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Plant Physiology and Biochemistry 43 (2005) 83–89
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Short communication

Cloning of dehydrin coding sequences from *Brassica juncea* and *Brassica napus* and their low temperature-inducible expression in germinating seeds

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Received 13 June 2004; accepted 20 December 2004

Available online 18 January 2005

Abstract

A novel subclass of dehydrin genes, homologous to the *Raphanus sativus* late embryogenesis-abundant (LEA) protein (RsLEA2) and the *Arabidopsis thaliana* dehydrin, was isolated from *Brassica juncea* and *Brassica napus*, here designated *BjDHN1* and *BnDHN1*, respectively. The cDNA of *BjDHN1* and *BnDHN1* genes share 100% nucleotide identity. The encoded protein is predicted to consist of 183 amino acid residues (molecular mass of 19.2 kDa and p*I* of 7.0). It shares 85.3% and 65.4% amino acid sequence identity with the RsLEA2 and *Arabidopsis* dehydrin, respectively. This *Brassica* dehydrin also features a "Y_3SK_2" plant dehydrin structure. Expression analysis indicated that the *Brassica* dehydrin gene is expressed at the late stages of developing siliques, suggesting that the gene expression may be inducible by water-deficit. Analysis of gene expression also indicated that in germinating seeds the gene expression was inducible by low temperature. Seed germination under low temperature was compared between *B. juncea* and *B. napus*. The results showed that *B. juncea* seeds germinated faster than *B. napus* seeds. Expression of *Brassica* dehydrin gene was also examined as a function of seed germination under low temperature.

Keywords: *B. juncea*; *B. napus*; Dehydrin; Gene expression; Low temperature; Seed germination

1. Introduction

Dehydrins have been identified as one of the five major classes of late embryogenesis-abundant (LEA) proteins from many plant species. More specifically, dehydrins are the D-11 family of the super LEA protein family [2]. Plant dehydrins are encoded by multigene families and many distinct subclasses have been observed [3,4]. It is well known that the expression of dehydrin genes can be induced by environmental stresses, such as dehydration or low temperature [2–4]. Although the fundamental biochemical and physiological roles of the dehydrin proteins remain to be elucidated, the involvement of dehydrins in low temperature tolerance seems to be clear. For example, it has been found that the abundance of WCOR410, an acidic dehydrin, is positively related to freezing tolerance of wheat seedlings, which provides a potential marker for freezing tolerance of different wheat varieties [5,6,8]. Other reports indicated that there is a correlation between the 35 kDa cowpea dehydrin and the chilling tolerance during cowpea seedling emergence [9,10]. The study showed that allelic variation of the dehydrin gene locus co-segregates with the chilling tolerance trait [10]. These studies suggested that exploration of dehydrin genes might provide useful markers for low temperature tolerance in other crops as well.

A whole spectrum of cold-responsive genes has been isolated from plants. Although many of these genes encode proteins with known functions, most of them encode either newly discovered proteins such as the *Arabidopsis* COR6.6, COR15 and COR78 or homologs of LEA proteins such as

Abbreviations: BjDHN, *Brassica juncea* dehydrin; *BnDHN*, *Brassica napus* dehydrin; LEA, late embryogenesis-abundant; RT-PCR, reverse transcription polymerase chain reaction.

☆ The cDNA and genomic DNA sequences of *BjDHN1* gene reported here have been deposited in GenBank/EMBL/DDBJ databases under accession nos. **AY130998** and **AY130999**, respectively.

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doi:10.1016/j.plaphy.2004.12.006

Table 1
Comparison of germination rates between *B. napus* and *B. juncea* seeds under different temperatures. Q2 and EC excel are *B. napus* species commercially available; PC98-44 and PC-105 are canola *B. juncea* breeding lines proprietary to the Saskatchewan Wheat Pool. Germination rate was counted after seeds were germinated at 22 °C for 3 d or at 5 °C for 5 and 10 d. The average of two independent germination experiments was shown for each treatment

Species	Cultivar	Germination rate (%)		
		22 °C/3 d	5 °C/5 d	5 °C/10 d
B. napus	Q2	>98	4	53
	EC Excel	>98	15	57
B. juncea	PC98-44	>98	63	84
	PC-105	>98	55	96

the *Arabidopsis* COR47 [17]. *Brassica* homologues of the *Arabidopsis* gene family COR6.6 and COR15 were isolated [17], however, no isolation of *Brassica* homologues of the *Arabidopsis* dehydrin gene (*COR47*) has been reported.

Through extensive breeding efforts, the original *Brassica juncea*, which contains unfavorable traits including high erucic acid, high glucosinolate and low oleic acid has been converted to the new canola quality *B. juncea* [11,13,14]. The canola quality *B. juncea* not only possesses oil and meal qualities identical to *B. napus* and *B. rapa* but also is more heat- and drought-tolerant. It, therefore, provides an alternative canola crop species to the canola industry. In this study, we report the cloning of dehydrin genes from both *B. napus* and *B. juncea*. We also investigated whether the isolated *Brassica* dehydrin gene is involved in cold tolerance as in the case of cowpea during seedling emergence. Analysis of gene expression indicated that the *Brassica* dehydrin gene isolated in the current study may be involved in low temperature tolerance only during seed germination.

2. Results and discussion

2.1. Comparison of Brassica seed germination at low temperature

As part of our continued research efforts, we investigated the canola quality *B. juncea* seed vigor and made comparisons with *B. napus* by testing seed germination under cold temperature [18]. Seed germination is defined as a physiological process that commences when the quiescent dry seed begins to take up water and is completed when embryonic axis elongates. Therefore, what we observed in these germination tests is the actual penetration by the radicle of structures surrounding the embryo, which is often called the visible germination [1]. As show in Table 1, over 98% of *B. juncea* and *B. napus* seeds germinated after 3 d at 22 °C. However, germination rate was quite different for *B. juncea* and *B. napus* when seeds were germinated at 5 °C. In particular, both lines of *B. juncea* germinated much faster at 5 °C than did the two *B. napus* varieties. Specifically, after 5 d of germination at 5 °C, the germination rates were 55% and 63%, respectively, for the two *B. juncea* lines. However, under the same conditions, only 4% and 15% germinated for the two *B. napus* varieties. From these germination tests we conclude that *B. juncea* seeds are more cold-tolerant during germination under low temperature than *B. napus* seeds.

2.2. Cloning of Brassica dehydrin coding sequences

The mechanisms involved in plant cold tolerance are very complex and many genes are believed to be involved [17]. Our research interest was inspired by one particular report that a cowpea dehydrin gene is associated with chilling tolerance during cowpea seedling emergence under low temperature [10]. We questioned whether the expression of any dehydrin gene(s) from *B. juncea* and *B. napus* could also be correlated to cold tolerance during seed germination at low temperature in a similar way as in the case of cowpea seedlings. It was our hope that successful identification of a candidate gene or genes that are responsible for the differences in cold germination between *B. juncea* and *B. napus* could lead to the development of a genetic marker for future plant breeding.

To examine if any *Brassica* dehydrin gene expression is correlated to the cold tolerance during seed germination under low temperature, we decided to clone dehydrin gene(s) from both *B. juncea* and *B. napus* and compare their expression under cold temperature. Total RNAs isolated from germinating seeds (5 °C for 3 d) of both *B. juncea* and *B. napus* were used to perform reverse transcription polymerase chain reactions (RT-PCRs) in order to clone the cDNAs. Because there was no previously reported *B. juncea* or *B. napus* dehydrin gene(s), we designed degenerate primers P1 and P2 on the basis of the reported dehydrin genes from *Raphanus sativus* (*RsLEA2*, GenBank accession number **X56280**) and *Arabidopsis thaliana* (*AtDHN*, GenBank accession number **X91920**), which belong to the *Brassicaceae* family.

Cloning and sequencing of the RT-PCR products (partial cDNA, 528 bp) confirmed that one unique dehydrin gene was cloned from each of *B. juncea* and *B. napus*. Comparison with sequences of the *R. sativus RsLEA2* gene and the *Arabidopsis AtDHN* gene indicated that the first ATG at the 5′ end of the cDNA is the translation initiation codon (Fig. 1A). The missing 3′ end of the cDNA (24 bp) was determined by genomic walking technique using a TOPO Walker Kit (Invitrogen) in order to obtain the full-length cDNA. Analysis of the genomic walking product showed that there is an in-frame stop codon, followed by a perfect polyadenylation signal. Therefore, the full-length cDNA for both dehydrin genes of *B. juncea* (designated *BjDHN1*) and *B. napus* (designated *BnDHN1*) are predicted to be 552 bp in size.

Analysis of the nucleotide sequence indicated that the cDNAs of *BjDHN1* and *BnDHN1* are 100% identical and encode polypeptides of 183 amino acid residues. Alignment with the *R. sativus* RsLEA2 and the *Arabidopsis* AtDHN indicated that the *Brassica* dehydrin shares 85.3% and 65.4%