한국식물병리학회지 14(3) : 240-246(1998)
Korean J. Plant Pathol. 14(3) : 240-246(1998)

Molecular Characterization of a Chinese cabbage cDNA, *C-DH*, Predominantly Induced by Water-Deficit Stress and Plant Hormone, ABA

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수분부족 및 식물호르몬, ABA에 의하여 발현이 유도되는 배추의 *C-DH* cDNA에 대한 분자적 특성

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ABSTRACT: A cDNA encoding desiccation-related protein was isolated from a flower bud cDNA library of Chinese cabbage (C-DH) and its nucleotide sequence was characterized. It contains 679 bp nucleotides with 501 bp open reading frame. The amino acid sequence of the putative protein showed the highest amino acid sequence homology (79 % identity) to dehydrin protein in *Gossypium hirsutum*. Also, the C-DH shares 48~52% amino acid sequence identity with the other typical dehydrin proteins in plant cells. When the amino acid sequence of their proteins were aligned, several peptide motifs were well conserved, of which function has to be solved. Particularly the C-DH contains 15 additional amino acids at its N-terminus. Genomic Southern blot analysis using the coding region of C-DH showed that the C-DH consists of a single copy gene in Chinese cabbage genome. The C-DH mRNA, whose transcript size is 0.7 kb, was expressed with a tissue-specific manner. It was highly expressed in seed, flower buds and low expression was detected in root, stem or leaf tissues of Chinese cabbage. And the transcript level of C-DH was significantly induced by the treatment of plant hormone, abscisic acid and water-deficit conditions.

***Key words*: dehydrin gene in Chinese cabbage, expression of the dehydrin cDNA, flower cDNA library, southern blot analysis, Lea proteins.**

When the water available to a plant falls below a critical level, the plant begins to experience water-stress and exhibit several physiological and biochemical changes. Numerous changes in gene expression that take place in plants to compensate for a water-deficit or to tolerate and recover from dehydration have been documented (15, 22). The amount of mRNAs induced upon drought conditions increase dramatically during late seed development when embryos begin to desiccate and remain at high levels in dry seeds. At this period, the level of abscisic acid (ABA) reaches a maximum (16). The most prevalent mRNA species in dry seeds are encoded by a group of genes designated as late embryogenesis-abundant (Lea) genes, because desiccation stress is a normal aspect of seed formation (9). Mostly, the predicted polypeptides of dehydrins share similarity with those of Lea proteins and contain structural elements in common with other proteins which are induced by desiccation and ABA. The dehydrin mRNAs are detected at low levels in leaves or roots of non-stressed plants and in young immature seed tissues. However, when the plant hormone, ABA is applied to immature embryos, the dehydrin mRNAs can be induced to high levels (23). Thus, there may be a functional linkage between desiccation stress, ABA, and dehydrin proteins (26). To promote cellular tolerance of dehydration (3), ABA appears to modulate the response of plants to water-stress (21). This defense is augmented by the increases in the concentrations of various water-absorbing materials including proline, sugars, and or-

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ganic acids (27). Even though the action mode of the ABA hormone remains unclear, a model has been proposed involving calcium as a second messenger modulating ABA-induced responses (24). In this model, the stimulus induces the activation of a protein kinase C resulting in the phosphorylation of discrete sets of proteins. Subsequently, many phosphorylated proteins transduce the foreign signals into the cellular effector molecules and activate self-defense systems in plant cells. Even though several Lea and desiccation-related genes have been isolated from plants, the physiological function of these gene-products in drought conditions remains unclear. To better understand the potential link between environmental water-stress and the function of these genes, it is necessary to isolate much more desiccation-related genes and characterize their properties.

Towards this end, the present work describes the cloning of a cDNA, which is critically induced by ABA or drought conditions from a flower bud cDNA library of Chinese cabbage.

MATERIALS AND METHODS

Materials. Chinese cabbage (*Brassica campestris* L. ssp. pekinensis) was grown in a growth chamber under various growth conditions. Different tissues of Chinese cabbage were dissected and used for the preparation of genomic DNA, RNA and a cDNA library.

Cloning of a dehydrin gene. To isolate tissue-specifically regulated new genes from Chinese cabbage flower buds, we prepared a phagemid-based flower buds cDNA library and carried out random sequencing of flower bud-specific cDNA clones (18). It led to identification of a partial cDNA clone Expressed Sequence Tag (EST)-F0392, which encodes a dehydrin-like protein in Chinese cabbage. To get a full length cDNA clone, the EST-F0392 clone was labeled with [α-^{32}P] ATP to a specific activity of ~5×10^8 cpm/μg by the random-primer labeling method (8) and used as a screening probe. Hybridization was performed in the solution containing 5×SSC, 5×Denhart's reagent, 0.1% SDS and 50 μg/ml yeast total RNA at 55°C for 1 day with the probe at a concentration of 5×10^6 cpm/ml. The filters were washed three times in 2×SSC, 0.1% SDS at 55°C, each for 15 min. And subsequent washings were done at 55°C with 1×SSC, 0.1% SDS twice, each for 15 min. After washing the filters, they were dried and autoradiographed for appropriate time at -70°C. From the positive clones, the longest *Eco*RI/*Xho*I-digested cDNA insert was sequenced by dideoxy sequencing methods using the Taq dye primer on an automated DNA sequencer (Applied Biosystems Model 373A). Sequence analyses were performed by the public database using Blastx program provided by NCBI e-mail server (1, 7, 25).

Southern and Northern blot analysis. Genomic DNA was prepared from Chinese cabbage leaves as described by Dellaporta et al (6) and further purified by a cesium chloride/ethidium bromide density gradient ultracentrifugation (4). Ten μg of purified DNA was digested with appropriate restriction enzymes, size fractionated on a 0.8% agarose gel, and transferred onto a Hybond-N+ membranes (Amersham). Hybridization was carried out in the buffer of 6×SSPE, 5×Denhardt's reagent, 1% SDS, 100 μg/ml denatured calf thymus DNA, 5×10^6 cpm/ml probe at 65°C. The probes used were the full cDNA coding region of the clone labeled with [α-^{32}P] ATP by the random primer labeling method. The hybridized blots were finally washed in 0.1×SSC, 0.1% SDS at 65°C. After washing the filters, they were dried and autoradiographed for 10 h at -70°C. For northern blot analysis, various tissues of seven-day old Chinese cabbage seedlings were divided into sections as described previously (17). Total RNA was extracted from these sections by guanidium thiocyanate/phenol/chloroform extraction method and further purified by ultracentrifugation. Twenty μg of total RNAs were separated on 2% formaldehyde agarose gels and transferred onto Gene Screen Plus membranes (New England Nuclear). Hybridization conditions were analogous to those used for Southern blots except for hybridization temperature of 60°C and final washes in 0.5×SSC, 0.1% SDS at 55°C.

RESULTS AND DISCUSSION

Isolation of the C-DH gene and comparison of the deduced amino acid sequence with other dehydrin proteins. A partial cDNA clone (EST-F0392) closely matched with drought-induced cDNA of Lea gene in *Gossypium hirsutum* was obtained from ESTs of Chinese cabbage flower buds cDNA library. The *Gossypium hirsutum* Lea gene shows a typical characteristics of dehydration-resistant property and acts as a desiccation protectant (10). To obtain the full length of this cDNA, Chinese cabbage cDNA library was screened with the ^{32}P-labeled EST-F0392 clone as a probe. After screening about 5×10^6 colonies, 5 candidate clones could be

obtained. From the positive clones, we isolated a cDNA having the longest cDNA insert and directly sequenced. The amino acid sequence comparison of the clone with the entries in the EMBL and Swiss-Prot sequence database revealed that it belongs to a dehydrin protein group, thus it was named C-DH, representing for Chinese cabbage dehydrin gene. The C-DH gene encodes mRNA of 679 bp cDNA insert with 501 bp open reading frame, thus it would encode 167 amino acids. The gene contains 32 bp of 5'-untranslated region and 146 bp 3'-untranslated sequence as shown in Fig. 1.

Fig. 2 shows a comparison of the amino acid sequence of C-DH with other typical dehydrin proteins cloned to date. The putative translation product of C-DH showed 79% sequence identity with the drought-induced Lea protein of *Gossypium hirsutum* (10). And it also shows high amino acid sequence similarity to dehydrin proteins of *Arabidopsis* (accession #; Y12776), soybean (20) and *Cruterostigma plantagineum* (19). The *Cruterostigma plantagineum* is a desiccation-resistant African resurrection plant (12). The amino acid sequences of all dehydrin proteins contained several highly conserved peptide domains. When we introduced gaps to maximize the fit of amino acid sequences, the peptide of KAK (amino acid residues positioned from 22 to 24 in C-DH), DVD (43-45), NPY (64-66), PDPGSL (93-98), DIDY (129-132) and KLP (158-160) were perfectly conserved, suggesting that these regions may have important roles in the protection against water-deficit stress. The peculiar characteristic of C-DH clone is that it contains 15 additional amino acids at its N-terminus compared with other dehydration proteins, of which role is remained to be solved. The high sequence similarity of C-DH with the dehydrin-related Lea proteins, which are mostly expressed at Lea period of the plants, suggests that the C-DH may have an important role at the stage of late embryogenesis of Chinese cabbage and at the water-deficit condition as a drought-resistant protective protein.

Southern blot analysis. To see the copy number of C-DH gene in Chinese cabbage genome, Southern blot analysis was carried out by using the full length cDNA insert of C-DH as a probe. When genomic DNA digested with *Eco*RI, *Bam*HI or *Hind*III restriction enzymes that do not cleave within the coding sequence was hybridized under the stringent washing conditions, only one band in each restriction fragment hybridized to the probe of C-DH (Fig. 3). Considering the result of Southern analysis, the C-DH gene consists of a single gene family in Chinese cabbage genome.

Transcript levels of C-DH gene in different tissues. To identify possible physiological function of C-DH

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                                        GGATTCCAAAAGAAAAAAAAGACAGAGAAAGA    32
ATGGCAGAAACGGAGCAAAAGGAGGTAGAAGAAAAGGGATCGTTGATCTCAGGCTTGTTG  92
 M  A  E  T  E  Q  K  E  V  E  E  K  G  S  L  I  S  G  L  L   (20)
GATAAAGCCAAAGGTTTCTCCGCAGAGAAGCTTGCTAATATTCCGACGCCGGAAGCCACC  152
 D  K  A  K  G  F  S  A  E  K  L  A  N  I  P  T  P  E  A  T   (40)
GTGGACGACGTAGACTTCAAAGGTGTGGCTCGTCAAGGAGTTGATTATCACGCCAAGGTC  212
 V  D  D  V  D  F  K  G  V  A  R  Q  G  V  D  Y  H  A  K  V   (60)
TCCGTCAAGAACCCTTACCCTCAGGCCATCCCTATTTGCCAGCAATCTTACATCCTCAAG  272
 S  V  K  N  P  Y  P  Q  A  I  P  I  C  Q  Q  S  Y  I  L  K   (80)
AGTGACACAAGGATGATTAGCGTCTTGGCACGATTACCGGATCCGGGTTCGTTGATCGAA  332
 S  D  T  R  M  I  S  V  L  A  R  L  P  D  P  G  S  L  I  E  (100)
CGGGTCGACGGTTCCTGGACCGTACCGGTGAAGGTGCCTTATAGCATAGCGGTGAGTTTG  392
 R  V  D  G  S  W  T  V  P  V  K  V  P  Y  S  I  A  V  S  L  (120)
ATGAAGGACATGTGTTTGGACTGGGACATTGACTATCAACTCGACATTGGACTGACCATC  452
 M  K  D  M  C  L  D  W  D  I  D  Y  Q  L  D  I  G  L  T  I  (140)
GACATTCCTATTGTTGGTGACATTACCATTCCTGTCTCTACTCAGGGTGAGGATAAGCTC  512
 D  I  P  I  V  G  D  I  T  I  P  V  S  T  Q  G  E  D  K  L  (160)
CCTTCCCTTCGCGACTTCTTTTAATCATTCTATAAGTTATAATCTGATTTTTCAATAAGT  572
 P  S  L  R  D  F  F  *                                        (167)
ACGATCCGTAAACGAGATAAACGATCGCTGGATGTTTCGGTTGTGGACACTTATGTTTGT  632
TGGTTATATGTATTTGTTGCTTTGAAAAAAAAAAAAAAAAAAAAAA                679
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the Chinese cabbage C-DH cDNA. The nucleotides are numbered starting with the first nucleotide of the insert. The amino acid numbering starts with the first methionine residue.

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C-DH      MAETEQKEVEEKGSLISGLLDKAKGFSAEKLANIPTPEATVDDVDFKGV   49
GH-DH     MSQ---------------LLEKAKDFVVDKVANIKKPEASVSDVDLKHV   34
A-DH      MAS---------------LLDKAKDFVADKLTAIPKPEGSVTDVDLKDV   34
S-DH      MSQ---------------LLDKAKNYVAEKVTNMPKPEASVTDVDFKRV   34
CP-DH     MAQ---------------LMNKAKNFVAEKVANVEKPKASVEDVDLKDV   34
          *.                 *..***.. ..*.... .*...*.***.* *

C-DH      ARQGVDYHAKVSVKNPYPQAIPICQQSYILKSDTRMISVLARLPDPGSL   98
GH-DH     SRECVEYGAKVSVSNPYSHSIPICEISYNFRSAGRGIAS-GTIPDPGSL   82
A-DH      NRDSVEYLAKVSVTNPYSHSIPICEISFTFHSAGREIGK-GKIPDPGSL   82
S-DH      SRDSVEYLAKVSVSNPYSTPIPICEIKYSLKSAGKEIAS-GTIPDPGSL   82
CP-DH     GRHGITYLTRICVENPYSASIPVGEIKYTLKSAGRVIVS-GNIPDPGSL   81
          .*. ..* ....*.***. .**. . .. ..*... *   . .******

C-DH      IERVDGSWTVPVKVPYSIAVSLMKDMCLDWDIDYQLDIGLTIDIPIVGD   146
GH-DH     KASDTTMLDVPVKVPYNILVSLVKDIGADWDIDYELELGLTIDLPIVGN   130
A-DH      KAKDMTALDIPVVVPYSILFNLARDVGVDWDIDYELQIGLTIDLPVVGE   130
S-DH      KASDTTMLDVPVKVPHSILLSLAKDIGADWDIDYQLDLGLVIDLPVIGN   130
CP-DH     KGNDKTMLEPAIKVPHSALVSLIKDIGADMDIDYVLELGLVVDLPVIGN   129
           .  .   . .. **..   .* .*.  * **** *..**..*.*..*.

C-DH      ITIPVSTQGEDKLPSLRDFF-   167 (100%)
GH-DH     FTIPLSQKGEIKLPTLSDIF-   151(78.8%)
A-DH      FTIPISSKGEIKLPTFKDFF-   151(52.3%)
S-DH      FTIPLSQKGEIKLPTLSDMFA   152(51.9%)
CP-DH     FTIPLSHKGEMKLPGLSDIF-   151(48.4%)
          ...*.* .** ***...  *
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Fig. 2. Comparison of the deduced amino acid sequences of C-DH with other typical dehydrin proteins. The deduced amino acid sequence of dehydrin proteins in Chinese cabbage (C-DH, this paper), *Gossypium hirsutum* (GH-DH, 10), *A. thaliana* (A-DH, accession #; Y12776), Soybean (S-DH, 20) and *Cruterostigma plantagineum* (CP-DH, 19) are aligned for comparison. Asterisks denote positions perfectly conserved and dot (•) mark well conserved positions. The alignment was computed with the CLUSTAL program of the PCGENE software package (Genofit SA, Geneva, Switzerland/IntelliGenetics Inc. Mountain View, CA). Gaps are introduced for maximizing the fit.

gene, we have measured its RNA expression levels in different tissues using northern blot analysis. It was performed by using total RNA isolated from various tissues of Chinese cabbage such as seed, leaf, root, stem and flower buds. Total RNA isolated from different tissues were hybridized with full-length cDNA of C-DH as a probe. Fig. 4 shows that the single gene hybridized to a transcript of approximately 0.7 kb. The transcript level of C-DH gene was expressed with a tissue-specific manner, specifically high in seeds, flower buds and low expression was detected in root, stem, and leaf tissues (Fig. 4, panel A). This result strongly suggests that the product of C-DH gene may exert its function at seeds and flower organs. In the majority of higher plants, tolerance to protoplastic dehydration is strictly restricted to the seed. During embryogenesis the developing embryo acquires desiccation tolerance, but the onset of germination interrupts the state of tolerance. And both the seed and emerging seedling rapidly lose the ability to survive desiccation. Thus, if dehydrin proteins, or a set of them, are part of a water stress response system, the system is a normal part of the seed development program, but on-call in tissues at other times or tissues in the life cycle of plants. Furthermore, to analyze the effects of external signals on the expression of C-DH gene, we treated several stimuli and analyzed the transcript levels of C-DH. The transcript levels of C-DH in leaves were significantly induced by the treatment of plant hormone, ABA (50 μM) or dehydration (Fig. 4, panel B) compared to well-watered control plant. Since the PEG is not readily taken up by cells and reduces the external free water concentration without altering the ionic composition of the cell, we treated 5% PEG to Chinese cabbage seedlings to mimic the drought condition. Even though the effects of PEG and salt (0.2 M-NaCl) were not so severe as desic-

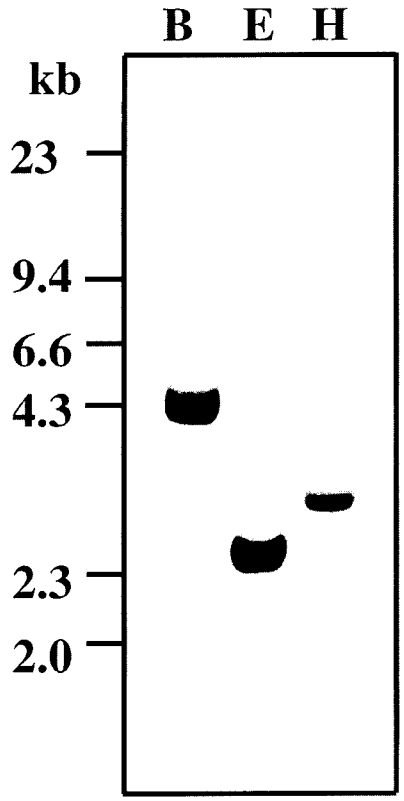

Fig. 3. Genomic Southern blot analysis of the C-DH gene. Genomic DNA was isolated from mature leaves of Chinese cabbage. 10 g of purified genomic DNA was digested either with *Bam*HI (B), *Eco*RI (E) or *Hind*III (H), size fractionated on a 0.8% agarose gel and transferred to a Hybond N+ nylon membrane. The filter was hybridized with the ^{32}P-labeled C-DH coding region probe. Numbers indicate the sizes of standard DNA in kilobases (kb).

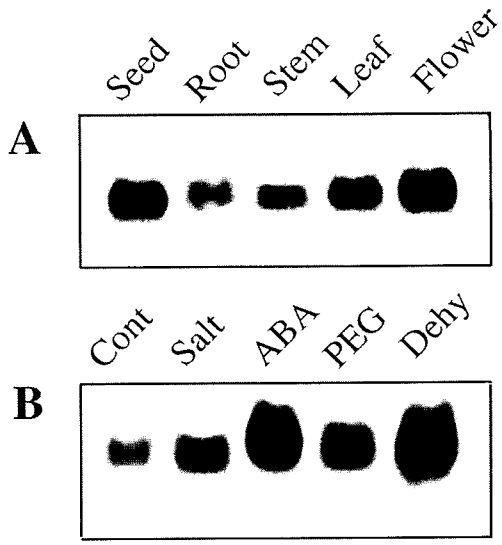

Fig. 4. Northern blot analysis of the C-DH expression. Twenty ug of total RNA from various tissues of Chinese cabbage were separated on a 2% formaldehyde/agarose gel and transferred to a Hybond N+ nylon membrane. The blot was hybridized with the ^{32}P-labeled coding sequence of C-DH. Panel A; The transcript level of the C-DH gene in various tissues of Chinese cabbage. Panel B; Accumulation of the C-DH mRNAs in 7 day-old Chinese cabbage seedlings following addition of 50 μM abscisic acid (ABA), 0.2 M NaCl (Salt), 5% polyethylene glycol (PEG), air-drying (Dehy), and well-watered (Contol) whole plants for 12 hr at 25°C. Drying was accomplished by removing plants from growth media and air-drying on a laboratory bench. After these treatments, leaves were deteched, mRNAs isolated and their transcript levels analyzed.

cation, they slightly increased the mRNA level of C-DH gene. As reported previously (11, 28), there should be a functional linkage between desiccation stress and ABA, and the ABA plays a critical role on the desiccation conditions as a signal transducing molecule (26).

Like other plant hormones, ABA has multiple roles during the life cycle of a plant (5). Recently, genes have been isolated from cereal embryos which are responsive to ABA and expressed in water-stressed leaf tissues (14). This result showed that the increased ABA level could coordinate the activation of genes related to desiccation tolerance at the transcriptional and/or translational level to cope with the water-deficit stress (28). Exposure of many plants to water-limiting conditions results in the synthesis of new mRNAs. Therefore, several water-deficit stress induced mRNAs were identified in excised tomato leaves, pea shoot (13) and in *Zea mays* seedlings (2) and so on. These transcripts are synthesized as a response to drying but it has not been demonstrated that they are directly involved in the mechanism of desiccation tolerance (21). Using the C-DH clone, these questions and the physiological role of the dehydrin gene in plants have to be investigated in the future.

요 약

배추의 꽃봉오리 cDNA library로부터 가뭄의 조건에서 발현되는 유전자 (C-DH)를 분리하고 이 유전자의 특성을 분석하였다. 이 유전자는 679 bp의 염기서열로 구성되었으며 501개의 단백질을 코딩하는 염기서열을 지니고 있었다. 이 유전자로부터 유추한 아미노산의 염기서열은 *Gossypium hirsutum*에서 발현되는 수분부족에 대한 저항성을 나타내는 dehydrin 유전자와 가장 유사한 배열(79%)을 나타내었으며, 또한 다른 식물의 dehydrin 유전자들과도 48~52% 정도의 아미노산 서열 유사성을 보였다. 이들 유전자의 아미노산 서열을 비교해 보았

을 때, 이들 dehydrin 유전자는 아직까지 기능규명이 되지않은 여러개의 conserved 펩타이드 domains를 가지고 있었다. 특히, C-DH는 다른 유전자와 비교해서 N-terminus에 15개의 아미노산이 더 많이 존재하였다. 유전자의 Southern 분석실험결과, C-DH 유전자는 배추 genome에서 single copy gene으로 구성되어 있음을 알 수 있었다. 그리고 0.7 kb의 transcript size를 갖는 C-DH 유전자의 발현 pattern을 조사한 결과, 이 유전자는 조직특이적인 발현양상을 보임을 알았다. C-DH 유전자는 종자 및 꽃 봉우리에서 특히 많은 발현을 보였으며, 잎과 줄기 및 뿌리 등에서는 발현정도가 약함을 알 수 있었다. 그리고 C-DH의 mRNA는 식물 호르몬인 abscisic acid의 처리와 수분부족상태의 조건하에서 상당히 발현량이 증가됨을 알 수 있었다.

ACKNOWLEDGEMENT

This research was supported by SPECIAL FUND for UNIVERSITY RESEARCH INSTITUTE, Korea Research Foundation (1994).

NOTE

The nucleotide sequence data will appear in the EMBL, GenBank, DDBJ, and NCBI nucleotide sequence database under the accession number of AF060884.

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(Received April 30, 1998)