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Promoter Analysis of Cold-responsive (COR) Gene from *Capsella bursa-pastoris* and Expression Character in Response to Low Temperature

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Abstract

Capsella bursa-pastoris is well adapted to different environments, especially low temperature suggesting that it possesses relatively strong tolerance against cold stress. Cold tolerance ability is associated with the accumulation of several cold-induced transcripts. By using a cloning technology, we isolated and sequenced the corresponding COR15 gene from *C. bursa-pastoris*. The putative promoter of CbCOR15 contains *cis-acting* elements that have been shown to mediate expression of cold-responsive genes of *Arabidopsis thaliana*. In this study, we analyzed the *CbCOR15a* and *CbCOR15b* promoter sequence shown that there are two or one *cis-acting* elements in between -305 and -149, or -207 and -128, respectively. Deletion sequence of CbCOR15 promoter region were fused to the GUS reporter gene and introduced into *A. thaliana* plants. The analysis of independent transgenic lines using histochemical GUS staining method indicated that the *CbCOR15a* promoter sequences from -305 to -149, *CbCOR15b* promoter sequences from -207 to -128 is as necessary for gene expression of low temperature regulated. *CbCOR15* promoter displayed a slight activity in seedlings, mature rosette leaves, stem leaves, flowers and mature siliques, and after cold treatment, the promoter activity increased greatly in all tissues. *CbCOR15a* being more sensitive to cold than *CbCOR15b*. After cold treatment, the *AtCOR15* promoter activity was greatly induced in leaves, flowers and siliques, but not roots. In comparison with character of *AtCOR15* promoter indicated that *CbCOR15* being more sensitive to cold. The character may be related to strong cold acclimation ability of *C. bursa-pastoris.* © 2016 Friends Science Publishers

Keywords: *Capsella bursa-pastoris*; Cold Regulated (COR) gene; Promoter; Histochemical staining experiments

Introduction

Chilling stress has negative impact on plant growth and development. Based on plant tolerance, cold can be divided into freezing (<0°C) and chilling (0-15°C) temperatures (Zhou *et al*., 2011). During the evolution history, plants have developed some adaptability to resist cold stress. These abilities known as cold acclimation which increase the response of tolerance to low non-freezing temperatures (Thomashow, 1999) and modify variety of carbohydrate composition including in lipid, protein during cold acclimation (Steponkus and Lynch, 1989; Guy, 1990; Thomashow, 1990). Among these adaptive changes, Cold Regulated (COR) and Dehydrin (DHN) proteins have a key structural role to stabilize membrane structure (Thomashow 1999; Koag *et al.*, 2009) or these proteins may have important roles in the acclimation process (Thomashow, 1990). In *Arabidopsis thaliana*, five pairs *COR* genes have been found, including *COR6.6* (Kurkela and Borg-Franck, 1992), *COR15* (Lin and Thomashow, 1992; Wilhelm and Thomashow, 1993), *COR47* (Gilmour *et al*., 1992), *COR78* (Horvath *et al*., 1993) and *COR413* (Hajela *et al*., 1990). Each gene pair has a high identity of nucleic acid sequence and is physically tandem linked array. In addition, the individual members of each gene pair showed some differences in gene regulation level. Among these proteins, COR15 was the first to be found and provides the first direct evidence for cold tolerance which is associated with constitutive and continued expression of cold-induced genes. The COR15a and COR15b from *A. thaliana* is a chloroplast- targeted protein, its molecular weight is 15 kDa (Wilhelm and Thomashow, 1993). The *COR15a* and *COR15b* homologs gene are present in tandem on the chromosome 2, but their expression level response to cold stresses is differ temporally or spatially (Wilhelm and Thomashow, 1993). Similarly, *COR15* two copies have

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been discovered in the *Brassica napus* species (Weretilnyk *et al*., 1993), *Hordeum vulgare* (Cattivelli and Bartels, 1990; Crosatti *et al*., 1996), *Triticum aestivum* (Shimamura *et al*., 2006), *Chorispora bungeana* (Si *et al*., 2009), *Cucurbita moschat* and *Citrullus lanatus* (Kang *et al*., 2009), *B. oleracea* (Hadi *et al*., 2011), *Capsella bursa pastoris* (Zhou *et al*., 2012; Wu *et al*., 2012). COR15a and COR15b protein are necessary for Arabidopsis to attain comprehensive freezing tolerance during cold acclimation; its function in *A. thaliana* seems to protect chloroplast membranes through binding and folding during freezing (Artus *et al*., 1996; Thalhammer *et al*., 2014). After exposing plants to low temperature, a number of *COR* genes transcript levels increase dramatically and remain elevated for as long as remain plants in the low temperature environments. In spite of this, studies have shown that expression changes indeed occur during cold acclimation in plants (Guy, 1990). But character of gene expression is different in various plants specific, such as, the *COR15a* gene promoter is inactive (or only low active) in many plants grown organs or tissues, exception anther, at 22°C temperatures using gene fusion experiments. When plant moved to cold temperature, these genes become activated in grown tissues and organs, exception in the roots (Baker *et al*., 1994). In addition, the regulated pattern of *CORl5b* and *CORl5a* is different (Wilhelm and Thomashow, 1993). *BnCOR25* gene from *B. napus* was weakly expressed in leaves or roots and strong expressed in flowers, stems, hypocotyls and cotyledons using RT-PCR analysis. *BnCOR25* gene transcripts were highly accumulated in roots during cold treatment (Chen *et al*., 2011).

In comparison with *A. thaliana*, *C. bursa-pastoris* possesses relatively strong tolerance ability to cold. However, cold tolerance ability associated with the accumulation of COR15 cold-induced transcript is not well understood. In *C. bursa-pastoris*, some COR genes, such as *CbCOR15a*, *CbCOR15b* have been characterized and are found to be low temperature induced gene (Wu *et al*., 2012; Zhou *et al*., 2012). In order to clarify the possible mechanisms involved in cold expression regulation of COR gene, which demonstrated the low temperature induced of *COR15a/b* gene requires the CRT/DRE/LTRE element. We investigated the induced and tissue specific expression of *COR15a/b* from *C. bursa-pastoris* using the promoter-reporter gene (GUS) fusions in transgenic *A. thaliana*. The results show that the CRT/DRE/LTRE element is a key element of *CbCOR15a* and *CbCOR15b* to cold response. The results confirm that the *cis-acing* regulatory element is essential to adjust the expression of cold-regulated gene. Further, to investigate the different transcript regulation of *COR15a/b* from *A. thaliana* or *C. bursa-pastoris*, we carried out some transgenic lines using *COR15a/b* promoter from *A. thaliana* or *C. bursa-pastoris* using b-glucuronidase (GUS) fusions, respectively. Under the cold treatments, the increase in GUS activity was found in transgenic plant lines, which indicate that cold induction of *COR15a* and *COR15b* from *C. bursa-pastoris* occurs at the transcriptional level.

Materials and Methods

Plants Growth and Treatments with Low Temperature

The generations of *C. bursa-pastoris* seeds was purchased from Shanghai Baiyulan Vegetable Seed Ltd. and were grown in the greenhouse. The conditions of greenhouse contain 16-h-light/8-h-dark cycle long photoperiod, 140 to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ cool-white fluorescent illumination and 70% relative humidity at 22ºC. Before germination, the seeds of *A. thaliana* (ecotype Columbia) were treated for 3 days at 4ºC. The seeds were grown in growth chambers under at $100 \mu mol \cdot m^{-2} \cdot s^{-1}$ constant light and 50% relative humidity. To imposelow temperature treatments, *C. bursa-pastoris* and *A. thaliana* seedlings were grown on MS solidified medium at 22°C, 2-week-old seedlings were transferred to a cold room (4°C; 8/16 h light/dark) and plants collected after 2 d. Unless otherwise specified, three seeding constituted experimental unit.

Construction of the Plant Expression Vectors and Transformation of Plant

The total genomic DNA from *C. bursa-pastoris* and *A. thaliana* was isolated using the improve CTAB program (Murray and Thompson, 1980). The *Cbcor15a* or *Cbcor15b* promoter sequences were reported in our previous study reports (Wu *et al*., 2012; Zhou *et al*., 2012). The *AtCOR15a* or *AtCOR15b* promoter sequences from *A. thaliana* were reported in NCBI databases under gene number At2g42540 (*AtCOR15a*) or At2g42520 (*AtCOR15b*). To clone the *CbCOR15a*, *CbCOR15b*, *AtCOR15a*, *AtCOR15b* promoter sequence, four pair specific primers listed in Table 1 were used to amplify the four promoter sequence from *C. bursa-pastoris* or *A. thaliana* by PCR. To facilitate cloning into the vector pCAMBIA1301, a restriction enzyme site of *Pst*I was incorporated into a forward primer, whereas a *Bgl*II site was flanked with a reverse primer. The PCR was performed using genomic deoxyribonucleic acid (DNA) isolated from young leaves of *C. bursa-pastoris* or *A. thaliana* as template under the following condition: The template was denatured 5 min at 94°C followed by amplification 30 cycles, 1 min at 94°C, 30 s at 60°C, and 1 min at 72°C for program followed by 10 min at 72°C. After digestion with *Pst*I and *Bgl*II, the CaMV35S promoter of pCAMBIA1301 (CAMBIA, Australia) be replaced by the amplified fragment. The plasmid was named pCbCOR15aP::GUS, pCbCOR15bP::GUS, pAtCOR15aP: GUS or pAtCOR15bP: GUS. Construction of vector model is shown in Fig. 1. To clone the -459 to +97 bp, -335 bp to +97 bp, -149 to +97 bp fragments of CbCOR15a or -207 to +97 bp, -128 to +97 bp fragments from CbCOR15b, five specific primers listed in Table 1 were used to amplify the

five sequences by PCR. To facilitate cloning into the vector pCAMBIA1301, a restriction enzyme site of *Pst*I was incorporated into a forward primer. Construction diagram is shown in Fig. 1. Agrobacterium LBA4404 strain was transformed with these plant expression vectors by the freeze-thaw program and *A. thaliana* transformation by the floral dip program (Clough and Bent, 1998).

Molecular Analysis of Transgenic Plants

The transgenic T_0 seeds were selected on hygromycin (20 mg L^{-1}) and further were tested using PCR program to detect the specific fragment of the various constructs vector. After genomic DNA of leave was isolated, the gene-specific primers, hygromycin-specific primers or GUS-specific primers were used to amplify a specific fragment, hygromycin gene fragment GUS fragment, respectively, which demonstrated the presence of the *CbCOR15a* or *CbCOR15b* fragments, the hygromycin gene or GUS gene. These primers were listed in table 1.

RT-PCR Method

Total RNA was isolated from leave tissues of 200 mg, treated with the deoxyribonuclease I to remove any DNA contamination, was reversely transcribed using Plant RNA Mini Kit (Watson Biotechnologies, Inc, China), DNase I Kit (Promega, Madison, WI, USA), or PrimeScript® RT Master Mix Perfect Real Time Kit (TaKaRa, China), respectively. For PCR amplifications, first cDNA strand was used as template using specific GUS primers described in table 1. The *A. thaliana* tublin gene (Genbank number: M17189) was used as the control.

Histochemical Staining

GUS staining program was carried out as described by Jefferson *et al*. (1987). Briefly, various tissues were incubated in staining solution and vacuum infiltrated for 15 min. The staining buffer containing 7.2 mM ethylene diamine tetraacetic acid (EDTA), 3 mM potassium ferrocyanide, 42.3 mM sodium phosphate, 57.7 mM disodium phosphate, 0.005% Triton X-100 and 0.075% 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc). Afterward the tube was put in incutabor overnight at 37°C and de-stained using 75% ethanol for five times at 60°C until their chlorophyll were clear. The GUS activity was observed using microscope (Zeiss Scope A1, Zeiss, Germany).

Statistical Analysis

The effects of the tissue type and treatments were analyzed with the analysis of variance (ANOVA). P values < 0.05 were considered significant.

Result

Sequence Analysis of CbCOR15a and CbCOR15b Promoters

The gene expression was driven by its promoter. There weresome regulatory elements in their promoter's sequences that control these gene expressions. Several genes to the freezing- or cold-induced responsive contain one or more *cis-element* in their promoter region (Wang and Hua, 2009). These elements contain C-repeat element (CRT), DRE (dehydration responsive element) or LTRE (low temperature responsive element) (Baker *et al*., 1994). The CCGAC sequence, conserved core sequence is the binding region to the cold specific CBFs/DREBs transcriptional activators (Stockinger *et al*., 1997) that enhance the expression level of cold responsive COR genes which subsequently increase plants cold resistance (Mantas *et al*., 2010). To examined whether *CbCOR15a* and *CbCOR15b* gene is induced by low temperature through the CRT/DRE/LTRE element in the promoter, we analysis the *cis-element* by the PLANTCARE database (http:// bioinformatics.psb.ugent.be/webtools/plantcare/html/). These results showed that *CbCOR15a* and *CbCOR15b* promoter sequences contain two or one CRT/DRE/LTRE elements, respectively. We analyzed a series of transgenic positive plants to test whether, this CRT/DRE/LTRE element is also involved in low temperature induction of *CbCOR15a* and *CbCOR15b*. Lines CbCOR15aP, CbCOR15aP3, CbCOR15aP2 and CbCOR15aP1 contain -939/+97, -459/+97, -335/+97 and -149/+97 of the *CbCOR15a* promoter sequences from transcription initiation site, respectively. Lines CbCOR15bP, CbCOR15bP2 and CbCOR15bP1 contain -1037/+97, -207/+97 and -128/+97 of the *CbCOR15b* promoter sequences from transcription initiation site, respectively (Fig. 2). These plants were grown on plates for about 14 days at 22°C, and then seedlings were shifted to 4°C. To GUS activity sufficiently detection, we stain the tissue in a longer induction time (2 days). There was no GUS activity in any of these lines grown at 22°C. However, CbCOR15aP, CbCOR15aP2, CbCOR15aP3 and CbCOR15bP and CbCOR15bP2, but not CbCOR15aP1 and CbCOR15bP1, showed GUS activities at 4ºC (Fig. 3). The sequence of CbCOR15aP and CbCOR15aP3 contain two CRT/DRE/LTRE elements, the sequence of CbCOR15bP and CbCOR15bP2 contain one CRT/DRE/LTRE element, respectively. CbCOR15aP1 and CbCOR15bP1 do not contain any CRT/DRE/LTRE element. CbCOR15aP2, CbCOR15aP3 contain one CRT/DRE/LTRE element, both are not active at room temperature, but active at at low temperature. CbCOR15aP contain two CRT/DRE/LTRE elements, which are less active at room temperature, but activation was induced by low temperature. The active CbCOR15aP was high than CbCOR15aP2 and CbCOR15aP3 in low temperature. These

Table 1: Primers used in this work

Primers	Sequence(5'---3')
CbaP-Pst	TActgcagGTGAACATGGGGAGAGTGTA
CbaP-Bgl	CGagatctACCATCGCCATGAGTTATCTCTTTTAAGTTTTTTTGGGTGCA
CbbP-Pst	CGctgcagGATCAGTCTGTTGTAATGA
CbbP-Bgl	CGagatctACCATCGCCATGAGAGATATG
Atap-Sac	CGCTGCAACTTTTCAGAGCTCGC
Atap-Nco	CCCATGGGAGAGAGATCTTTAAGATGTGA
Atbp-Sac	GTTTAAGTGCTTGAAATTGAGCTCGC
Atbp-Nco	CCCATGGGAGGACTCTTAAAGATGTT
CbaP1-Sac	GCgagctcCACGTGGCCAAAA
CbaP2-Sac	GCgagctcCACGTGAAGAGAATGAG
CbaP3-Sac	GCgagctcATTTACGGACCAATGTT
CbbP1-Sac	GCgagctcAACGAACAAAAACTCTT
CbbP2-Sac	GCgagctcTGGCCGACCTCTTTT
GUS-F	GCTCTACACCACGCCGAACACCTG
GUS-R	TCTTCAGCGTAAGGGTAATGCGAGGTA
tublin-F	ATGCGTGAGATTCTTCACATCC
tublin-R	TGGGTACTCTTCACGGATCTTAG

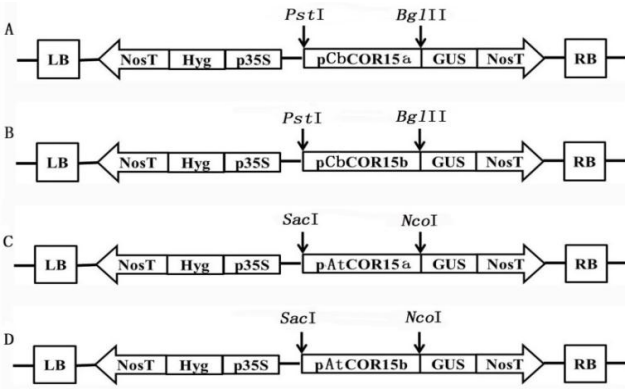

Fig. 1: The construction model of plant expression vectors
A: Construction model of CbaP::GUS, B: Construction model of CbbP::GUS, C: Construction model of AtaP::GUS, D: Construction model of AtbP::GUS

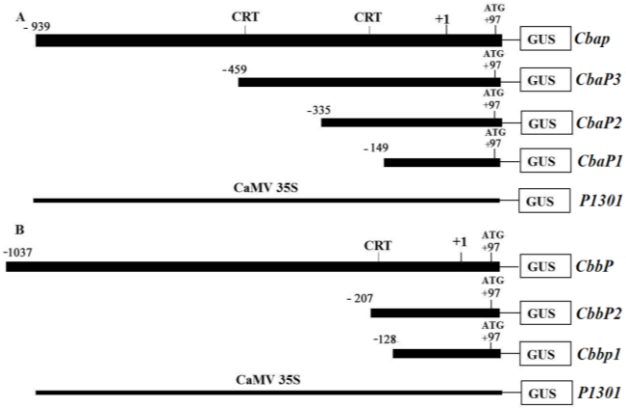

Fig. 2: Regions of *corl5* included in the *corl5a-gus* fusions. A. The *cor15a* sequences that were fused to *gus* are indicated. B. The *cor15b* sequences that were fused to *gus* are indicated. The numbers refer to the nucleotide sequence. The locations of the CRT, transcription initiation site and translation initiation site (AUG) are shown

results show that the CRT/DRE/LTRE element is a key element of *CbCOR15a* and *CbCOR15b* cold response.

Comparing Analysis of COR15 Promoters from *C. bursa-pastoris* and *A. thaliana*

Previous experiments about histochemical staining indicated that the *COR15a* promoter at normal temperature can be stained deeply blue only in anther of plants, in contrast, in the leaves, at cold-treated can be stained deeply blue in stem, leave and apical meristem. However, the roots and mature ovules of these seedlings were detected no blue (Baker *et al*., 1994). The nucleic acid sequence of *COR15b* has a high degree identity to *COR15b*, but different in regulation (Wilhelm and Thomashow, 1993). In the study, four expression vectors, including CbCOR15aP::GUS, CbCOR15bP::GUS, AtCOR15aP:: GUS and AtCOR15bP::GUS, were constructed and transferred into *A. thaliana* in order to analyze the promoter tissue-specific expression activity in normal temperature or cold treatment. Seeds of T_2 lines (transgenic CbCOR15aP, CbCOR15bP, AtCOR15aP and AtCOR15bP positive Arabidopsis plants) were plants in MS_0 solid medium. For the histochemical GUS staining assay, the 6-week-old seedlings were collected at 22ºC and 4ºC treatment for 2 d, respectively. The results showed that *CbCOR15a* promoter displayed a slight activity at 22ºC in seedlings, mature rosette leaves, stem leaves, flowers and mature siliques, and after 4ºC cold treatment, the activity increased greatly in all tissues. *CbCOR15b* promoter was detected inactive at 22ºC, but after 4ºC cold treatment, the activity was greatly induced in leaves, flowers, siliques and roots. The active of *AtCOR15b* promoters are same to *AtCOR15a*, after 4ºC cold treatment, the promoter activity was no induced in roots (Fig. 4). This result is consistent with RT-PCR analysis (Zhou *et al*., 2012). At the same time the transcript levels of the GUS genes were determined in transgenic plants using low temperature treatment and control by

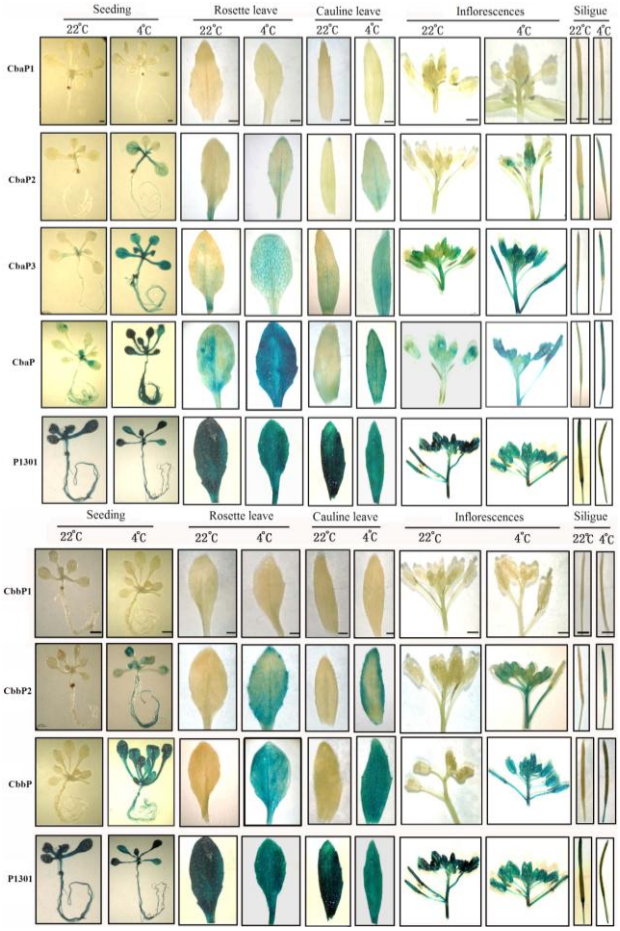

Fig. 3: Tissue-specific expression of the various *gus* chimeric gene in control and cold-treated plants. A. Plants transformed with the -930/+97 (CbaP), -459/+97 (CbaP3), -335/+97 (CbaP2) and -149/+97 (CbaP1) *cor15a-gus* chimeric gene were grown under different conditions and subjected to histochemical staining to localize GUS activity. B. Plants transformed with the -1037/+97 (CbbP), -207/+97 (CbbP2) and -128/+97 (CbbP1) *cor15b-gus* chimeric gene were grown under different conditions and subjected to histochemical staining to localize GUS activity. Transgenic plants grown for 21 days at 22°C then cold-treated at 4°C for 1 day. Transgenic plants carrying the CaMV 35S-gus fusion (CaMV 35S) as control. The scale bar represents 2 mm

RT-PCR method, the result is shown in Fig. 4. The results showed the GUS fusions containing AtCOR15aP, AtCOR15bP, CbCOR15aP and CbCOR15bP were strongly responsive to cold. To cold-treated plants, GUS transcripts accumulation was high than the control plants. In contrast, the lever of GUS expression is higher containing CbCOR15aP or CbCOR15bP than containing AtCOR15aP or AtCOR15bP (Fig. 5). The result shown that the *COR15* genes differ in their cold sensitivity: *COR15* from *C. bursa-pastoris* being more sensitive to cold than *COR15* from *A. thaliana; COR15b* from *C. bursa-pastoris* is being more sensitive to cold than *COR15a.*

Discussion

The COR gene has been described from many plants, little is known about the gene expression patter from various species and its multiple members. The promoters of many COR genes such as *COR15a/b* (Wilhelm and Thomashow, 1993; Baker *et al.*, 1994), *RAB18* (Lång and Palva, 1992), *COR78* (Yamaguchi-Shinozaki and Shinozaki, 1993), *KIN1/2* (Kurkela and Franck, 1990; Kurkela and Borg-Franck, 1992) from *A. thaliana* contain highly conserved *cis-elements* such as CRT, DRE or LTRE element (Stockinger *et al.*, 1997), and consequently, expressions of COR genes are mediated by cold. Under cold stress, the upstream regulators named inducers of CBF expression (ICE) act as a positive regulator of CBFs, while CBFs regulate the cold responsive (COR) genes by binding to the CRT/DRE element (Lissarre *et al.*, 2010). Our promoter-fusion experiments found that *CbCOR15a* contain two *cis-acting* CRT/DRE elements between nucleotides -939 and +97 and *CbCOR15b* contain one *cis-acting* CRT/DRE element between nucleotides -1037 and +97 that can drive strong cold regulated gene expression. This evidence confirms that *CbCOR15* genes contain a cold-regulatory element in their promoter. Previous study shown expression in almost all plants tissues about *AtCOR78-gus* (Horvath *et al*., 1993) and *AtRD29a-gus* gene (Yamaguchi-Shinozaki and Shinozaki, 1993) was either very low or undetectable in non-acclimated condition. However, relatively high expression in the *AtCOR15a-gus* can be detected in the anther of control plants. Our result showed relatively high expression levels of the *CbCOR15a-gus* that can be detected in the anthers at 22°C. The molecular basis for lack of expression of the *COR* promoter in some tissues at normal growth temperature is unclean. In addition, these results indicated that the expression level of various *COR* gene is up-regulated by cold in most plant tissues, but not whole. Such as, after 4°C cold treatment, the activity of *AtCOR15a* promoter was greatly induced in leaves, flowers and siliques, but not roots (Baker *et al*., 1994). But, the activity of *AtCOR78* promoter was greatly induced in the flower sepals, leaves, stems, roots of plants by cold-treated. However, no activity was observed in some plants tissue, such as stigmas, anthers, styles of flowers or ovaries (Horvath *et al*., 1993). Our study showed that activity of *CbCOR15* promoter can be induced greatly in all tissues, which includes seedlings, rosette leaves, cauline leaves, inflorescence and siliques. The results indicated that different *cis-acting* elements are responsible for the different expression patterns under conditions of normal or low temperature.

The main function of COR15a protein in plant is stabilizing thylakoid membranes against freezing-induced damage, and as a result, constitutive overexpression of the

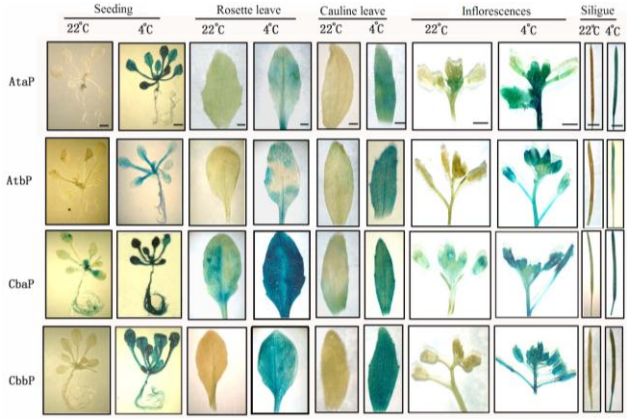

Fig. 4: Tissue-specific expression of the four *gus* chimeric gene in control and cold-treated plants. Plants transformed with the -930/+97 (CbaP), -1037/+97 (CbbP), -335/+97 (AtaP) and -149/+97 (AtbP) *cor15-gus* chimeric gene were grown under different conditions and subjected to histochemical staining to localize GUS activity. Transgenic plants grown for 21 days at 22℃ then cold-treated at 4℃ for 2 day. Transgenic plants carrying the CaMV 35S-gus fusion (CaMV 35S) as control. The scale bar represents 2 mm

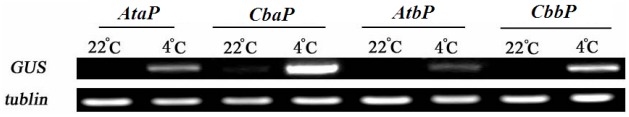

Fig. 5: Expression *of corl5-gus* chimeric genes in response to low temperature using RT-PCR analysis. Transgenic plants carrying the indicated *cor15-gus* fusions (see Fig. 4) were grown in pots at control temperature (22°C) and then treated at low temperature (4°C) for 1 day. Total RNA was isolated from the transgenic *Arabidopsis* leaves

COR15 gene resulted in a significant increase in cell freezing-tolerance of plant (Artus *et al*., 1996). It was indicated that the accumulation of COR proteins is related with plant frost resistance. Under field conditions, plant accumulated more COR proteins in winter than spring (Grossi *et al*., 1998). So COR15 are necessary for plant to attain full freezing tolerance during cold acclimation. Our study showed that activity of *CbCOR15* can be induced greatly in all tissues by cold, the CbCOR15 gene plays a positive role in conferring freezing/cold tolerance, suggesting that it may function in *C. bursa-pastoris* response and tolerance to cold stress. Our findings for increasing the freezing tolerance of crop species have potential application using transgenic technology. It is favorable to drive those genes expression by use plant endogenous stress-inducible promoter instead of the 35S promoter to prevent the 35S promoter leading to side effect, such as dwarfism or delay flowering. The cold inductive activities of *CbCOR15a/b* promoter provide a potential element in improvement of cold resistance crops using transgenic technology.

Conclusion

The ability of plants to tolerate cold stress is associated with the expression of COR gene. The *cis-acting* element is necessary for cold-regulated gene expression. This study showed that the 5' region of *CbCOR15a* and *CbCOR15b* contains two and one *cis-acting* element, respectively. The *CbCOR15* promoter displayed a slight activity in seedlings, mature rosette leaves, stem leaves, flowers and mature siliques, and after cold treatment, the promoter activity increased greatly in all tissues. But after cold treatment, the *AtCOR15* promoter activity was greatly induced in leaves, flowers and siliques, but not in roots. The *C. bursa-pastoris COR15* is more sensitive to cold than *A. thaliana COR15* and *C. bursa-pastoris COR15b* is more sensitive to cold than *C. bursa-pastoris COR15a.* The character may be related to strong cold acclimation is ability of *C. bursa-pastoris.*

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References

Artus, N.N., M, Uemura, P.L. Steponkus, S.J. Gilmour, C. Lin and M.F. Thomashow, 1996. Constitutive expression of the cold regulated *Arabidopsis thaliana* COR15a gene affects both chloroplast and protoplast freezing tolerance. *Proc. Natl. Acad. Sci. USA*, 93: 13404–13409

Baker, S.S., K.S. Wilhelm and M.F. Thomashow, 1994. The 5'-region of *Arabidopsis thaliana* COR15a has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. *Plant Mol. Biol.*, 24: 701–713

Cattivelli, L. and D. Bartels, 1990. Molecular cloning and characterization of cold-regulated genes in barley. *Plant Physiol.*, 93: 1504–1510

Chen, L., H. Zhong, F. Ren, Q.Q. Guo, X.P. Hu and X.B. Li, 2011. A novel cold-regulated gene, COR25, of Brassica napus is involved in plant response and tolerance to cold stress. *Plant Cell Rep.*, 30: 463–471

Clough, S.J. and A.F. Bent, 1998. Floral dip: a simplified method for *Agrobacterium*- mediated transformation of *Arabidopsis thaliana*. *Plant J.*, 16: 735–743

Crosatti, C., E. Nevo, A.M. Stanca and L. Cattivelli, 1996. Genetic analysis of the accumulation of COR14 proteins in wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare*) barley. *Theor. Appl. Genet.*, 93: 975–981

Gilmour, S.J., N.N. Artus and M.F. Thomashow, 1992. cDNA sequence analysis and expression of two cold-regulated genes of *Arabidopsis thaliana*. *Plant Mol. Biol.*, 18: 13–21

Grossi, M., E. Giorni, F. Rizza, A.M. Stanca and L. Cattivelli, 1998. Wild and cultivated barleys show differences in the expression pattern of a cold-regulated gene family under different light and temperature conditions. *Plant Mol. Biol.*, 38: 1061–1069

Guy, C.L., 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 41: 187–223

Hadi, F., M. Gilpin and M.P. Fuller, 2011. Identification and expression analysis of CBF/DREB1 and COR15 genes in mutants of *Brassica oleracea* var. botrytis with enhanced proline production and frost resistance. *Plant Physiol. Biochem.*, 49: 1323–1332

Hajela, R.K., D.P. Horvath, S.J. Gilmour and M.F. Thomashow, 1990. Molecular cloning and expression of *cor* (cold regulated) genes in *Arabidopsis thaliana*. *Plant Physiol.*, 93: 1246–1252

Horvath, D.P., B.K. McLarney and M.F. Thomashow, 1993. Regulation of *Arabidopsis thaliana* L. (Heyn) *cor78* in response to low temperature. *Plant Physiol.*, 103: 1047–1053

Jefferson, R.A., T.A. Kavanagh and M.W. Bevan, 1987. GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.*, 6: 3901–3907

Kang, G.Z., Z.H. Zhu, T.C. Guo and J.P. Ren, 2009. Isolation and expression pattern of COR15b and KIN1 genes in watermelon and pumpkin. *Afr. J. Biotechnol.*, 8: 5666–5672

Koag, M.C., S. Wilkens, R.D. Fenton, J. Resnik, E. Vo and T.J. Close, 2009. The K-segment of maize DHN1 mediates binding to anionic phospholipid vesicles and concomitant structural changes. *Plant Physiol.*, 150: 1503–1514

Kurkela, S. and M. Borg-Franck, 1992. Structure and expression of *kin2*, one of two cold- and ABA-induced genes of *Arabidopsis thaliana*. *Plant Mol. Biol.*, 19: 689–692

Kurkela, S. and M. Franck, 1990. Cloning and characterization of a cold- and ABA-inducible *Arabidopsis* gene. *Plant Mol. Biol.*, 15: 137–144

Lång, V. and E.T. Palva, 1992. The expression of a rab-related gene, *rab 18*, is induced by abscisic acid during the cold acclimation process of *Arabidopsis thaliana* (L.) Heyn. *Plant Mol. Biol.*, 20: 951–962

Lin, C. and M.F. Thomashow, 1992. DNA sequence analysis of a complementary DNA for cold-regulated *Arabidopsis* gene cor15 and characterization of the COR15 polypeptide. *Plant Physiol.*, 99: 519–525

Lissarre, M., M. Ohta, A. Sato, and K. Miura, 2010. Cold-responsive gene regulation during cold acclimation in plants. *Plant Signal Behav.*, 5: 948–952

Mantas, S., H. Pekka and P.E. Tapio, 2010. Genes and gene regulation for low temperature tolerance. *In*: *Genes for Plant Abiotic Stress*, pp: 187–209. Mathew, A.J. and J.W. Andrew (eds.). Willey-Blackwell Publishing, UK

Murray, M.G. and W.F. Thompson, 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, 8: 4321–4325

Shimamura, C., R. Ohno, C. Nakamura and S. Takumi, 2006. Improvement of freezing tolerance in tobacco plants expressing a cold responsive and chloroplast- targeting protein WCOR15 of wheat. *J. Plant Physiol.*, 163: 213–219

Si, J., J.H. Wang, L.J. Zhang, H. Zhang, Y.J. Liu and L.Z. An, 2009. CbCOR15, a cold-regulated gene from alpine *Chorispora bungeana*, confers cold tolerance in transgenic tobacco. *J. Plant Biol.*, 52: 593–601

Steponkus, P.L. and D.V. Lynch, 1989. The behavior of large unilamellar vesicles of rye plasma membrane lipids during freeze/thaw-induced osmotic excursions. *Cryo. Lett.*, 10: 43–50

Stockinger, E.J., S.J. Gilmour and M.F. Thomashow, 1997. *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc. Natl. Acad. Sci. USA*, 94: 1035–1040

Thalhammer, A., G. Bryant, R. Sulpice and D.K. Hincha, 2014. Disordered cold regulated 15 proteins protect chloroplast membranes during freezing through binding and folding, but do not stabilize chloroplast enzymes *in vivo*. *Plant Physiol.*, 166: 190–201

Thomashow, M.F., 1990. Molecular genetics of cold acclimation in higher plants. *Adv. Genet.*, 28: 99–131

Thomashow, M.F., 1999. Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu. Rev. Plant Physiol.*, 50: 571–599

Wang, Y. and J. Hua, 2009. A moderate decrease in temperature induces COR15a expression through the CBF signaling cascade and enhances freezing tolerance. *Plant J.*, 60: 340–349

Weretilnyk, E., W. Orr, T.C. White, B. Iu and J. Singh, 1993. Characterization of three related low-temperature-regulated cDNAs from *Brassica napus*. *Plant Physiol.*, 101: 171–177

Wilhelm, K. and M.F. Thomashow, 1993. *Arabidopsis thaliana corl5b*, an apparent homologue of *corl5a*, is strongly responsive to cold and ABA, but not drought. *Plant Mol. Biol.*, 23: 1073–1077

Wu, L.H., M.Q. Zhou, C. Shen, J. Liang and J. Lin, 2012. Transgenic tobacco plants expressing *CbCOR15b* from *Capsella bursa-pastoris* show enhanced accumulation and tolerance to cold. *J. Plant Physiol.*, 169: 1408–1416

Yamaguchi-Shinozaki, K. and K. Shinozaki, 1993. Characterization of the expression of a desiccation-responsive *rd29* gene of *Arabidopsis thaliana* and analysis of its promoter in transgenic plants. *Mol. Gen. Genet.*, 236: 331–340

Zhou, M.Q., C. Shen, L.H. Wu, K.X. Tang and J. Lin, 2011. CBF-dependent signaling pathway: A key responder to low temperature stress in plants. *Crit. Rev. Biotechnol.*, 31: 186–192

Zhou, M.Q., L.H. Wu, J. Liang, C. Shen and J. Lin, 2012. Expression analysis and functional characterization of a novel cold-responsive gene CbCOR15a from *Capsella bursa-pastoris*. *Mol. Biol. Rep.*, 39: 5169–5179

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