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Cloning and Expression Pattern Analysis of Flowering Related Gene *ApVIN3* from Baizilian (*Agapanthus praecox* ssp. *Orientalis*)

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Abstract A core fragment highly homologous with *VERNALIZATION INSENSITIVE* 3(*VIN3*) was obtained on the basis of preliminary *Agapanthus praecox* ssp. *Orientalis* transcriptome sequencing results. The full length sequence of cDNA, *VIN3* gene of *A. praecox* was gained by the RACE method, which was named *ApVIN3* with the accession number of KJ472789. The results of sequence analysis showed that the *ApVIN3* gene length of cDNA was 1 943 bp, which contained an open reading frame of 1 680 bp, 559 encoding amino acids, untranslated region (UTR) 5' and 3' with the length of 88 bp and 175 bp, respectively. *ApVIN3* encoding is a PHD-finger protein with a conserved sequence structure of C4HC3 (cys4-His-cys3), which is highly similar to the *VIN3* protein of *Brachypodium distachyum* (L.) Beauv (XP_003573553.1). Quantitative Real-time PCR show: The regulation of gene expression in quantity by temperature in *Agapanthus africanus* Hoffmgg. shoot apex, with prolonging the time of low temperature rising. This gene may play an important role in the process of brucella induction, and its function and regulation mechanism can be studied deeply.
Keywords *Agapanthus praecox* ssp. *Orientalis*; *ApVIN3*; Clone; Gene expression

Baizilian (*Agapanthus praecox* ssp. *Orientalis*), also known as "Lanbaihe" in Chinese, is a perennial rhizome flower of the genus *Agapanthus* in the family of Agapanthaceae. It blooms from July to August, likes warm, moist and sufficient sunshine, and has high ornamental value. *A. praecox* has beautiful plant types, upright flower stems, umbrella-shaped inflorescence, funnel-shaped small flowers, beautiful flowers, mostly white or blue, and elegant flowers. It is suitable for cultivation and viewing in parks, green spaces, courtyards, and other roadside, rock sides, and walls, and can also be potted and used as fresh cut flower materials. So far, there are more than 300 varieties of *A. praecox* cultivated in the world, and their flower shape, color, inflorescence, plant height and other characteristics are very abundant. China's research on *A. praecox* has started relatively late. Since its introduction into China in 2001, it has carried out a series of studies on embryology (Zhang, 2011), flowering biology (Sun, 2009; Zhuo, 2009), tissue culture and rapid propagation (Liu et al., 2011; Hu and He, 2011), flowering regulation (Shi et al., 2016), and landscape application (Chen et al., 2016). This study is based on previous studies, digging out the important flowering genes in its flowering pathway, will lay a theoretical foundation for promoting the flowering and improving the flowering quality of *A. praecox*, thus expanding the application of *A. praecox* in gardens.

As an important process in plant life history, flowering time will be affected by different signals. Typical ones are photoperiod pathway, vernalization pathway, gibberellin pathway and autonomous pathway. *FT* (*FLOWERING LOCUS T*), *CO* (*CONSTANS*) and *VIN*3 (*VERNALIZATION INSENSITIVE* 3) genes are key regulatory factors in photoperiod pathway and vernalization pathway, respectively. The expression of *CO* gene in plant leaves can induce the expression of *FT* gene (Kobayashi and Weigel, 2007). *FT* is the integration factor and determinant gene of plant flowering regulation. Each signal pathway ultimately starts the flowering process by influencing the expression of *FT*. Therefore, *FT* protein is considered as "florigen" (Abe et al., 2005; Notaguchi et al., 2008; Li et al., 2009). Vernalization pathway and environmental temperature are particularly important in more temperate

species. Vernalization needs to ensure flowering after a long low-temperature process (Kim et al., 2009). The environmental temperature pathway is to regulate flowering through short-term temperature changes (Wigge, 2013). *VIN3*, the key gene in the vernalization pathway, can sense the time course of low temperature, and can judge the time of low temperature treatment in the process of plant vernalization, thus inhibiting the expression of the vernalization related gene *FLC*. However, no annotated *FLC* gene has been found in the transcriptome sequencing data of *A. praecox*, while *CO* and *FT* homologous genes have been reported in the monocotyledonous plant of *A. praecox* (Shi et al., 2014a; Shi et al., 2014b). On the basis of previous studies, this study obtained the full-length cDNA sequence of *ApVIN3*, and analyzed the regulation of flowering related gene *ApVIN3* under different temperature treatments by real-time fluorescence quantitative qRT-PCR, in order to study the effect of this gene on the flowering and other characteristics of *A. praecox,* and lay a theoretical foundation for further study of the vernalization mechanism and molecular mechanism of flowering in the genus *Agapanthus*.

1 Results and Analysis

1.1 Acquisition of the full-length sequence of *ApVIN3* gene of *A. praecox*

After the core sequence 1 636 bp of homologous gene of *ApVIN3* gene in *A. praecox* was detected by ORF, it was found that this sequence had a starting codon, starting at 89 bp, and no ending codon. Primer5 software was used to design primers VIN3-F1 and VIN3-R1. The 885 bp core fragment was obtained by PCR amplification using the cDNA in the shoot tips of *A. praecox* as a template (Figure 1A). According to BLASTp comparison on NCBI website, it had high homology with *VIN3* of many plants. Two 3' RACE-PCR specific primers were designed according to the target fragment, and the 3' sequence was 411 bp (Figure 1C). After splicing the whole sequence, the mRNA full-length sequence was 1 943 bp, 3' non-coding region was 175 bp, 5' non-coding region was 88 bp, coding region sequence was 1 680 bp, encoding of a 559 amino acid residue (Figure 2). According to the design of full-length primers at both ends of the coding region, 1 680 bp band was obtained (Figure 1D). The full-length sequence of mRNA was registered into NCBI, named *ApVIN3*, and the accession number was KJ472789.

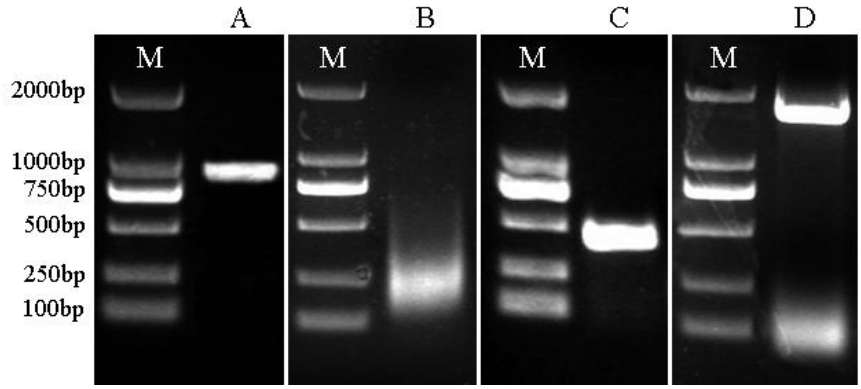

Figure 1 Agarose electrophoresis of the *ApVIN3* gene RT-PCR and RACE products

Note: A: Target fragment product; B: 3' First-round product; C: 3' Second round of nested PCR product; D: Amplification of *ApFT* ORF; M: DL2000 plus Marker

1.2 Amino acid sequence analysis of *ApVIN3*

According to the website (http://www.expasy.org/tools/protparam.html), it was predicted that the molecular weight of *ApVIN3* protein was 62 956.5 kD, and the isoelectric point (pI) was 8.50. SOPMA software was used to analyze the secondary structure of the amino acid of *ApVIN3* protein. Results suggested that alpha helix, extended strand, beta turn, and random coil accounted for 39.18%, 12.70%, 4.47% and 43.63%, respectively. α-helix and random coil were the structural elements with the largest proportion of the protein (Figure 3). The instability coefficient of *ApVIN3* protein was 45.67, which was an unstable hydrophilic protein (instability coefficient>40), and its grand average of hydrophobicity (GRAVY) value was -0.373.

```
1    TCTCTCGTTAAAGAATCAAAGGCCTCTTTTTCCTTTCCTTTTCTCTTCTCTCCTCCTCCATTTTGGAAGGGGGTAGGGATCTCAAACTATGGAACCCAACTTCTCAGGTTTTGTAATAGA
1                                                                                                   M  E  P  N  F  S  G  F  V  I  D
121  TCCCGCCAAATGCAGCGAGTTAAGTTTGGAGGAGAAAAGAGGTTTAGTGCACGAGCTTTCGTGCTGGGCAGACAACGCGCCTGAAATTCTCCAGTCCTTTAGTCGCCGAGAGCTCATTCA
12     P  A  K  C  S  E  L  S  L  E  E  K  R  G  L  V  H  E  L  S  C  W  A  D  N  A  P  E  I  L  Q  S  F  S  R  R  E  L  I  Q
241  ACTCATATGCGCAGAGCTGGGAAAAGAAAGGAAATACACCGGTGTCACGAAGCCTAAAATGATCGCACATCTCCTTAGGTTAGTTTCAGAAAAGAAAAATGGGAAAAAAGTCGAAGTTGG
52     L  I  C  A  E  L  G  K  E  R  K  Y  T  G  V  T  K  P  K  M  I  A  H  L  L  R  L  V  S  E  K  K  N  G  K  K  V  E  V  G
362  AATCAAAAAGAAAAGGAAGAAAGAAATTTCGCTGCAATTCACTGATCATACGGCCCAGGTTCACTCTCAAATCAGCAAATCAGAGGAAATCAAGACCTTGGTTTGTCAAAATCTTGCTTG
92     I  K  K  K  R  K  K  E  I  S  L  Q  F  T  D  H  T  A  Q  V  H  S  Q  I  S  K  S  E  E  I  K  T  L  V  C  Q  N  L  A  C
481  CCGAGCTACTTTGAATCAATGTGATAAAATATTGCAAGAGGTGTTCTTGTTGTATATGTTATCAATTTGACGATAACAAGGATCCGAGCCTATGGTTGGTTTGTAGTTCTGACGCTCCATA
132    R  A  T  L  N  Q  C  D  K  Y  C  K  R  C  S  C  C  I  C  Y  Q  F  D  D  N  K  D  P  S  L  W  L  V  C  S  S  D  A  P  Y
601  TTGCGGCGAATCTTGTGGAATGTCGTGTCATTTGAAATGTGCTCTTAAGAGTGAGAAGGCTGGCATTTCAAGGCATGGACATTACACGAAACTGGATGGTAGTTTCTATTGTGTATGCTG
172    C  G  E  S  C  G  M  S  C  H  L  K  C  A  L  K  S  E  K  A  G  I  S  R  H  G  H  Y  T  K  L  D  G  S  F  Y  C  V  C  C
721  CGGGAAGGTGAATTGGCTTATAGGGAGTTGGCGTAAACAACTGCAAGTGGCAAAGGAAGCTAGGAGAGTAGACATACTATGTGATCGCTTGTCTCTGAGCCAGAAAATCCTCAAAGGAAC
212    G  K  V  N  W  L  I  G  S  W  R  K  Q  L  Q  V  A  K  E  A  R  R  V  D  I  L  C  D  R  L  S  L  S  Q  K  I  L  K  G  T
841  AGAACAATACAAAGAACTCCATAACATTGTGAATGAGGCTGTTAAAAAACTCAAGAAAGAACTTGGACCACTCGACAAAGTTTCTCTAGTGATGGCGAGGGGCATTGTGAACAGGCTCGC
252    E  Q  Y  K  E  L  H  N  I  V  N  E  A  V  K  K  L  K  K  E  L  G  P  L  D  K  V  S  L  V  M  A  R  G  I  V  N  R  L  A
961  TTGTGGTGCAGAGGTTCAGAAACTCTGTGCTTTGGCCGTTGATGCATTGGACGCTATGCTCTCTGGAACATTTCAAGGCTTGGTTGATACTGGTCACCCAAAGATCCTAGGTCCCCAATC
292    C  G  A  E  V  Q  K  L  C  A  L  A  V  D  A  L  D  A  M  L  S  G  T  F  Q  G  L  V  D  T  G  H  P  K  I  L  G  P  Q  S
1081 GTTCCAAATCCATTTCGAAGACATATCTCCAACCTCACTCGTCGTCTCTCTCCAATTCAAAGACGAATCATTTGAAGAAACAATCATTGGTTGCACACTCTGGCATCAATCCTCAAATTC
332    F  Q  I  H  F  E  D  I  S  P  T  S  L  V  V  S  L  Q  F  K  D  E  S  F  E  E  T  I  I  G  C  T  L  W  H  Q  S  S  N  S
1201 TTCAGACTACCCTCAAGATCCCACATTCATAATCCAAAGACCCGAAACAAAATGTAAAATCTCTGGGCTCACTCCTTCAACTGAATACCTCTTCAAAGCTTCGCCTTTCAGCAGCACAAA
372    S  D  Y  P  Q  D  P  T  F  I  I  Q  R  P  E  T  K  C  K  I  S  G  L  T  P  S  T  E  Y  L  F  K  A  S  P  F  S  S  T  K
1321 AGAGCTAGGAAAATGGGAGGCCCGGTGCTCGACTTTGAAATCTCCGAAAAAAGATCAAGAAATTCAATCTGACTCGCAGAAAGGCTCGACAAATACAAGCGATAATAATCAAGCACCAAA
412    E  L  G  K  W  E  A  R  C  S  T  L  K  S  P  K  K  D  Q  E  I  Q  S  D  S  Q  K  G  S  T  N  T  S  D  N  N  Q  A  P  N
1441 CCTTAAGAACGTCGTGACGCCGTTAGTTGACACGCCGAGCAATGCGAATAAAGAAAAGGTTGACGAGAGGCAATATGAATATTGTGTGAAAGTGGTAAGGTGGTTGGAATGCGAAGGGTA
452    L  K  N  V  V  T  P  L  V  D  T  P  S  N  A  N  K  E  K  V  D  E  R  Q  Y  E  Y  C  V  K  V  V  R  W  L  E  C  E  G  Y
1561 TATGGAAAAAGAGTTCCGAGTAAAGTTTCTGACTTGGTTTAGTTTGAAAGCGACGGCTCGAGAGAGGAGAGTTGTGAGTGCATTCATCGATGTGTTGATCGATGAGCCTGAGAGTTTGGT
492    M  E  K  E  F  R  V  K  F  L  T  W  F  S  L  K  A  T  A  R  E  R  R  V  V  S  A  F  I  D  V  L  I  D  E  P  E  S  L  V
1681 TGCACAGCTGGCCGATGCTTTCATGGATGGTATTTGTACTAGGGAGGAGGTTGCTAGAAAAGGTCTGTGCACTAGATTGTTTAGCTGAAAGGTTTTTTTAGTTCCGTTACAATTTGGGTC
532    A  Q  L  A  D  A  F  M  D  G  I  C  T  R  E  E  V  A  R  K  G  L  C  T  R  L  F  S  *
1801 TTTTGGGATGCCATTTATTTTGGAAAAGAAATTGTTGATACAATCTTTTGAATAGTTCTCAGTTGTAACCGATTCAGAAATTGGAGACTAATCTATAGTTAAGTTATTCATCTTTCTCTA
1921 AAAAAAAAAAAAAAAAAAAAAAA
```

Figure 2 The nucleotide sequence and the deduced amino acid sequence of *ApVIN3*

Note: ATG in the underline means initiator codon; TGA with * in the box means terminator codon; Numbers at left means numbers of nucleotide and amino acid

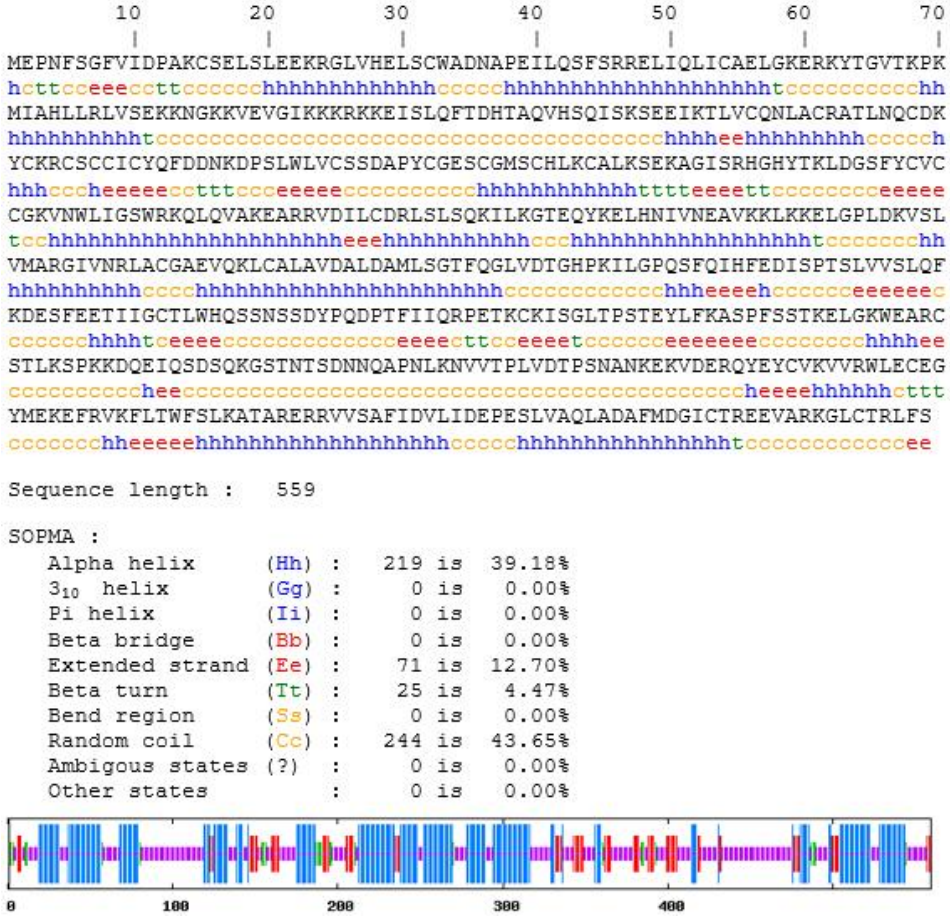

Figure 3 Secondary structure features of *ApVIN3* protein

1.3 Homology comparison and phylogenetic tree analysis of *ApVIN3*

After obtaining the protein sequence according to the coding region sequence of *ApVIN3* gene, BLAST homology analysis of *ApVIN3* protein was performed in NCBI. The amino acid sequence of *ApVIN3* protein was compared and analyzed with the protein amino acid sequence encoded by six plants' *VIN3* homologous genes, including wheat (ABM81546.1) and tomato (ABM81546.1). The results showed that *ApVIN3* protein encoded a PHD-finger protein, and the PHD domain was a relatively small protein domain, composed of about 60 amino acid residues, with a typical C4HC3 (cys4-His-cys3) conservative sequence structure (Figure 4), highly conservative in the process of evolution. From the phylogenetic tree, it could be seen that *ApVIN3* had the highest homology with *VIN3* of the *Brachypodium distachyon* (XP_003573553.1), and relatively low homology with *VIN3* protein of wheat (ABM81546.1), cucumber (XP_004172547.1) and grape (XP_002281346.2) (Figure 5).

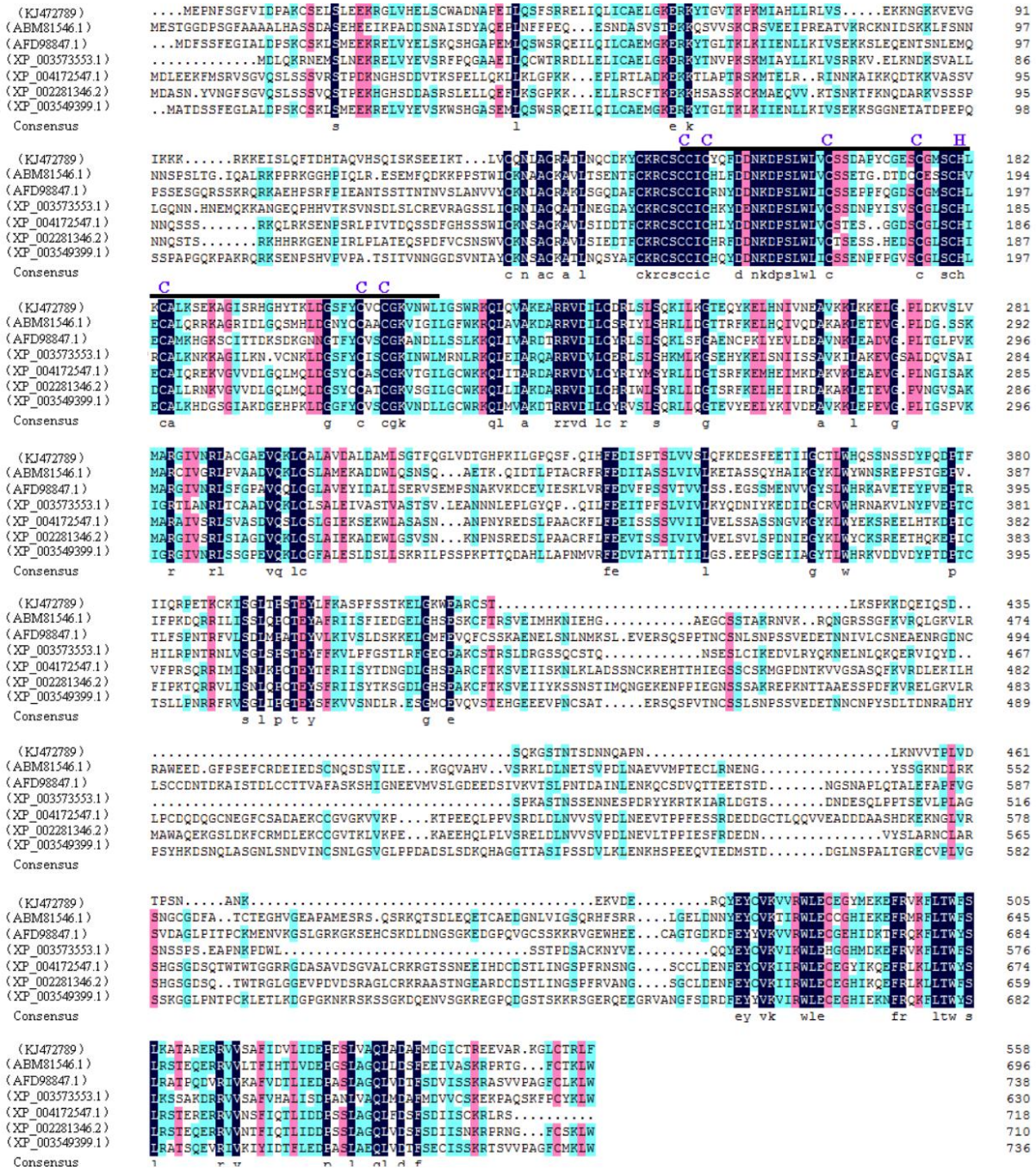

Figure 4 Comparison of the amino acid sequences deduced by *ApVIN3* gene and the multi sequences deduced by the *VIN3* protein from other plants

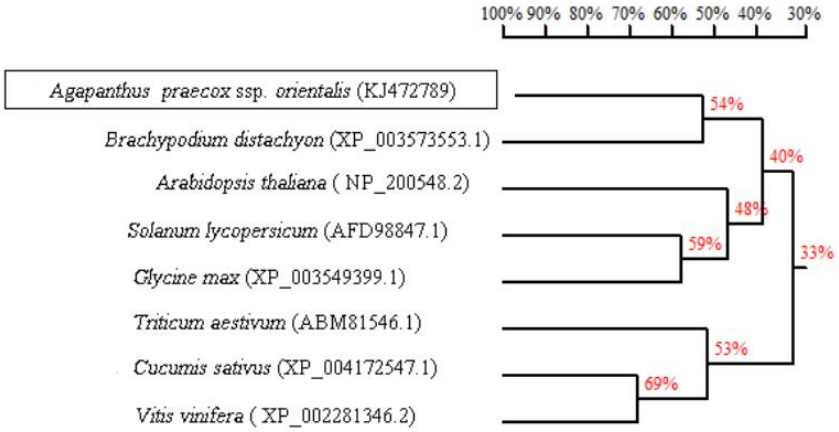

Figure 5 Phylogenetic tree analysis of the deduced amino acid sequences of *VIN3* in different plant species

1.4 The expression of *ApVIN3* in different tissues and organs of *A. praecox*

Tissue-specific analysis was carried out on *A. praecox* under different temperature treatment conditions. Through real-time quantitative PCR detection and analysis, the expression of *ApVIN3* in the shoot tips continued to increase with the extension of low temperature (10 °C/6 °C), and the highest expression was 2.46 times of the lowest expression. However, under the condition of variable temperature treatment (25 °C/15 °C), the expression trend first increased and then decreased (Figure 6), which indicated that low temperature treatment induced the expression of this gene. In *A. thaliana* research, low temperature treatment could induce the expression of the key vernalization gene *VIN3*. *VIN3* could not be detected under normal temperature conditions. It could only be induced under long-term low temperature treatment, but *FCL* still appeared to be inhibited (Schultz et al., 2002). In this study, *FLC* (the core gene of the vernalization pathway), the downstream gene of the vernalization pathway *VIN3*, was not detected in *A. praecox*. Therefore, the expression of *VIN3* and whether *A. praecox* have vernalization need further study.

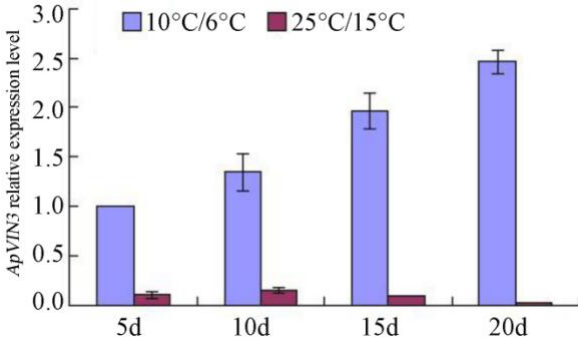

Figure 6 The differential expression of *ApVIN3* in the shoot tips detected by the fluorescence quantitative method

2 Discussion

Flowering is an essential stage in the plant life cycle, and is also a highly complex process. It involves many ways, multiple signals, and the participation of multiple genes, which determines whether the plant can reproduce successfully, and is also closely related to human life. With the in-depth study of the molecular mechanism of temperature affecting plant flowering by domestic and foreign scholars, the temperature sensing pathway, vernalization pathway and environmental temperature pathway of temperature affecting plant flowering have been revealed (Liu et al., 2020), so that we have a clearer understanding of vernalization. In the process of vernalization, the plant body generally feels the low temperature through the shoot tips. After a certain period of low temperature process, the plant needs to continue to grow for several weeks under normal temperature conditions before flowering. There are usually spatiotemporal differences between the induction process of low temperature and the flowering time of the plant, indicating that the plant has the ability to remember the intensity and time of low temperature (Li and Xu, 2014). Previous studies showed that the appropriate temperature and temperature

difference between day and night are conducive to the differentiation and development of the floral bud of *A. praecox*, and the temperature control culture can advance the overall flowering period of *A. praecox* by 66 days (Shi et al., 2016).

During the vegetative growth period of plants, low temperature is used to achieve negative regulation of FLC transcription and protein expression, which is the key gene of vernalization, so as to promote plant flowering (Dennis and Peacock, 2007). FLC protein mainly maintains the vegetative state at the top of plant buds, and can also bind to the promoter of SOC1, the upstream of the translation start site of FD and the first intron of FT to inhibit their expression (Searle et al., 2006; Helliwell et al., 2006). The level of temperature affects the flowering of plants to varying degrees. Previous studies have shown that the *VIN3* level is higher at the fluctuating 14.2 ℃ than at the constant 14.2 ℃, indicating that *VIN3* independently inhibits FLC (Hepworth et al., 2018). Vernalization can not only inhibit the activity of FLC protein and promote plant flowering, but also change the expression of key genes through the change of methylation level. It may be that the sensitivity of methyltransferase to temperature reduces the degree of DNA methylation. After vernalization, *Arabidopsis thaliana* show early flowering. Because *VIN3* has the characteristic of sensing the time course of low temperature, the PHD-finger encoded by *VIN3* participates in the interaction between proteins, such as the methylation and deacetylation of nucleosome histones, and can also reshape the chromatin structure (Sung et al., 2006; Greb et al., 2007), resulting in an increase in the level of dimethylation and trimethylation of FLC chromatin histones H3K27 and H3K9. In this study, the expression of *ApVIN3* in the shoot tips will gradually increase with the extension of low temperature time. The protein encoded by *ApVIN3* also contains a conserved PHD domain. It is speculated that *ApVIN3* may play an important role in the process of induction of flower formation, and it has the function of histone modification and chromatin remodeling at different stages of flower bud differentiation.

In this study, *ApVIN3*, the key gene of the vernalization pathway, was isolated from *A. praecox*. Through nucleotide and amino acid sequence alignment analysis, it was found that *ApVIN3* has high homology with other plant *VIN3*. Using real-time fluorescence quantitative qRT-PCR technology to analyze the expression pattern of *ApVIN3* in the shoot tips of *A. praecox* under different temperature treatments has great differences. The gene may be involved in the process of flower induction of the *A. praecox*. As for how *ApVIN3* directly or indirectly affects the flowering of *A. praecox*, and the molecular evidence of *ApVIN3* expression and the occurrence of upstream and downstream genes still needs further in-depth study.

3 Materials and Methods

3.1 Test materials and reagents

In this study, the seedlings of 3~4 years old *A. praecox* were used as the experimental materials. The materials in the vegetative growth period were put into the artificial climate box. The average temperature was set at 8 °C (day/night, 10 °C/6 °C) for 20 days, the day/night light duration was 10 h/14 h, the light intensity was 10 000 lux, and the humidity was 70%~80%. With the same temperature change treatment group (day/night, 25 °C/15 °C) as the control, the shoot tips of *A. praecox* were taken every 5 days and frozen in liquid nitrogen, store in refrigerator at -80 ℃ for standby. Total RNA was extracted by RNA small extraction kit (Shanghai LifeFeng Biotechnology Co., Ltd.), and engineering bacteria (DH5 α *Escherichia coli*), carrier (PMD18-T carrier), etc. were purchased from TaKaRa; The primer was purchased from Sangon Biotech (Shanghai) Co., Ltd; RACE kit, reverse transcriptase, Taq enzyme, etc. were purchased from Clontech.

3.2 Obtaining fragments of target genes

According to the core sequence fragment (1 636 bp) of the *VIN3* homologous gene obtained from the transcriptome sequencing of *A. praecox*, the primer (VIN3-F1, VIN3-R1) was designed using Primer5 software, and the target fragment was amplified using the cDNA of *A. praecox* leaf as the template, and the primer was designed (Table 1). Amplification procedure: pre-denaturation at 94 °C for 3 min; 94 °C denaturation for 30 s, 65 °C annealing for 30 s, 72 °C extension for 90 s, and the number of cycles was 35; Hold at 72 °C for 5 min. PCR products were detected by 1% agarose gel electrophoresis (120 V, 30 min), purified and recovered with DNA recovery kit (Sangon Biotech (Shanghai) Co., Ltd).

Table 1 Sequence of primers and description

Primer		Sequence (5'-3')	Annealing temperature (°C)	Description
UPM (10X)	Long (0.4 μmol/L)	CTAATACGACTCACTATAGGGCAAGCA GTGGTATCAACGCAGAGT	70.3	RACE-PCR Universal primers
	Short (2 μmol/L)	CTAATACGACTCACTATAGGGC	58.2	
NUP(10 μmol/L)		AAGCAGTGGTATCAACGCAGAGT	60.2	
VIN3-F1 (10 μmol/L)		TTGCGGCGAATCTTGTGGAATGT	60.2	Fragment detection
VIN3-R1 (10 μmol/L)		GCATTGCTCGGCGTGTCAACTAA	62.0	
VIN3-3'-GSP1 (10 μmol/L)		GCATTGTGAACAGGCTCGCTTGTGGTG	66.6	*ApVIN*3 3' RACE
VIN3-3'-GSP2 (10 μmol/L)		TGGTAAGGTGGTTGGAATGCGAAGGG	64.9	
VIN3-sense (10 μmol/L)		ATGGAACCCAACTTCTCAGGTTTTG	60.3	Amplification of
VIN3-antisense (10 μmol/L)		TCAGCTAAACAATCTAGTGCACAGAC	60.4	CDS sequence
qVIN3-F (10 μmol/L)		GCGATAATAATCAAGCACCA	53.7	Real-time PCR
qVIN3-R (10 μmol/L)		GCATTCCAACCACCTTAC	55.8	analysis
Actin-F (10 μmol/L)		CAGTGTCTGGATTGGAGG	55.3	Internal reference
Actin-R (10 μmol/L)		TAGAAGCACTTCCTGTG	53.6	gene primer

3.3 Acquisition of full length of *ApVIN3* gene

Construction of a *A. praecox* 3' RACE library: The total RNA of *A. praecox* tissue was used as a template and amplified with SMARTer ™ RACE cDNA Amplification Kit (Clontech) kit. The 3' RACE amplification primers were VIN3-F1 and UPM, and the 3' RACE cDNA library of *A. praecox* was obtained 1 μL. The 3' end sequence was obtained according to the operation of RACE amplification kit. Splice the target fragment sequence with 3' RACE sequence, design the full-length specific primers VIN3-sense and VIN3-antisense of *ApVIN3* gene, and then carry out PCR amplification, cloning and sequencing to obtain the full-length sequence of *ApVIN3* gene.

3.4 Bioinformatics analysis

Use the BLASTx and GenBank ORF finder programs on the NCBI website to analyze the sequence similarity and predict the open reading frame. Use DNAman software and Swiss model prediction website (http://swissmodel.expasy.org/), comparative analysis of homology and prediction analysis of hydrophobicity, hydrophilicity, secondary structure and tertiary structure of protein were carried out, and the phylogenetic tree was constructed using MEGA4 software.

3.5 Fluorescent quantitative PCR

The expression of *ApVIN3* in the stoop tips of *A. praecox* was analyzed by real-time fluorescence quantitative analysis (qRT-PCR) under different temperature conditions. Use Beacon Designer 7 software to design qRT-PCR primers qVIN3-F and qVIN3-R (134 bp). Real-time fluorescence quantitative PCR instrument (FTC-3000™ SYSTEM) was used for fluorescence quantitative PCR reaction. The reaction system: SYBR Premium Ex Taq II (2X) 10 μL, cDNA 2.0 μL, primer (10 μmol/L) each 0.5 μL, RNase-free H_2O up to 20 μL. Reaction procedure: 94 ℃ pre-denaturation for 60 s; Denaturation at 94 ℃ for 10 s, annealing at 53.5 ℃ for 15 s, extension at 72 ℃ for 25 s, and the number of cycles was 40. Data analysis adopted $2^{-\Delta\Delta Ct}$ method, the internal reference gene was *Actin*, the fragment size was 117 bp (Zhang et al., 2011), and three repeats were set.

Authors' contributions

SYB is the designer, principal and executive of the project, wrote the first draft of the paper. WCZ and ZY participated in the experimental design and data processing. All authors read and approved the final manuscript.

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References

Abe M., Kobayashi Y., Yamamoto S., Daimon Y., Yamaguchi A., Ikeda Y., Ichinoki H., Notaguchi M., Goto K., Arati T., 2005, FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex, Science, 309(5737): 1052-1056.
https://doi.org/10.1126/science.1115983

Chen X.B., Lu L., Qian Y.Y., and Fan Y.T., 2016, Recent advances in germplasm and landscape application of *Agapanthus* spp., Zhongguo Yuanlin (Chinese Landscape Architecture), (8): 99-105.

Dennis E., and Peacock W., 2007, Epigenetic regulation of flowering, Current Opinion in Plant Biology, 10(5): 520-527.
https://doi.org/10.1016/j.pbi.2007.06.009

Greb T., Mylne J.S., Crevillen P., Geraldo N., An H.L., Gendall A.R., Dean C., 2007, The PHD finger protein VRN5 functions in the epigenetic silencing of *Arabidopsis FLC*, Curr. Biol., 17(1): 73-78.
https://doi.org/10.1016/j.cub.2006.11.052

Helliwell C.A., Wood C.C., Robertson M., Peacock W.J., Dennis E.S., 2006, The *Arabidopsis* FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex, The Plant Journal, 46(2): 183-192.
https://doi.org/10.1111/j.1365-313X.2006.02686.x

Hepworth J., Antoniou-Kourounioti R.L., Bloomer R.H., Selga C., Berggren K., Cox D., Collier Harris B.R., Irwin J.A., Holm S., Säll T., Howard M., and Dean C., 2018, Absence of warmth permits epigenetic memory of winter in Arabidopsis, Nat. Commun., 9(1): 639.
https://doi.org/10.1038/s41467-018-03065-7

Hu Z.Y., and He Y.Q., 2011, Study on tissue culture and plantlet regeneration of *Agapanthus praecox* ssp. *Orientalis* 'Big Blue', Beifang Yuanyi (Northern Horticulture), (10): 118-120.Kim D.H., Doyle M.R., Sung S.B., and Amasino R.M., 2009, Vernalization: winter and the timing of flowering in plants, Annu. Rev. Cell. Dev. Biol., 25(1): 277.
https://doi.org/10.1146/annurev.cellbio.042308.113411

Kobayashi Y., and Weigel D., 2007, Move on up, it's time for change-mobile signals controlling photoperiod-dependent flowering, Genes & Development, 21(19): 2371-2384.
https://doi.org/10.1101/gad.1589007

Li C.Y., Zhang K., Zeng X.W., Stephen J., Zhou Y., Hong YG., 2009, A cis element within flowering locus T mRNA determines its mobility and facilitates trafficking of heterologous viral RNA, Journal of Virology, 83(8): 3540-3548.
https://doi.org/10.1128/JVI.02346-08

Liu F.Y., Gao Y.H., and Shang A.Q., 2011, Tissue culture and plant regeneration of A. *praecox* ssp. *Praecox* 'Floribunda', Beifang Yuanyi (Northern Horticulture), (13): 121-124.Liu J., Li L., Lu B.C., Deng P., and Ai X., 2020, Research progress on the temperature-regulated flowering of plants, Yingyong yu Huanjing Shengwu Xuebao (Chinese Journal of Applied & Environmental Biology), 26(3): 713-721.

Li W., and Xu J.Q., 2014, Epigenetic research progress on flowering time and flower organ development in angiosperms, Yuanyi Xuebao (Acta Horticulturae Sinica), 41(6): 1245-1256.

Notaguchi M., Abe M., Kimura T., Daimon Y., Kobayashi Y., Yamafuchi A., Tomita Y., Dohi K., Mori M., Araki T., 2008, Long-distance, graft-transmissible action of *Arabidopsis* FLOWERING LOCUS T protein to promote flowering, Plant Cell Physiology, 49(11): 1645-1658.
https://doi.org/10.1093/pcp/pcn154

Schultz D.C., Ayyanathan K., Negorev D., 2002, ETDBI: a novel KAP-1-assoeiated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatin genes by KRAB zinc-finger proteins, Genes Dev., 16: 919-932.
https://doi.org/10.1101/gad.973302

Searle I., He Y., Turck F., 2006, The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*, Genes & Development, 20(7): 898-912.
https://doi.org/10.1101/gad.373506

Shi Y.B., Qian C.G., Zhang P., and Zhuo L.H., 2016, Study on the regulation of florescence of *Agapanthus praecox* ssp.*orientalis*, Shanghai Nongye Xuebao (Acta Agriculturae Shanghai), 32(6): 133-136.

Shi Y.B., ZHANG D., Shen X.H., Wang L., and Zhuo L.H., 2014, Cloning and expression pattern analysis of flowering related gene *ApFT* from *Agapanthus praecox* ssp.*orientalis*, Yuanyi Xuebao(Acta Horticulturae Sinica), 41(4): 726-734.

Shi Y.B., Zhang D., Shen X.H., Wang L., and Zhuo L.H., 2014, Molecular cloning and expression pattern analysis of a *CONSTANS* homolog from *Agapanthus praecox* ssp.*orientalis*, Beijing Linye Daxue Xuebao (Journal of Beijing Forestry University), 36(4): 113-120.

Sung S., Schmitz R.J., and Amasino R.M., 2006, A PHD finger protein involved in both the vernalization and photoperiod pathways in *Arabidopsis*, Gene Dev., 20(23): 3244-3248.
https://doi.org/10.1101/gad.1493306

Sun Y., 2009, The studies on reproductive biology of *Agapanthus Praecox* ssp. *Orientalis* 'Big Blue', Dissertation for Ph.D., Northeast Forestry University, Supervisors: pp.

Wigge P.A., 2013, Ambient temperature signalling in plants, Curr. Opin. Plant Biol., 16(5): 661-666.
https://doi.org/10.1016/j.pbi.2013.08.004

Zhang D., 2011, Flower Bud Development and Flowering Mechanism in *Agapanthus Praecox* ssp. *orientalis*, Dissertation for Ph.D., Northeast Forestry University, Supervisors: Zhuo L.H., pp.114-115.

Zhang D., Shen X.H., and Zhuo L.H., 2011, Studies on the blossom physiology in the different development stage of *Agapanthus praecox* ssp.*orientalis*, Shanghai Jiaotong Daxue Xuebao (Journal of Shanghai Jiaotong University (Agricultural Science)), 29(3): 6-13.

Zhuo L.H., and Sun Y., 2009, Floral syndrome and breeding system of *Agapanthus praecox* ssp. *orientalis* 'Big Blue', Yuanyi Xuebao (Acta Horticulturae Sinica), 36(11): 1697-1700.