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*Full Length Article*

# Molecular Characterization and Transcription Profiling of NAC Genes in *Lilium pumilum* under Abiotic Stresses

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## Abstract

The NAC transcription factors family is one of the plant specific families, which plays a key role in plant biological processes, including the responses to environmental stimuli. However, there are few studies on the *NAC* gene in *Lilium pumilum*. In the present study, a total of 21 NAC genes were used to encode the NAC protein. Most NACs contained a complete *NAC* DNA-binding domain. The RT-PCR indicated that the *LpNAC* genes were expressed in roots, bulbs and leaves of *L. pumilum* under salt, drought, low temperature, and abscisic acid (ABA) treatment. The results indicated an important role of *NAC* in abiotic stress tolerance and ABA-dependent stress signal transduction pathway. Under various abiotic stresses, the *LpNAC9* and *LpNAC17* were highly expressed in roots. The expression of *LpNAC5*, *LpNAC10*, *LpNAC19* and *LpNAC20* in bulbs increased more than twice as much as that in control group. Similarly, the *LpNAC2*, *LpNAC8*, *LpNAC17* and *LpNAC20* in leaves were up-regulated by all stress. In conclusion, the resistance of *L. pumilum* to stress environment maybe partly related to the expression of *NAC* gene. This is first studies to report the *NAC* gene family of *L. pumilum*. It clarifies the functions of *NACs* when plants are subjected to abiotic stress and provides a basis for selecting suitable genes to improve plant stress resistance by molecular techniques. 

**Keywords:** Abiotic stress; Gene expression; *LpNAC*; *Lilium pumilum;* ABA

## Introduction

Cold, drought, high salinity and other environmental factors, are key triggers for altering gene expression patterns and plant growth and metabolism. NAC transcription factors play an important role during plant development, growth and stress response (Le *et al.* 2011). Transcription factors in plants are considered as stress response genes, which encode important metabolic or regulatory proteins, for instance MYB, DERB, bZIP, WRKY, NAC and AP2/ERF (Alves *et al.* 2013; Yao *et al.* 2016). NAC (NAM.ATAF1/2 and CUC1/2) domain proteins, a family of transcription factors, play a role in stress response, growth and development of plant (Jensen *et al.* 2010). NAC transcription factors contain a highly conservative N-terminal DAN domain and differentiated C-terminal domain. The N-terminal region consists of about 150 amino acids, including five subdomains A, B, C, D and E (Ooka *et al.* 2004). So far, many NAC genes have been identified in different plants, including 88 members of pigeon pea (Satheesh *et al.* 2014), 32 members of ramie (Liu *et al.* 2014a), 204 members of Chinese cabbage (Liu *et al.* 2014b), 37 members of pine (Pascual *et al.* 2015) and 86 members of common bean (Wu *et al.* 2016).

It has been documented that NAC proteins participated in all aspects of plant development, including flowering (Yu *et al.* 2014), leaf senescence (Shah *et al.* 2014), seed germination (Han *et al.* 2015), cell wall synthesis (Liu *et al.* 2014a), cell death (Wang *et al.* 2015), xylogenesis (Yang *et al.* 2015) and hormone signaling (Han *et al.* 2015). These are involved in abiotic and biological stress responses.

NAC proteins have their own characteristics in many plants. Transgenic plants overexpressing *CarNAC2* in chickpea have lower germination vigor and later flowering than wild chickpea (Yu *et al.* 2014). The productivity of transgenic plants expressing the barley NAC transcription factor *HvSNAC1* increased significantly (Abdallat *et al.* 2014). In addition, ectopic wall deposition was observed in overexpressed *MusaVND2* or *MusaVND3* transgenic banana plants (Negi *et al.* 2015). NAC family genes in *Gossypium hirsutum* L. may be involved leave aging (Shah *et al.* 2014). *SlNAC1* acts as a NAC protein of stress-response and participates in ABA-dependent signaling pathway (Li *et al.* 2014). In *Arabidopsis*, *ANAC019*, *ANAC055* and *ANAC072* are associated with high salinity, drought and ABA treatment (Tran *et al.* 2004). ATAF1, as a transcriptional regulator, negatively regulates the expression of stress response genes in *Arabidopsis* under drought stress (Lu *et al.* 2007). The overexpression of *CarNAC4* improves the expression of stress response

genes, such as COR15A, ERD10, RD29A, KIN1, COR47 and DREB2A, suggesting that *CarNAC4* acts as a transcription factor induced by the regulation of salt and drought stress response (Yu *et al*. 2016). Of 57 NAC genes in the two elites rice, 23 are regulated by NaCl (García-Morales *et al.* 2014).

*L. pumilum* originated in the cold area of northern China. It has a high ornamental value because of its beautiful flowers and bright red color. *L. pumilum* has strong tolerance to abiotic stresses such as cold, drought, salt and alkalinity and has edible and medicinal value (Zhang *et al.* 2016). These traits make *L. pumilum* is a good source for studying the mechanism of stress tolerance. Cold stress has a negative impact on growth, and cold tolerance is a complex trait. Its expression depends on the interaction of different physiological, molecular and morphological characteristics. The study of plant tolerance mechanism to low temperature stress can provide information for improving plant cold tolerance through genetic modification. Therefore, the cold stress produced by 4°C treatment creates a good model of natural low temperature. In this study, four transcripomes from *L. pumilum* bulb treated at 4°C were constructed by high-throughput sequencing. Real-time PCR polymerase chain reaction was used to further study NAC response patterns to low temperature, drought, salinity and ABA expression. Furthermore, 21 *LpNAC* genes with complete NAM domain and complete open reading frames were explored.

## Materials and Methods

### Identification of NAC family genes in *L. pumilum* transcripts

The experimental materials were the *L. pumilum* bulbs aged 2–3 year (the circumference was 4–6 cm). *L. pumilum* was planted in the nursery of Northeast Forestry University. In mid-October 2014, single-headed *L. pumilum* bulbs without pests or diseases were harvested, treated with carbendazim WP for about 30 min, then washed and dried. Bulbs were stored in wet perlite which has been sterilized by high temperature and preserved in the 4°C refrigerator. In previous studies, we found that it took about 90 days for *L. pumilum* bulbs to complete dormancy release from 4°C (low temperature) treatment. The superficial structure of bulb organs showed that 0, 30, 60, and 90 days were the key period for releasing dormancy of *L. pumilum* bulbs. Therefore, four transcriptomes from *L. pumilum* bulbs were constructed by high-throughput sequencing using RNA-seq technology after 0, 30, 60 and 90 days of treatment at 4°C. The keywords "NAC", "no apical meristem" or "NAM" were used to query these single gene annotations for identifying NAC genes. The ORF of all NAC genes was analyzed by NCBI ORF finder (https://www.ncbi.nlm.nih.gov/orffinder//) and the NAC domain was examined by NCBI CDD searcher (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Baranwal and Khurana, 2016; Bhattacharjee *et al.* 2017). NAC genes with these domains in the *Arabidopsis* genome were downloaded from the TAIR databases (http://www.arabidopsis.org/) (Wei *et al.* 2016).

### Bioinformatics analysis of NAC family

NAC proteins and *Arabidopsis* were used for studying phylogenetic tree. These sequences were aligned through BioEdit software with Clustal W. A bootstrapped Neighbor-Joining (NJ) tree was prepared in MEGA 5.0 software. LpNAC MTFs were predicated using the TMHMM server 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). In order to evaluate the evolutionary relationship among identified *L. pumilum* NACs (Clement *et al.* 2008; Hall, 2013), the isoelectronic point prediction for each *LpNAC* protein and theoretical molecular weight were calculated using the ExPASy compute tool (http://expasy.org/tools/protparam.html/) (Shang *et al.* 2013). The protein secondary structure of each LpNAC was predicted byhttp://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html/ (Combet *et al.* 2000; Lindemose *et al.* 2014). The transmembrane domain was predicted by the TMHMM server 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) (Wang *et al.* 2013; Shang *et al.* 2016). We used the online Plant-PLoc server 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) for subcellular localization prediction (Bailey and Elkan, 1994).

### Plant growth and stress treatment

Shoot regeneration was collected directly from bulb scales on Murashige and Skoog (MS) medium (MS + 6 BA 1.5 mg/L + NAA 0.5 mg/L). The explants were cultured on bud induction medium for 30 days. The multiple shoots or bulblets (3–5 cm in height) induced clumps were divided into single bulblets or shoots and transferred to sub-culture medium (MS + 6BA 1.0 mg/L + NAA 0.2 mg/L) for shoot and bulblet proliferation. When seedlings reached 5–6 cm, they were placed in semi-intensive MS (1/2 MS + IBA 0.5 mg/L) medium and rooted in the greenhouse (16:8 h light-dark;75–80% relative humidity; 23°C). The rooted shoots were placed in fresh water for 7 days. Then, the samples were treated with 200 m*M* NaCl (salt stress), 20% PEG6000 (drought stress),150 $\mu M$ ABA and low temperature (2°C) for 1, 3, 6, 12, 24 and 48 h respectively. Fresh-water control was also carried out. After processing, the bulbs, leaves and roots of each sample seedling were collected at a specified time after treatment, collected and frozen immediately in liquid nitrogen and stored at -80°C until they were needed.

### RNA isolation and first strand DNA synthesis

Total RNA of each sample was extracted by the CTAB

method and a few modifications were made. RNA was treated with RQ1 RNase-Free DNase (ReverTra Ace qPCR RT Master Mix with gDNA Remover. TOYOBO, Japan) before the synthesis of cDNA. Follow the instruction manual, make sure there is no genomic DNA contamination and the first-strand cDNA was synthesized using the Rever Tra Ace qPCR RT Kit (TOYOBO, Japan).

**Quantitative real-time PCR**

Real-time PCR was performed in the Poche Light Cycler96 and the genes was used as internal references. Three biological replicates were used for each sample and three technical replicates were used for each biological replica. All primers were designed with Primer3 web version 4.0.0 (http://primer3.ut.ee/), as shown in Table 1. The reaction mixture (20 $\mu$L) contained 10 $\mu$L of SYBR Green Real-time PCR master Mix, each primer 0.5 $\mu$L and 1 $\mu$L of cDNA template. The amplification was completed and the cycle parameters were as follows: initial denaturation at 94°C for 30 s; denaturation at 94°C for 5 s; annealing at 58°C for 15 s; and extension at 72°C for 10 s for 45 cycling parameters. In order to determine the specificity of the reaction, the melting curve analysis of the product was analyzed immediately after the last PCR cycle, using 97°C for 10 s, 55°C for 60 s and 97°C for 1 s. The relative expression level was calculated as the transcription level under stress treatment divided by the transcription level without treatment (Hussain *et al.* 2017).

## Results

**Identification and sequence analysis of NAC genes in *L. pumilum***

Four transcriptomes were constructed from *L. pumilum* bulbs treated at 4°C. Through single gene annotation, 11 genes were annotated as "NAC transcription factor" and 38 genes were annotated "no apical meristem". NAC genes with overlapping sequences were deleted. Finally, out of 49 NAC genes, 21 NAC genes containing the full-length ORF were identified, named as *LpNAC1* to *LpNAC21*. Except for *LpNAC18*, all the *LpNACs* genes were annotated as "NAM superfamily" in the NCBI CDD searcher. *LpNAC18* was annotated as "NAC superfamily". These *LpNACs* encoded proteins ranging from 51 to 890 amino acids, predicted sizes from 5.97 to 10KD, pI *values* from 4.26 to 9.61 amino acid and hydrophobicity from -8.24 to -1.553. *LpNACs* gene wasa hydrophobic protein. Exceptfor *LpNAC1* and LpNAC15, all *LpNACs* genes were classified as unstable proteins. The protein structure of *LpNACs* was mainly composed of irregular curls, α-helix and β-angle, which was dispersed in the protein. Among 21 *LpNAC* proteins, only one TM was found in *LpNAC19* and *LpNAC20* and transmembrane sequences were found in C-terminal regions, 626–648 and 632–654.

**Phylogenetic analysis of LpNAC protein**

All *LpNACs* had a high conservative N-terminal DNA-binding domain, which is a typical NAC domain with five common subdomains (A–E) (Fig. 1). In addition, *LpNACs* were localized in the nucleus. All *LpNAC* proteins were divided into seven groups together with the *Arabidopsis* NAC proteins (designated as NAC-a to NAC-g) (Fig. 2).

**Expression profiles of *LpNACs* genes under various abiotic stresses**

*LpNAC1* showed significant upward regulation at 12 h in roots (Fig. 3). In bulbs and leaves, *LpNAC8* showed up-regulated at all point times of salt stress treatment and the expression level in roots was gradually increased but down-regulated at 48 h. *LpNAC10* was obviously up-regulated at all point times of stress in bulbs and roots. In the three tissues, *LpNAC20* showed a significant regulatory effect at all time points after salt stress treatment, different from *LpNAC20*, the *LpNAC3* and *LpNAC16* showed a significant down-regulation effect. At 48 h, *LpNAC21* in the bulbs was significantly up-regulated. Under stress, the total gene expression level of NAC-c and NAC-d groups was up-regulated, while that of NAC-b and NAC-f groups was down-regulated (Fig. 4A). After 6 h of salt treatment, the overall expression levels of NAC-e and NAC-g groups decreased (Fig. 4B). The overall expression level of NAC-a group was up-regulated except for 6 h (Fig. 4C).

Under cold treatment (Fig. 5), in three organs, the expression of *LpNAC1* was up-regulated at 12–48 h, the expression of *LpNAC10* and *LpNAC17* was significantly induced at all treatment time points, and the expression of *LpNAC20* was up-regulated at 6–48 h. The expression of *LpNAC5* was significantly up-regulated at 12 h in bulbs. The expression of *LpNAC16* was inhibited in bulbs and roots and LpNAC13 was down-regulated in leaves and bulbs during the whole treatment. The expression levels of NAC-a, NAC-c, NAC-d, NAC-f and NAC-g were up-regulated (Fig. 6A and 6B).

Under drought stress (Fig. 7), the expression levels of *LpNAC1*, *LpNAC8*, *LpNAC13*, *LpNAC16* and *LpNAC20* in roots and leaves increased at all stress times. *LpNAC5* in the bulbs was up-regulated. *LpNAC10* showed a regulatory effect at all treatment time points of three organs, while *LpNAC11* showed an up-regulated effect after 3 h of stress treatment. *LpNAC17* expression in roots was up-regulated, reaching the highest level at 24 h. The total expression levels of NAC-b, NAC-c and NAC-d in bulbs were up-regulated at all stress time points (Fig. 8B). The overall expression levels of NAC-a, NAC-c,

**Table 1:** Primer sequences of LpNAC gene designed by using Primer3 web version 4.0.0 (http://primer3.ut.ee/)

| Gene name | Nucleotide sequence | Gene name | Nucleotide sequence |
|---|---|---|---|
| LpNAC1 F | GCTTCAGGTAGGTATCCGCT | LpNAC11 R | CACTTTGTCTTCGTCCCCAC |
| LpNAC1 R | TGAACCCTCCAGCTCAAGAG | LpNAC12 F | AGGGCCACAATAGCAGGTTA |
| LpNAC2 F | ACTTCAAGTATCCCCGTCGG | LpNAC12 R | TGCACTCGTTCGGATCCATA |
| LpNAC2 R | TCCTCCCATTCAACCCCTTC | LpNAC13 F | CGGCAAGTCATGGATTCTGG |
| LpNAC3 F | GCAGATGGGTGATGCATGAG | LpNAC13 R | CTCTCTCTGAAACCACCCGT |
| LpNAC3 R | GGTACATGTGAGGTGGGGAT | LpNAC14 F | GCTTCCGACAATGGCTACTG |
| LpNAC4 F | GTTTCTGCTGCACCTTTGGA | LpNAC14 R | CTCCTTCCACTACTGCTGCT |
| LpNAC4 R | GATGGAATCGGAAACCAGGC | LpNAC15 F | TCACCAGTCTCCCACATCAG |
| LpNAC5 F | GCTTCCGATTTCATCCCACC | LpNAC15 R | TCACGAAGACTCACACCCTC |
| LpNAC5 R | CTTCCCAGTCACCTTCCAGT | LpNAC16 F | ACTTCTTCTGCCAGCGAGAC |
| LpNAC6 F | CGAGCCAAACATGATGCAGT | LpNAC16 R | TTGGGGGCTCTACCCATGTA |
| LpNAC6 R | ATCCCTCCCAGCCTGATAGA | LpNAC17 F | GAAGGCGCTGGTGTTCTATG |
| LpNAC7 F | AACGGCAGTGGTGGATTCTA | LpNAC17 R | AACCAAACTATGCCGCGTAC |
| LpNAC7 R | AGTCTTTCTCATGCCGACGA | LpNAC18 F | TATGTGAGGCAGCTTCCACA |
| LpNAC8 F | TTTCGATTCCATCCCACCGA | LpNAC18 R | AAAACTGAAGGGTGCTGAGC |
| LpNAC8 R | TTCCAGTCGCCTTCCAGTAG | LpNAC19 F | CGGAGAGCAGGGTCAATTTG |
| LpNAC9 F | AGGTTTCATCCCACTGACGA | LpNAC19 R | AAGGCCTGTGAGTTCTCCTG |
| LpNAC9 R | GTTGCTCGGTTGGTACGAAA | LpNAC20 F | CCCTAGTTCACCTTGCCAGA |
| LpNAC10 F | TGGTGTTCTACATGGGGAGG | LpNAC20 R | CGAGGTCCAGATCCTTTGGT |
| LpNAC10 R | ACGTTGATGGCATTGTCTCG | LpNAC21 F | CAATGTTCGGATCCCAGCAG |
| LpNAC11 F | ACCGAGCAGTGGTTCTTCTT | LpNAC21 R | AGAGGGCCAGACTTGTCTTC |

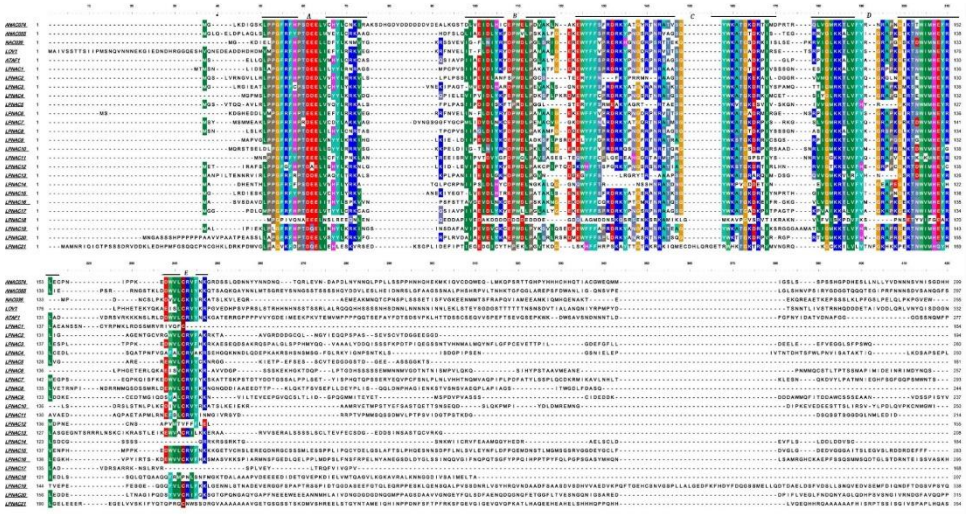

**Fig. 1:** Identification of conserved NAC subdomains. Multiple sequence alignments of 21 *LpNACs* and five representative *Arabidopsis* NACs were performed with Clustal W using BioEdit software. The consensus NAC subdomain (A-E) are indicated by lines above the sequence

NAC-d and NAC-g in leaves were up-regulated by all stress treatment (Fig. 8C).

### Effect of abscisic acid on the expression of the *LpNACs* genes in *L. pumilum*

In roots (Fig. 9A), genes increased rapidly at 1 h of ABA stress, such as *LpNAC1*, *LpNAC2*, *LpNAC3*, *LpNAC4*, *LpNAC5*, *LpNAC6* and *LpNAC20* and decreased after 3–6 h. In bulbs (Fig. 9B), except for *LpNAC6* and *LpNAC18*, the other *LpNAC* genes were obviously induced within 48 h. In roots and leaves (Fig. 9C), the expressions of *LpNAC16* and *LpNAC17* were up-regulated. The overall expression levels of NAC-c were up-regulated before 3 h of stress, but after 12 h of stress, the expression level of NAC-f was up-regulated (Fig. 10A). The total expression level of NAC-a was up-regulated after 12 h of stress, while the expression level of NAC-b and NAC-c were up-regulated at 48 h of stress (Fig. 10B). The overall expression level decreased gradually in all groups (Fig. 10C).

## Discussion

In order to survive under various environment conditions, plants adopt different strategies to cope with adverse conditions. Previous research has indicated that NAC gene plays an important role in abiotic stress response. In present study, several members of the *LpNAC* gene were up-regulated by various abiotic stresses (cold, salt and drought), suggesting that they might be crucial

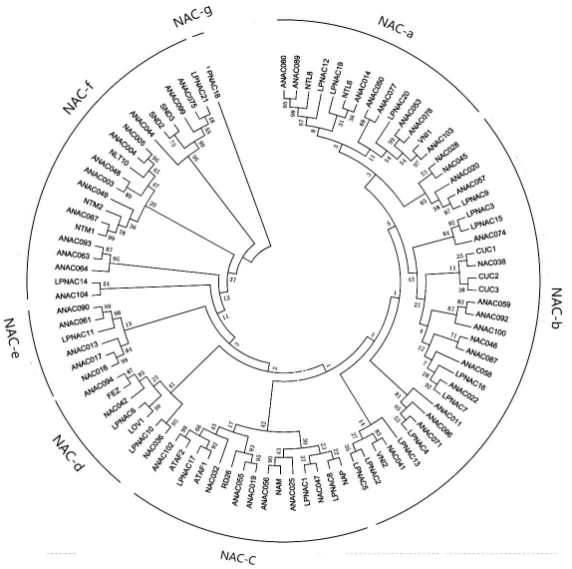


**Fig. 2:** Phylogenrtic analysis of NAC protein sequence. Phylogenrtic relationship of NAC protein from *L. pumilum* and Arabidopsis. The deduced 21 NAC protein sequence and 76 *NAC* genes were aligned by Clustalx and the un-rooted NJ tress was constructed using MEGA 5.0 with 1,000 bootstrap replicates. The sequence of *Arabidopsis* NAC domain proteins was downloaded from the *Arabidopsis* genome TAIR

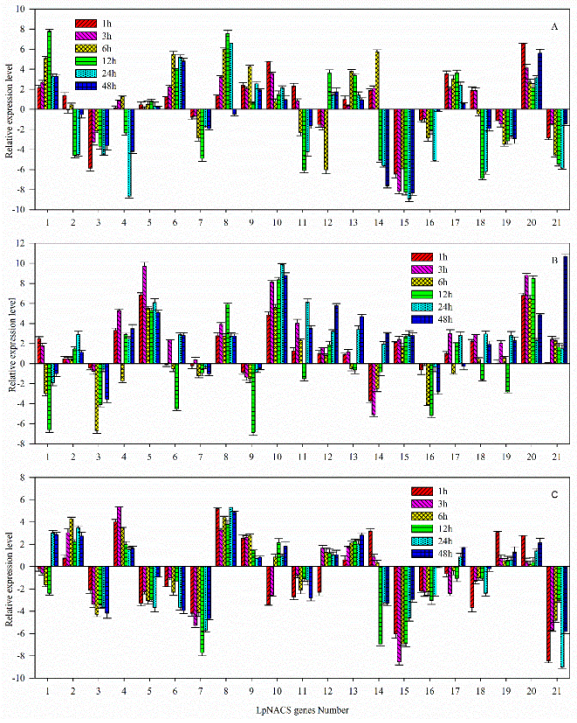


**Fig. 3:** Expression patterns of the 21 *LpNACs* genes in roots (**A**), bulbs (**B**), and leaves (**C**) of the *L. pumilum* seedlings subjected to salt stress (200 m*M* NaCl) with different stress hours. Relative expression level = $\log_2$ (transcription level under stress treatment/transcription level under control conditions). Error bars were obtained from multiple replicates of the real-time PCR

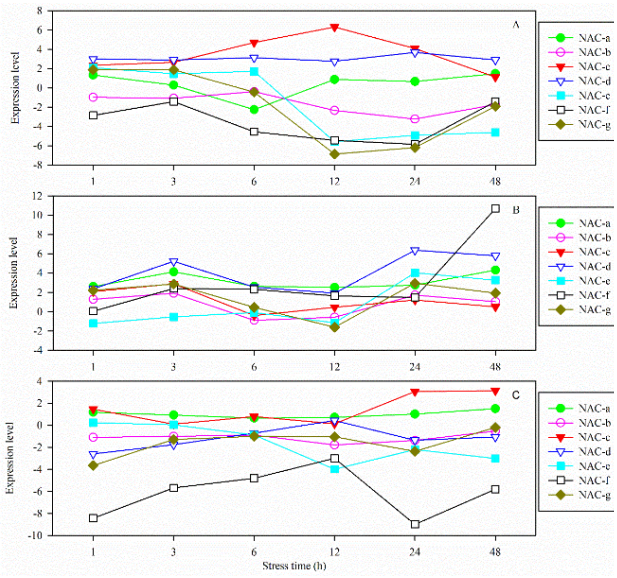


**Fig. 4:** Expression patterns variations with stress time for the six groups *LpNACs* genes in roots (**A**), bulbs (**B**), and leaves (**C**) of the *L. pumilum* seedlings subjected to salt stress (200 m*M* NaCl). Relative expression level = $\log_2$ (transcription level under stress treatment/transcription level under control conditions)

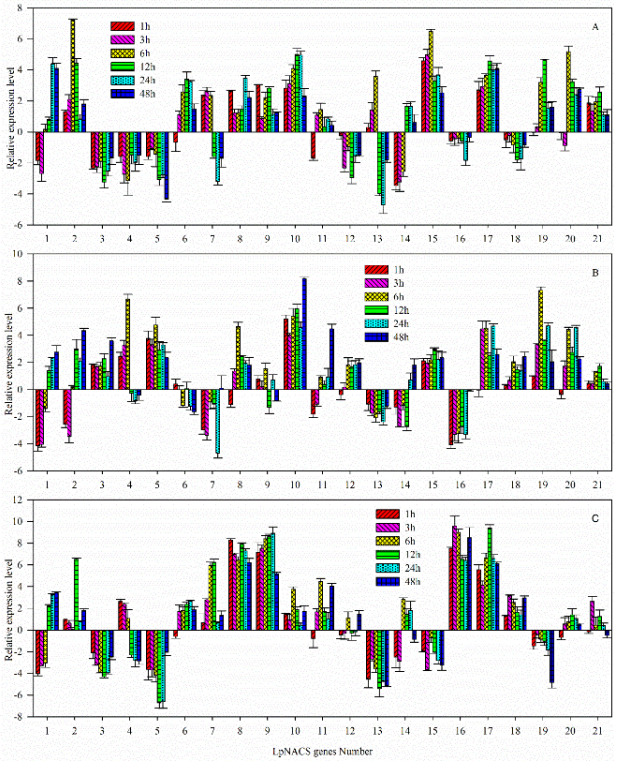


**Fig. 5:** Expression patterns of the 21 *LpNACs* genes in roots (**A**), bulbs (**B**) and leaves (**C**) of the *L. pumilum* seedlings subjected to low temperature stress (2°C) with different stress times. Relative expression level=$\log_2$ (transcription level under stress treatment/transcription level under control conditions). Error bars were obtained from multiple replicates of the real-time PCR

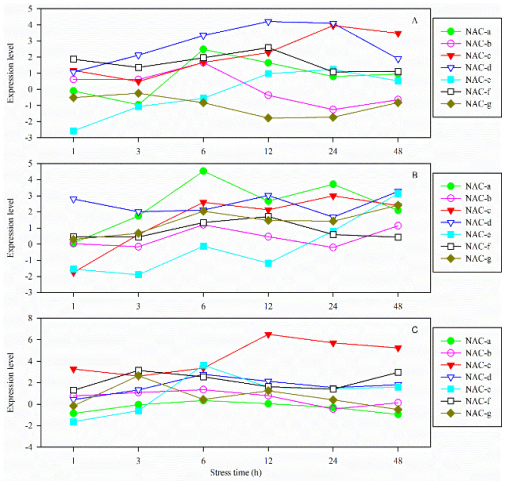


**Fig. 6:** Expression patterns variations with stress time for the six groups *LpNACs* genes in roots (**A**), bulbs (**B**), and leaves (**C**) of the *L. pumilum* seedlings subjected to low temperature stress (2°C). Relative expression level = $\log_2$ (transcription level under stress treatment/transcription level under control conditions)

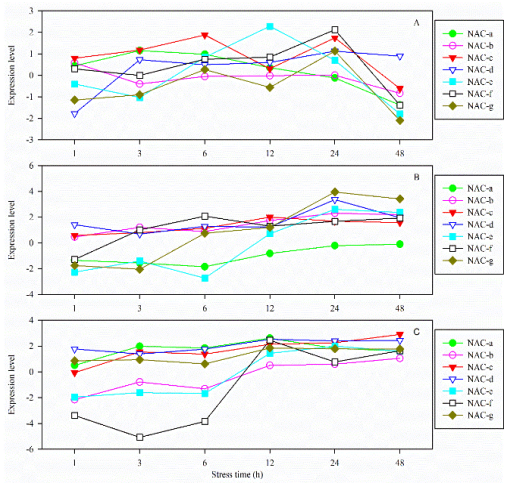


**Fig. 8:** Expression patterns variations with stress time for the six groups *LpNACs* genes in roots (**A**), bulbs (**B**), and leaves (**C**) of the *L. pumilum* seedlings subjected to PEG drought stress (20% PEG6000). Relative expression level = $\log_2$ (transcription level under stress treatment/transcription level under control conditions)

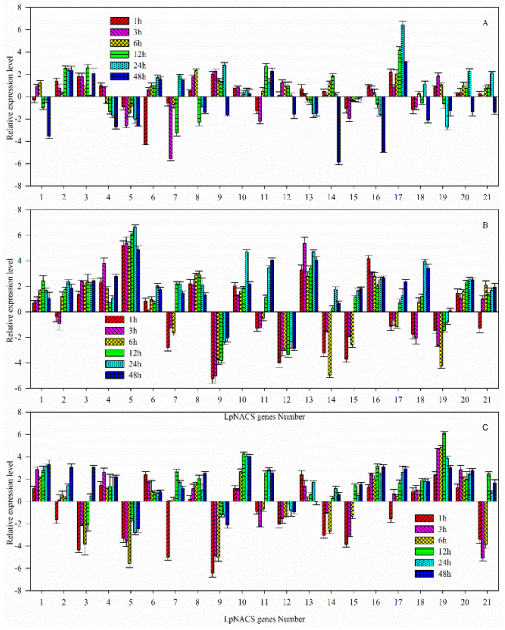


**Fig. 7:** Expression patterns of the 21 *LpNACs* genes in roots (**A**), bulbs (**B**), and leaves (**C**) of the *L. pumilum* seedlings subjected to PEG drought stress (20% PEG6000) with different stress times. Relative expression level = $\log_2$ (transcription level under stress treatment /transcription level under control conditions). Error bars were obtained from multiple replicates of the real-time PCR

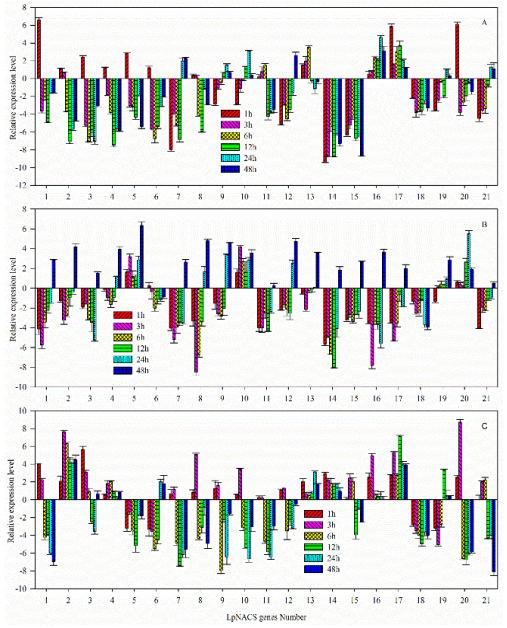


**Fig. 9:** Effect of ABA on the expression of the 21 *LpNACs* genes in roots (**A**), bulbs (**B**), and leaves (**C**) of the *L. pumilum* seedlings with various times. Relative expression level = $\log_2$ (transcription level under stress treatment/transcription level under control conditions). Error bars were obtained from multiple replicates of the real-time PCR

factors participating in the response of various signal transduction pathways to abiotic stress.

Transcriptional responses are regulated by transcriptional, posttranslational and trans-locational mechanisms (Kim *et al.* 2009). Genome analysis found that there were at least 18 and 5 NAC MTFs (Membrane-bound transcription factors) in rice and *Arabidopsis* respectively, including a α-helical tranmembrane motif in C-terminal regions. NAC MTFs of *Arabidopsis* mediate cytokinin signaling during endoplasmic reticulum stress responses (Kim *et al.* 2007). MTFs were stored in the cytoplasm in the form of dormancy (Kim *et al.* 2007). They are involved in various environmental stimuli. Several MTFs located in the nucleus regulate the expression of target genes. Phylogenetic

analysis of *LpNACs* and *Arabidopsis* indicated that *LpNAC19 and LpNAC20* may play a role in stress responses. Genes with related functions tend to fall into one group. The unique function of *LpNAC* protein in *L. pumilum* was predicted. NAC-a and NAC-e groups include most stress-related genes of NAC family in *Arabidopsis* (*NTL6*, *NTL8*, *ANAC013* and *ANAC061*), which are membrane-bound transcription factors that regulate both biological and abiotic stress signal transduction or endoplasmatic reticulum stress responses (Seo and Park, 2010; Kim *et al.* 2012; Clercq *et al.* 2013; Yang *et al.* 2014). These studies suggest that *LpNACs* may also play a potential role in biological and abiotic stress signals. *LpNAC9*, *LpNAC3* and *LpNAC15* were separated from other *LpNACs* genes and aggregated into NAC-b group together with CUC1, CUC2, *ANAC053* and *ANAC045*, which played an important role in shoot organ boundary delimitation (Furuta *et al.* 2014; Kamiuchi *et al.* 2014). The expression of *NAC* genes of *Arabidopsis* during petal differentiation and expansion indicates that *LpNACs* genes may also be involved in plant multicellular organismal development and organ initiation differentiation (Kamiuchi *et al.* 2014). *LpNAC1*, *LpNAC8* and *LpNAC17* belong to the NAC-c group and contain most stress-related genes, such as *ATAF1*, *ATAF2* and *ANAC047*, which are central regulators of plant defense and hormone metabolism development (Delessert *et al.* 2005; Wang and Culver, 2012; Liu *et al.* 2016). Therefore, *LpNACs* genes may play a role in regulating responses to abiotic stresses and hormone signaling. *LpNAC6* and *LpNAC10* were divided into the NAC-d group, including *LOV1*, *ANAC36* and ANC042, which were induced in leaf and inflorescence stem morphogenesis and flower development (Kato *et al.* 2010). In particular, *ANAC042* is involved in the regulation of phytoalexin biosynthesis, a key transcription factor in *Arabidopsis* (Saga *et al.* 2012). The *LpNAC* gene in this group may be involved in flower and leaf development. The sequence was clustered with *SND2*, *SND3* and *ANAC075* genes in *Arabidopsis* (the NAC-f group), which were involved in secondary cell wall formation (Zhong *et al.* 2008; Grant *et al.* 2010; Hussey *et al.* 2011; Sakamoto and Mitsuda, 2015). *LpNAC21 and LpNAC14* may be involved in the formation of secondary cell wall. The NAC-g group contained *LpNAC18* and no *NAC* genes was collected from *Arabidopsis*. However, the functions of *LpNAC18* needs further verification. Our phylogenetic analysis of *LpNACs* can identify potential stress response *LpNAC* genes, which can be preferentially used for further research, especially in the NAC-a group. Therefore, we further explored the role of *LpNACs* in stress response.

In this study, the stress- responsive *LpNACs* were identified by qRT-PCR. Different expression patterns provide important information for the functions of *LpNAC* genes. These results suggest that NAC family genes may be negatively or positively related to the stress response of *L. pumilum*. Our results suggest that *LpNAC20* may play an active effect in responding to salt stress in *L. pumilum*,

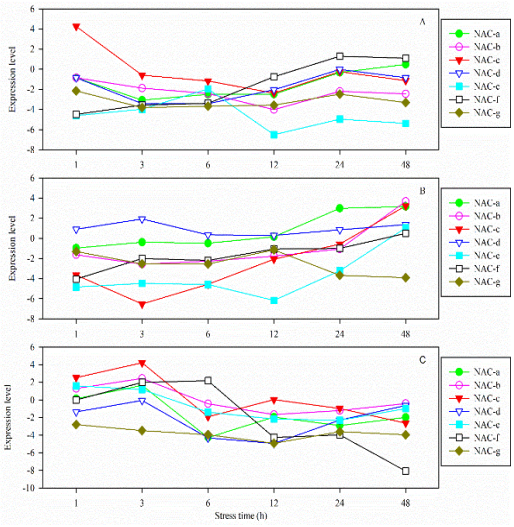


**Fig. 10:** Expression patterns variations with stress time for the six groups *LpNACs* genes in roots (**A**), bulbs (**B**), and leaves (**C**) of the *L. pumilum* seedlings subjected to ABA. Relative expression level = $\log_2$ (transcription level under stress treatment/transcription level under control conditions)

because it was up-regulated under stress. Previous reports had indicated that NAC genes play an active role in response to salt stress, such as *ATAF1*, *NAC57* and *BoNAC019* (Liu *et al.* 2016; Yao *et al.* 2018; Wang *et al.* 2018a). In contrast, *LpNAC3* and *LpNAC16* may play a negative regulatory role in salt stress of *L. pumilum*. It is reported that some NAC genes, including *SlNAC35*, *MdNAC029/MdNAP* and *PbeNAC1*, are induced under cold stress (Jin *et al.* 2017; Wang *et al.* 2018b; An *et al.* 2018). Similar to those genes, *LpNAC10* and *LpNAC17* are highly correlated with cold stress. They may play an important role in the low temperature stress of *L. pumilum.* Other NAC genes, *CarNAC2*, *CarNAC4* and *SlNAC8*, play an active regulatory role in plant drought stress (Yu *et al.* 2014, 2016; Wu *et al.* 2018). The expression of *LpNAC10* and *LpNAC20* in roots, bulbs and leaves was induced by drought treatment, which indicated that they might be involved in the drought stress response of *L. pumilum*. Almost all *LpNAC* groups responded to various stress. In particular, the NAC-a, NAC-c and NAC-d groups suggest their role in abiotic stresses resistance. For example, *LpNAC19* and *LpNAC20*(NAC-a group), *LpNAC17*(NAC-c group) and *LpNAC10*(NAC-d group) were highly expressed in *L. Pumilum* under abiotic stresses. Especially under drought, high salinity, cold and ABA treatments, the expression of *LpNAC10* and *LpNAC17* in roots, leaves and bulbs was induced. This indicated that they might play a role under abiotic stress and be closely influenced by ABA concentration. In bulbs, *LpNAC5* was highly involved under NaCl, PEG, cold and ABA stress. However, in leaves and roots, *LpNAC5* expression was down-regulated or unchanged under stress. This suggests that *LpNAC5* may be involved in the development of bulbs.

ABA plays a crucial role in signal transduction pathways of different environmental stimuli and stress response (Ha *et al.* 2014). We analyzed the expression level of each *LpNAC* gene in *L. pumilum* seedlings treated with different ABA levels. Under ABA stress, most of the *LpNACs* genes in different tissues were significantly induced. It is speculated that these *LpNACs* genes may play a role in ABA-dependent abiotic stress signal transduction pathway.

## Conclusion

The sequence, phylogenetic and comprehensive expression patterns of 21*LpNAC* genes of *L. pumilum* under different stress conditions was comprehensively analyzed. We founded that some genes had high research value for abiotic stress of *L. pumilum*, such as *LpNAC5, LpNAC10, LpNAC17* and *LpNAC20*. The LpNACs genes of *Lilium pumilum* can be clearly improved in leaves, bulbs or roots by NaCl, PEG and cold stresses. *LpNAC5* may be involved in bulb development. The role of NAC genes in *L. pumilum* in responding to abiotic stress is related to ABA dependent stress signaling pathway. Our results suggested that the role of NACs in abiotic stress is helpful to select excellent transcription factors for laying a foundation for lily resistance breeding. In conclusion, the comprehensive expression patterns of 21 *LpNACs* genes in *L. pumilum* under different stress conditions provide new information for determining tissue-specific expression under abiotic stress.

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