted proteins coded by the Wo gene showed 73, 75, 78, and 79% amino acid sequence identity to the four Wo homologs in tobacco, which were further clustered as NtHD-ZIP-IV-1 and NtHD-ZIP-IV-2 (Additional file 1: Figure S2). Tissue-preferential expression pattern is an indication of the specific gene function. We found that NtHD-ZIP-IV-1 and NtHD-ZIP-IV-2 were trichome-specific genes. Moreover, NtHD-ZIP-IV-1 and NtHD-ZIP-IV-2 were strongly upregulated
under MeJA application, followed by the initiation of secreting trichomes. Our results indicate that NtHD-ZIP-IV-1 and -2 may act crucial roles in the induction of secreting trichomes, similar to the role of Wo in tomato.

Diverse HD-ZIP IVs were implicated in hormone and abiotic stress response

In the present study, JA could induce the generation of glandular trichomes. Recently, HDG11 in Arabidopsis, a homologous gene of NtHD-ZIP-IV-11, has been reported to control the JA biosynthesis [48]. However, NtHD-ZIP-IV-11 was not responsive to JA in our present study. The transcripts of most NtHD-ZIP-IVs were not respond to MeJA treatment, except for NtHD-ZIP-IV-1, -2, -5, and -7, which may play important roles in the induction of secreting trichomes. Surprisingly, most NtHD-ZIP IVs responded to ABA, GA, 6-BA, and SA. These hormones are key signaling regulators in plant responses to abiotic stresses [49].

This study primarily focused on determining the dynamic transcriptional changes in NtHD-ZIP IV genes under various abiotic stresses. The results indicate that most NtHD-ZIP IVs were sensitive to heat, but
insensitive to cold and osmotic stress; each NtHD-ZIP IV gene had distinct functions; and NtHD-ZIP IVs were implicated in a complex network of responses to abiotic stress. The NtHD-ZIP IV genes might be good target genes for improving abiotic-stress tolerance in crop plants.

Conclusions

Fifteen HD-ZIP IV genes were identified from N. tabacum genome. These NtHD-ZIP-IVs showed differential tissue-specific expression patterns. Jasmonates could induce the generation of glandular trichome, and four NtHD-ZIP-IVs were implicated in glandular trichome induction. Each NtHD-ZIP IV gene had a distinct role in abiotic stress and phytohormone response. The present study provides evidence to elucidate the gene functions of NtHD-ZIP IVs in epidermal development and stress responses.

Methods

Analysis of the HD-ZIP IV gene family in N. tabacum

The sequence of the Solanum lycopersicum and Oryza sativa HD-ZIP IV gene family was obtained at the Solanaceae Genome Network (https://solgenomics.net/) and the Rice Genome Database (http://rice.plantbiology.msu.edu/), respectively. The A. thaliana HD-ZIP IV proteins were obtained using the Arabidopsis Information Resource (http://www.arabidopsis.org/). The Arabidopsis HD-ZIP IV proteins were used as query seeds to identify the N. tabacum HD-ZIP IV proteins (https://solgenomics.net/), via a BlastP search (e < 1×10^-10). These predicted HD-ZIP IV proteins were further confirmed and analyzed using the Pfam tool and SMART web server. The biophysical properties of the HD-ZIP IVs were estimated with the ExPASy ProtParam tool.
To estimate the phylogeny of the **HD-ZIP IV** genes, phylogenetic analysis was carried out using MEGA 7.0 with 1000 replicates, using the HD-ZIP IVs in tobacco, tomato, rice and **Arabidopsis**. Sequences were aligned with ClustalW program. Gene structure was visualized with the Gene Structure Display Server 2.0. The Multiple Expectation Maximization for Motif Elicitation tool was performed to identify the conserved motif. To determine synteny, the synteny blocks containing **HD-ZIP IVs** in the **N. tabacum** genome were scanned using the MCScanX project. The position of each gene in the corresponding chromosome and its synteny relationship were generated using Circos (http://circos.ca/).

### Tissue-specific expression analysis

**N. tabacum** ‘K326’ seedlings were raised in a growth chamber at 22 °C with a 12/12 h light-dark photoperiod. For the tissue-specific expression analysis, cotyledons were sampled from one-week-old seedlings, and the leaf, root, stem epidermis, and the stem with its epidermis removed were sampled from three-week-old seedlings. Total RNA was extracted and removed the residual DNA using DNase I. Quantitative real-time PCR (qRT-PCR) and semi-quantitative RT-PCR were employed to determine the relative mRNA transcriptions of **HD-ZIP IVs** in five tobacco tissues using the gene-specific primers (Additional file 1: Table S1). L25 gene was selected as an internal control. q-PCR reaction was performed on an ABI PRISM 7000 system (Applied Biosystems, USA) with the SYBR Green RT-PCR Kit (Takara, China). Each reaction was run in triplicate, and analysis was performed using the 2^ΔΔCT method [50].

### Induction of long-stalk glandular trichomes by MeJA

**N. tabacum** T.I.1112 without long-stalked glandular trichomes was developed by the Oxford Tobacco Research Station. Seedlings at the four-leaf stage were sprayed with 5.0 mM methyl jasmonate (MeJA). Plants were sprayed until all plants were saturated. Three applications were repeated every one week. Three weeks later, three plants from each treatment were selected, and the youngest terminal leaflet at least 5 cm in length on each plant was sampled for the trichome morphology observation. The area of glandular head, and trichome density on the upper leaf surface were counted using an Axioplan 2 microscope (Zeiss, Oberkochen, Germany). The morphological data were analyzed using one-way ANOVA. Moreover, the leaf epidermis of plants exposed to the 5.0 mM MeJa treatment, and of the control, was removed to analyze the expression level of **HD-ZIP IVs**.

### Abiotic stress and hormone treatments

To test the effects of abiotic stress, K326 tobacco seedlings at the four-leaf stage were stressed by placing the plants under one of four treatments: application of 300 mM NaCl or PEG-6000 (~ 0.5 MPa); solutions; and exposure to low (4 °C) or high (42 °C) temperatures. In preliminary studies, we found that these treatments caused significant stress to the plants. Control plants were cultured normally without treatment.

To test the effects of exogenous hormone treatment, seedlings at the four-leaf stage were sprayed separately with 100 μM abscisic acid (ABA), 100 μM 6-benzylaminopurine (6-BA), 2.0 mM salicylic acid (SA), and 150 μM gibberellic acid (GA). Control seedlings were sprayed with distilled water. True leaves were collected at 0, 1, 3, 6, 12, 24, 48, and 72 h post treatment for q-PCR analysis.

### Abbreviations

- 6-BA: 6-benzylaminopurine; ABA: Abscisic acid; GA: Gibberellin; **HD-ZIP IV**: homeodomain-leucine zipper class IV family; MeJa: Methyl jasmonate; ORF: Open reading frame; q-PCR: Quantitative real-time PCR; RT-PCR: Reverse transcription-polymerase chain reaction; SA: Salicylic acid

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### Authors’ contributions

HYZ and XDM conceived and write the manuscript. HCY and YFY participated to the data analysis. HC projected de-sign and supervision. All authors carefully checked and approved this version of the manuscript.

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### Availability of data and materials

All data generated in this study is available as Additional files.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-019-2023-4.

### Additional file 1: Table S1.

Specific primers of **HD-ZIP IV** in qRT-PCR. Figure S1. Motif analysis of the NtHD-ZIP IV proteins. The 20 motifs were analyzed using the MEME online tool. Different letters represent the abbreviation of various amino acids. The higher the letter height, the stronger the conservation of the amino acid at that position. Figure S2. Sequence alignment of Nt-HD-ZIP IV proteins and Wo from *S. lycopersicum*. Alignments were performed using Megalign program of DNAStar. Identical amino acid residues are shared in black background. Dashed lines represent gaps that were introduced to maximize alignment. (DOCX 568 kb)
Competing interests
The authors declare that they have no competing interests.

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