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独行菜冷上调基因 *LaNHR2B* 的低温耐受性研究

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摘　要:新疆北部早春短命植物独行菜幼苗期能够在早春的低温条件下生长,具有良好的耐受低温胁迫的能力。前期研究获得了独行菜幼苗冷诱导上调表达基因片段,通过同源克隆获得该基因的全长 cDNA 序列(*LaNHR2B*),运用生物信息学软件预测分析该基因编码蛋白的性质及其构象,采用荧光定量 RT-PCR 技术分析其表达量与幼苗生长阶段、冷诱导处理以及外源 ABA 处理间的关系,通过转化拟南芥研究过表达该基因对植物幼苗低温耐受性的影响。结果表明:(1)*LaNHR2B* 基因全长 1 035 bp,编码 344 个氨基酸,蛋白质分子质量为 85 791.16 kD,理论等电点为 5.06,分子式为 $C_{3129}H_{5225}N_{1035}O_{1307}S_{235}$;其蛋白主要由丙氨酸、苏氨酸、甘氨酸及半胱氨酸组成,具有 3 个跨膜结构,功能未知;在十字花科植物中保守性强,其他科的植物中未见同源基因。(2)*LaNHR2B* 受 4 ℃低温诱导显著上调表达,但随幼苗生长受低温诱导上调表达有所降低,与幼苗随发育耐受低温能力下降变化一致。(3)外源 ABA 可诱导 *LaNHR2B* 表达上调;过表达拟南芥幼苗低温耐受性明显增强。该研究初步证明,*LaNHR2B* 表达量与独行菜幼苗耐受低温密切相关,可能是独行菜幼苗经过冷驯化后能够增强植株抗寒能力的功能性基因。这为进一步开发利用该基因进行油菜等作物抗寒育种提供理论依据。

关键词:独行菜;冷胁迫;*LaNHR2B*;过表达;抗寒相关性;ABA

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Evaluating the Function of a Novel Gene *LaNHR2B* of *Lepidium apetalum*, Up-regulated by Low Temperature for Cold Tolerance

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Abstract: Ephemeral plant *Lepidium apetalum* Willd. seedlings grow well at low temperatures in early spring in the north of Xinjiang, China, and have a perfect tolerance to low temperature stress. *LaNHR2B* (cold up-regulated expression gene in *L. apetalum*), a gene found in *L. apetalum*, was previously reported as being up-regulated in seedlings by cold stress. In this study, we obtained the full-length cDNA sequence of *LaNHR2B* using homology-based cloning. We predicted and analyzed the nature and conformation of its encoding protein with bioinformatics software, the relationship between its expression and the growth stages of seedlings. We also studied its expression in response to cold induction and exogenous ABA treatment with quantitative RT-PCR, and further explored the effects of *LaNHR2B* over-expression

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in *Arabidopsis thaliana* on cold tolerance. Results showed that: (1) the full-length of *LaNHR2B* gene is 1 035 bp, encoding a protein consisting of 344 amino acid with a molecular weight of 85 791.16 kD, a theoretical PI of 5.06 and a formula of $C_{3129}H_{5225}N_{1035}O_{1307}S_{235}$. The protein contains alanine, threonine, glycine and cysteine, which has three transmembrane structures with unknown functions and strongly conserved in cruciferous plants. (2) Expression of *LaNHR2B* was up-regulated by cold induction at 4 ℃, but this increase gradually decreased with the growth and development of seedlings, consistent with the decreasing trend of cold tolerance with growth. (3) Exogenous ABA induced *LaNHR2B* expression, and over-expression of *LaNHR2B* in *A. thaliana* enhanced cold tolerance. With these results we have proved that *LaNHR2B* expression has a close relationship with the cold tolerance of *L. apetalum* seedlings. Moreover, *LaNHR2B* may be a functional gene enhancing cold resistance after cold acclimatization, providing a certain theoretical basis and reference for further developing cold-resistant breeding of crops with this gene.

Key words: *Lepidium apetalum* Willd.; cold stress; *LaNHR2B*; over-expression; chilling resistance; ABA

Low temperature is one of the most important environmental factors limiting and affecting the production of crops[1]. In particular, low temperature stress is more apparent to plants in middle and high latitudes[2]. Plants directly or indirectly increase the cold resistance of plants by regulating the unsaturated fatty acids, protective enzymes, functional genes and regulatory genes of membrane lipids[3]. And utilizing cold tolerance functional genes to improve the cold resistance of plants has become an important and effective approach in modern plant breeding[4]. Enhanced resistance of plants has been reported in many plants transformed with cold tolerance functional genes[5]. At present, the available effective functional genes are still limit, therefore obtaining cold-resistant functional gene becomes a key goal in molecular-assisted breeding. Many studies also focus on the screening functional verification of cold tolerance functional genes[6-7], endeavoring to enrich this resource of important genes.

Lepidium apetalum Willd. belongs to the *Lepidium* of the Brassicaceae, widely distributed in provinces north and south of China, with different ecotypes that adapt to the surrounding environment. The *L. apetalum* present in the north of Xinjiang has typical characteristics of spring ephemerals[8]. They can tolerate cold to germinate and grow in early spring, having an important position in the area as a pioneer plant[9]. Previous studies found that the seeds of *L. apetalum* could not germinate at low temperature (below 4 ℃), but they would grow normally at the temperature below 4 ℃ after germinating at 8 ℃ to the stage that radical grows out. In addition, the seeds can germinate at 4 ℃ after treated at 25 ℃ for a short time (60 min). It is inferred that the higher temperature acts as a signal to start the expression of some genes which are related to the germination of seeds of *L. apetalum*[10-14], but there is still a lack of report about the cold tolerance functional genes of *L. apetalum* seedlings.

In previous studies, a cold induced gene segment *TDF*119 was screened by analyzing the transcriptome data of *L. apetalum*[15], whose sequence is almost exactly the same with AT4G25030 in *Arabidopsis thaliana*. It was reported that the AT4G25030 is a serine/threonine protein kinase, locating on *A. thaliana* chromosome 4 and encoding 344 amino acid residues[16]. Seki found that this protein possesses an exactly similar amino sequence to GSLTFB57ZD01 in plant growth and development[17]. And Boaz Kaplan reported that the promoter of AT4G25030 contained multiple ABRE or ABRE-CE motifs, which suggesting that the gene participates in Ca^{2+} regulation in *A. thaliana* biotic and abiotic stresses pathway[18]. Amazingly, and the transcription processing characteristics of this gene about AT4G25030 have also been studied and it is considered an alternative splicing gene[19].

The latest research advances in biology, the AT4G25030 was named *AtNHR2B* (*A. thaliana* nonhost resistance 2B), it is a chloroplast-localized protein and plays an important role in deposition of callose in nonhost disease resistance[20]. Therefore, the cold-up-regulated gene TDF119 was named *LaNHR2B* (*Lepidium apetalum* nonhost resistance 2B, GenBank accession number KF362124.1) in *L. apetalum* in this test. We analyzed the response of gene expression to low temperature stress and tested the effect of gene overexpression on tolerance to low temperature stress in plants, in order to investigate the role of *LaNHR2B* in resisting abiotic stress of low temperature in plants.

1　Materials and methods

1.1　Seeds

L. apetalum seeds were collected from the Carp Mountain in Urumqi Municipality in June 2012, mature and full grains were selected, and the experiments were carried out in the laboratory of molecular biology at Xinjiang Normal University.

After the common routinely sterilization with ethanol (70% for 30 s) and NaClO (0.5% for 20 min), the seeds were cultured in dishes at 25 ℃ with light (Irradiance of 14.5 $W \cdot m^{-2}$) for germination, until the stages of radicle protrusion, radicle protraction, hypocotyl protrusion and three-leaf stage were reached, for further use[10].

1.2　Cloning and sequence analysis of *LaNHR2B* full-length cDNA of *L. apetalum*

The seedling was collected at the cotyledon expansion stage for cold acclimation at 4 ℃ for 6 h. Total RNA from seedlings (about 100 mg) was extracted with Trizol™ total RNA extraction kit (Invitrogen, Carlsbad, CA, USA), and cDNA synthesized with The RNA PCR Kit (AMV Ver. 2.1, TaKaRa) according to the manufacturer's instructions. According to homology comparison results between the *LaNHR2B* core sequence obtained previously with the known *A. thaliana* gene sequence (AT4G25030) in NCBI, PCR primers were designed as follows: C-F (5′-GCCAAGCTTATGGATAATTGTACTGGA-3′) and C-R (5′-GCACCCGGGTTAGCAGACAACAGTAGC-3′). The above cDNA was been used as a template, and the cDNA sequence of full-length *LaNHR2B* of *L. apetalum* was cloned and ligated into a pMD18-T vector, named as T-*LaNHR2B* for sequencing. The sequence of its encoding protein were predicted with DNAMAN, the protein properties were analyzed with ProtParam, structure prediction was performed with SMART, and the presence of signal peptides was analyzed with Signal P 4.1.

1.3　Effects of low temperature treatment on *LaNHR2B* gene expression in *L. apetalum* seedlings

The 180 *L. apetalum* seedlings were taken at the cotyledon expansion stage, divided into 6 groups, and treated at 4 ℃ for 0, 1, 3, 6, 12 and 24 h. Half of each group was sampled, and cultured at 25 ℃ for 6 h, with a total of 12 samples. The effects of cold acclimation on *LaNHR2B* gene expression were analyzed with fluorescence quantitative RT-PCR. *eEF*-1α gene (Elongation factor-1α) was used as an internal control[21]. The *LaNHR2B* detection primers were designed as follows: *LaNHR2B*-F (5′-GTTCAATGTCATCTTCTTC-3′), *LaNHR2B*-R (5′-TAGTTCATGCCTGATGTC-3′), with an expected product size of 108 bp. The *eEF*-1α detection primers were designed as follows: ef1-F (5′-CAAGGCTAGGTACGAT-3′), ef1-R (5′-CAATCATGTTGTCTCCCT-3′), the expected product size was 119 bp. All primers were synthesized by Beijing Huada Co., Ltd. Reverse transcription was used to synthesize cDNA, *eEF*-1α was taken as an internal control to calibrate the mRNA levels, and fluorescence quantitative RT-PCR analysis was carried out in real-time with cDNA as template. Due to the extremely low background expression of *LaNHR2B*, it was unable to be detected on polyacrylamide gel electrophoresis with silver staining. For convenience, the gene expression quantity in *L. apetalum* seedlings treated at low temperature for 1 h was used as the reference standard, defined as one unit, and relative quantitative analysis was performed for samples in the other 5 groups.

1.4 Relationship between *LaNHR2B* gene expression and cold resistance of *L. apetalum* at different development stages

The seedlings of *L. apetalum* were taken at the stages of the radicle protrusion, radicle protraction, hypocotyl protrusion and three-leaf stage (180 seedlings were selected in each growth stage), for cold induction at 4 ℃ for 12 h. The seedlings were then divided into two groups for stress treatment at −5 ℃ and −10 ℃ for 10 min. After 2 h, the temperature was continuously increased slowly (up to 4 ℃ after 12 h), finally reaching 25 ℃. The survival rate was calculated after 48 h, and the ability to continue to grow was taken as the survival standard. Additionally, seedlings at the above four developmental stages were divided into two groups, with a total of eight samples, one group was treated at 4 ℃ for 12 h and the other was not treated. Fluorescence quantitative RT-PCR analysis was adopted to analyze the effects of different developmental stages of *L. apetalum* on cold induced up-regulated *LaNHR2B* gene expression. The quantitative method was the same as that described above.

1.5 Effects of exogenous ABA treatment on *LaNHR2B* gene expression in *L. apetalum* seedlings

The 180 seedlings of *L. apetalum* were taken at the stage of cotyledon expansion and divided into three groups treated with ABA (100 μmol · L^{-1}) for 0, 1 and 6 h. Half of each group was sampled, rapidly washed with sterile water, and then cultured with exogenous ABA-free medium for 24 h. Fluorescence quantitative RT-PCR was used to analyze the effects of exogenous ABA treatment on *LaNHR2B* gene expression. The quantitative method was the same as that described above.

1.6 Effects of *LaNHR2B* over-expression on the cold tolerance of *A. thaliana* seedlings

1.6.1 Expression vector construction, *A. thaliana* transformation and identification The cloned T-*LaNHR2B* and pBI121 were digested with both of *Bam*H Ⅰ and *Hind* Ⅲ respectively. And the target fragments digested were extracted from gel, ligated with T_4 ligase, recombined into the plant expression vector named as pBI-p*LaNHR2B*, and then transformed pBI-p*LaNHR2B* into competent *Agrobacterium* strain *GV*3101 cells. The engineered *Agrobacteria* were transformed into *A. thaliana* using the floral dip method to obtain the first generation (T_0) of seeds. Seedlings were selected with kanamycin, and after PCR identification of transformants, the plants were cultured and T_1 seeds harvested. Planting T_1 seeds to harvest a large number of T_2 seeds for subsequent experiments.

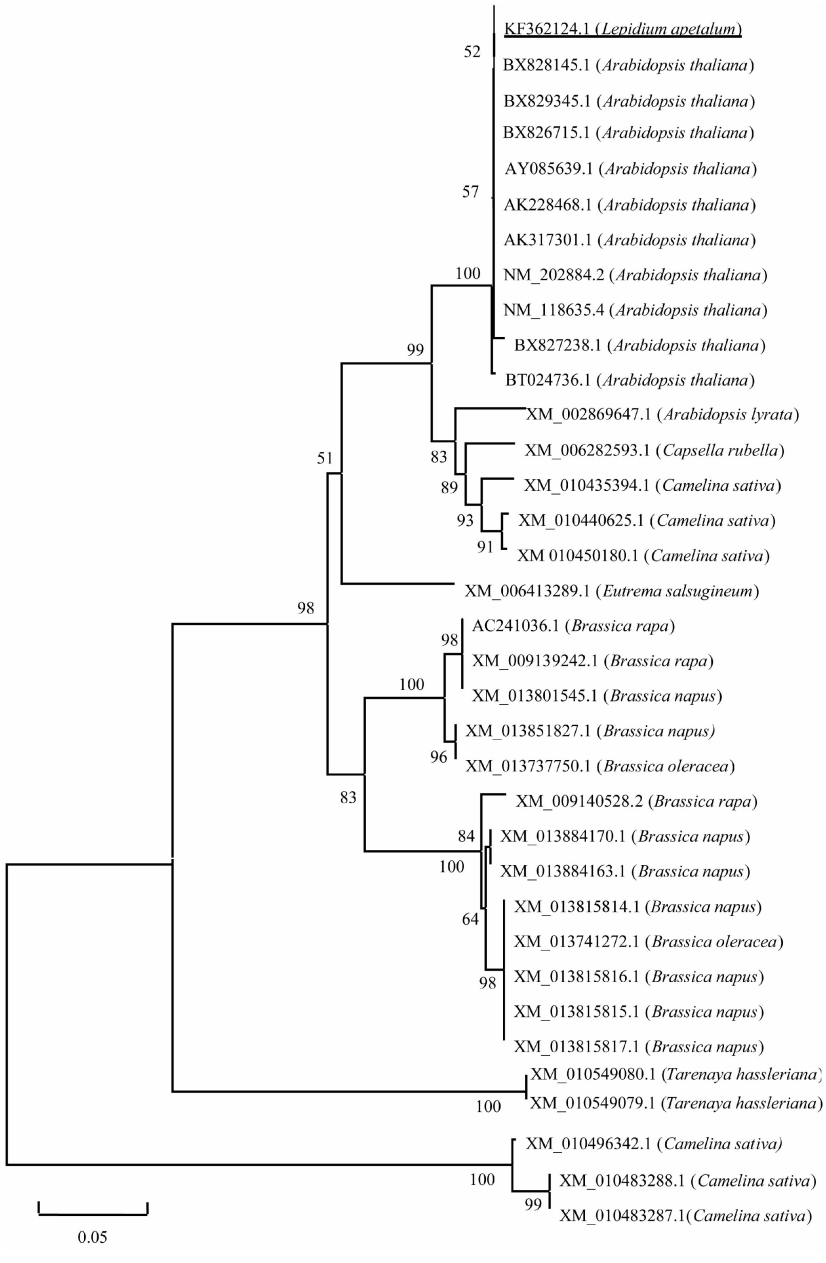
1.6.2 Cold tolerance test of *LaNHR2B*-transformed *A. thaliana* seedlings About 120 T_2 seeds were collected, divided into groups Ⅰ and Ⅱ, and 60 seeds for each group were tiled into three clusters on culture dishes containing MS solid culture medium. Meanwhile, three clusters of wild type *A. thaliana* seeds were sown in every dish. After vernalization at 4 ℃ for 2 days, the seeds were transferred into an incubator for light culture (light intensity: 80−100 μmol · m^{-2} · s^{-1}; temperature: (21±1) ℃; light cycle: 24 h continuous illumination). The seedlings were cultivated for about 7 days for germination, and when the true leaf was going to unfold, group Ⅰ was placed at 5 ℃ for 2 h, then back to 4 ℃ for 6 h. After this, growth was observed after cultivating in the incubator for 5 days. Group Ⅱ was induced at 4 ℃ for 12 h, then placed at 5 ℃ for 2 h, and the growth was observed after cultivation in the incubator for 5 days.

All of the above experiments were repeated three times with similar results.

2 Results and analysis

2.1 Cloning and sequence analysis of *LaNHR2B* full-length cDNA of *L. apetalum*

Total RNA was extracted from the samples of *L. apetalum* treated at 4 ℃ for 6 h during the period from seeds germination to cotyledon expansion. RT-PCR amplification was performed with C-F/R primers, and sequencing results showed that the fragment was 1 035 bp, encoding a complete reading frame with 344 amino acid. A total of



GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences. Use MEGA6 make evolutionary tree

Fig. 1　Cluster analysis of *LaNHR2B* and homologous sequences retrieved from the database of GenBank

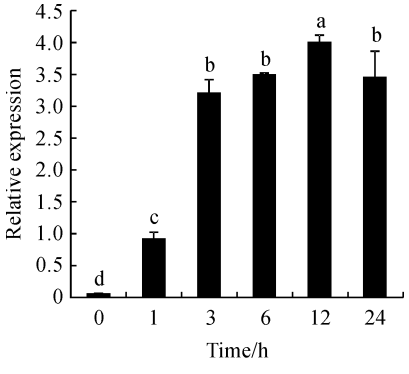
40 homologous sequences were found in GenBank, derived from plants belonging to nine species in the cruciferae family. Homology and cluster analysis results of *LaNHR2B* with homologous genes in the database are shown in Fig. 1. *LaNHR2B* is very conservative in cruciferous plants. The nucleotide sequence of *LaNHR2B* (KF362124.1) of *L. apetalum* is 100% to its homology of *A. thaliana* (AT4G25030), and the amino acid sequence is exactly the same as the corresponding protein of *Arabidopsis*.

The encoded protein sequence of *LaNHR2B* was predicted with DNAMAN, and was found to consist of 344 amino acid residues. ProtParam (http://web.expasy.org/protparam/) was used to analyze the protein properties encoded by *LaNHR2B*. The result showed that the theoretical isoelectric point of the predicted protein is 5.06, the molecular weight of the protein is 85 791.16 kD, the formula of the protein is $C_{3129}H_{5225}N_{1035}O_{1307}$

S_{235}, and the protein contains alanine, threonine, glycine and cysteine. SMART structure prediction showed that the protein encoded by this gene contains three transmembrane helices are located between 207－224, 231－248 and 258－275 amino acid residues, and three low complexity regions located between 31－40, 101－115, and 119－134 amino acid residues. No signal peptide was found using Signal P 4.1 software analysis.

2.2 Low temperature stress induced up-regulation of *LaNHR2B* gene expression in *L. apetalum* seedlings

With *eEF*-1α gene as an internal control, relative quantitative comparative analysis was carried out for samples in the other five groups, including one group without low temperature stress, and four groups with different treatment time. The result is shown in Fig. 2. Expression analysis showed up-regulation with increasing time within 0－24 h of low temperature stress, and this especially increased rapidly within 1－3 h. After this time there was a slight decrease in expression, maintaining high expression levels with a significant difference when compared with the control samples. Numerous studies have shown that low temperature induction can effectively enhance the cold resistance ability of plants[22-24]. *LaNHR2B* expression is up-regulated in *L. apetalum* after low-temperature induction, however whether it is one of the main factors enhancing the cold resistance of plants is still to be further investigated.



Data are means from three biological replicates with three technical replicates each. The error bars show the means ± SD. The same as below

Fig. 2 Expression profile analysis of *LaNHR2B* in response to chilling temperature of *L.* seedlings using qRT-PCR

2.3 Up-regulated expression pattern of *LaNHR2B* is consistent with the change in cold resistance of plants after cold acclimation

2.3.1 Cold resistance decreases with the growth of *L. apetalum* seedling *L. apetalum* is vulnerable to low temperature stress during the stages of seed germination and seedling growth under the natural environment in the north of Xinjiang in China, where the low temperature is in early spring. As the plant grows up, the temperature becomes warmer. Thus when *L. apetalum* seedlings gradually grew up, the possibility of suffering from low temperature damage also decreased. In this study, it was showed that the cold resistance capability of early stage seedlings of *L. apetalum* was strong, but gradually weakened along with the growth of seedlings (Fig. 3). Cold acclimation at different temperatures and of different durations was given to the *L. apetalum* seedlings at four different developmental stages, following which the survival rates were measured. Results showed that the bud swelling-white petals forming stage had very strong tolerance to low temperature stress. The overall survival rate slightly decreased at the hypocotyl elongation stage, but with a strong cold tolerance, while the tolerance to cold was further lowered at the cotyledon expansion stage, and was minimal at the three-leaf stage. In summary, the cold tolerance capability of *L. apetalum* seedlings

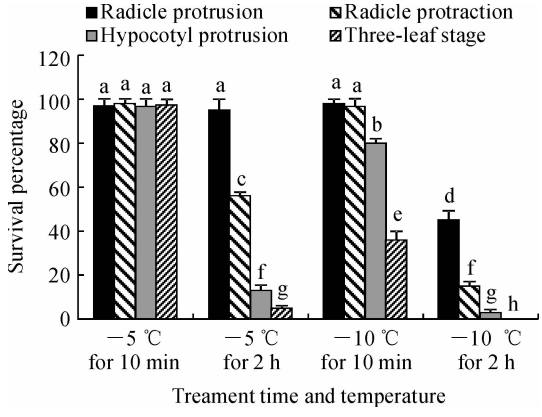


Fig. 3 Cold tolerance analysis of *L. apetalum* seedlings at different growth stages

decreased along with its growth.

2.3.2 Decreased effects of low temperature treatment on *LaNHR2B* gene expression with the seedling growth The low temperature induction conditions on *L. apetalum* at different developmental stages were set at 4 ℃ for 12 h, due to the maximal expression of *LaNHR2B* under these conditions (as described above). The result is shown in Fig. 4 revealed that the expression quantity of *LaNHR2B* was significantly higher at four stages after low temperature induction than that of controls without treatment, including the stages of radicle protrusion, radicle protraction, hypocotyl protrusion and three-leaf stage. But there were differences in the expression quantities amongst different developmental stages, being extremely high expression levels at the stages of radicle protrusion and radicle protraction, there was no significant difference between them. However there was a slightly decreased up-regulation at the hypocotyl protrusion stage compared with the above two stages, and an obviously decreased amplitude at the three-leaf stage compared with the above three stages, even still maintaining a certain high degree of up-regulated expression.

Combined with the cold tolerance changes of *L. apetalum* seedlings during growth, we have shown that the changing trend of cold induced up-regulation of *LaNHR2B* gene expression was consistent with the changing trend of the cold tolerance of the seedlings, implying that there is a certain correlation between both. *LaNHR2B* may be one of the important regulators in the cold signaling pathways of *L. apetalum*, playing an important function in low temperature resistance at seedling stages. Currently, it is acknowledged that there are two kinds of cold signaling pathways, namely, ABA-dependent and ABA-independent pathways[25-26]. In order to explore the function of *LaNHR2B* in cold signaling pathways of *L. apetalum* seedlings, we further determined exogenous ABA induced *LaNHR2B* gene expression in this study.

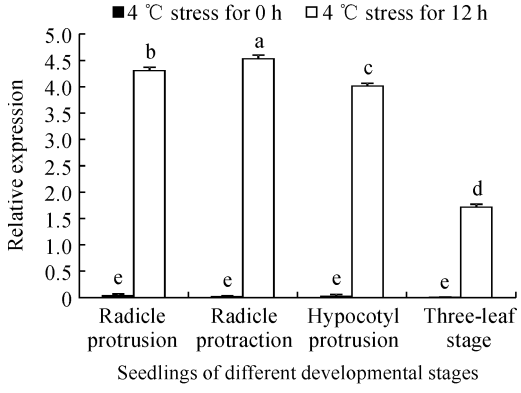


Fig. 4 Expression analysis of *LaNHR2B* following low temperature stress at different developmental stages of *L. apetalum*

2.4 Exogenous ABA treatment significantly induced up-regulated expression of *LaNHR2B* in *L. apetalum* seedlings

Relative quantitative analysis of *LaNHR2B* gene expression was performed on *L. apetalum* seedlings treated with ABA (100 μmol · L^{-1}) for 1 h and 6 h (Fig. 5). The results showed that *LaNHR2B* expression was significantly up-regulated after ABA treatment for only 1 h, and doubled in quantity after 6 h treatment. Considering that ABA treatment can usually induce the expression of stress resistance genes, the above results further increased the possibility that *LaNHR2B* is associated with cold resistance of *L. apetalum*, and proved that *LaNHR2B* may play an important role in an ABA-dependent cold signaling pathway.

2.5 *LaNHR2B* over-expression significantly enhanced the low temperature tolerance of *Arabidopsis thaliana* seedlings

Arabidopsis transgenic lines overexpressing

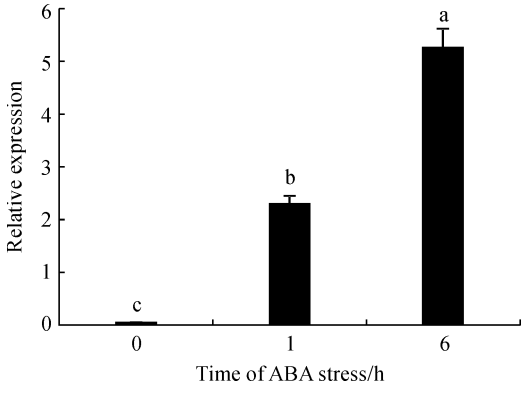


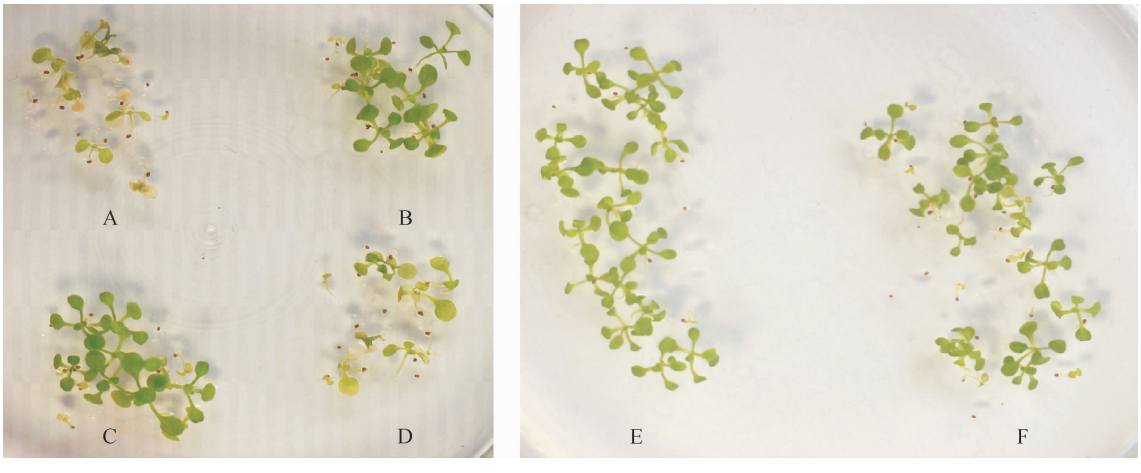
Fig. 5 Expression profile analysis of *LaNHR2B* in response to ABA stress in *L. apetalum* seedlings with qRT-PCR

LaNHR2B were also generated to investigate its function on the resistance to low temperature. The preparation and screening of *Arabidopsis* transgenic lines of *LaNHR2B* are described in Online Resources 1—3. The cold tolerance of T_2 seedlings at different developmental stages was subsequently investigated and the results are shown in Fig. 6. Wild type *Arabidopsis* seedlings without low temperature induction were treated at −5 ℃ for 2 h, then, cultivated at 4 ℃. Almost all the seedlings were dead after 5 days at 21 ℃ (Fig. 6 A, D), but the transformed seedlings grew well after the same treatment (Fig. 6 B, C). The wild type and transformed *Arabidopsis thaliana* seedlings after low temperature induction also grew well after the same treatment (Fig. 6 E, F), showing that *LaNHR2B* transgenic plants have stronger cold resistance, whether treated with cold induction or not. Therefore, constitutive *LaNHR2B* over-expression substantially improved the cold resistance of transgenic *Arabidopsis thaliana* seedlings, its effect can be equated to cold acclimatization training. It is well known that cold acclimation can greatly increase the cold resistance capability of plants. Here, the heterologous expression of *LaNHR2B* makes the *A. thaliana* seedlings without cold acclimatization to have a cold resistance capability equal to that with cold acclimatization, implying that *LaNHR2B* can really enhance the cold resistance function of *A. thaliana*.

The above results mainly support a conclusion that *LaNHR2B* plays an important role in cold resistance. Firstly, the cold resistance trend of *L. apetalum* seedlings at different growth stages was consistent with the expression trend of *LaNHR2B* being up-regulated by low temperature. Secondly, ABA treatment induced a significant up-regulated expression of this gene. Finally, and also the most compelling evidence was that the cold resistance capability was significantly enhanced in *LaNHR2B* transgenic *A. thaliana*. Therefore, *LaNHR2B* may be an important gene functional in the cold resistance of *L. apetalum* during the processes of germination and seedling growth.

3 Discussion

Low temperature stress is an adverse environment often encountered by early spring ephemeral plants. Adaptation to the microthermal climate in early spring is one pre-requisite characteristic of early spring ephemeral plants for seed germination and seedling growth[27]. *L. apetalum* in northern Xinjiang of China belongs to the group of typical early spring ephemeral plants, and can tolerate the cold stressing to germinate and grow, by having suitable response mechanisms to low temperature[28-29].



A—D is *A. thaliana* seedlings without cold induction. E—F is *A. thaliana* seedlings with cold induction. A, D and F are wild type *A. thaliana* seedlings. B, C and E are *LaNHR2B* over-expressing transgenic *A. thaliana* seedlings

Fig. 6 The tolerance to low temperature stress of *LaNHR2B* over-expressing *Arabidopsis* seedlings of T_2 generation

Scientific studies have shown that cold induction, namely, cold acclimatization, can improve the cold resistance of plants. At present, a series of genes related to cold acclimation signaling pathways have been obtained through all kind methods of screening and cloning functional genes. Meanwhile, the cold tolerance of plants has been greatly improved through transforming the key genes involved in the cold acclimation process[30-37].

LaNHR2B is a new cold induced gene, it is closely related to the expression of *LaNHR2B* gene and cold tolerance of *L. apetalum* and transgenic *A. thaliana*. The homologous gene *AtNHR2B* of *LaNHR2B* can enhance plant resistance to bacteria and pathogens, whether *LaNHR2B* also has the ability of disease resistance? It is a long way to go to study how to enhance tolerance of a variable splicing gene *LaNHR2B* for biotic and abiotic stresses, and what the molecular mechanism of the gene is in the process of cold tolerance in plants for further study.

After the treatment by 4 ℃, *L. apetalum* seedlings could tolerate the temperature below the 0 ℃. The tolerance to low temperature gives *L. apetalum* the ability to survive in frigid zone. What with the research concerned are the functional genes which underlay the ability of *L. apetalum* to adapt to the environment. *LaNHR2B* is a cold inducible expression gene and its high level expression could increase the cold tolerance of its transgenic *Arabidopsis*. It is just what we concern with results in this study showed that *LaNHR2B* expression can be up-regulated by cold induction in *L. apetalum* during the seed germination stage, implying that this gene plays an important role in low temperature tolerance. This speculation is also verified by results of the stress hormone ABA, which participates in ABA-dependent signal transduction pathways of cold stress, and further confirmed by the significantly enhanced cold resistance capability of *LaNHR2B* overexpressing transgenic *A. thaliana*. As the temperature is gradually increased during the mature seedling stage, the low temperature stress damage is consequently reduced, and *LaNHR2B* gene expression high for resistance to low temperature is no longer required, especially for the flowering stage of *L. apetalum* *LaNHR2B* gene expression is gradually weakened until there is no response to low temperature induction.

In conclusion, the *LaNHR2B* gene of *L. apetalum* is likely to play an important role in cold tolerance of *L. apetalum* seedlings. Given that it can significantly enhanced the cold resistance of *A. thaliana*, this gene is considered to have the potential of a cold-resistant gene, at least in the transgenic breeding of cruciferous crops; however its actual effect, specific function and mechanism of action are worthy to be further studied.

After analyzing *LaNHR2B* gene expression of *L. apetalum* seedlings during low temperature stress, and the effects of *LaNHR2B* over-expression on the cold resistance of *A. thaliana*, it was found that *LaNHR2B* expression was different at the different growth stages of *L. apetalum*, and was only largely expressed at the seedling stage, beneficial to the adaptation of seedlings to frigid weather in early spring. The ability of tolerate low temperature was higher significantly in *A. thaliana* overexpressing *LaNHR2B* than wild type, proving that *LaNHR2B* is a functional gene of plants tolerating cold for germination. Moreover, whether it has direct correlation with cold resistance of *L. apetalum* during the processes of germination and seedling growth, and how it works, still need to be further explored in our future work.

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