Regulatory role of DREB transcription factors in plant drought, salt and cold tolerance

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Abstract *rd29A* gene of *Arabidopsis* encodes a LEA-like hydrophilic protein, its expression is induced by drought, high-salt and cold stress. In the promoter region of *rd29A* gene, there are 2 DRE *cis*-acting elements involved in responses to these environmental stresses. 5 cDNAs (*DREB1A*～*C* and *DREB2A*～*B*) encoding DREB transcription factors, which specifically bind to DRE element and control the expression of reporter gene under drought, high-salt and stress, have been isolated by One-Hybrid screening method and with DRE element of *rd29A* promoter. DREB transcription factors and DRE element function in signal transduction of drought, high-salt and cold stress. One DREB transcription factor can control the expression of several target functional genes involved in plant tolerance to drought, high-salt and cold stress. Thus, it may be an effective strategy to achieve ideal, multiple and fundamental effect for improving plant stress-resistance by DREB

gene transfer.

Keywords: promoter, *cis*-acting element, transcription factor, signal transduction, gene expression.

The environmental stresses such as drought, high-salt and cold cause increasingly great damages to crop production. Although these environmental stresses come in various forms, they have in common effect on plant water status. Drought, high-salt and cold stress decrease the water status of plant cells and make plants injure. Under serious condition, these adverse environmental stresses can result in death of plant. Land plants grow in soil and they cannot move by themselves. They must respond and adapt to these adverse environmental conditions to avoid or decrease cell injury caused by water deficit. Present studies are focused on plant molecular responses and signal transductions when subjected to water stress. According to the recent reports, many plant genes are induced by drought, high-salt and cold[1−5]. These inducible genes are classified into two groups according to the functions of their encoding products.

The gene products of the first group include functional proteins that directly protect macromolecules and membranes (LEA proteins, osmotin, antifreeze proteins, chaperones and mRNA binding proteins, etc.) and maintain water movement through membranes (water-channel proteins and membrane transporters, etc.); the enzymes catalyzing the biosynthesis of various osmoregulators (proline, betaine and surgars, etc.); and the detoxification enzymes enabling cellular phsiological or biochemical metabolism to maintain a normal level (glutathione S-transferase, soluble epoxide hydrolase, catalase, superoxide dismutase and ascorbic peroxidase, etc.). The exact functions of the gene products of this group have been extensively paid attention to. Plant tolerance to drought or high-salt can be improved by transformation of genes encoding LEA protein, proline synthetase or betaine synthetase etc. Many reports indicate that these gene products really function in stress tolerance.

The gene products of second group include transcriptional factors (bZIP, MYC, MYB and DREB, etc.); protein kinases (MAP kinase and CDP kinase, receptor protein kinase, ribosomal-protein kinase and transcription-regulation protein kinase, etc.); proteinases (phosphoesterase and phospholipase C, etc.) which are involved in signal transductions of stresses and the expression controls of stress-tolerant genes. Recently, two-component system genes have been found in *Arabidopsis* and tobacco. The two-component gene encodes protein kinase which is composed of a "sensor" and a "response regulator" involved in acceptance and transduction of stress signal. Two of these protein kinase genes, *ATRR1* and *ATRR2* of *Arabidopsis*, are induced by drought, high-salt and cold[6]. The *NTHK1*, a two-component system gene of tobacco, is also induced by high-salt[7].

It is still unclear for the molecular mechanism how plant cells sense the water deficit resulting from drought, high-salt or cold, how these stress signals are transduced to nuclear transcription factors, and how the expression of the downstream functional genes are controlled. Thus, the expression characteristics of the genes of the second group and the functions of their encoding products have been becoming the important research contents in plant molecular biology. The molecular mechanism of plant response to drought, high-salt and cold is reviewed here, and the control role of DREB transcription factors in plant response to these stresses is analyzed, according to the expression of *Arabidopsis rd29A* gene[8], and the function of dehydration responsive element (DRE) *cis*-acting element[9] and DRE-binding protein (DREB) transcription factors[10,11].

rd29A gene was primarily isolated from drought-treated *Arabidopsis* plants by differential hybridization screening method[8]. The expression of *rd29A* gene is differentially induced under the condition of drought, high-salt and cold, or under the treatment with exogenous abscisic acid (ABA). This suggests that *rd29A* promoter has at least two kinds of *cis*-acting elements, one is involved in ABA-associated response and the other in ABA-independent response. Because the expression of *rd29A* gene is distinctive from other *rd* (responsive to dehydration) genes, we analyzed its promoter and defined a DRE *cis*-element (TACCGACAT) involved in the first rapid expression of *rd29A* gene under drought, high-salt and cold stress and an ABRE (ABA responsive element) *cis*-element involved in the second slow expression of *rd29A* gene under these stress conditions[9]. DRE *cis*-element functions in the first rapid response. *rd29A* gene can also be induced by these stresses when ABRE *cis*-element is deleted from its promoter region. There are 2 DRE *cis*-acting elements in *rd29A* promoter. As shown in

fig. 1, the core sequence of DRE is CCGAC. DRE *cis*-element or its core sequence has been found in *Arabidopsis rd17*, *kin1* and *cor6.6* genes which are also induced by drought, high-salt or cold stress[12,13]. A similar motif (C-repeat, TGGCCGAC) has been found in the promoter region of cold-inducible *cor15a* gene in *Arabidopsis*[14]. The core sequence CCGAC has also been found in the promoter of cold-inducible *BN115* gene in *Brassia rapus*, and termed low-temperature-responsive element (LTRE)[15]. These findings suggest that DRE *cis*-element (or its core sequence) is very conserved and widely exists in plant genes induced by drought, high-salt and cold stress.

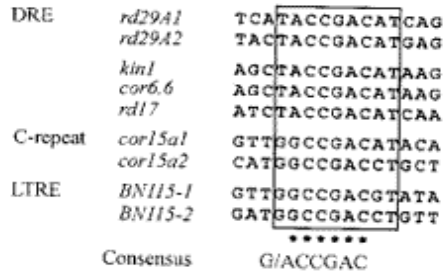

Fig. 1. The sequences of DRE *cis*-acting element and DRE-like elements involved in plant response to drought, high-salt and cold stress.

In order to understand the molecular mechanism how the different stress signals are transmitted in plant cells to activate DRE-dependent transcription and the gene expression under drought, high-salt and cold stress, we cloned 5 cDNAs encoding DRE-binding proteins (named DREB1A—C, DREB2A—B), using DRE *cis*-element of *rd29A* promoter and One-Hybrid screening method[16,17]. *DREB1A*, *DREB1B* and *DREB1C* were isolated from low-temperature treated *Arabidopsis* cDNA library, and *DREB2A* and *DREB2B* were isolated from dehydrated *Arabidopsis* cDNA library[10]. *In vivo* experiments in yeast cells and in protoplast of leaf cells, and *in vitro* gel-shift analyses indicate that both *DREB1A* and *DREB2A* encoding products specifically interact with DRE *cis*-element and activate *GUS* reporter to express. All these 5 DREB proteins contain a basic region in their N-terminal regions which might function as nuclear localization signal (NLS), and an acidic activation region (AAR) in their C-terminal regions. In addition, all DREB proteins contain a conserved DNA-binding domain (termed EREBP/AP2 domain) which is composed of 58 amino acid residues and presents in a large family of plant genes encoding transcription factors[18,19]. The expressions of *DREB1A* and *DREB2A* genes were analyzed and compared with the expression of *rd29A* gene. *DREB1A* was induced within 10 min under 4℃ low-temperature treatment. *DREB2A* was also induced within 10 min under drought or 250 mmol/L NaCl high-salt treatment. Moreover, both *DREB1A* and *DREB2A* genes were not induced by exogenous ABA.

Compared with *DREB* genes, the distinct increase in expression of *rd29A* happens in 40 min when subjected to drought, high-salt or cold stress. Present studies suggest that the expression of *Arabidopsis rd29A* gene under low-temperature, drought and high-salt stress is regulated by *DREB1A* and *DREB2A* transcription factors in two separate signal transduction pathways, respectively. In the characterization of DREB transcription factors, the transgenic plants of *Arabidopsis* transferred by *DREB1A* or *DREB2A* genes enhanced their tolerances to cold or drought and high-salt[10]. The transgenic plants with an enhancement to cold tolerance have been obtained with *CBF1* (namely *DREB1B*) gene transformation in *Arabidopsis* by Jaglo-Ottosen et al.[20,21].

LEA proteins appear in the later development stage of embryos and their contents increase along with desiccation and maturation of seeds. *LEA* genes are also induced by drought, high-salt and cold stress in the vegetative tissues of various plants[4,22]. LEA proteins are quite hydrophilic and directly protect macromolecules and membranes for avoiding or decreasing cell injury by these stresses. Among known *Arabidopsis* genes induced by drought, high-salt and cold stress, *rd29A* encodes a hydrophilic protein similar to LEA protein[23]; both *rd17* and *erd10* genes encode Group 2 LEA proteins[22]; and both *kin1* and *cor6.6* genes encode the proteins that are structurally similar to alanine-rich fish antifreeze proteins[13]. These similarities in protein structure and property and in gene expression character suggest that the products of these genes may have similar functions involved in plant tolerance to drought, high-salt and cold stress. Another cold-inducible *cor15a* gene of *Arabidopsis* encodes a protein that disperses in stromal compartment of the chloroplasts and can enhance the freezing tolerance of leaf protoplast[23]. Because DRE element (or its core sequence) has been found in the promoters of these stress-inducible genes, it is conceived that if one of the 5 *DREB* genes can express under normal condition or its

expression further increases under stress conditions, this DREB transcription factor can simultaneously activate the expression of *rd29A*, *rd17*, *kin1*, *cor6.6*, *cor15A* and *erd10* genes under normal condition or further enhances their expression under stress conditions, which would result in multiple improvement of plant stress-tolerance. We used the strong constitutive 35S cauliflower mosaic virus (CaMV) promoter to drive *DREB1A* gene in transgenic plants of *Arabidopsis*. The above inference has been confirmed by analyzing the expressions of *DREB1A* and its target genes (*rd29A*, *rd17*, *kin1*, *cor6.6*, *cor15a* and *erd10*) and examining the tolerance of transgenic plants to cold, drought and high-salt stress. As shown in fig. 2, *DREB1A*, *rd29A*, *rd17*, *kin1*, *cor6.6* and *cor15a* genes are not expressed in the wild type under normal condition. However, in *DREB1A* transgenic plants, the overexpression of *DREB1A* not only activates the expression of *rd29A*, *kin1*, *cor6.6*, *cor15A* and *erd10* genes under normal condition, but also further enhances the expression of *rd29A*, *rd17*, *kin1*, *cor6.6*, *cor15a* and *erd10* under drought and cold conditions in contrast with wild types. This suggests that DREB1A transcription factor of transgenic plants specifically interacts with DRE element (or DRE core sequence) and induces the expression of these stress-tolerant genes, and then results in multiple improvement of plant tolerance to drought, salt loading and freezing[11]. In contrast experiments, some drought-inducible genes (such as *P5CS*, *erd1*, *rd22* and *rd29B*) which contain no DRE element in their promoter regions are not induced by *DREB1A* overexpression in the transgenic plants. By transformation of a cold-inducible *cor15a* gene, only the freezing tolerance in transgenic plants of *Arabidopsis* has been improved[23]. Although the drought, salt and cold tolerances in transgenic plants *Arabidopsis* have been improved by transfer of *rd29A* gene induced by these stresses[9], its effect is not as good as by gene transfer of a single stress-inducible *DREB1A*[11]. In addition, the gene transfers by another single gene such as proline synthetase gene or betain synthetase gene can enhance plant tolerance to high-salt, but cannot make plant stress-tolerance achieve ideal and multiple improvement[1]. Compared with the correlated characters of plant insect-resistance and disease-resistance, the character of plant stress-resistance is far more complicated. The capability of plant tolerance to drought, high-salt and cold is independent of a single functional gene, the character of plant stress-resistance is influenced by many functional genes. Thus, it is important to enhance the control capability of one or some key regulatory factor(s) to make the plant achieve ideal, multiple and fundamental improvement for stress-resistance.

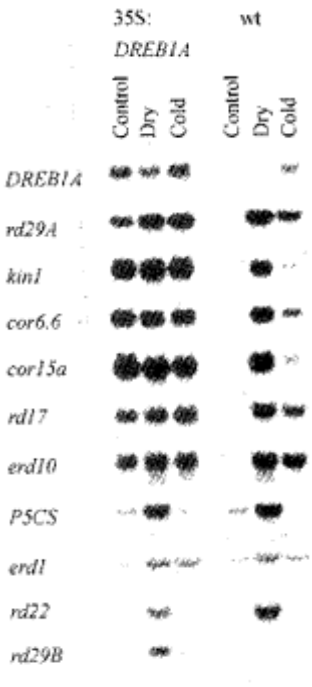

Fig. 2. Expression of *DREB1A* target genes in 35S: *DREB1A* transgenic plants and in wild type plants. *rd29A*, *kin1*, *cor6.6*, *cor15a*, *rd17* and *erd10* genes contain DRE *cis*-acting element (or DRE-like element) in their promoters, while *PSC5*, *erd1*, *rd22* and *rd29B* genes have not DRE element in their promoters. The full-length fragments of *DREB1A*, *kin1*, *cor6.6*, *cor15a*, *rd17*, *erd10*, *PSC5*, *erd1* and *rd22* cDNAs, and 3′-terminal non-coding regions of *rd29A* and *rd29B* cDNAs were used as probes in Northern blot analysis. (Dry: dehydration for 5 d; cold: treated at 4℃ for 5 h).

Fig. 3 illustrates the regulatory function of DREB transcription factor and a series of events from acceptance of drought, salt and cold-stress signal and signal transductions to transcription control of DREB transcription factor and DRE *cis*-acting element, expression of target genes with DRE element, accumulation of stress-induced gene products, physiological and biochemical regulations, enhancement of plant stress-tolerances. The study of DREB transcription factors suggests that a transcription factor may control the expression of many genes involved in correlated characters. In molecular breedings to improve crop stress-resistance, it may be a more effective strategy to improve or enhance the control capability of a key transcription factor for the ideal and multiple effect, compared with the conventional

methods which transfer a single functional gene (the gene of the first group) for improving a single trait. If the regulatory ability of a key transcription factor is enhanced, it can activate the expressions of many target genes controlling correlated characters, and then make a fundamental improvement of multiple characters of crop.

It is worthwhile to mention the EREBP/AP2 DNA-binding domain in DREB transcrip-tion factors. The EREBP/AP2 domain is quite conserved, and the transcription factors containing it are widespreadly found in *Arabidopsis*[24–29], tomato[30], tobacco[19,31], rice[32,33] and maize[34]. It functions in signal transductions involved in cell development[24,25,27,29], ethylene hormone[19,31,35–37], pathogenesis[30], cold stress[10,11,21], drought and salt-stress[10, 11], and it also plays an important role in the transcriptional regulation of many genes. Researchers of plant molecular biology have attached importance to its conserved structure, widespread distribution and specificity binding to special *cis*-element. At present, we are molecularly and biophysically studying the molecular mechanism how the EREBP/AP2 domains recognize and bind to specific sequence of various *cis*-elements, according to the difference of three-dimensional structures between wild type and mutants. Besides, we transferred a chimeric gene into rice, which was composed of the stress-inducible *rd29A* promoter containing two DRE *cis*-acting elements and *GUS* reporter. Under high-salt treatment, *GUS* activity in transgenic rice was clearly observed (unpublished data). This suggested that there was a (or were some) salt-inducible DREB-like transcription factor(s) which could bind to DRE *cis*-element and activate *GUS* reporter gene to express under high-salt condition. It is in progress in several research groups that isolates and characterizes rice, maize and tomato *DREB*-like genes. A practicable prospect will be expected in the extensive applications of *DREB* genes and *rd29A* promoter in improving plant stress-resistance gene engineering.

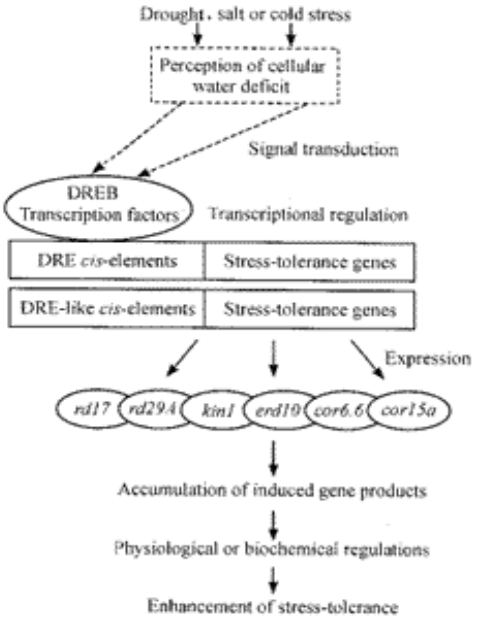

Fig. 3. A stress-regulatable DREB transcription factor can activate the expression of DRE-element-containing target genes involved in plant tolerance to drought, high-salt and cold stress.

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