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Research article

Tobacco transcription repressors NtJAZ: Potential involvement in abiotic stress response and glandular trichome induction

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ABSTRACT

Members of the Jasmonate ZIM domain (JAZ) proteins act as transcriptional repressors in the jasmonate (JA) hormonal response. To characterize the potential roles of JAZ gene family in plant development and abiotic stress response, fifteen *JAZs* were identified based on the genome of *Nicotiana tabacum*. Structural analysis confirmed the presence of single Jas and TIFY motif. Tissue expression pattern analysis indicated that *NtJAZ-2*, *-3*, *-5*, and *-10* were highly expressed in roots and *NtJAZ-11* was expressed only in the cotyledons. The transcript level of *NtJAZ-3*, *-5*, *-9*, and *-10* in the stem epidermis was higher than that in the stem without epidermis. Dynamic expression of *NtJAZs* exposed to abiotic stress and phytohormone indicated that the expression of most *NtJAZs* was activated by salicylic acid, methyl jasmonate, gibberellic acid, cold, salt, and heat stresses. With abscisic acid treatment, *NtJAZ-1*, *-2*, and *-3* were not activated; *NtJAZ-4*, *-5*, and *-6* were up-regulated; and the remaining *NtJAZ* genes were inhibited. With drought stress, the expression of *NtJAZ-1*, *-2*, *-3*, *-4*, *-5*, *-6*, *-7*, and *-8* was up-regulated, whereas the transcript of the remaining genes was inhibited. Moreover, high concentration MeJA (more than 1 mM MeJA) had an effect on secreting trichome induction, but inhabited the plant growth. Nine *NtJAZs* may play important role in secreting trichome induction. These results indicate that the JAZ proteins are convergence points for various phytohormone signal networks, which are involved in abiotic stress responses.

1. Introduction

Jasmonate (JA) is a crucial signaling molecule that regulates various developmental processes and multiple stress responses in plants (Wasternack and Hause, 2013; Howe, 2018; Chini et al., 2016). The JA signaling pathway is a highly complex process, containing the JA biosynthesis and signaling transduction (Wasternack and Song, 2017; Wasternack and Feussner, 2018; Wasternack and Hause, 2018; Chini et al., 2018; Wasternack and Strnad, 2019). Increasing evidence indicates that SCF^{COI1}-JAZ-MYC2 is the core signaling module in the JA signaling pathway (Thines et al., 2007; Chini et al., 2007; Sheard et al., 2010a). The JAZ proteins act as transcriptional repressors of JA-responsive genes, and they can be used for functional characterization of JA regulation in relation to immunity and development.

Most JAZ proteins include a TIF[F/Y]XG domain (also called the ZIM motif) and a Jas domain (also known as CCT_2) (Pauwels and Goossens, 2011). The TIF[F/Y]XG domain located at the N-terminus is a large conserved region (approximately 28 amino acids) (Vanholme et al., 2007). It has been reported that the TIF[F/Y]XG domain functions in the dimer formation, especially in the interaction between the JAZ protein and its co-suppressor (Pauwels et al., 2010; Chini et al., 2009; Chung et al., 2009). The Jas domain located at the C-terminus is characterized by an S-L-X(2)-F-X(2)-K-RX(2)-R core sequence. It has been suggested that the Jas domain might mediate the interaction of JAZ with SCF^{COI1} and with most transcription factors (Sheard et al., 2010b; Zhang et al., 2017a; Fernandez-Calvo et al., 2011). Moreover, some other motifs have been found in the JAZ of angiosperms. According to seven specific motifs, the JAZ proteins are further grouped into five subgroups (Zhang et al., 2017b; Bai et al., 2011). In fact, different JAZ proteins can bind to different transcription factors according to specific motifs (Fernández-Calvo and Solano, 2011). Thus, it is important to understand the origin and structure of these *JAZ* genes.

Increasing evidence suggests that the temporal and tissue specific expression of *JAZ* genes have important roles in the plant

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Table 1
JAZ gene family in *Nicotiana tabacum*.

Gene locus	Location	Gene Length (bp)	Exon number	CDS (bp)	Length AA	Mw (kDa)	pI
Nitab4.5_0000343g0360	Nt02	1247	3	564	188	20.49	9.42
Nitab4.5_0002046g0020	Nt06	3043	7	1182	394	41.70	8.98
Nitab4.5_0004234g0080	Nt08	1013	2	516	172	19.38	10.22
Nitab4.5_0000238g0080	Nt09	3246	7	1050	350	36.64	8.98
Nitab4.5_0001531g0020	Nt15	1579	3	366	122	13.77	9.83
Nitab4.5_0001531g0040	Nt15	2617	3	471	157	18.10	10.13
Nitab4.5_0001531g0050	Nt15	1060	2	303	101	11.65	9.86
Nitab4.5_0000787g0240	Nt19	1315	5	540	180	20.40	9.95
Nitab4.5_0000073g0270	Nt21	1469	2	735	245	27.18	10.00
Nitab4.5_0001044g0010	Nt22	3507	8	1011	337	37.36	5.78
Nitab4.5_0009891g0020	Nitab4.5_0009891	1716	4	747	249	25.85	10.11
Nitab4.5_0006232g0010	Nitab4.5_0006232	2179	5	1008	336	36.62	9.16
Nitab4.5_0004322g0050	Nitab4.5_0004322	1307	4	525	175	19.18	8.22
Nitab4.5_0009991g0010	Nitab4.5_0009991	3781	3	378	126	14.31	10.51
Nitab4.5_0001546g0010	Nitab4.5_0001546	3477	7	1032	344	35.93	8.98
Nitab4.5_0000201g0130	Nitab4.5_0000201	4596	7	636	212	24.29	9.63
Nitab4.5_0015056g0010	Nitab4.5_0015056	3549	7	996	332	36.56	7.39
Nitab4.5_0007295g0020	Nitab4.5_0007295	1326	5	567	189	21.51	9.39
Nitab4.5_0001264g0110	Nitab4.5_0001264	3124	7	1206	402	42.62	8.70
Nitab4.5_0000189g0060	Nitab4.5_0000189	503	2	390	130	14.17	10.14
Nitab4.5_0021182g0010	Nitab4.5_0021182	2421	5	915	305	32.96	9.62
Nitab4.5_0005246g0010	Nitab4.5_0005246	1361	3	399	133	15.25	9.77
Nitab4.5_0011970g0020	Nitab4.5_0011970	1954	6	1110	370	39.20	10.40

developmental processes and various stresses responses. In rice, the JAZ proteins exhibit different biological functions. For example, over-expressing *OsTIFY11b* in rice led to the effective carbohydrate assimilates (Hakata et al., 2012). *OsJAZ8* has been reported to function in JA-induced resistance to bacterial blight (Yamada et al., 2012). Inhibition and over-expression of *OsJAZ9* induced opposite effects in ion homeostasis, and over-expressing *OsJAZ9* in rice enhanced the salt stress tolerance (Wu et al., 2015). In tobacco, increasing evidences indicate that *JAZs* are important regulatory components of JA-inducible alkaloid biosynthesis (Shoji et al., 2008; Oh et al., 2012; Zhang et al., 2012). Recent study indicated that the nicotine biosynthesis was regulated by ZTL targeting a JAZ-MYC2 module in *N. attenuate* (Li et al., 2018). Silencing of *NaJAZi* could increase JA accumulations and up-regulated JA-responsive genes in *N. attenuata* flowers, resulting in stronger budworm resistance (Li et al., 2017).

In addition to the model plant *Arabidopsis*, *Nicotiana tabacum* serves as excellent models to elucidate the exact function of each *JAZ* gene in dicotyledons. Tobacco is a significant economic crop with high yields and planting areas. Various environmental stresses challenge tobacco production and quality. Therefore, the molecular technique is an effective approach to improve the production and quality of tobacco. In the present study, 23 *JAZ* genes were screened based on genomic information of three tobacco species. We analyzed their chromosomal location, gene structure, phylogenetic relationship, tissue expression profile, and expression pattern under various phytohormone treatments and abiotic stresses.

2. Material and methods

2.1. Obtaining of JAZ gene family members

The JAZ proteins of *Arabidopsis thaliana* were obtained from TAIR database (http://www.arabidopsis.org/). The sequence of *Oryza sativa JAZs* and *Solanum lycopersicum JAZs* was achieved from TIGR database (http://www.tigr.org/tdb/ezkl) and the Solanaceae Genome Database (https://solgenomics.net/), respectively. A Hidden Markov Model (HMM), including TIFY and Jas domain, were extracted from the Pfam database (http://pfam.sanger.ac.uk) to the scan the *JAZ* sequences of *N. tabacum* (https://solgenomics.net/) via a BlastP search. The identified *JAZ* sequences were determined using the SMART web server (http://smart.emblheidelberg.del).

2.2. Phylogenetic analysis and motif analysis

To understand the phylogenetic relationship between JAZ proteins, phylogenetic analysis was conducted with a neighbor joining method in MEGA 6.0 using the sequences of *Arabidopsis* (Thines et al., 2007; Chini et al., 2007), rice (Ye et al., 2009), tomato (Chini et al., 2017), *N. attenuata* (Oh et al., 2012) and *N. tabacum.* Multiple sequence alignments of JAZ amino acid sequences were performed using ClustalW. The conserved motif was determined via the MEME tool (http://meme-suite.org/tools/meme).

2.3. Gene structure, chromosome distribution, and synteny analysis

To illustrate the gene structure of *JAZs*, the open reading frame of each *JAZ* was aligned with its genomic sequence using GSDS tool (http://gsds.cbi.pku.cn). The chromosome distribution information of the *NtJAZ* genes was extracted from the Sol Genomics database. The syntenic chromosome regions containing the *JAZ* genes were determined with MCScanX program. The synteny relationship and chromosome distribution of each *JAZ* gene and were displayed with circos (http://circos.ca/).

2.4. Effect of phytohormone on induction of glandular trichomes

Tobacco Introduction 1112 (*N. tabacum*) characterized by non secreting trichomes was developed by the Oxford Tobacco Research Station. Fourth-leaf T.I.1112 plants were separately sprayed with 1.0, 3.0 and 5.0 mM methyl jasmonate (MeJA), or control solution (0.8% ethanol and water) every seven days for a total of three applications. After three weeks, stem epidermis were stained using 2% rhodamine, and photographed using a microscope. The experiment was in triplicates. Experiments were performed in triplicate.

For 5.0 mM MeJA treatment and control, the stem epidermis was removed for qPCR analysis at 6, 12, and 24 h after MeJA treatment.

2.5. Tissue-specific expression analysis

Six tissues of tobacco plants (*N. tabacum* L. 'Kentucky 326'), *viz.*, the cotyledon, leaf, root, seed, stem epidermis, and stem without epidermis, were collected for tissue-specific expression analysis. Stem with its epidermis removed was collected as stem without epidermis. Reverse

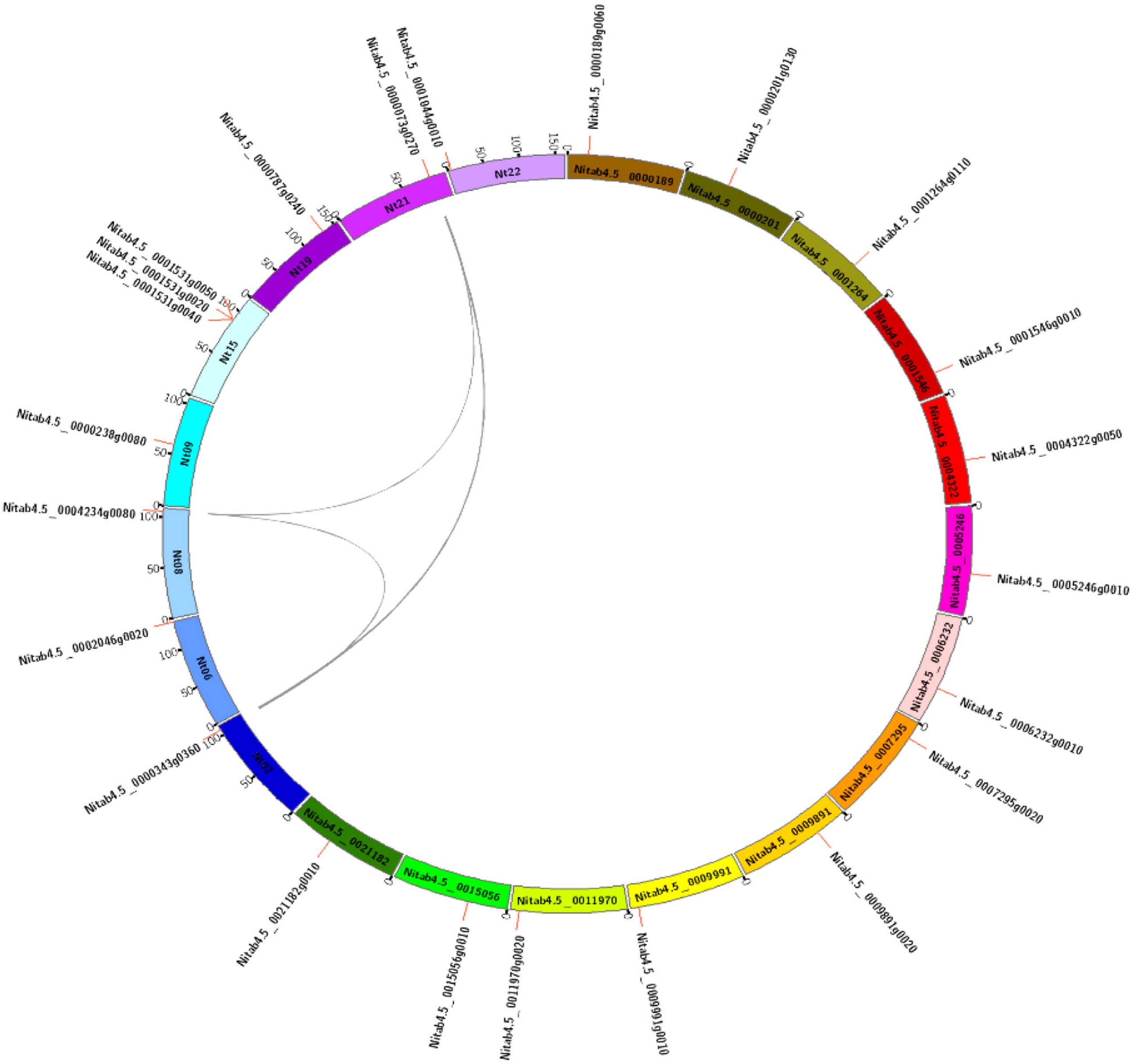

Fig. 1. Chromosomal location of *JAZ* genes in *Nicotiana tabacum* genome. Gray lines represent gene duplications.

transcription-polymerase chain reaction (RT-PCR) was used to determine the tissue expression patterns of *NtJAZs* in tobacco. The transcript of tobacco ribosomal protein gene *L25* was used as a reference gene.

2.6. Abiotic stress and hormone treatments

For abiotic stress treatment, ten four-leaf seedlings were separately stressed with 300 mM NaCl solution, PEG-6000 (−0.5 MPa) solution, and low (4 °C) and high (42 °C) temperatures. For exogenous hormone treatment, ten four-leaf seedlings were separately sprayed with 100 μM MeJA, 150 μM GA, 100 μM abscisic acid (ABA) and 2.0 mM salicylic acid (SA). Controls were cultured without any treatment. Leaves were sampled at 0, 1, 3, 6, 12, 24, 48, and 72 h post treatment for RNA isolation. Experiments were performed in triplicate.

2.7. Expression pattern of the NtJAZ genes

Total RNA was extracted from the samples using the TRIzol method, and then treated with Nase-free DNase (Takara, China). Complementary DNA was synthesized using the reverse transcription kit (Takara, China). The expression pattern of *NtJAZs* in various tobacco tissues was evaluated using the reverse transcription-polymerase chain reaction (RT-PCR). The quantitative real-time PCR (q-PCR) was performed to detect the transcription level of *NtJAZ* under different abiotic stresses and phytohormone treatments. Quantitative assays were performed in triplicates for each sample with the SYBR Green Master Mix (Takara, China) on an ABI PRISM 7000 system. The relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The CT (cycle threshold) value is the mean of the triplicate independent PCRs. The *L25* transcript was used to quantify the relative transcript levels. Gene-specific primers are listed in Table S1.

2.8. Statistical analysis

Data was analyzed by Student's *t*-test using SAS 9.2 software to determine any significant differences between means.

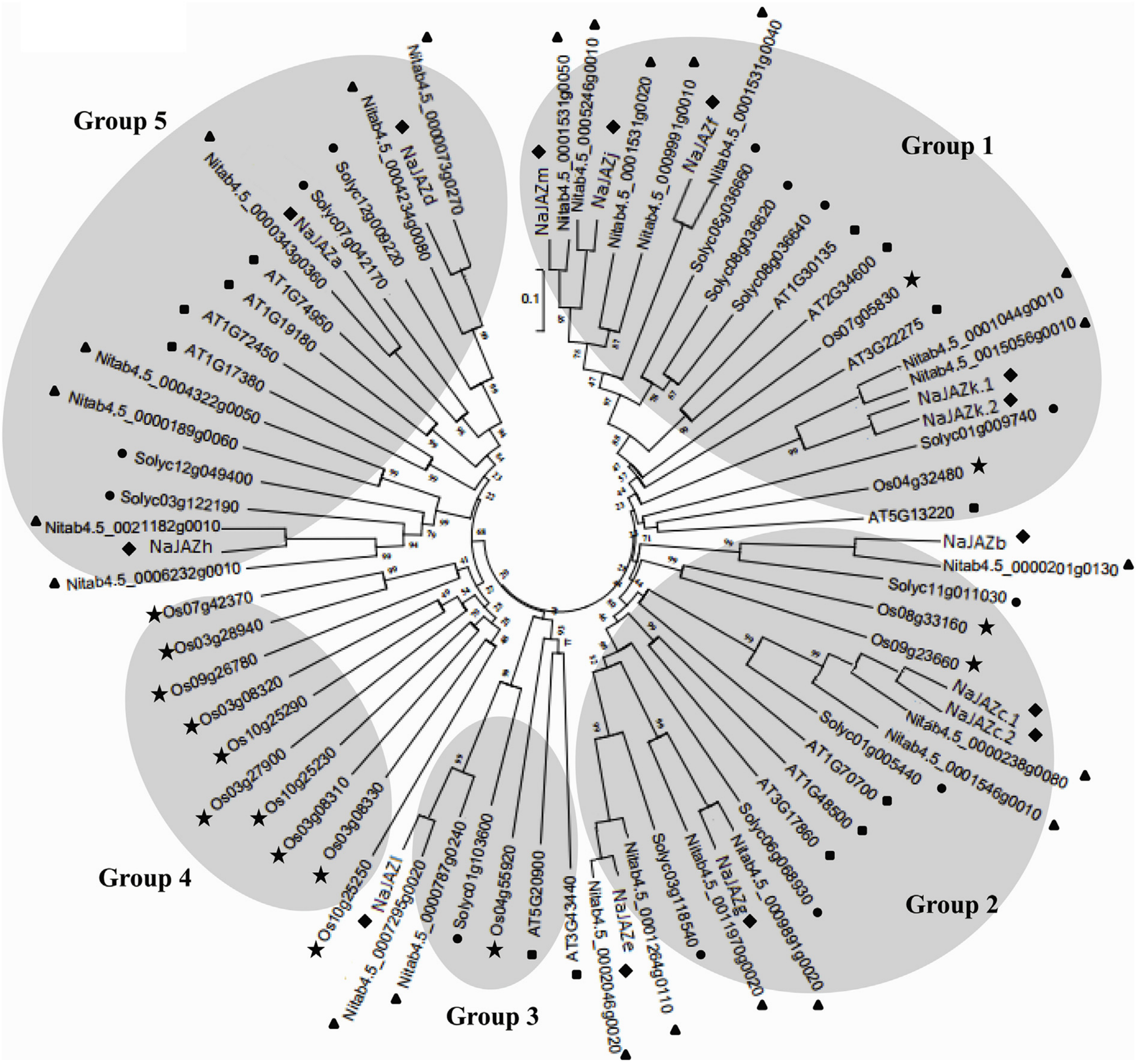

Fig. 2. Phylogenetic analysis of JAZ proteins among tobacco and other species. The full-length amino acid sequences of 13 *Arabidopsis*, 13 tomato, 15 rice, 14 *N. attenuata* and 23 tobacco JAZ proteins were aligned by using ClustalX and the phylogenetic tree was constructed using MEGA 6.0 by the neighbor-joining method with 1000 bootstrap. ▲,*N. tabacum*; ◆, *N.attenuata*; •, *Solanum lycopersicum*; ■, *Arabidopsis thaliana*; ★, *Oryza sativa.*

3. Results

3.1. Determination of the JAZs in Nicotiana tabacum

Based on the tobacco genome data, 23 candidate JAZ family proteins in tobacco were identified. The 23 *JAZs* were unevenly distributed among tobacco chromosomes, of which 10 were mapped to eight chromosomes and 13 could not be localized to a certain chromosome (Table 1, Fig. 1). Further, chromosome 15 had three *NtJAZ* gene copies, whereas other chromosomes contained only one copy. In addition, there were at least three pairs of *NtJAZ* genes that were duplicated.

The length of coding *NtJAZ* sequences ranged from 303 to 1206 bp, resulting in NtJAZs of different amino acid sequences (Table 1). The molecular weight of NtJAZs was also low ranging from 11.65 to 42.62 kDa. The exon number of NtJAZs ranged from two to eight. The isoelectric point of all NtJAZs, except Nitab4.5_0001044g0010, was alkaline, indicating that NtJAZs are rich in alkaline amino acids.

3.2. Phylogenetic analysis of the JAZ gene families

As shown in Fig. 2, 78 JAZ proteins derived from different plants, including 13 from *Arabidopsis*, 13 from tomato, 15 from rice, 14 from *N. attenuata* and 23 from tobacco, were clustered into five groups. Three groups, *viz.*, groups 1, 2, and 3 contained the JAZ proteins from *Arabidopsis*, rice, tomato, and tobacco. Group 5 included 15 JAZ proteins from *Arabidopsis*, tomato, and tobacco. Notably, only 10 OsJAZ proteins were clustered into group 4. In addition, the JAZ proteins from the same species were commonly grouped into the same clade. A comparison of phylogeny of JAZ proteins showed that the tobacco JAZs shared high similarity with those in tomato.

3.3. Structure and phylogenetic analysis of the tobacco JAZ gene family

Comparison of gene structure is a key method for exploring genetic evolution (Yamada et al., 2012). phylogenetic analysis can verify whether the exon–intron distribution profiles correlate with their phylogenetic classification. The tobacco *JAZs* can be divided into 15 categories (*NtJAZ-1* to *NtJAZ-15*) (Fig. 3A). The *JAZs* in the same

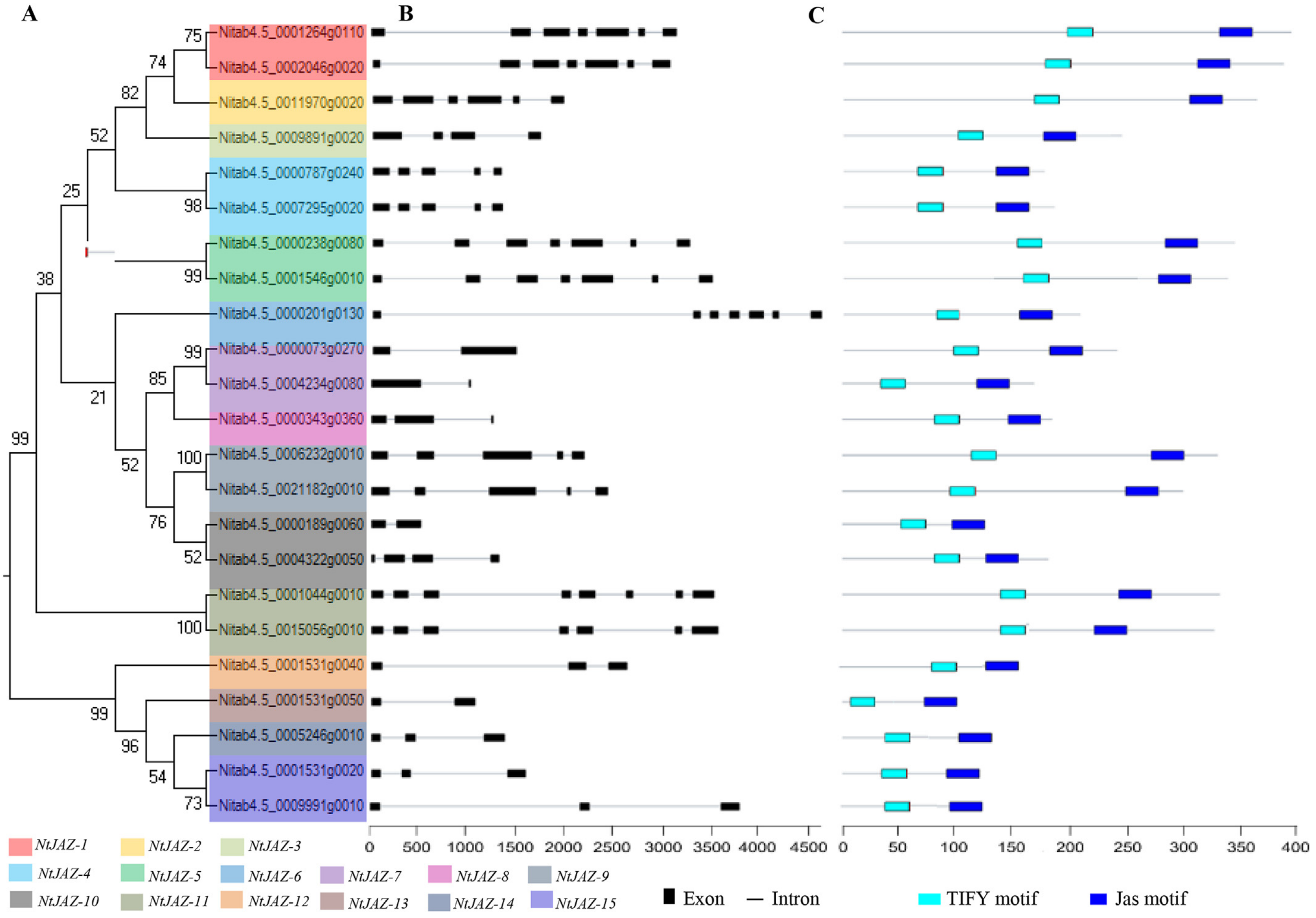

Fig. 3. Phylogenetic tree, exon-intron structure, and motif analysis of *JAZ* genes. (A) Phylogenetic analysis of JAZ proteins in tobacco. (B) Gene structure analysis of *JAZs*. Exons and introns are showed in black rectangles and black lines, respectively. (C) Conserved motifs analysis of JAZ proteins using MEME tools. Conserved motifs are showed in different colored boxes.

branch showed a similar exon–intron distribution profile, except *NtJAZ-7*, *NtJAZ-10*, and *NtJAZ-11* (Fig. 3B). Although all *NtJAZs* contained introns in the genomic sequences, the number of introns varied significantly from one to seven, indicating that *NtJAZs* usually varied in the exon–intron distribution profile and gene length in the tobacco genomic sequences.

As shown in Fig. 1C, single TIFY (motif 1 + 3) and Jas motifs (motif 2) were distributed in all NtJAZ proteins. The finding is consistent with those of previous reports, suggesting that the components of JAZ proteins are conserved (Chini et al., 2016; Wu et al., 2015; Shoji et al., 2008).

3.4. Jasmonic acid had an effect on secreting trichome induction

To address the effect of JA, SA and GA on the induction of glandular trichomes, tobacco T.I.1112 with no long-stalked glandular trichomes were separately sprayed with different phytohormone (0–5 mM/L). The results showed that SA or GA had no significant effect on the induction of glandular trichomes (data not shown). Under high concentration of MeJA treatment from 600 to 900 μM, a few long-stalk glandular trichomes were induced, whereas low concentration MeJA (less than 600 μM MeJA) had no effect on glandular trichome induction (Fig. S2). When treated using high concentration MeJA (more than 1 mM MeJA), long-stalk glandular trichomes were mostly induced, and their density was positively related to the MeJA concentration; this was not the case for the short stalked glandular trichome (Fig. 4A and B). Rhodamine staining patterns showed that application of 5 mM MeJA to tobacco plants induced obvious screting trichomes after treatment, and had an increased glandular trichome density. However, the plant growth and development were obviously inhibited by high concentration MeJA.

Analyzing the expression level of the *NtJAZ* genes in the stem epidermis showed that nine *NtJAZs* (*NtJAZ-1*, *-3*, *-5*, *-7*, *-8*, *-9*, *-10*, *-12* and *-15*) were up-regulated under 5.0 mM MeJA application (Fig. 4C, Table S2). Notably, the transcription level of *NtJAZ-7* increased rapidly and strongly after MeJA treatment. *NtJAZ-5*, *-8* and *-9* were induced gradually, while *NtJAZ-1*, *-3*, *-10*, *-12* and *-15* were up-regulated weakly compared with other four *NtJAZs*.

3.5. Tissue expression pattern of the NtJAZ genes

To assess the potential functions of *NtJAZs* in tobacco development, the expression profiles of all 15 *NtJAZ* genes were investigated in six tissues. As shown in Fig. 5A, no trichome was existed on cotyledons, while lots of trichomes were present on the surface of leaf and stem epidermis. Tissue expression pattern analysis showed that three *NtJAZ* genes, namely, *NtJAZ-4*, *NtJAZ-14*, and *NtJAZ-15*, showed no significant tissue-related differences in expression, which might play a more ubiquitous role in tobacco (Fig. 5B). *NtJAZ-1* was constitutively expressed in tobacco, with high expression in the cotyledon, seed, root, and stem without epidermis. *NtJAZ-2*, *NtJAZ-3*, *NtJAZ-5*, and *NtJAZ-10* were expressed at high levels in the roots compared with those in other tissues. Compared with that in other tissues, the expression of four *NtJAZs*—*NtJAZ-7*, *NtJAZ-8*, *NtJAZ-12*, and *NtJAZ-13*—were weak in the cotyledons, whereas, *NtJAZ-11* was expressed only in the cotyledons.

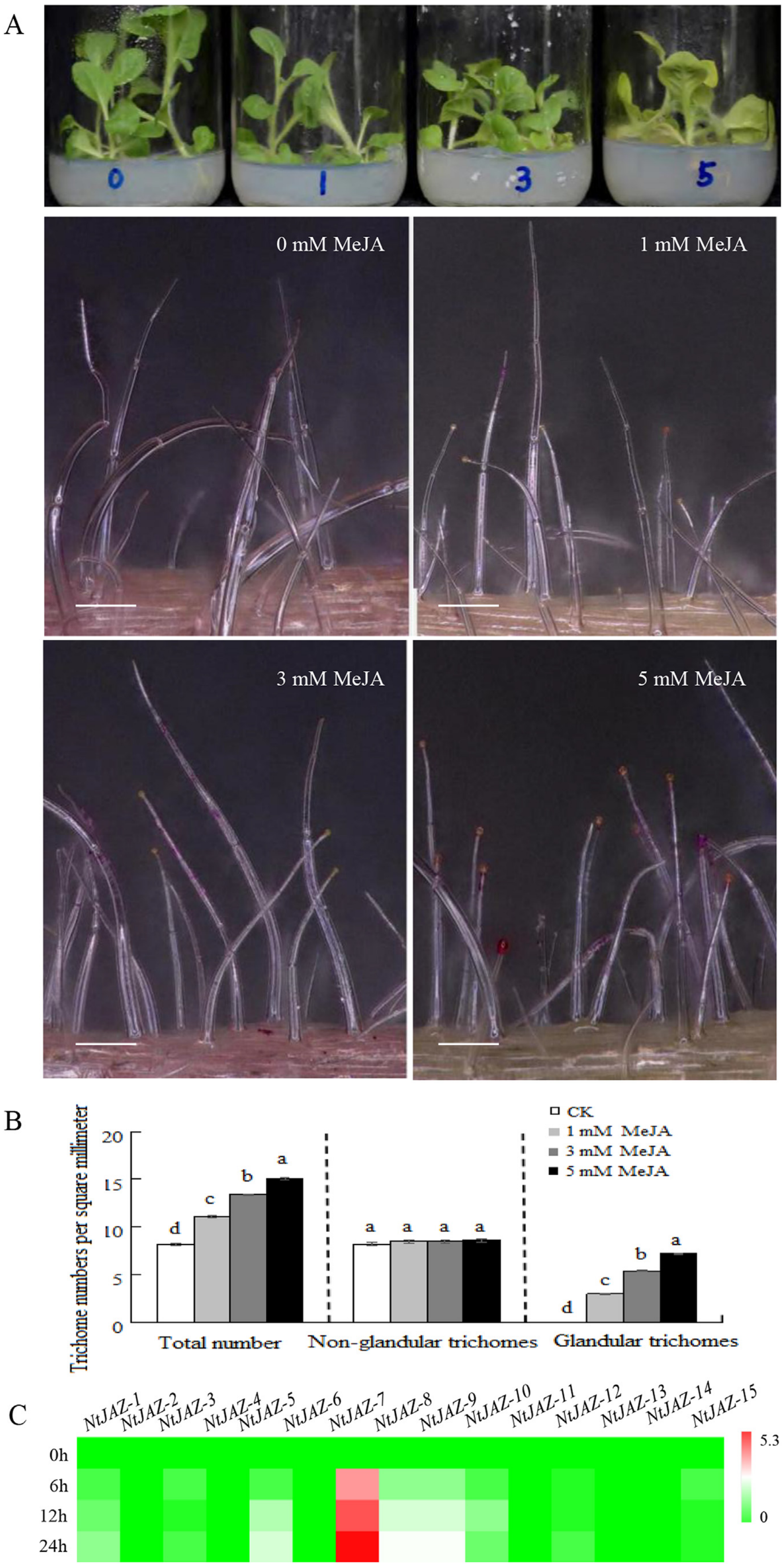

Fig. 4. Effects of 5 mM MeJA treatment on glandular trichome. (A) MeJA had an effect on secreting trichome induction. Scale bar = 100 μm. (B) Effects of MeJA treatment on trichome density. Different letters indicate statistically significant differences at $P \leq 0.05$. (C) Expression pattern of 15 *NtJAZ* genes under 5 mM MeJA treatment. The expression level uner no MeJA treatment was regarded as a standard for each gene.

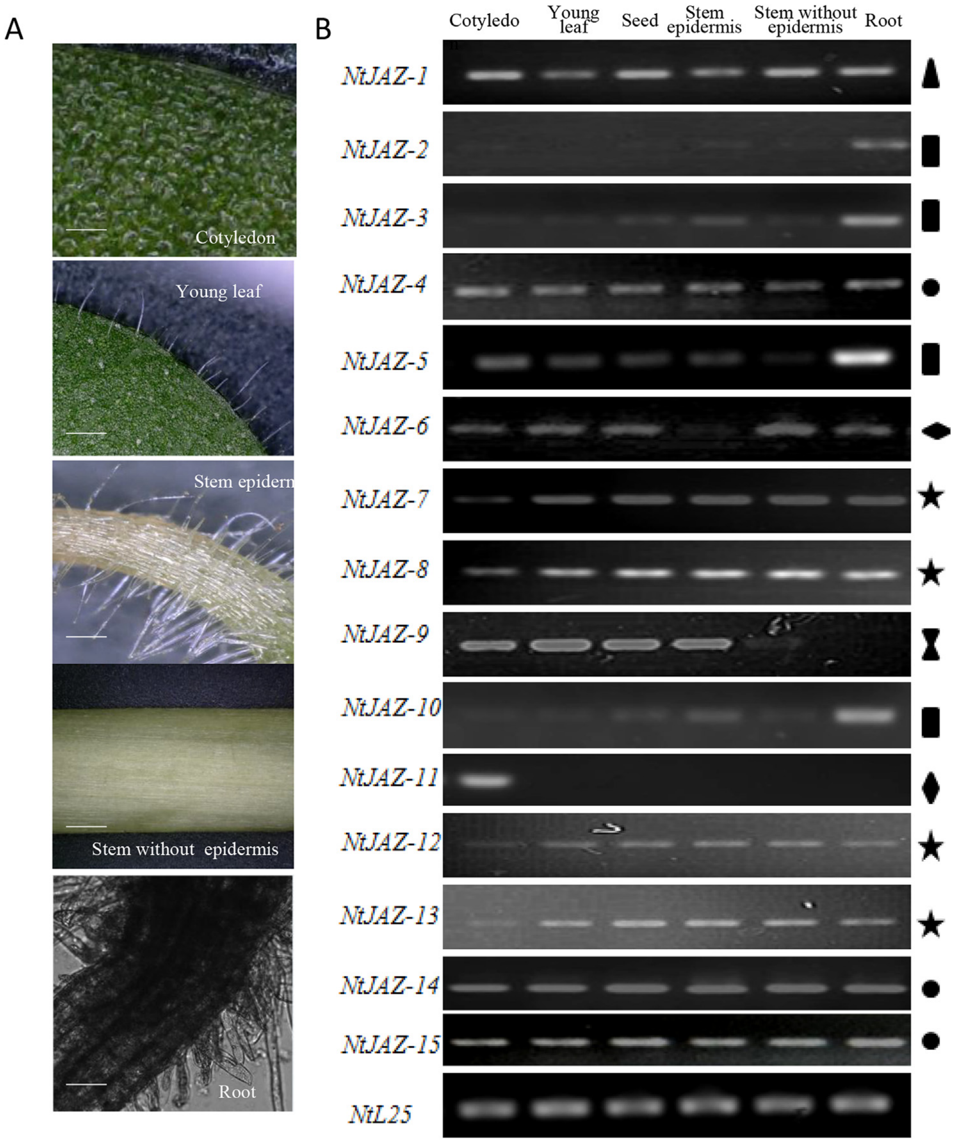

Fig. 5. Expression pattern of 15 *NtJAZ* genes in different tissues. (A) The epidermal phenotype of different tobacco tissues. Scale bar = 20 µm. (B) Gene expression levels in different tissues. Different symbols shows different expression patterns.

NtJAZ-6 was weakly expressed in the stem epidermis. However, the transcript level of *NtJAZ-3*, *NtJAZ-5*, *NtJAZ-9*, and *NtJAZ-10* in the stem epidermis was higher than that in the stem without epidermis, indicating that the four *NtJAZ*s might be specifically associated with epidermis development.

3.6. *Expression pattern of the NtJAZ genes under abiotic stress and phytohormone treatments*

In order to obtain the expression profiling of *NtJAZ* genes under phytohormone and abiotic stresses, qRT-PCR was performed to gain the relative expression pattern of each *NtJAZ* gene. As shown in Fig. 6, the transcripts of most *NtJAZ* genes increased significantly, whereas *NtJAZ-1* was only marginally up-regulated with SA treatment. The treatment with ABA influenced the expression dynamics of *NtJAZ* genes, except *NtJAZ-1*, *-2*, and *-3*, which were not activated. The expression of three *NtJAZ* genes, namely, *NtJAZ-4*, *-5*, and *-6*, was up-regulated by ABA treatment, but the expression of the remaining *NtJAZ* genes were inhibited by ABA treatment. With 100 µM MeJA, the expression pattern of most *NtJAZ* genes in leaves was consistent with those in stem epidermis under 5.0 mM MeJA application. The transcripts of ten *NtJAZs* (*NtJAZ-1*, *-2, -3*, *-5*, *-7*, *-8*, *-9*, *-10*, *-12* and *-15*) were activated by MeJA treatment. With GA treatment, the transcript of *NtJAZ*-5 was inhibited, whereas the expression level of other *NtJAZs* was increased. These results revealed that all the *NtJAZ* genes may be involved in intricate signaling pathways, and each gene has acquired distinct regulatory properties.

With high salinity treatment, the expression of all the *NtJAZ* genes was up-regulated. Moreover, the transcript level of *NtJAZ-1*, *-10*, *-12*, and *-15* was high at 24–72 h post treatment (Fig. 6, Table S3). Under drought stress, the expression of eight *NtJAZ* genes (*NtJAZ-1*, *-2*, *-3*, *-4*, *-5*, *-6*, *-7*, and *-8*) was up-regulated, whereas the transcript level of the remaining genes was inhibited by drought stress. Under cold stress, all *NtJAZ* genes were activated. The expression of five *NtJAZ* genes (*NtJAZ-6*, *-7*, *-8*, *-10*, and *-11*) was up-regulated considerably by cold stress. Compared with that of other stresses, the transcripts of all *NtJAZ* genes were increased under high (42 °C) temperature stress. In addition, the transcript level of five genes (*NtJAZ-2*, *-6*, *-7*, *-8*, and *-9*) was high at each sampling time after treatment.

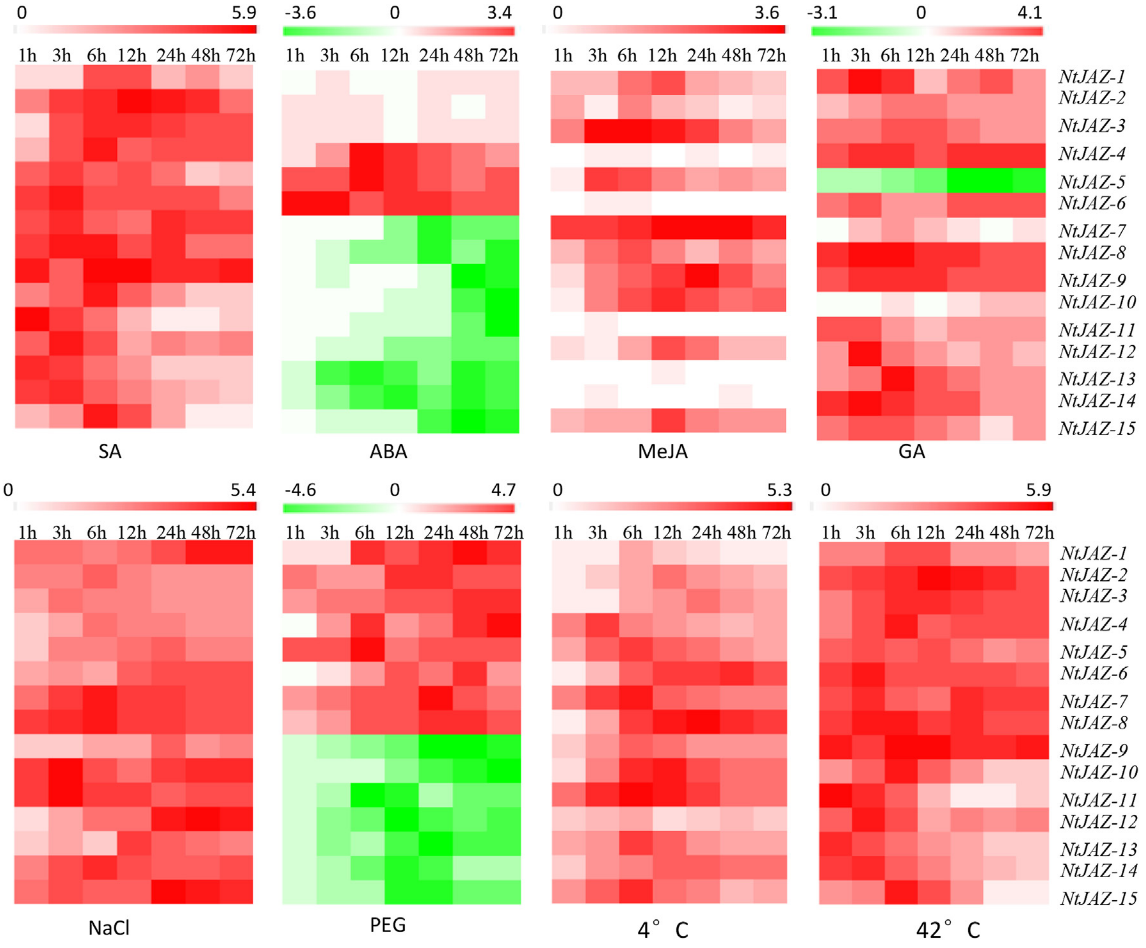

Fig. 6. Expression heat maps of 15 *NtJAZs* under four phytohormones, salt, drought, cold and hight high temperature stress. qRT-PCR strategy was used to analyze the relative expression level of each *NtJAZ*. The transcription of tobacco ribosomal protein gene *L25* was used as an internal reference gene to standardize the RNA samples for each reaction, and the expression at 0 h was set as 0 (data not show in figure).

4. Discussion

As key key regulators of the jasmonate hormonal response, the JAZ gene family has been identified in several plants, such as *T. aestivum* (Wang et al., 2017), *A. thaliana* (Thines et al., 2007; Chini et al., 2007), and *O. sativa* (Ye et al., 2009). The gene structure, gene expression profile, protein characteristics and functions have already been confirmed in these plants. However, studies on JAZ gene family in *N. tabacum* are limited. According to the latest draft of the tobacco genome data, 15 *JAZ* gene families were identified in the present study.

During gene evolution, many duplicated genes functioned in plant growth and development might have evolved as resistance genes to overcome environmental stress (Kim et al., 2006). Here, the phylogenetic analysis revealed that the orthologous genes from *Arabidopsis*, tobacco, tomato, and rice were claded in the same group, indicating the occurrence of *NtJAZ* genes before separation of dicots and monocots.

Tissue transcription profiling can indicate a gene's involvement in functional or differential events. Nicotine exclusively synthesized in tobacco roots, and then translocated to the leaves through the xylem (Li et al., 2018; Shoji et al., 2000). Jasmonate-induced nicotine formation in tobacco was mediated by tobacco *COI1* and *JAZ* genes, and tobacco *NtJAZ1* and *NtJAZ3* repressors (*NtJAZ-2* and *NtJAZ-3* in the study) could regulate the nicotine biosynthesis (Shoji et al., 2008). Tobacco transcription factors *NtMYC2a* and *NtMYC2b* formed nuclear complexes with the *NtJAZ1* repressor (*NtJAZ-2* in the study) and regulated multiple jasmonate-inducible steps in nicotine biosynthesis (Zhang et al., 2012). A detailed analysis revealed that *NtJAZ1*, *NtJAZ3*, *NtJAZ7*, and *NtJAZ10* (*NtJAZ-2*, *NtJAZ-3*, *NtJAZ-5*, and *NtJAZ-10* in the present study) were highly expressed in untreated BY-2 cells and relatively weakly expressed upon MeJA treatment. RNAi-induced silencing of the four *JAZ* genes specifically reduced the nicotine content (Yang et al., 2015). In the present study, the four *JAZ* genes had a similar expression profile, with high transcripts in the roots compared with that in other tissues, and their expression was activated by MeJA treatment. Another study was reported that *NaJAZh* (*NtJAZ-9* in the study) regulated a subset of defense responses against herbivores and spontaneous leaf necrosis in tobacco (Oh et al., 2012). Here, *NtJAZ-9* was highly expressed in the cotyledon, seed, root, and stem epidermis, while not expressed in the root. Recent study indicated that silencing of *NaJAZi* (*NtJAZ-4* in the study) could up-regulated JA-responsive genes in flowers, resulting in stronger budworm resistance (Li et al., 2017).

Trichomes are specialized cellular structures derived from the epidermal cells that act as barriers to protect plants against various environmental damage, insects, and herbivores (Ishida et al., 2008; Yoshida et al., 2009). Trichomes can be divided into glandular and non-glandular trichomes. Non-glandular trichomes do not have heads, while glandular trichomes have heads secreting lots of biochemical compounds, such as diterpenoids, waxes, and sucrose esters. JA regulates various plant developmental processes, *viz.*, tendril coiling, embryo maturation, and tuber and trichome growth (Browse, 2005; Li et al., 2004). Increasing evidence indicates that JA regulates trichome initiation in a COI1-dependent manner (Ramsay and Glover, 2005; Pesch and Hulskamp, 2009; Zhang et al., 2018). Untill now, the specific role of each *JAZ* gene in the induction of glandular trichomes remain enigmatic. In this study, we found that high concentration MeJA specifically induced the initiation of long stalk glandular trichome. Analyzing the expression pattern of *NtJAZs* under 5 mM MeJA application indicated that *NtJAZ-1*, *-3*, *-5*, *-7*, *-8*, *-9*, *-10*, *-12* and *-15* may play important role in secreting trichome induction. Consistent with tissue expression pattern, these genes were expressed highly in stem epidermis. Notably, the transcript level of *NtJAZ-3*, *NtJAZ-5*, *NtJAZ-9*, and *NtJAZ-10* in stem epidermis was higher than that in the stem without epidermis, suggested that they might be specifically associated with epidermis development.

In this study, the expression profile of *NtJAZ*s with plant hormone treatments revealed that the expression of most *NtJAZs* was up-regulated by SA, MeJA and GA treatments, but inhibited by ABA treatment. Furthermore, each gene had a different expression profile. To accomplish various developmental and physiological processes, the JA response is intertwined with other phytohormone and developmental pathways to regulate the expression of a large number of target genes negatively or positively (Ito et al., 2007; Zhao et al., 2018). Increasing evidence indicates that the JAZs play essential roles in mediating the JA-ABA, JA-SA, and JA-GA crosstalk during the development and stress tolerance of plants (Song et al., 2011; Leon-Reyes et al., 2009; Kunkel and Brooks, 2002).

Several researches have demonstrated that JAZs function in various abiotic stress responses. Here, the dynamic expression patterns of *NtJAZs* under abiotic stresses were determined. It was revealed that the transcripts of all the *NtJAZ* genes were up-regulated under salinity, cold, and high temperature stresses. However, the expression of eight *NtJAZs* (*NtJAZ-1*, *-2*, *-3*, *-4*, *-5*, *-6*, *-7*, and *-8*) was up-regulated, whereas that of seven *NtJAZs* (*NtJAZ-9*, *-10*, *-11*, *-12*, *-13*, *-14*, and *-15*) was inhibited by drought stress. These results suggest that *NtJAZs* are part of a complex network involved in various environmental stress responses. They might be good targets to strengthen environmental stress tolerance of plants via genome editing with CRISPR-Cas9. Our study lays a foundation for exploring the molecular mechanism of JA signaling and stress responses mediated by the tobacco *JAZ*s. These findings indicate that each *NtJAZ* has a distinct expression pattern; the JAZs are convergence points for various phytohormone signal networks, which are involved in abiotic stress responses.

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Author contributions

HYZ and WJL conceived and write the manuscript. DXN performed the expression pattern experiment. ZJW and XXY performed the genome-wide characterization. XLY and YFY participated to the data analysis. HC projected design and supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.06.021.

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