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Isolation of genes similar to DREB1/CBF from sweet cherry (*Prunus avium* L.)

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Summary

The C-repeat (CRT) dehydration responsive element (CRT/DRE) is a *cis*-acting DNA element, which stimulates transcription in response to low temperature and drought stress. Recently, the DREB1/CBF (regulatory factor that binds to the CRT/DRE elements and promotes expression of multiple low-temperature-induced genes resulting in cold-acclimation) has been identified in *Arabidopsis thaliana* L. We have isolated a genomic clone from *Prunus avium* L. (sweet cherry) that was predicted to include three *DREB1/CBF*-like genes. Two (*D2A* and *D2B*) of the genes were deduced to have a complete open reading frame (ORF) and the other (*D2Ctr*: truncated *D2C*) was deduced to have an incomplete ORF. Each putative protein had an EREBP/AP2 DNA binding domain motif and a potential nuclear localization signal in the N-terminal region. Furthermore, the D2A and the D2B proteins had an acidic C-terminal region. The D2A and the D2B proteins were found to be 53 and 53.5% identical to DREB1B/CBF1, respectively. However, the EREBP/AP2 conserved domain of the three D2 proteins showed a high identity (74-79%) with that of each DREB1/CBF. In addition, the TATA-box and G-box sequences, and MYB- and MYC-recognition sites were predicted along with in the 5' other conserved sequences of all three genes. The expression of the *D2* genes was found to be induced by low temperature in *P. avium*.

Key Words: DREB1/CBF, low temperature, *Prunus avium* , sweet cherry.

Introduction

Freezing temperatures cause significant losses in plant productivity and thus limit the geographical distribution of plants. Under freezing conditions plants vary greatly in their ability to survive. Plants that originate from tropical regions have virtually no capacity to survive even under a slight freeze and, in contrast, plants from temperate regions generally survive under freezing temperatures (Thomashow, 1994). This freezing tolerance of plants in temperate regions is not constitutively induced but is induced in response to low, nonfreezing temperatures. This phenomenon is known as cold acclimation (Thomashow, 1994).

Low temperature induces biochemical and physiological changes and alternations in gene expression (Guy, 1990; Thomashow, 1994). Most genes induced by low temperature encode either newly discovered proteins such as the *A. thaliana* COR6.6/KIN2, COR15a and COR78/RD29a polypeptides or homologs to LEA (late-embryogenesis abundant) proteins such as *A. thaliana* COR47/RD17. These low-temperature-induced genes have been reported in many species (Thomashow, 1998). Especially, COR15a was reported to enhance freezing tolerance (Artus et al., 1996). Further analysis of these low-temperature-induced genes in *A. thaliana* led to the identification of a DNA regulatory element which was the C-repeat (CRT) dehydration responsive element (CRT/DRE). Yamaguchi-Shinozaki and Shinozaki (1994) first demonstrated that the CRT/DRE is a significant element for responsiveness to low temperature and dehydration. Subsequently, transcriptional activators that bind to the CRT/DRE were identified and were designated as either DREB1A (dehydration responsive element binding protein 1A), DREB1B and DREB1C (Liu et al., 1998) or CBF3 (C-repeat/DRE binding factor 3), CBF1 and CBF2 (Stockinger et al., 1997; Gilmour et al., 1998). Constitutive overexpression of the *DREB1A/CBF3* (Liu et al., 1998; Kasuga et al., 1999) or *DREB1B/CBF1* (Jaglo-Ottosen et al., 1998) genes in transgenic *A. thaliana* plants induces the expression of multiple CRT/DRE-containing genes without a low-temperature stimulus, and as a result, they are more tolerant to freezing than the control plants (Jaglo-Ottosen et al., 1998; Liu et al., 1998; Kasuga et al., 1999).

Sweet cherry (*Prunus avium* L.) growing in a temperate region also acquires freezing tolerance by cold-acclimation in the late fall and retains it during winter. Previously, in order to investigate freezing injury many studies that focused on the mechanism of ice formation

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(Ishikawa and Sakai, 1985; Warmund et al., 1988; Rodrigo, 2000), the monitoring of freezing temperature (Kader and Proebsting, 1992; Kang et al., 1998; Kaneda et al., 2001), and the system of peroxide scavenging (Nakagawara and Sagisaka, 1984; Kuroda and Sagisaka, 1998) in the flower buds of woody plants including fruit trees have been reported. Our purpose is to investigate the relationship between the tolerance to freezing and the expression of multiple low temperature responsive CRT/DRE-containing genes and their regulators in woody plants. In this study, we report the first isolation of the *DREB1/CBF* homologous genes from the sweet cherry.

Material and Methods

Amplification and sequencing of a genomic DNA fragment encoding DREB1/CBF

Genomic DNAs were isolated from the young leaves and inflorescences of *Arabidopsis thaliana* L. ecotype Columbia according to the instructions of the manufacturer of the DNeasy™ Plant Maxi Kit (QIAGEN, Germany). Two primers were designed to amplify the ORF region of *DREB1/CBF*s (Shinwari et al. 1998): the 5' end sequences are 5'-CAATGAACTCATTTTCTGC-3', and the 3' end sequences are 5'-TWTTARTAACTCCAWARCG-3'. The polymerase chain reaction was carried out in a 50 μl reaction volume containing 20 ng of genomic DNA, 50 pmol of each primer, 20 pmol of each dNTP and 2.5 units of ExTaq DNA polymerase in 1 × PCR buffer (TaKaRa, Japan) using a DNA thermal cycler (Perkin Elmer, Japan). Amplification was carried out at 94 ℃ for 1 min, 55 ℃ for 2 min, and 72 ℃ for 3 min (30 times). A 6 μl aliquot of the PCR reaction mixture was ligated into the pCRII vector using a TA Cloning Kit (Invitrogen, Netherlands) followed by introduction into *E. coli* cells using the One Shot Kit (Invitrogen, Netherlands). The double-stranded plasmid DNA was purified and sequencing was carried out by the dye-terminator method using a PRISM™ 377 (Applied Biosystem, U.S.A.) automated DNA sequencer.

DNA gel blot analysis

In vitro cultured *Prunus avium* L. cv. Benisyuhou was used as a source of plant material. 'Benisyuhou' is the cultivated variety, crossing 'Satounishiki' × 'Tenkounishiki', which was bred at the Yamagata Horticultural Experimental Station. The total DNA was isolated from the young leaves of cv. Benisyuhou and *A. thaliana* plants according to the instructions of the manufacturer of the DNeasy™ Plant Maxi Kit (QIAGEN, Germany). Either the complete ORF DNA region of *DREB1B/CBF1* or the DNA segment corresponding to the EREBP/AP2 region of *D2Ctr* was used as a probe. The probes were labeled with digoxigenin (Roche, Switzerland). Hybridization, washing and detection were performed as described by Kitashiba et al. (2001).

Construction and screening of a genomic library

The total DNA was isolated as described above and digested with *Sau* 3AI, and cloned into the *Xho* I half-site arms of the Lambda GEM-12 vector (Promega, USA). The complete ORF DNA region of *DREB1B/CBF1* was used as a probe. The library was screened by DIG-labeled *DREB1B/CBF1* DNA as described by Suzuki et al. (1995). Positively hybridizing phages were purified using the liquid culture method (Sambrook et al., 1989). The partial fragments of the phage DNA inserts were subcloned into the pBluescript SK(-) vector or pBI121. DNA sequencing was carried out as described above.

RNA gel blot analysis

The *in vitro* cultured 'Benisyuhou' plants were exposed to a low temperature (0 ℃ or -3 ℃) for 3 hr. The total RNA was extracted from leaves as described by Tao et al. (1999). After denaturation in glyoxal, 15 μg of total RNAs were electrophoresed on 1 % (w/v) agarose gel and transferred to a nylon membrane (Schleicher & Schuell, Germany) by blotting with 20 × SSC. The DNA fragment corresponding to EREBP/AP2 region of *D2Ctr* was labeled with digoxigenin (DIG, Roche, Switzerland) and used as a probe. The membranes were hybridized as described by Watanabe et al. (1994) followed by two washings in 0.1 × SSC, 0.1 % SDS at 65 ℃ for 20 min.

Results and Discussion

Isolation of DREB1/CBF-like genes from P. avium

To confirm the existence of the *DREB1/CBF*-like genes in *P. avium*, we first performed a DNA gel blot analysis. For this experiment, we carried out an amplification of the ORF region of *DREB1/CBF* DNA by PCR as described in the Material and Methods section. The amplified fragments were cloned and three clones were sequenced. Since all three of the sequenced clones were found to be *DREB1B/CBF1*, we used a DNA fragment containing *DREB1B/CBF1* as a probe for the DNA gel blot analysis. The genomic DNAs of *P. avium* cv. Benisyuhou were digested with *Eco* RI or *Xho* I, and those of *A. thaliana* were digested with *Hin*dIII. Although the hybridization and washing were at low stringency, several bands were detected in both lanes of *P. avium* (Fig. 1). In *A. thaliana*, several bands were detected (Fig. 1) similar to those described in a report by Liu et al. (1998). These results suggest that the *DREB1/CBF*-like genes exist in the *P. avium* genome. Therefore, the next step was to isolate the *DREB1/CBF*-like genes from *P. avium*.

A genomic library was constructed from the total DNA of the *P. avium* cv. Benisyuhou plant. A total of 2.0×10^5 recombinants were screened using the ORF

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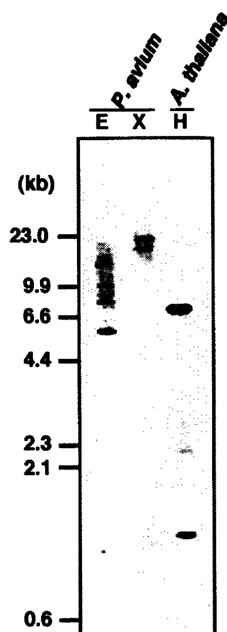

Fig. 1. DNA gel blot analysis of *DREB1/CBF*-like genes in *P. avium* and in *A. thaliana*.
Genomic DNA was digested with *Eco* RI (E), *Xho* I (X), or *Hind*III (H). A DNA fragment containing the open reading frame region of *DREB1B/CBF1* from *A. thaliana* was used as a probe. Membrane was washed twice in 0.5 × SSC, 0.1% SDS at 50℃ for 20 min. Molecular markers are indicated in kb on the left.

region of *DREB1B/CBF1* as a probe. In the primary screening, 6 positive clones and 5 weakly hybridizing clones were obtained. The six positive clones were purified by secondary and tertiary screenings, and finally one clone (named *D21*) was further analyzed. Fig. 2a indicates a restriction map of the *D21* clone. DNA gel blot analysis was performed in order to outline the region encoding *DREB1/CBF*-like gene. A 0.8 kb-*Bam* HI fragment and both the 4.8 kb- and the 2.4 kb-*Bam* HI/*Sac* I fragments (Fig. 2a) that hybridized were subsequently subcloned and sequenced. In addition, the DNA sequences around the *Bam* HI or the *Sac* I junction were also confirmed. A sequence of 8073 bp was finally obtained and analyzed. Since it was reported that the *DREB1/CBF* genes have no introns (Shinwari et al., 1998), we performed the ORF-search within this 8073 bp region, postulating that the *DREB1/CBF*-like genes of *P. avium* may also have no introns. The three longest translated regions were found by the ORF-search. Two of them had both an initiation codon and a terminal codon with a deduced size of 232- and 240-amino acids and were designated as *D2A* (GenBank accession no. AB080965) and *D2B* (AB080966), respectively (Fig. 2). The other had a 130 amino acid long-translated region including a putative initiation codon but no termination codon was found in this region because of an incomplete C-terminal region at the cloning site of the lambda phage vector. Therefore, it was designated as *D2Ctr* (truncated *D2C*, accession no. AB080967; Fig. 2). Although the full-length of the *D2Ctr* clone was not found, it is predicted that the three clones are tandemly arrayed over the approximate 8.0 kb region of the *P. avium* (Fig. 2a). A homology search indicated that the deduced amino acid sequences of the *D2A*, *D2B* and *D2Ctr* included a significant region similar to DREB1/CBF. Each DREB1/CBF protein (DREB1A/ CBF3, DREB1B/ CBF1 and DREB1C/ CBF2; Stockinger et al., 1997; Gilmour et al., 1998; Liu et al., 1998) has a conserved DNA binding domain of 58 amino acids referred to as the EREBP/AP2 (ethylene response element binding protein/ APETALA2) domain (Ohme-Takagi and Shinshi, 1995; Weigel, 1995). The EREBP/AP2 domain was also conserved in each deduced D2 protein (D2A, D2B, and D2Ctr; Fig. 2b). A basic region in the N-terminal that might function as a nuclear localization signal was found in the D2 proteins (Fig. 2b), and an acidic C-terminal region that might act as an activation domain for transcription was found in the D2A and the D2B protein (pIs of 3.7 and 4.4, respectively). The D2A and the D2B were found to be 53% and 53.5% identical to DREB1B/CBF1, respectively. In addition, D2A was 53.2% and 53.4% identical to DREB1A/ CBF3 and DREB1C/ CBF2, respectively, and D2B was 54.0% and 52.0% identical to DREB1A/ CBF3 and DREB1C/ CBF2, respectively. However, the EREBP/AP2 conserved domain of the three deduced D2 proteins showed a more conserved identity (74-79%) with that of each DREB1/CBF (Fig. 2b). Recently, *DREB1/CBF*-like genes have been isolated from other annual plants, for example, *Brassica napus* (accession no. AF370733), tomato (AY034473), rye (AF370730), wheat (AF376136) and barley (AF298230). Comparisons of the entire D2 protein sequences with these sequences indicated about 50% identity with DREB1/CBF-like proteins of *B. napus* and tomato, and about 40% identity with DREB1/CBF-like proteins of the others. However, for the EREBP/AP2 conserved domain, the D2 proteins showed a higher level of identity (71-76%) with the DREB1/CBF-like proteins of *B. napus* and tomato as is the case with DREB1/CBFs. In contrast this region of the D2 proteins showed 58 to 65% identity with the others.

In the 5'-region of the three D2 regions (*D2A*, *D2B*, *D2Ctr*) typical TATA box sequences were predicted (Fig. 2c). A further analysis of the 5'-region revealed the sequence motif CATGTGGC in *D2A* and the sequence motif CACGTGGC in *D2B* and *D2Ctr* which are similar to the G-box and ABRE-related sequences (T/CACGTGG/TC; Marcotte et al., 1989, Yamaguchi-Shinozaki et al., 1989; Fig. 2c). The motifs related to the MYB (C/TAACNA/G; Biedenkapp et al., 1988, Nakagoshi et al., 1990) and MYC recognition sites (CANNTG; Murre et al., 1989) were also found in the 5'-regions of the three D2 regions. A comparison among the 5'-regions of the three revealed that the

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DNA gel blot analysis

The number of genes (*D2* genes) associated with *D2A*, *D2B* and *D2Ctr* in the *P. avium* genome was estimated by DNA gel blot analysis (Fig. 3). Genomic DNAs from *P. avium* was digested with *Sac* I, because we expected to detect an about 10 kb and an over 3.0 kb band based upon the D21-restriction map (Fig. 2a). Genomic DNAs from *A. thaliana* was digested with *Hin*dIII as described by Liu et al. (1998). Each digested DNA was hybridized under both high- and low-stringency conditions using the EREBP/AP2 region of *D2Ctr* as a probe. Since the probe we used showed more than 90% identity with the corresponding region of *D2A* and *D2B*, it was expected that the probe should recognize all *D2* genes in *P. avium*. Under the low-stringency hybridization condition, a 10 kb band and three bands over 3.0 kb were detected in *P. avium*, while several bands were detected in *A. thaliana* (Fig. 3), which display a very similar pattern to that observed by Liu et al. (1998). On the other hand, under the high-stringency condition, the same bands were detected as observed under the low stringency conditions in *P. avium*, but no bands were detected in *A. thaliana* (Fig. 3). These results suggest that there may be a few additional *D2*-related genes in the *P. avium* genome other than *D2* genes that we identified.

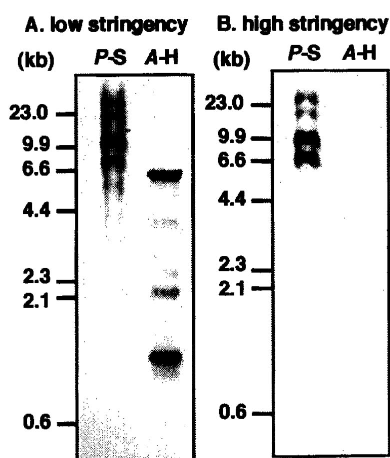

Fig. 3. DNA gel blot analysis of *D2* genes in *P. avium* and in *A. thaliana*.
Genomic DNA of *P. avium* was digested with *Sac* I (P-S), and that of *A. thaliana* was digested with *Hin*dIII (A-H). Membrane was washed twice in 0.5 × SSC, 0.1% SDS at 50 ℃ for 20 min (A. low stringency), or twice in 0.1 × SSC, 0.1% SDS at 65 ℃ for 20 min (B. high stringency). A DNA fragment containing the conserved region including the EREBP/AP2 domain of *D2Ctr* was used as a probe. Molecular markers are indicated in kb on the left.

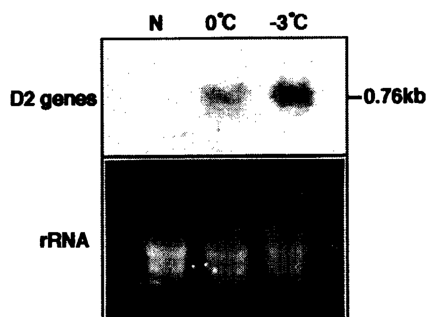

Fig. 4. Expression of genes corresponding to *D2* family in response to low temperature. The conserved region including the EREBP/AP2 domain of *D2Ctr* was used as a probe. rRNA, ribosomal RNA; N, grown under normal condition (16-h day/ 8-h dark, 20 ℃).

RNA gel blot analysis

The expression pattern of the *D2* genes was analyzed by RNA gel blot hybridization using the same probe in the DNA gel blot analysis. A band was detected at 0 ℃ after 3 hr and also strongly detected at -3 ℃ after 3 hr (Fig. 4). In contrast, no bands were detected under normal condition (Fig. 4). This result suggests that the expression of one, two or all of three *D2* genes was induced by the low temperature. This expression pattern by low temperature is almost similar to that of *DREB1/CBF* in *A. thaliana* (Liu et al., 1998) and that of *DREB/CBF*-like genes in other annual plants (Jaglo et al., 2001).

In this report, the existence of several genes that have some features similar to *DREB1/CBF* was found in the *P. avium* genome. Recently, *DREB1/CBF*-like genes have been isolated from some annual plants. To our knowledge, our finding of the *DREB1/CBF*-like gene in a fruit tree is the first report. We suggest that *DREB1/CBF*-like gene may be extensively conserved in plants. The *D2* genes will be useful for helping us to understand the mechanisms of freezing tolerance in woody plants at the molecular level, and for studying of evolution of the *DREB1/CBF* genes. For these purposes, further analyses, for example, the expression pattern analysis of each *D2* gene, the production of transgenic plants containing the *D2* genes and other experiments should be examined. In addition, it is reported that DREB1/CBF has a significant effect against freezing, drought, and salt stress (Kasuga et al. 1999). Therefore, further analyses described above will lead us to reveal the ability of the D2 protein against various stresses in the near future.

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Literature Cited

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. U.S.A. 93: 13404-13409.

Biedenkapp, H., U. Borgmeyer, A. E. Sippel and K. H. Klempnauer. 1988. Viral *myb* oncogene encodes a sequence-specific DNA-binding activity. Nature 335: 835-837.

Gilmour, S. J., D. G. Zarka, E. J. Stockinger, M. P. Salazar, J. M. Houghton and M. F. Thomashow. 1998. Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced *COR* gene expression. Plant J. 16: 433-442.

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

Ishikawa, M. and A. Sakai. 1985. Extraorgan freezing in wintering flower buds of *Cornus officinalis* Sieb. et Zucc. Plant Cell Environ. 8: 333-338.

Jaglo-Ottosen, K. R., S. J. Gilmour, D. G. Zarka, O. Schabenberger and M. F. Thomashow. 1998. *Arabidopsis CBF1* overexpression induces *COR* genes and enhances freezing tolerance. Science 280: 104-106.

Jaglo, K. R., S. Kleff, K. L. Amundsen, X. Zhang, V. Haake, J. Z. Zhang, T. Deits and M. F. Thomashow. 2001. Components of the Arabidopsis C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. Plant Physiol. 127: 910-917.

Kader, S. A. and E. L. Proebsting. 1992. Freezing behavior of *Prunus*, subgenus *Padus*, flower buds. J. Amer. Soc. Hort. Sci. 117: 955-960.

Kaneda, M., Y. Hara, T. Suzuki and T. Shiraishi. 2001. Chilling injury of sweet cherry flower buds with and without freezing. J. Japan. Soc. Hort. Sci. 70: 516-518.

Kang, S. K., Motosugi, H., Yonemori, K. and Sugiura, A. 1998. Supercooling characteristics of some deciduous fruit trees as related to water movement within the bud. J. Hort. Sci. Biotech. 73: 165-172.

Kasuga, M., Q. Liu, S. Miura, K. Yamaguchi-Shinozaki and K. Shinozaki. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotech. 17: 287-291.

Kitashiba, H., R. Ota and K. Toriyama. 2001. Analyses of the genomic sequence and promoter activity of a gene for a protein similar to Tat binding protein from *Brassica rapa*. Plant Biotech. 18: 33-38.

Kuroda, H. and S. Sagisaka. 1998. Participation of hydrogen peroxide in the dysfunction of peroxide-scavenging systems in apple flower buds associated with freezing injury. J. Japan. Soc. Hort. Sci. 67: 161-165.

Liu, Q., M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki and K. Shinozaki. 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell 10: 1391-1406.

Marcotte, W. R. Jr., S. H. Russell and R. S. Quatrano. 1989. Abscisic acid-responsive sequences from the em gene of wheat. Plant Cell 1: 969-976.

Murre, C., P. S. McCaw and D. Baltimore. 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and Myc proteins. Cell 56: 777-783.

Nakagawara, S. and S. Sagisaka. 1984. Increase in enzyme activities related to ascorbate metabolism during cold acclimation in poplar twigs. Plant Cell Physiol. 25: 899-906.

Nakagoshi, H., T. Nagase, C. Kanei-Ishii, Y. Ueno and S. Ishii. 1990. Binding of the c-*myb* proto-oncogene product to the simian virus 40 enhancer stimulates transcription. J. Biol. Chem. 265: 3479-3483.

Ohme-Takagi, M. and H. Shinshi. 1995. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7: 173-182.

Rodrigo, J. 2000. Spring frosts in deciduous fruit trees - morphological damage and flower hardiness. Scientia Hortic. 85: 155-173

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press, New York.

Shinwari, Z. K., K. Nakashima, S. Miura, M. Kasuga, M. Seki, K. Yamaguchi-Shinozaki and K. Shinozaki. 1998. An Arabidopsis gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. Biochem. Biophys. Res. Commun. 250: 161-170.

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. U.S.A. 94: 1035-1040.

Suzuki, G., M. Watanabe, K. Toriyama, A. Isogai and K. Hinata. 1995. Molecular cloning of members of the S-multigene family in self-incompatible *Brassica campestris* L. Plant Cell Physiol. 36: 1273-1280.

Tao, R., H. Yamane and A. Sugiura. 1999. Molecular typing of S-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. J. Amer. Soc. Hort. Sci. 124: 224-233.

Thomashow, M. F. 1994: *Arabidopsis* thaliana as a model for studying mechanisms of plant cold tolerance. p. 807-834. In: E. Meyerowitz and C. Somerville (eds.). Arabidopsis. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Thomashow, M. F. 1998: Role of cold-responsive genes in

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plant freezing tolerance. Plant Physiol. 118: 1 - 7.

Warmund, M. R., M. F. George and B. G. Cumbie. 1988. Supercooling in 'Darrow' blackberry buds. J. Amer. Soc. Hort. Sci. 113: 418 - 422.

Watanabe, M., T. Takasaki, K, Toriyama, S. Yamakawa, A. Isogai, A. Suzuki, T. Takeuchi and K. Hinata. 1994. A high degree of homology exists between the protein encoded by *SLG* and the *S* receptor domain encoded by *SRK* in self - incompatible *Brassica campestris* L. Plant Cell Physiol. 35: 1221 - 1229.

Weigel, D. 1995. The APETALA2 domain is related to a novel type of DNA binding domain. Plant Cell 7: 388 - 389.

Yamaguchi - Shinozaki, K., J. Mundy and N. H. Chua. 1989. Four tightly linked *rab* genes are differentially expressed in rice. Plant Mol. Biol. 14: 29 - 39.

Yamaguchi - Shinozaki, K. and K. Shinozaki. 1994. A novel *cis* - acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low - temperature, or high - salt stress. Plant Cell 6: 251 - 264.

DREB1/CBF様遺伝子の甘果オウトウ (*Prunus avium* L.)からの単離

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摘　要

CRT/DRE とは C-repeat (CRT) dehydration responsive element の略で，低温や乾燥ストレスに応答し遺伝子の転写を促進する DNA シスエレメント配列のことである．近年，CRT/DRE 配列に結合し，複数の低温誘導性の遺伝子発現を促進し，植物に低温馴化を獲得させるように働く制御因子 DREB1/CBF がシロイヌナズナで同定された．今回，*DREB1/CBF* 様の遺伝子 (*D2A*, *D2B*, *D2Ctr* と名付けた)を含むゲノム DNA 断片を甘果オウトウ (*Prunus avium* L.)より単離した．この断片内には完全な ORF を含むと推測される領域が 2つ (D2A, D2B) と，ORF は推定出来ないがアミノ酸に翻訳できる領域 (D2Ctr) が存在していた．これら 3つの推定アミノ酸配列を解析した結果，EREBP/AP2 と呼ばれる DNA 結合性の領域，N 末端側には核局在性シグナル配列が存在した．また D2A, D2B に関しては C 末端側が酸性に富む領域であることが分かった．D2A, D2B 推定アミノ酸配列と DREB1/CBF 群アミノ酸配列との比較の結果，全体にわたっては 53% 程度であったが，D2A, D2B, D2Ctr の EREBP/AP2 領域では 74 - 79% と高い類似性を示した．*D2A*, *D2B*, *D2Ctr* の 5' - 領域においては，TATA - box，G - box，MYB - や MYC - 認識配列が見られた．また，3つの間で共通な保存配列が 4箇所見出された．*D2* 遺伝子群は低温により誘導された．

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