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Classification and expression diversification of wheat dehydrin genes

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ABSTRACT

Dehydrins (DHNs) are late embryonic abundant proteins characterized by the dehydrin domains that are involved in plant abiotic stress tolerance. In this study, fifty-four wheat DHN unigenes were identified in the expressed sequence tags database. These genes encode seven types of dehydrins (KS, SK_3, YSK_2, Y_2SK_2, K_n, Y_2SK_3, and YSK_3) and separate in 32 homologous clusters. The gene amplification differed among the dehydrin types, and members of the YSK_2- and K_n-type DHNs are more numerous in wheat than in other cereals. The relative expression of all of these DHN clusters was analyzed using an *in silico* method in seven tissue types (*i.e.* normal growing shoots, roots, and reproductive tissues; developing and germinating seeds; drought- and cold-stressed shoots) as well as semi-quantitative reverse transcription polymerase chain reaction in seedling leaves and roots treated by dehydration, cold, and salt, respectively. The role of the ABA pathway in wheat DHN expression regulation was analyzed. Transcripts of certain types of DHNs accumulated specifically according to tissue type and treatment, which suggests their differentiated roles in wheat abiotic stress tolerance.

1. Introduction

Dehydrins, also called group 2 late embryonic abundant (LEA) proteins, are among the most studied dehydration-induced water-soluble proteins. The dehydrin family is characterized by a highly conserved Lys-rich motif that consists of 15 amino acid residues (consensus EKKGIMDKIKEKLPG), referred to as the K-segment [1–3]. K-segments are predicted to form amphipathic α-helices [2,4] and are usually found in more than one copy and in combination with domains rich in Gly and a Ser stretch, the S-segment. The N terminal region of many dehydrins contains another conserved sequence (V/T) DEYGNP, the Y-segment, which shares significant homology with the nucleotide-binding site of plants and bacterial chaperones [5]. K-segments are essential for enzyme protection by their supposed function of preventing abiotic stress-imposed protein aggregation [6]. Dehydrins are subdivided into several classes according to a combination of these conserved segments [2].

Conserved expression of plant *DHNs* was revealed in certain abiotic stress response. The expression of YSK_2-type *DHNs* in barley was revealed to be up-regulated by drought stress but not cold stress, while the expressions of SK_3-, K_n-, and KS-type *DHNs* were induced by both low-temperature stress and drought stress [7,8]. Similar results were revealed in rice and wheat [9–16], Experiment GSE6901, Rice Genome Annotation Project, http://rice.plantbiology.msu.edu/cgi-bin/generate_experiment_page.pl?experiment=GSE6901]. It was noted that *DHN* expression levels were higher in the more tolerant cultivar than that in the less tolerant cultivar [17]. During long-term cold acclimation, the accumulation of dehydrin is significantly affected by Vrn1/Fr1 locus and the expression of the major vernalization gene *VRN1*, respectively [18]. The transcript abundance of wheat *DHNs* was correlated with tissue water content and acquired frost tolerance [12,18]. The role of dehydrins in plant abiotic stresses has been verified by transgenic experiments. Overexpression of wheat YSK_2-type *DHNs* in rice and *Arabidopsis* enhanced their tolerance to salt and osmotic stresses [19,20]. The overexpression of cold-induced *DHNs WCOR410* (SK_3-type) and *CuCOR19* (K_3S-type) in strawberry and tobacco plants, respectively, resulted in improved cold tolerance [21,22].

Gramineous food crops including wheat, rice, maize, sorghum, and barley provide >90% of the human food supply throughout the world. Unlike summer crops such as rice, maize, and sorghum, wheat experiences a low temperature phase in the winter during its seedling stage and usually experiences hot and dry weather in its late growth stages. As such, wheat has broader climate adaptability, especially strong resistance to low temperatures during the

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seedling stage. However, knowledge about the molecular basis for wheat abiotic stress tolerance is limited [23]. Plants usually possess a dehydrin family. For example, Wang et al. [10] identified eight *DHNs* in the rice genome by scanning pseuduomolecules of the japonica genome (release 4) from TIGR (http://www.tigr.org). Thirteen *DHNs* have been identified in barley [7,8,17], while ten *DHNs* have been identified in *Arabidopsis* [24]. Although nearly ten *DHNs* have been identified in wheat [11–13,15,25,26], the composition of the gene family in this crop remains unclear. The objective of this study was to explore the composition and diversification of wheat *DHNs* and analyze their expression diversification under abiotic stress conditions. The composition of *DHNs* among the major cereals was also compared.

2. Materials and methods

2.1. Plant materials, growth conditions, and treatments

Spring wheat cv. Kehan 15, a drought tolerance variety bred in the Wheat Research Institute of Heilongjiang Academy of Agricultural Sciences, China, was used for the gene abiotic stress responsive analysis in this study. This cultivar was selected for its higher tolerance to dehydration at the seedling stage as demonstrated in our pre-experimental analysis.

Kehan 15 seedlings were grown in petri dishes at 25/18 °C (day/night) with a 15-h photoperiod for the tissue harvest. For the dehydration treatment, Kehan 15 seedlings grown under hydroponic condition for 10 days were placed on dry filter paper under 70% humidity and dim light; for the cold treatment, 10-day seedlings were transferred to 4 °C with a water supply; and for the salt treatment, 10-day seedlings were transferred to petri dishes with 250 mM NaCl. In each treatment, the leaves and roots were harvested at 24 and 48 h, respectively, after the treatment. In ABA treatment, 10-day seedlings of Chinese Spring grown in petri dishes were transferred to petri dishes with 0.5 mM ABA. Leaf and root tissues were harvested 24 h after the transfer. For all of the experiments, the corresponding tissues were collected from untreated plants as controls.

2.2. RNA extraction, first-strand cDNA synthesis, and PCR

RNA was extracted with the Trizol kit (GIBCO BRL, the United States of America/USA). First-strand cDNA was synthesized using 2 μg total RNA and M-MLV RT reverse transcriptase (Promega, USA) according to the manufacturer's protocol. Reverse transcription-polymerase chain reaction (RT-PCR) for the expression analyses was conducted in 25-μL reactions containing 5 ng of the template, 5 pmol of each primer, 5 nmol of each dNTP, 37.3 nmol of $MgCl_2$, 0.5 U rTaq DNA polymerase (Takara, Japan) and 1× PCR buffer (supplied with the enzyme). The thermal cycle was 94 °C 3 min, then cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 50 s, followed by 72 °C for a 5-min extension. PCR products were resolved in 1.5% gels and visualized with ethidium bromide staining. RT-PCR amplification of the *β-tubulin* gene was used to indicate the amount of cDNA employed in the PCR reactions. The RT-PCR reactions were repeated independently at least three times to ensure reproducibility. The contig (Unigene) cluster-specific primers (Table 1) were designed with Macvector 9.0 (Accelrys, Oxford, USA) using the default parameters. Since *DHN* clusters can share high homology at the nucleotide level, some primers may be shared by different clusters, but at least one primer was cluster-specific within each primer pair to ensure cluster-specific amplification.

2.3. Expressed sequence tags database (dbEST) mining and in silico expression analysis

To collect EST coding information for the wheat dehydrins, the 50 Group 2 LEA proteins (dehydrins) [27] were used to query against the GenBank wheat dbEST with the cutoff parameter of >30% identity in a >20-amino acid overlap. After removing vector sequence contamination and adaptors of the hits, contigs were assembled using the parameters of >100-bp overlap and >97% identity. Contigs derived from cross hits in different queries were removed. In the *in silico* analysis, wheat *DHN* contigs were queried against the wheat dbEST using the criteria of >97% identity and >100-bp overlap. All hits were checked to avoid multiple counting of the same clones caused by double sequencing or repetitive submission. EST hits were then classified according to tissue type. Those from SSH and normalized libraries were excluded. The hierarchical clustering method [28] was employed to compare the EST tissue distribution profiles among the contig clusters. The EST tissue distribution profile is displayed based on the frequency of EST members within a contig cluster.

2.4. Open reading frames (ORFs) and dehydrin domain prediction

The ORF of each contig was predicted with Macvector 9.0 (Accelrys) using the default parameters. The dehydrin domain within each predicted protein was detected using the PFAM domain prediction method (http://smart.embl-heidelberg.de/). Those predicted proteins that lacked a dehydrin domain were removed.

2.5. Multiple sequence alignment and phylogenetic tree construction

Peptide sequences were aligned with Macvector 9.0 (Accelrys) using the Clustal W method [29]. Phylogenetic trees were constructed using the neighbor-joining method, and pictures of them were drawn using the MEGA4 program (http://www.megasoftware.net/mega.html) [30].

3. Results

3.1. Identification of wheat DHNs

We identified the wheat *DHNs* by querying the GenBank wheat dbEST using the 50 different *DHN* sequences representing all known *DHN* types [27] as the query sequences. The unique hits were assembled into 54 dehydrin contigs (Supplemental Table S1), 51 of which had full ORFs of 279–1410 bp. The predicted proteins were sorted into seven types (KS, SK_3, YSK_2, Y_2SK_2, K_n [n = 1, 2, 3, 4, 6 and 7], Y_2SK_3, and YSK_3 types) according to the combination of the conserved segments (Table 2). These dehydrins were grouped into 32 homologous clusters, among which one had five contigs, seven had three contigs, four had two contigs, and the rest had only one member each. We designated these contig clusters as *TaDHNx.y*, where *x* represents the cluster accession and *y* represents the contig accession in each cluster. Among the members in each cluster, the coding regions have >89% identity and the coded peptide sequences have >90% homology. Sequence alignment revealed that the contigs/unigenes included the 14 wheat *DHNs* registered in the NCBI database (Table 2).

3.2. Comparing the DHN family between wheat and other major cereals

As shown in Table 3, the KS, SK_3, and Y_2SK_2 types are common in the five cereals, but the K_n- and Y_2SK_3-type dehydrins exist in wheat and barley only, and the K_n-type *DHNs* exist in higher