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Allelic variation in the dehydrin gene family of 'Himalaya' barley (*Hordeum vulgare* L.)

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Abstract Variation in the dehydrin gene family of 'Himalaya' barley (*Hordeum vulgare* L.) was studied using 3′ fragments of dehydrin cDNA clones (DHN1–4) as hybridisation probes. Each fragment specifically hybridised to the corresponding cDNA clone, but the 3′ fragment of DHN1 also hybridised to the very closely related DHN7 genomic clone. The same fragments were hybridised to Southern blots of genomic DNA prepared from a pool of 100 plants and digested with different restriction enzymes. DHN1 and DHN2 probes hybridised to one or two fragments, but only single bands were seen with DHN3 and DHN4 probes. When DNA was prepared from individual plants, the DHN1 and DHN2 probes each hybridised to single bands, representing one or other of the two bands in the pooled DNA sample. The 'Himalaya' population is therefore polymorphic, with two types of individual, designated P1 and P2. A cross between P1 and P2 types yielded an F_1 with the same hybridisation profile as the pooled DNA. Segregation observed in the F_2 generation was consistent with a 1 : 2 : 1 ratio. Therefore, P1 and P2 individuals contain RFLP alleles detected by DHN1 and DHN2 probes, with the alleles occurring in specific associations consistent with linkage between *Dhn1* and *Dhn2* loci. Allelic variation was also detected within the *Dhn1* locus. Dehydrin RNA from P1 and P2 individuals was analysed by reverse transcription/polymerase chain reaction using primers specific for DHN1/DHN7 sequences. Sequencing of the amplified and cloned products indicated that P1 individuals expressed only the DHN1 sequence, and P2 individuals only DHN7. These results indicate that the DHN1 and DHN7 clones represent allelic alternatives at the *Dhn1* locus (now designated *Dhn1-1* and *Dhn1-2*, respectively). A stock that contained only P2 individuals was generated from a single seed, allowing hormonal regulation of *Dhn1* gene expression to be studied without complications arising from polymorphism.

Key words Allelic variation · Barley (*Hordeum vulgare* L. 'Himalaya') · Dehydrin · Gene family · Polymorphism

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Introduction

Dehydrins (DHN), also called *l*ate *e*mbryogenesis-*a*bundant (LEA, group 2) or *r*esponsive to *AB*A (RAB) proteins, are a family of related proteins that accumulate in developing embryos, and in response to abscisic acid (ABA) application and environmental stresses such as dehydration, low temperature, salt and mannitol (reviewed by Close 1996). Characteristic to all members of the DHN/LEA/RAB family of proteins from different plant species is a highly conserved, lysine-rich repeating motif (2–11 copies). In addition, a stretch of serine residues in the middle of the polypeptide and a repeated DEYGNP amino acid motif in the N-terminal region are present in some members of this family. Despite extensive investigation of *Dhn*/*Lea*/*Rab* gene expression, the physiological role of the encoded proteins remains unknown. Of particular interest is whether such proteins are directly involved in stress tolerance.

In 'Himalaya' barley, dehydrin genes exist as a multigene family (Close and Chandler 1990). Many different dehydrin clones have been sequenced, but seven distinct dehydrin sequences are now recognised, including four cDNA clones (Close et al. 1989) and three genomic

clones (Close et al. 1995; Robertson et al. 1995; Kleinhofs et al. 1993). The seven distinct dehydrin sequences potentially represent seven different genetic loci. Three chromosomal loci have been defined by restriction fragment length polymorphism (RFLP) mapping: *Dhn1* and *Dhn2* loci are tightly linked and map to the minus arm of barley chromosome 7 (5H), *Dhn3*, *Dhn4* and *Dhn5* are also linked and map to the minus arm of chromosome 6 (6H) (Close and Chandler 1990; van Zee et al. 1995) and *Dhn6* has been mapped to the plus arm of chromosome 4 (4H) (van Zee et al. 1995). The putative *Dhn7* locus has not been mapped. In rice, (*Oryza sativa* L.) four distinct dehydrin (*rab*) genes have been cloned and mapped in a 30-kb region of the genome (Yamaguchi-Shinozaki et al. 1989).

The occurrence of cross-hybridisation between related dehydrin sequences has limited the analysis of expression of individual dehydrin loci. Hybridisation conditions have been manipulated to allow specific detection of mRNAs encoded by DHN3, 4, 5 and 6 (Close et al. 1989; van Zee et al. 1995), however it has not yet been possible to obtain specific hybridisation for mRNAs encoded by the more closely related DHN1 and DHN2 loci (Close et al. 1989). Even more problematic is the extremely close sequence relationship between DHN1 and DHN7, since their coding sequences differ in only six bases and in one short (6-bp) deletion (Robertson et al. 1995). Screening of a barley genomic library for a DHN1 genomic clone was unsuccessful, but two independent DHN7 genomic clones were isolated, and the promoter used to study the hormonal control of dehydrin gene expression (Robertson et al. 1995). Since no DHN7 cDNA clones have been isolated, the relationship between expression of DHN1 and DHN7 is unclear. Our current assessment of the regulation of DHN1 and DHN2 gene expression by dehydration and hormone treatment (Close et al. 1989) is therefore based on a composite hybridisation profile that also potentially includes DHN7 sequences (if expressed). Obviously there could be considerable differences between the patterns of expression of these three genes.

The general aim of the study presented here was to investigate whether part of the variation observed in dehydrin sequences from 'Himalaya' barley represented allelic variation, since in a parallel study in pea (*Pisum sativum* L.) considerable allelic variation in dehydrin genes was observed (Grosselindemann et al. 1998). Many genes have now been cloned from barley, including from the variety 'Himalaya'. The inbreeding nature of barley means that the degree of homozygosity is high. cDNA clones that encode related, but non-identical, sequences probably represent the products of different genetic loci. However, the potential for outcrossing, either naturally occurring or during breeding, at an earlier stage in the development of a variety means that allelic variation may also contribute to sequence diversity among related clones. The results presented here demonstrate that the genomic clone originally assumed to represent a seventh dehydrin locus (*Dhn7*) actually represents an allelic variant at *Dhn1*. An RFLP allele was also observed at the linked *Dhn2* locus. 'Himalaya' barley is polymorphic for these variants, and by selection of homozygous types, lines were developed which, in conjuction with the use of 3′ specific hybridisation probes, allowed expression of these closely related gene products to be monitored individually.

Materials and methods

Plant material

Grains of the 1985 harvest of 'Himalaya' barley (*Hordeum vulgare*. L) were obtained from Washington State University, Pullman, Wash., USA.

Preparation of plant material for RNA extraction

For the reverse transcription-polymerase chain reaction (RT-PCR) analysis of dehydrin mRNAs, young seedlings (3–4 days after imbibition) were exposed to dehydration stress as previously described (Close et al. 1989). After 4 days of dehydration, shoot material was frozen and stored in liquid nitrogen. For northern blot analysis of aleurone RNA, de-embryonated half-grains from the homozygous P2 seed stock (see Results) were surface-sterilised and imbibed on sterile, moist filter paper for 4 days. Aleurone layers were separated from starchy endosperm and incubated in 10 m*M* $CaCl_2$ (control treatment) or 10 m*M* $CaCl_2$ containing either 1 μ*M* ABA, 10 μ*M* gibberellic acid (GA_3) or 1 μ*M* ABA + 10 μ*M* GA_3. ABA [(±), *cis*, *trans*-abscisic acid, 99 + %] and GA_3 (> 90% of total gibberellins) were from Sigma Chemical Co (Australia). After 24 h of treatment the layers were frozen and stored in liquid nitrogen.

DNA preparation and electrophoresis

DNA was extracted from individual plants or from a pool of 100 individual plants essentially as described by Dellaporta et al. (1983) but with an additional RNase treatment and phenol extraction. For Southern blot analysis, 80 μg of DNA was digested with *Eco*RI, *Eco*RV, *Hin*dIII or *Xba*I, loaded equally on four separate 0.8% agarose gels and electrophoresed overnight. DNA was transferred to Hybond-N membranes (Amersham) and fixed with UV light (150 mJ).

Radioactive probes and hybridisation

The dehydrin clones and the sequences they encode are systematically designated DHN1, DHN2, DHN3,. . . DHN7. Fragments from the 3′ end of DHN1–4 were generated by restriction enzyme cleavage and used as probes (see legend to Fig. 1). The 3′ probes represented the last 213 bp of DHN1, 219 bp of DHN2, 226 bp of DHN3 and 245 bp of DHN4. They were labelled with [^{32}P]-dCTP using the "Multiprime" method (Amersham) and were purified from unincorporated radioactivity through a Sephadex G-50 spun column. Membranes were prehybridised, hybridised, washed and exposed to X-ray film as previously described (Robertson and Chandler 1992), except for the omission of formamide; the temperature of pre-hybridisation and hybridisation was accordingly increased to 65°C.

RNA preparation, RT-PCR and northern analysis

RNA was extracted from shoots of dehydrated seedlings and from aleurone layers by the hot phenol-LiCl method (Verwoerd et al. 1989). For RT-PCR, reverse transcription was performed with MMLV enzyme according to the manufacturer's (GibcoBRL) recommendations using 5 µg of total RNA, 200 ng of oligoT_{18} primer and 20 U RNAsin. As controls, reactions were also performed using 0.5 ng of in vitro-transcribed RNAs prepared from the cDNA clones DHN1 (positive control) and DHN2–4 (negative controls). To specifically amplify DHN1/DHN7 sequences, we mixed 2 µl of the RT products (obtained from in vivo or in vitro RNAs) in a final volume of 10 µl with oligonucleotide primers ("THGAA" sense: 5′-GACGCACGGAGCTGC-3′; "KKGMKE" antisense: 5′-TCCTTCATCCCCTTCTTC-3′, final concentration of each primer 0.2 m*M*), 1 µl 10 × *Taq* buffer and 0.4 U *Taq* DNA polymerase (Promega). Following PCR, products (4 µl) were analysed on 7% polyacrylamide gels stained with ethidium bromide. For northern analysis, total RNA (5 µg/lane) and in vitro-transcribed RNAs (0.5 ng/lane) from cDNA clones DHN1–4 were electrophoresed on 1.5% agarose/formaldehyde gels and transferred to Hybond-N membranes (Amersham). The 3′ probe fragments for cDNA clones DHN2–4 were as described above. The α-amylase probe used was the insert of clone pHV19 (Chandler et al. 1984). Labelling of the fragments, hybridisation, washing and the detection of radioactive bands were as described above.

DNA sequencing and analysis

Following RT-PCR, fragments (155 bp from P1 individuals and 149 bp from P2 individuals) were cloned into pGEM®-T vector (Promega) and sequenced using DyeDeoxy™ terminator cycle sequencing kit (Applied Biosystems). The sequences were analysed using the University of Wisconsin Genetics Computer Group programs (Devereux et al. 1984).

Results

Specificity of hybridisation of probes from 3′ regions of dehydrin cDNA clones

Fragments from the 3′ ends of cDNA clones DHN1–4 were prepared, radioactively labelled and hybridised to filters containing inserts of each cDNA clone as well as the DHN7 genomic clone. Although the probe sequences show considerable relatedness (Fig. 1a), the hybridisation results (Fig. 1b) showed that each probe had a high degree of specificity for the corresponding cDNA clone. The 3′ probe prepared from DHN1 also

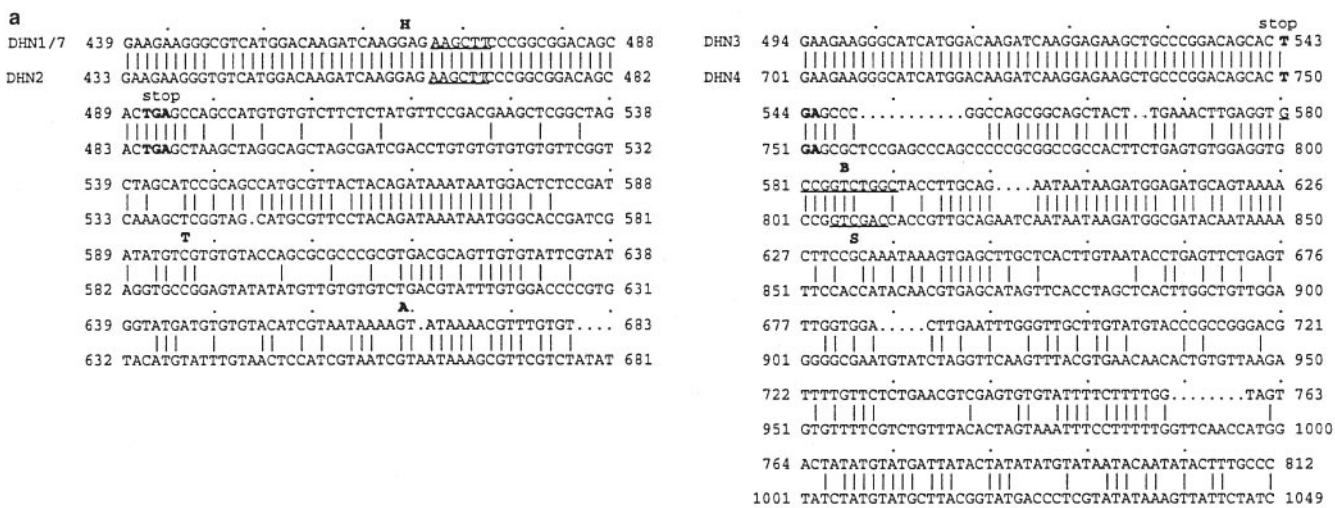

Fig. 1a, b Hybridisation between different dehydrin clones. **a** Pairwise comparisons of the two most closely related dehydrin 3′ regions, DHN1/7 versus DHN2, and DHN3 versus DHN4. The 2 nucleotides that differ between the DHN1 and DHN7 sequences are indicated (*A* and *T* occurring in the DHN7 sequence). The first 36 (DHN1/7 and DHN2) or 33 (DHN3 and DHN4) nucleotides represent the end of the coding region including the conserved KIKEKLPG(G)QH region. The restriction enzyme sites used for the preparation of the DHN1–4 hybridisation probes are indicated (*H Hin*dIII; *S Sal*I; *B Bgl*I). **b** Specificity of hybridisation between dehydrin clones. The complete inserts of the DHN1–4 cDNA clones (*Eco*RI fragments) and the +42 to +961 DHN7 *Cla*I fragment were electrophoresed on 0.8% agarose gels, transferred to nylon membranes and hybridised with the 3′ probes for DHN1–4. Sizes are indicated in kilobases

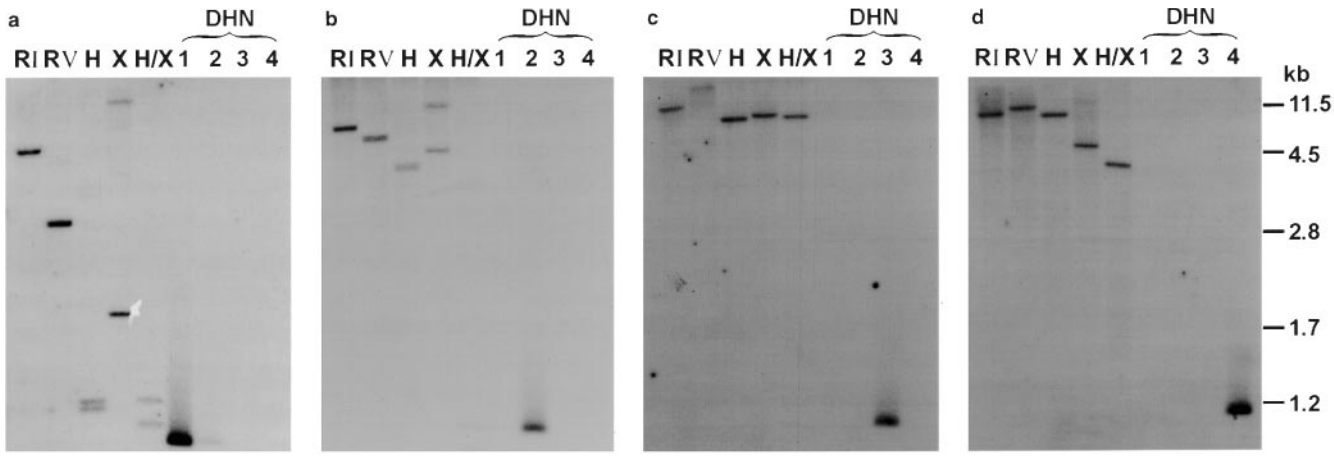

Fig. 2a–d Hybridisation of 3′ probes for DHN 1–4 to Southern blots of 'Himalaya' DNA. Each panel represents a filter containing barley DNA (digested with *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I, or *Hin*dIII + *Xba*I) and DNA of dehydrin clones DHN1–4 after digestion with *Eco*RI. 3′ probes were prepared from each DHN clone and hybridised with the appropriate filter. **a** DHN1, **b** DHN2, **c** DHN3, **d** DHN4

hybridised with the DHN7 fragment, as expected from the near identity of DHN1 and DHN7 sequences (Fig. 1a).

Detection of genomic sequences using 3′ dehydrin probes

Total DNA prepared from a pool of 100 plants was digested with restriction enzymes and analysed by Southern blots and hybridisation using 3′ probes prepared from DHN1–4 cDNA clones. Two fragments were detected with the DHN1 probe in samples digested with *Hin*dIII and *Xba*I (Fig. 2a). Two fragments were also detected with the DHN2 probe in *Hin*dIII- and *Xba*I-digested samples, although for the *Hin*dIII digest the two bands were a poorly resolved doublet (Fig. 2b). Neither 3′ sequence includes a *Hin*dIII or *Xba*I site, nor do any of the dehydrin genes that have been cloned have an intron in this position. We conclude that there must be two genes hybridising with these cDNA 3′ probes. Single fragments were detected with the DHN3 and DHN4 probes with each of the four enzymes (Fig. 2c and d). Hybridisation to inserts from cDNA clones on the same filter showed the high degree of specificity of the probes.

The nature of the two genes hybridising with the DHN1 and DHN2 probes was investigated by first preparing DNA from individual seedlings, followed by digestion with *Xba*I and analysis by Southern blots and hybridisation. Two distinct profiles (P1 and P2) were observed (Fig. 3). P1 individuals had the 11.5-kb DHN1 band in conjunction with the 5.0-kb DHN2 band. P2 individuals had the 2.0-kb DHN1 band and

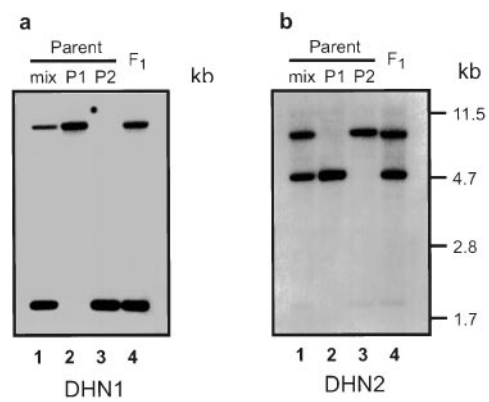

Fig. 3a,b Southern blot analysis of DHN1 and DHN2 in 'Himalaya' barley. A 50-μg aliquot of total DNA was digested with *Xba*I, loaded equally on two 0.8% agarose gels, transferred to nylon membranes and hybridised with 3′ probes for DHN1 (**a**) and DHN2 (**b**). *Lane1* DNA from a pool of 100 plants from the original 'Himalaya' Pullman 1985 seed stock, lanes *2*, *3* DNA from a P1 and a P2 individual (respectively) of Pullman 1985 seed stock, *lane4* DNA from a P1 × P2 F_1 plant. Sizes are indicated in kilobases

an 8.5-kb DHN2 band. For these two probes, P1 and P2 individuals contained alternative single bands representing the two bands in the pooled DNA sample. The observation that individual seedlings contained one or the other (but not both) of the bands detected in the pooled DNA sample indicates that the 'Himalaya' stock is polymorphic. There was no difference detected between P1 and P2 individuals in their DHN3 and DHN4 hybridisation profiles (data not shown), consistent with the lack of variation observed for these probes in the pooled DNA sample (Fig. 2c, d).

To confirm that differences in hybridisation pattern were due to RFLP variation, we crossed P1 and P2 plants and examined the F_1 and F_2 plants. Southern blot analysis of a representative F_1 plant is shown in lane 4 of Fig. 3a and b. DNA from F_1 plants contained both bands detected by each of the DHN1 and DHN2 probes and had the same profile as the DNA from the

pooled sample. DNA was also prepared from 26 individual F_2 plants derived from selfing of the F_1 plant. After *Xba*I digestion, Southern blot analysis using 3′ probes for DHN1 and DHN2 revealed 6 P1, 12 P1/P2 and 8 P2 types in the population (10 individuals shown in Fig. 4). This result is consistent with a 1:2:1 segregation ratio ($\chi^2_2 = 0.41$, $P = 0.82$). We conclude that the two different types of individual in the 'Himalaya' stock each contain, in a homozygous state, a particular combination of RFLP variants defined by the DHN1 and DHN2 hybridisation probes. Co-segregation of these markers in the F_2 generation confirmed that the *Dhn1* and *Dhn2* loci are tightly linked, as previously suggested by Pan et al. (1994). From the lack of non-parental types observed in the 26 F_2 progeny we estimate with a 95% probability that there is less than 5% recombination between the *Dhn1* and *Dhn2* loci (Brown 1980).

The DHN7 sequence represents an allelic variant at *Dhn1*

A specific aim of this study was to investigate the relationship of the two most closely related dehydrin clones, DHN1 (cDNA clone) and DHN7 (genomic clone), since they are almost identical in their overlapping region (Robertson et al. 1995). Given the RFLP allelic variation detected by DHN1, we tested whether the DHN7 sequence represented an allele at the *Dhn1* locus. 'Himalaya' barley seedlings were dehydrated to induce the expression of dehydrin genes. RNA was isolated from (1) a mixture of 20 individuals of the original 'Himalaya' barley seed stock (as in Fig. 3a,b, lane 1), (2) P1- and P2-type individual plants (as in Fig. 3a,b, lanes 2–3) and (3) an F_1 plant (as in Fig. 3a,b, lane 4). Reverse transcription reactions were performed on RNA from both well-watered and from dehydrated seedlings. As size markers and as controls for primer specificity, reverse transcription was also performed with in vitro-transcribed RNA derived from clones DHN1–4. PCR reactions were performed using cDNAs as templates and a pair of oligonucleotide primers that were specific for the DHN1/DHN7 sequence. The products of these reactions distinguish between the DHN1 and DHN7 sequences, because they encompass the region that contains a 6-bp deletion as well as two single base changes, one of which leads to an extra *Bsa*HI site in the DHN7 clone (Fig. 5a). Following PCR, fragments derived from DHN1 cDNA sequences (154 bp) were electrophoretically resolved from the 148-bp fragments derived from DHN7 cDNA sequences (Fig. 5b).

The RT-PCR results from well-watered controls revealed no fragments in the stained gel (Fig. 5b, lanes 1–4), while two fragments of slightly different size (approximately 150 bp) were obtained from the dehydrated seedlings (Fig. 5b, lanes 5–8). In the pooled material from the original 'Himalaya' barley seed stock, two fragments of approximately the same intensity

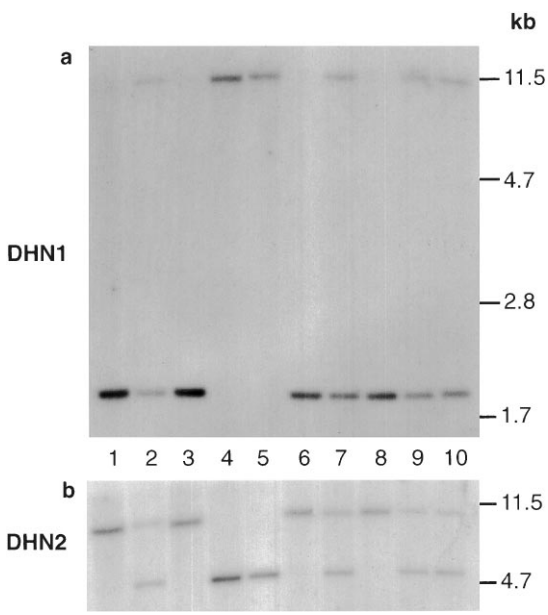

Fig. 4a, b Southern blot analysis of DNA from 10 individual F_2 plants derived from the P1 × P2 cross. A 50-µg aliquot of total DNA from each plant was digested with *Xba*I, loaded equally on two 0.8% agarose gels, transferred to nylon membranes and hybridised with 3′ probes of DHN1 (**a**) and DHN2 (**b**). Sizes are indicated in kilobases

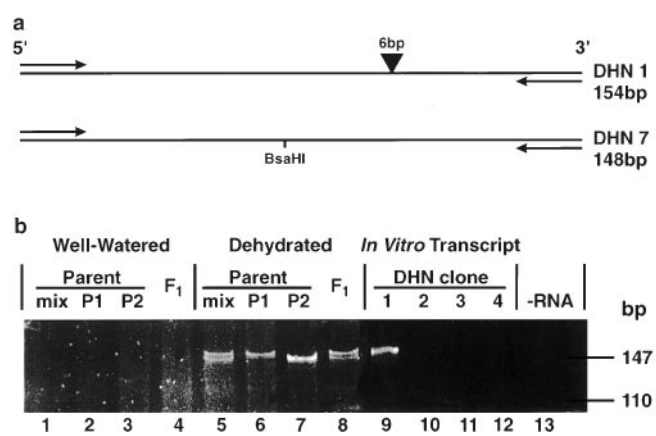

Fig. 5a, b Analysis of DHN1 and DHN7 sequences expressed in dehydrated barley seedlings. **a** Schematic representation of the expected sizes and specific features of DHN1- and DHN7-amplified fragments in P1 and P2 individuals, respectively. The extra 6 bp in the DHN1 sequence is absent from the DHN7 sequence. The extra *Bsa*HI site in the DHN7 sequence is created by a single base change relative to the DHN1 clone. **b** Amplified fragments from the RNA of dehydrated barley seedlings. *Lanes1–4* RNA extracted from well-watered control seedlings, *lanes 5–8* RNA extracted from seedlings dehydrated for 4 days, *lanes 9–12* in vitro-transcribed RNA from DHN1–4 cDNA clones, *lane 13* control reaction without RNA. *Mix* RNA from a mixture of 20 plants, *P*1 type 1, *P*2 type 2, F_1 P1 × P2 cross

were observed (Fig. 5b, lane 5). In the P1 individual, only a single fragment corresponding to the upper one in the mixed lane was detected (Fig. 5b, lane 6), whereas in the P2 individual, a single fragment corresponding to the lower band in the mixed lane was observed (Fig. 5b, lane 7). In the F_1 plant, two fragments corresponding to the sizes of the fragments observed in P1 and P2 individuals (Fig. 5b, lane 8) were observed. The control reaction using in vitro RNA derived from the DHN1 clone produced a fragment the same size as that produced by the P1 plant (Fig. 5b, lane 9). A comparable control using the DHN7 clone is not possible because an intron in the genomic clone makes this fragment much larger, and there is no cDNA clone available for DHN7. In vitro RNA from the other dehydrin clones (DHN2, 3 and 4) did not produce such fragments following amplification (Fig. 5b, lanes 10–12), neither did a reaction without any template (Fig. 5b, lane 13). The smaller-sized fragment amplified from P2 plants was consistent with the size expected for a product amplified from the DHN7 sequence. The two fragments obtained by RT-PCR from the P1 and P2 individuals were cloned into a pGEM-T vector and sequenced. The results from the sequencing confirmed that the P1 fragment corresponded to the DHN1 sequence, and the P2 fragment, the DHN7 sequence (data not shown). In addition, a *Bsa*HI digest of the two RT-PCR fragments confirmed that there is an extra *Bsa*HI site in the middle of the lower band, as expected from the sequence data of the DHN7 clone (data not shown). Taken together, the data indicate that DHN1 is derived from the *Dhn1* locus of a P1 individual and that DHN7 is derived from the *Dhn1* locus of a P2 individual. DHN1 is therefore renamed DHN1-1, and DHN7 is renamed DHN1-2. These allelic variants apparently exist in approximately equally proportions in the commercial 'Himalaya' barley seed stock.

ABA-induced expression of the *Dhn1-2* allele is reversed by GA_3 treatment

A seed stock from a P2 individual was established to study the expression of four individual members of the dehydrin gene family, extending the specificity of previous studies of dehydrin gene expression (Close et al. 1989; Robertson et al. 1995). Aleurone layers from P2-type grains were isolated and treated with hormones, and the RNA analysed by northern blot hybridisation. The results (Fig. 6) showed that hybridisation specificity of the 3′ probes was maintained in hybridisations to RNA, since in vitro-transcribed RNAs were detected only by the probe derived from the corresponding cDNA clone. mRNAs corresponding to sequences DHN1-2 (previously DHN7), DHN2, DHN3 and DHN4 were all induced by ABA. Also, DHN3 and DHN4 mRNAs accumulated to higher levels in response to ABA than did DHN1-2 and DHN2 mRNAs (consistent with earlier studies, Close et al. 1989). When ABA and GA_3 were added simultaneously, dehydrin mRNAs were at very low levels, or not detected, indicating that GA_3 treatment counteracted the ABA-induced accumulation of all four dehydrin mRNA sequence classes. The accumulation of α-amylase mRNA provides a positive control for the effectiveness of the GA_3 treatment. ABA reverses the induction of α-amylase mRNA by GA_3, although most studies use a 25-fold molar excess of ABA over GA_3. The limited reversal observed in Fig. 6 (lanes 7, 8) is probably due to the concentration of ABA being only one-tenth that of GA_3.

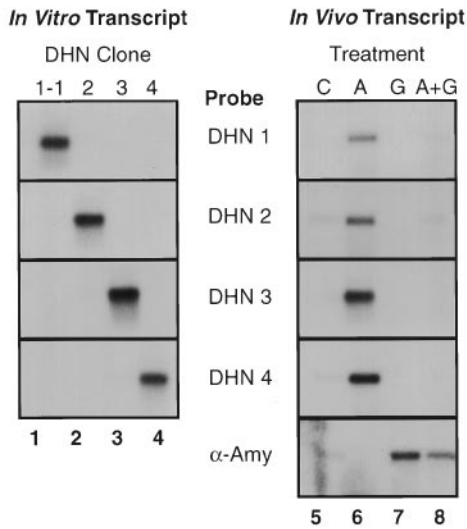

Fig. 6 Northern blot analysis of the expression of individual dehydrin clones. 0.5 ng of in vitro-transcribed RNA generated from DHN1-1 and DHN2–4 cDNA clones (*lanes 1–4*), and 5 µg of RNA extracted from control (*C*), ABA-treated (*A*), GA_3-treated (*G*) and ABA + GA_3-treated (*A* + *G*) aleurone layers (*lanes 5–8*) were electrophoresed on 1.5% agarose/formaldehyde gels, transferred to nylon membrane and hybridised with 3′ probes for DHN1–4 and with an α-amylase cDNA probe

Discussion

The use of clone-specific probes for dehydrin genes in 'Himalaya' barley has revealed that the Pullman stock of this variety is polymorphic and that one of the dehydrin clones previously assumed to represent a new dehydrin locus (*Dhn7*) encodes instead an allelic alternative at the *Dhn1* locus. The origin of the polymorphism in 'Himalaya' is not known. The history of this variety is obscure, although it is generally assumed that little, if any, deliberate outcrossing has occurred. Nevertheless, 'Himalaya' was grown commercially in the western part of the USA (Swenson 1940), so outcrossing in a breeding program could have occurred

and may account for the RFLP variation detected by the DHN1 and DHN2 probes. Alternatively, RFLP variation could be a result of a previous natural outcrossing, or mutation. The significance of our results is that even in varieties where there is little reason to expect previous natural or deliberate outcrossing, residual polymorphism from the original selection may still be present. Different dehydrin cDNA clones in 'Himalaya' barley generally represent distinct loci, but in one case it is apparent that they represent allelic variants at a single locus. This conclusion, together with a similar conclusion reached in a study of pea dehydrins (Grosselindemann et al. 1997), and the variation in maize dehydrins reported by Asghar et al. (1994) suggest that there is considerable variability within and surrounding dehydrin loci.

The identification of the DHN7 clone as an allelic variant at *Dhn1* has important implications for studying the regulation of expression of individual dehydrin genes. The *Dhn1-1* and *Dhn1-2* alleles are very similar in sequence, but even single base differences in regulatory regions may result in considerable differences in gene expression. The locus-specific (3′) probe for DHN1 detects both DHN1-1 and DHN1-2 transcripts. In 'Himalaya' grains from Pullman, both transcripts are present, and therefore a composite hybridisation result is obtained. If the two alleles were differentially regulated by hormones, the composite result might not equate with the promoter activity of either allele when studied in isolation, e.g. in transient expression assays. A similar situation may apply to the other *Dhn* loci, although only in the case of *Dhn*2 has RFLP allelism been documented in 'Himalaya'. The P1 and P2 seed stocks, homozygous for *Dhn1-1* and *Dhn1-2*, respectively, allow such complexities to be avoided.

The Canberra seed stock, which is homozygous for *Dhn1-2*, would also be expected to be homozygous at *Dhn2*, based on the linkage between *Dhn1* and *Dhn2* and the inbreeding nature of barley. No polymorphism was observed at *Dhn3* or *Dhn4* loci, but this may reflect the limited number of restriction enzymes used in the Southern analysis.

Using the gene-specific hybridisation probes we observed that all four dehydrin loci represented by the cDNA clones were up-regulated by ABA and that GA_3 prevented this induction. Whether the GA_3 down-regulation of dehydrin gene expression is occurring at the transcriptional level or post-transcriptionally is not yet known, but previous results from DHN1-2 promoter analysis suggest that the action of GA_3 may occur post-transcriptionally (Robertson et al. 1995). This observation can now be further investigated by nuclear run-on experiments using homozygous stocks.

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