Isolation and Characterization of a BBC1 cDNA from Common Wheat

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Abstract: A breast basic conserved 1 (BBC1) cDNA has been isolated from common wheat (Triticum aestivum L.). Analysis of amino acid sequence derived from the cDNA showed that the wheat BBC1 was highly hydrophilic and rich in alanine, lysine, glutamic acid and arginine residues. The transcription of wheat BBC1 mRNA was regulated by low temperature. Southern blotting analysis showed that BBC1 existed as a small family in common wheat genome.

Key words: breast basic conserved 1 (BBC1); cold induction; common wheat

Freezing temperature affects plant productivity in many parts of the world (Stone et al., 1993) and is one of the most important environmental factors limiting the geographic distribution of plants (Boyer, 1982). However, if exposed to a period of low, but non-freezing temperature, resistance to freezing is increased in many plants (Sáez-Vásquez et al., 1993). During this cold adaptation process, molecular and physiological changes in the plants have taken place (Duellet et al., 2001). A number of cold-induced genes have been isolated and many of them are found to encode hydrophilic polypeptides (Goldstein et al., 1990; Lin et al., 1990; Gilmour et al., 1992; Kazuoka and Oeda, 1992; Lang and Palva, 1992; Neven et al., 1992; Chauvin et al., 1993; Wolfraim et al., 1993; Kaye and Guy, 1995; Ghomashow, 1998).

The BBC1 gene is originally isolated from human being. In nucleic acid hybridization experiments under low stringency washing conditions, the probe prepared from BBC1 gene has been found to produce cross-hybridization signals in DNA samples from various eukaryotic phyla (Adams et al., 1992). It has been deduced that homologues of BBC1 gene are common in eukaryotic organisms (Bertauche et al., 1994). Human BBC1 gene shows about two-fold higher expression in benign breast fibroadenomas than in malignant breast carcinomas (Adams et al., 1992). In higher plants, the expression of AtBBC1 in Arabidopsis thaliana is positively correlated with active cell division (Bertauche et al., 1994), whereas that of BnC24 in Brassica napus is induced by cold (Sáez-Vásquez et al., 1993). In this paper, we report the cloning of the cDNA of a common wheat BBC1 homologue and its induced expression by cold treatment.

1 Materials and Methods

1.1 Plant material

Common wheat (Triticum aestivum cv. Xiaoyan 54) seeds were germinated at room temperature (22 °C) in the dark on wet filter papers. After 72 h, the seedlings were divided into two batches. One was grown at 4 °C, the other at 22 °C. Both treatments were conducted in the dark.

1.2 RNA and DNA extraction

Total RNA samples were prepared from leaves and roots using the Trizol Reagent (Gibco BRL, USA). For extracting total RNA sample from seeds, the method described by Gao et al. (2001) was followed. Genomic DNA was prepared from wheat seedlings of 14-day-old using the method described previously (Sáez-Vásquez et al., 1993).

1.3 Northern and Southern blotting analysis

Total RNA samples (30 µg each) were fractionated on 1.2% formaldehyde agarose gels and transferred to Nylon membranes (Hybond-N+, Amersham). The filters were air-dried and then baked for 2 h at 80 °C. Northern blot hybridization was performed according to the published method (Sambrook et al., 1989). Before capillary transfer, the gels were stained with ethidium bromide and were photographed to record the relative amount of RNA samples in each lanes. DNA samples (30 µg each) were digested with BamH1, EcoR1, and EcoRV, respectively. The digested DNA samples were fractionated on 0.8% agarose gels and transferred to Nylon membranes. Southern hybridization was performed according to Sambrook et al. (1989).

1.4 Reverse transcription and PCR

By aligning the DNA sequences of human BBC1 (accession number X64707), Drosophila melanogaster BBC1 (accession number X77926), BnC24 (accession number Z22620), AtBBC1 (accession number X75162), and OsBBC1 (accession number AB051074), a pair of primers, P1 (5'-CTTGGAGGCTTAAGTCGC-3') and P2 (5'-AATGGGCATTAGTCACCCTG-3'), were designed. Reverse transcription was done using a SMART™ cDNA
Synthesis Kit (Clontech) and superscript™ RT (Gibco BRL, USA). PCR was performed for 40 cycles (94 °C 30 s, 55 °C 60 s, 72 °C 60 s).

1.5 5'-RACE and 3'-RACE

For 5'- and 3'-RACE experiments, two additional primers, P3 (5'-CTTTGGGATGCCAGCGGACTTAAGC-3') and P4 (5'-CCCCAGGCAGTCGAAGGCTCAA-3') were designed. The reactions were carried out using the SMART™ RACE cDNA Amplification Kit (Clontech, USA). For amplification of the full-length of wheat BBC1 coding region, primers P5 (5'-AGACCCGCAAGTACAACATG-3') and P6 (5'-TCACCTTCTCTCCTTCTCCGCC-3') were synthesized. PCR was performed for 35 cycles (94 °C 30 s, 53 °C 60 s, 72 °C 60 s). PCR products were cloned using pGEM-T easy vector system (Promega, USA).

1.6 Expression of wheat BBC1 cDNA in bacterial cells

For bacterial expression experiments, the wheat BBC1 coding region was PCR-amplified using primers P7 (5'-CTCTCATATGATGAAGGCCAGGGCTGGCAGA-3', the italicized letters consist of the Nde I restriction site) and P8 (5'-CTCTCTGAGTCACTTCTCCTTCTCCGC-3', the italicized letters consist of the Xho I restriction site). After digestion of the PCR product with XhoI and NdeI, the cleaved fragment was cloned into the pET-30a (Invitrogen) vector that had been cut with XhoI and NdeI. The resulted construct was induced for protein expression according to the protocol provided by Invitrogen. Induced bacterial cells were harvested for SDS-PAGE analysis according to Sambrook et al (1989).

2 Results

2.1 Analysis of BBC1 cDNA and its derived amino acid sequence

The BBC1 cDNA sequence (The nucleotide sequence data reported have been deposited in the EMBL Nucleotide Sequence Databases under the accession number

![Fig.1. cDNA sequence of common wheat BBC1. The underlined sequences correspond to those of six primers (P1, P2, P3, P4, P5 and P6). The amino acid translation of the cDNA is shown in one-letter code below the nucleotide sequence. The stop codon is indicated by a dash symbol (-).]
AF487458) from common wheat contained a 429 bp open reading frame, starting at the ATG codon (from 190-192 bp) and terminating at the TGA codon (from 619-621 nt) (Fig. 1). Database searches indicated that the predicted amino acid sequence of wheat BBC1 was 89%, 62.4%, 61% identical to those of rice B. napus, and A. thaliana, respectively (Fig. 2).

The predicted wheat BBC1 protein contained 142 amino acid residues in size, which was shorter than those of BBC1 ones from human, D. melanogaster, B. napus, and A. thaliana. In contrast, wheat and rice BBC1 proteins were equal in their size. Computation analysis showed that wheat BBC1 was a basic protein of 16 kD with a predicted pI of 10.33. The deduced amino acid sequence of wheat BBC1 was rich in alanine (10.6%), lysine (14.1%), arginine (10.6%), glutamic acid (10.6%). Hydropathy analysis did not reveal any hydrophobic domain indicative of either a leader sequence or transmembrane domain (data not shown).

2.2 Genomic organization and expression pattern of wheat BBC1 gene

Upon Southern blotting analysis of common wheat genomic DNA samples digested using BamHI, EcoRI and EcoRV (these enzymes did not cut within the wheat BBC1 cDNA insert), the probe prepared from wheat BBC1 cDNA hybridized to 6-9 bands under low stringency washing conditions (Fig. 3). Thus, it appeared that BBC1 genes constituted a small gene family in the genome of common wheat.

Northern blotting analysis indicates that wheat BBC1 cDNA hybridized to a single transcript species present in the root of common wheat (Fig. 4A). The level of BBC1 mRNA in wheat root tissue was higher under 22 °C than that under 4 °C for 24-48 h. However, when wheat seedlings were cold-treated for 72-96 h, the level of BBC1 mRNA in the root tissue of the cold-treated seedlings was higher.
than that under 22 °C (Fig.4B). No BBC1 transcripts were detected in wheat leaf tissue regardless the temperature treatment (Fig.4A). BBC1 transcripts could not be found in dry wheat seeds either (Fig.4A).

2.3 Expression of common wheat BBC1 cDNA in *Escherichia coli*

After induction with IPTG, a 16 kD polypeptide was over-expressed in bacterial cells harboring the expression construct of wheat BBC1 cDNA (Fig.5). This suggests that the BBC1 cDNA was correctly expressed in *E. coli* cells.

![Fig.5](image-url)

Expression of common wheat BBC1 cDNA in bacterial cells. 1, 2, control bacterial cells harboring no expression construct; 3–6, bacterial cells harboring BBC1 cDNA expression construct; 1, 3, 5, samples not treated with IPTG; 2, 4, 6, samples induced with IPTG. The over-expressed polypeptide is marked with an arrow. The position and size of protein standards are shown in the left.

3 Discussion

When plants are grown under low temperature, water leaks out of cells. Consequently, freezing injury is primarily the result of freeze-induced dehydration (Artus et al., 1996). The freezing tolerance of a number of plants has been shown to be associated with an increase in resistance to dehydration stress (Yeh et al., 2000). The putative product of BBC1 gene was rich in alanine, lysine and arginine and was thus highly hydrophilic. This structural feature may make the BBC1 protein suitable as one of the components involved in preventing water from moving out of cells (Thomashow, 1998).

The human BBC1 gene has approximately two-fold higher expression in benign breast fibroadenomas than in malignant breast carcinomas (Adams et al., 1992). The *D. melanogaster* BBC1 mRNA is expressed at all developmental stages, with the highest levels of expression occurring during embryogenesis (Helps et al., 1995). The abundance of the *AtBBC1* mRNA is developmentally regulated during the course of fruit maturation (Bertauche et al., 1994). The expression of *BnC24* at the early stage of development of the etiolated seedlings is strictly regulated by cold temperature (Sáez-Vásquez et al., 1993). The available results suggest that the transcription of BBC1 genes in different organisms is regulated differently, indicating that BBC1 proteins may play a variety of roles in the biology of eukaryotes. In this study, we found that wheat BBC1 gene was expressed exclusively in the root tissue, and its transcription was regulated by cold treatment. This finding resembles cold regulation of BBC1 gene in *B. napus*, suggesting that, at least in higher plants, BBC1 genes may be commonly involved in cold adaptation.

The wheat BBC1 cDNA was correctly expressed in bacterial cells. Further studies are underway to purify the expressed protein and characterize its biochemical and biophysical properties.

References:


