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# RFLP mapping of a *Hordeum bulbosum* gene highly expressed in pistils and its relationship to homoeologous loci in other Gramineae species

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**Abstract** A cDNA sequence (Hbc8-2) isolated from pistils of the self-incompatible species *Hordeum bulbosum* was analysed for expression pattern and genetic map location. Hbc8-2 was expressed just prior to anthesis in mature pistils, and expression was maintained at a high level throughout anthesis. The same expression pattern was found in self-incompatible rye (*Secale cereale*), but no expression was detected in the self-compatible cereals wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) at comparable stages of development. However, three wheat expressed sequence tags from a pre-anthesis library had high homology to Hbc8-2. Southern blot analyses using Hbc8-2 as a probe detected hybridising bands in the genomes of various Gramineae species including rye, barley, bread wheat, wild wheat relatives (*Aegilops tauschii* and *Ae. speltoides*), oats (*Avena fatua* and *A. strigosa*), rice (*Oryza sativa*) and maize (*Zea mays*). This suggests that Hbc8-2-like sequences are present in many species but that high levels of expression may be associated with self-incompatibility. Hbc8-2 was mapped on the long arms of chromosome $2H^b$ of *H. bulbosum*, 2R of rye, and 2B and 2D of wheat and was assigned to chromosome 2H of barley using wheat/barley addition lines. On a *H. bulbosum* genetic map, *Xhbc8-2* was located between *Xbcd266* and *Xpsr87*, while in rye and wheat it was located in a 13.2-cM interval between *Xpsr331* and *Xpsr932*, consistent with previous comparative mapping studies of these species. Mapping in rye suggested that Hbc8-2 is probably proximal to the *Z* self-incompatibility locus which was previously shown to be tightly linked to *Xbcd266*.

**Keywords** *Hordeum bulbosum* · Self-incompatibility · Comparative maps · RFLP · Gene expression

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## Introduction

*Hordeum bulbosum* is a highly self-incompatible (SI) wild relative of barley (*H. vulgare*) which has been used to produce haploids in barley (Kasha and Kao 1970) and wheat (Barclay 1975) and as a source of resistance genes for powdery mildew (Xu and Snape 1989; Xu and Kasha, 1992; Pickering et al. 1995) and leaf rust (Pickering et al. 1998, 2000). In diploid *H. bulbosum*, the SI phenotype is determined by two loci (*S* and *Z*), and incompatibility occurs if both alleles carried by the pollen match alleles in the pistil (Lundqvist 1962). The *H. bulbosum* system is similar to that of other grasses including *Phalaris coerulesces* (Li et al. 1994) and rye (*Secale cereale*) (Wehling et al. 1994) and is probably common to all SI species of the Pooideae (Li et al. 1997; Baumann et al. 2000). The *S* and *Z* loci in rye have been mapped on chromosomes 1RS and 2RL, respectively (Voylokov et al. 1997; Börner and Korzun 1998). Restriction fragment length polymorphism (RFLP) maps of *H. bulbosum* chromosomes allow alignment with other Triticeae species (Jaffé et al. 2000; Salvo-Garrido et al. 2001), predicting that $1H^bS$ and $2H^bL$ will be the equivalent locations in *H. bulbosum*.

No candidate SI genes have been identified in *H. bulbosum*. One approach to isolating them is to identify sequences that are expressed in mature receptive pistils, but are not expressed in self-compatible (SC) relatives. The present paper describes the isolation and characterisation of one such sequence (Hbc8-2) and its mapping on the long arms of homoeologous group 2 chromosomes in *H. bulbosum*, rye and wheat. The relationship between Hbc8-2 and the *Z* locus is discussed.

**Table 1** Plant material used in the study, their sources and ploidy level (*SC* self-compatible, *SI* self-incompatible)

| Species | Ploidy | Accession/cultivar or mapping population (reference)[a] | Compatibility |
|---|---|---|---|
| *Aegilops tauschii* | 2*n* = 2*x* = 14 | 2220007 | SC |
| *Aegilops speltoides* | 2*n* = 2*x* = 14 | 2140008 | SC |
| *Avena fatua*[b] | 2*n* = 6*x* = 42 | Image | SC |
| *Avena strigosa* | 2*n* = 2*x* = 14 | S75 | SC |
| *Hordeum bulbosum* | 2*n* = 2*x* = 14 | GBC77[c] | SI |
| | | PB1 × PB11; 110 $F_2$ plants (1, 2) | |
| *Hordeum bulbosum* | 2*n* = 4*x* = 28 | PB201 | SI |
| *Hordeum vulgare* | 2*n* = 2*x* = 14 | Triumph | SC |
| | | H390-36[c] | SC |
| *Oryza sativa* | 2*n* = 2*x* = 24 | IR20 | SC |
| *Secale cereale* | 2*n* = 2*x* = 14 | 3030009 | SC |
| | | 3030025[d] | SI |
| | | Gazelle[c] | SI |
| | | DS2 × RXL10; 120 $F_2$ plants (3) | SC |
| *Triticum aestivum* | 2*n* = 2*x* = 42 | Chinese Spring (CS) | SC |
| | | CS nullisomic/tetrasomic lines | SC |
| | | Chris[c] | SC |
| | | SQ1 | SC |
| | | CS × Synthetic; 120 $F_2$ plants (4) | SC |
| | | CS × SQ1; 89 doubled haploid lines (5) | SC |
| *Triticum aestivum/Hordeum vulgare* | 2*n* = 44 | CS/Betzes addition lines | SC |
| *Zea mays* | 2*n* = 2*x* = 20 | CO159 | SC |
| | | Tx303 | SC |

[a] Reference (in parenthesis); 1, Jaffé et al. (2000); 2, Salvo-Garrido et al. (2001); 3, Devos et al. (1993); 4, Devos et al. (1993); 5, Quarrie et al. (1994)
[b] Cultivated form
[c] Materials from the Department of Crop Science, University of Guelph; the remainder were from the John Innes Centre
[d] Primarily self-incompatible

## Materials and methods

### Plant material

The list of plant materials used for mapping and RNA studies is given in Table 1. Chinese Spring wheat/Betzes barley addition lines (Islam et al. 1981) comprised a full set except for the group 1 addition line. The complete set of Chinese Spring nullisomic–tetrasomic lines (Sears 1954) was also used. *H. bulbosum* plants were vernalised (Gudu et al. 1993) for 8 weeks to induce flowering to obtain inflorescences for RNA extraction. The chromosomal position of Hbc8-2 relative to other markers in the various crosses was determined using MAPMAKER version 2.0 (Lander et al. 1987) or JOINMAP (Stam and Van Oijen 1995) using the Kosambi mapping function.

### Isolation of the Hbc8 clone

A cDNA library was constructed from poly(A)+ RNA extracted from *H. bulbosum* GBC77 pistils approximately 1 day before anthesis. cDNA was synthesised using Invitrogen kit L1088-15, and sized cDNA was ligated into the *Eco*RI site of the λgt10 vector (Huynh et al. 1988). Pistil-specific cDNA clones were identified by hybridising leaf mRNA and pistil mRNA to duplicate plaque lifts of the λgt10 library. A cDNA clone (Hbc8; accession L26917; 698 bp) was selected for detailed analysis. Hbc8 was found to be a chimeric clone with a 5′ region of 274 bp (Hbc8-1) showing 97% identify to the barley chloroplast psbA gene encoding the D1 protein of photosystem II. The remaining 424 bp of the sequence (Hbc8-2) was subcloned and used for all the work described in this paper. An additional sequence from the same library (Hbc14) was included in sequence comparisons. To confirm the pistil-specific expression of Hbc8-2, total RNA was extracted from leaves, stem, roots, mature flowers with pistils, mature flowers without pistils and pollen of *H. bulbosum* GBC77. The transcript was detected only in mature flowers with pistils (data not shown).

### Nucleic acid extraction, Southern blotting and Northern blotting

DNA was extracted using the techniques of Sharp et al. (1988), digested with restriction enzymes and blotted onto Hybond+ nylon membrane (Amersham) before hybridisation with random primer-labelled probes according to the procedures described by Devos et al. (1993) and Laurie et al. (1993). Filters were washed at a final stringency of 0.2× SSC, 0.5% SDS at 65 °C.

In order to determine the developmental regulation of Hbc8-2, we extracted total RNA from whole inflorescences of *H. bulbosum* accession GBC77, rye (Gazelle), wheat (Chris) and barley (H390-36) at five developmental stages (illustrated in Fig. 1) using the procedures of De Vries et al. (1988). Briefly, Stage 1 occurred soon after initiation of the inflorescence and represents early stages of growth and development of the floral organs. Stage 2 occurred after 20–30% spike emergence from the flag leaf and included mega- and micro-sporogenesis. Stage 3 occurred after complete inflorescence emergence from the flag leaf and marked the rapid transition from the green to yellow anther stages. Stage 4 occurred 3–4 days before anthesis and corresponded to the yellow bud stage. Stage 5 occurred 7 days after the initiation of anthesis. Twenty micrograms of total RNA from each sample was used per lane on Northern blots following the manufacture's protocols (Bio-Rad Zetaprobe membranes using alkaline capillary transfer, Bio-Rad, Hercules, Calif.). Membranes were pre-hybridised in buffer (0.5 *M* $NaH_2PO_4$ (pH 7.2), 1 m*M* EDTA, 7% SDS) at 65 °C for 5 min and hybridised overnight in the same conditions with labelled probes. Filters were given two 30-min washes in 40 mM $NaHPO_4$ (pH 7.2), 1 m*M* EDTA and 5% SDS at 65 °C and a third 30-min wash at 65 °C with SDS reduced to 1%. Membranes were wrapped in Saran wrap and exposed to Kodak XAR-5 film with an intensifying screen at –70 °C for 24–36 h.

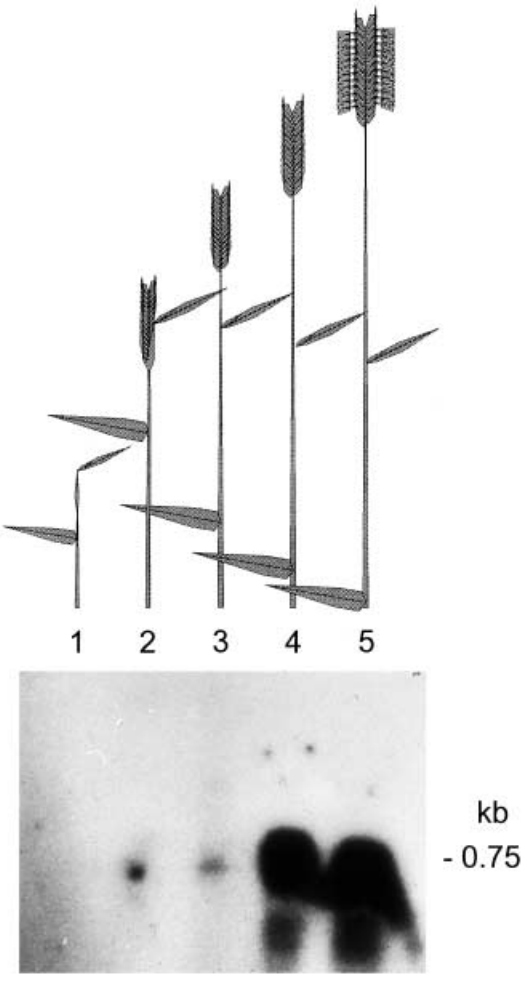


**Fig. 1** Northern blot analyses showing regulation of Hbc8-2 expression during inflorescence growth and maturation of *Hordeum bulbosum*. *1* Inflorescence initiation (early stages of growth and development of the floral organs), *2* 20–30% spike emergence from the flag leaf (mega- and micro-sporogenesis), *3* complete inflorescence emergence (transition from the green to yellow anthers), *4* 3–4 days before anthesis (anther yellow bud stage), *5* 7 days after initiation of anthesis

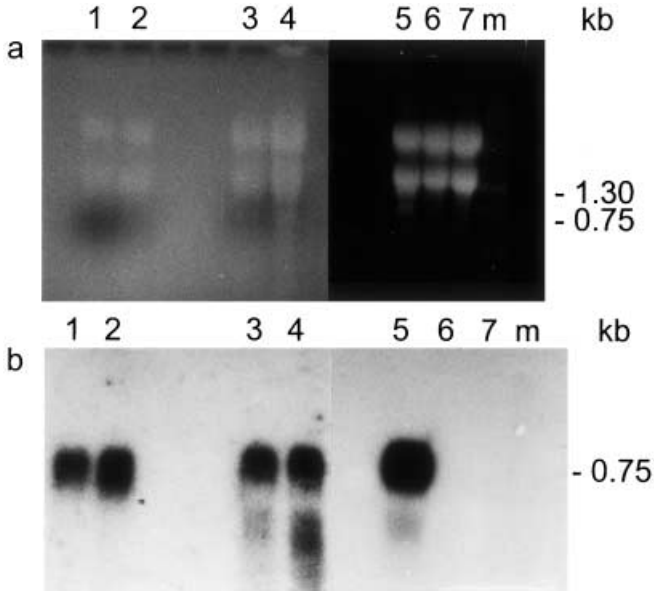


**Fig. 2a, b** Northern blot analyses using the Hbc8-2 cDNA clone. **a** Ethidium bromide-stained gels of RNA from mature inflorescences. *Lanes 1, 2* Rye, *lanes 3–5 H. bulbosum*, *lane 6* barley, *lane 7* wheat. *m* RNA size marker. **b** Northern blots of gels shown in **1a** probed with Hbc8-2. Hybridisation is to rye and *H. bulbosum* RNA only

## Results

### Expression analysis of the *Hordeum bulbosum* Hbc8-2 clone

Northern blots of *H. bulbosum* RNA showed Hbc8-2 to be pistil-specific and to have a tight developmental regulation. Abundant transcript appeared just prior to anthesis, and it was maintained at high levels throughout anthesis (Fig. 1). The transcript detected on Northern blots was estimated to be about 750 bp in size. To test the relationship between Hbc8-2 and self incompatibility, we used Hbc8-2 for Northern blot analyses of *H. bulbosum*, rye, wheat and barley. Hbc8-2 detected abundant transcript in mRNA prepared from mature inflorescences of SI rye (Gazelle) and *H. bulbosum* (GBC 77) but detected no transcript in SC wheat (Chris) or barley (H390-36) at comparable stages of growth and development (Fig. 2).

Expression was also investigated by searching expressed sequence tag (EST) databases using BLAST programmes provided at the NCBI web site (http://www.ncbi.nlm.nih.gov/BLAST/). These searches identified three highly homologous wheat EST sequences (GenBank accession numbers BE497693, BF484675 and BE500724) which were all from pre-anthesis spike tissue of Chinese Spring. Comparison of the wheat ESTs showed that BE500724 had an insertion of 115 bp with in frame stop codons and consensus intron splice sites at its termini (Fig. 3). The wheat EST sequences (after removal of the putative intron from BE500724) were 88% or 89% identical to Hbc8-2 and would therefore be expected to be detectable on Northern blots. Their absence indicates a much lower expression than in *H. bulbosum* and rye.

The wheat and *H. bulbosum* nucleotide sequences could be translated into short proteins, but the *H. bulbosum* sequences had single base differences from wheat (at positions 93 and 342 in Fig. 3) that would produce frame shifts in relation to wheat. As a result, the wheat and *H. bulbosum* sequences would produce different predicted peptides. This suggests that either the wheat or *H. bulbosum* sequences represent non-functional alleles. Database searches did not help to resolve which might be functional, as no known genes had significant homology to the nucleotide or predicted peptide sequences.

### Genetic mapping of Hbc8-2

Southern blot analyses of genomic DNA showed the presence of sequences homologous to Hbc8-2 in all of

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          1       *       *       *       *      50       *       *       *       *      100
   Hbc14  AACGAGAGAGAAAATGGCGGCCACAAAAGAAGCAGTGATGAAGTTAGCGGTTCTCCTCCTGGCCATG......CTCGTCCACATGGCGACGC.AACGGCGGATCC
  Hbc8-2  AACGAGAAAGAAAATGGCGGGCGCAAAAGCAGCAGTGATGAAGTTGGCGGTCCTCCTCCTGGCCATGAGCCAGCTCGTCCACATGGCGACGC.AACGGCGGATCC
BE497693  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~AGCAGTGATGAAGTTGGCGATCGTCCTCCTGGCGATGAGCCAGCTCGTCCACATGGCGACGCCAACGGCGGATCC
BE500724  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~AGCAGTGATGAAGTTGGTTATCGTCCTCCTGGCGATGAGCCAGCTCGTCCACATGGCGACGCCAACGGCGGATCC
BF484675  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~AGCAGTGATGAAGCTGGCGGTCGTCCTCGTGGCCATGAGCCAGCTCGTCCACATGGCGACGCCAACGGCGGATCC
consensus                                 AGCAGTGATGAAG T G   T  TCCTC TGGC ATG      CTCGTCCACATGGCGACGC AACGGCGGATCC

            110       *       *       *      150       *       *       *       *      200       *
   Hbc14  AACGACGGATCCAACGACTGCATCGTCTCTGCTGGTTACGAGCGTTTCCGCCCCTTCCAGCG.....................................
  Hbc8-2  AACGAC............TGCATCGTCTCTGCTGGTTACGAGCGTTTCCGCCCCTTCCAGCG.....................................
BE497693  AATGACT......ACTACATCATCGTCCCTACTGGTCACGAGCGTTTGCGGCCCTTCTAGCG.....................................
BE500724  AATGACT......ACTACATCATCGTCCCTACTGGTCACGAGCGTTTGCGGCCCTTCTAGCGgttagtcatcacctcaagttcatactagagcactggtaattat
BF484675  GACGACTGA............GTCGTCTCTGCTGGTTACGAGCGTTT...CCCCTTCT...G.....................................
consensus  A GAC               TCGTC CT CTGGT ACGAGCGTTT    CCCTTC    G

                220       *       *       250       *       *       *       *      300       *
   Hbc14.....................................................................AAGTCCATCCTACACCCAGCCATGGCATCA
  Hbc8-2  ...................................................................TGGAAGTCCATCCTACACCCAGCCATGGCATCA
BE497693.....................................................................GGGGAGTCAATCCTCAACCCAGTCATGGCATCA
BE500724  cttggttgattagttagctactgatgagaggtactacaagaaactgagatagttgatttttgtcttggccagGGGGAGTCAATCCTCAACCCAGTCATGGCATCA
BF484675  ...................................................................GGGGAGTCAATCCTCAACCCAGCCATGGCATCA
consensus                                                                    AGTC ATCCT  ACCCAG CATGGCATCA

            320       *       *       350       *       *       *       *      400       *
   Hbc14  GGGAAGAGAAGGTGTTGCTCGATGC.GCCAAGCCCAGCATCCCTGC...TCCGCCCCCTCCCCACCTCATCCTACCGTGCAAGGGAAGAGCCTGCTAG
  Hbc8-2  GGGAAGAGAAGGTGTTGCTCGATGC.GCCAAGCCCAGCATCCCTGC...TCCGCCTCCTCCCCACCTCATCCTACCGTGCAAGGGAAGAGCCTGCTAG
BE497693  GGGAAGAGAAGGTGCTGCTCGATGCGGCCAAGCCCAGCATCCCTGT...TCCGCCCCCTCCCCACCTCATCCTACCGTGCAAGGGAAGAGCCTGTTGA
BE500724  GGGAAGAGAAGGTGCTGCTCGATGCGGCCAAGCCCAGCATCCCTGT...TCCGCCCCCTCCCCACCTCATCCTACCGTGCAAGGGAAGAGCCTGTTGA
BF484675  GGGCAGTGAAGGTGTTGCTCGATGCGGCCAAGCCCAGCATCCCTGTCCCTCCGCCCCCTCCCCACCTCATCCTACCGTGCAAGGGAAGAACCTGCTAA
consensus GGG AG GAAGGTG TGCTCGATGC GCCAAGCCCAGCATCCCTG    TCCGCC CCTCCCCACCTCATCCTACCGTGCAAGGGAAGA CCTG T
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**Fig. 3** Nucleotide sequence alignment of *H. bulbosum* (Hbc8-2 and Hbc-14) and wheat (BE and BF) cDNA sequences. The sequences are not shown in full but are trimmed at the 3′ end to a candidate stop codon. A putative intron in the BE500724 sequence is shown in *lower case* and conserved nucleotides are shown in *bold*

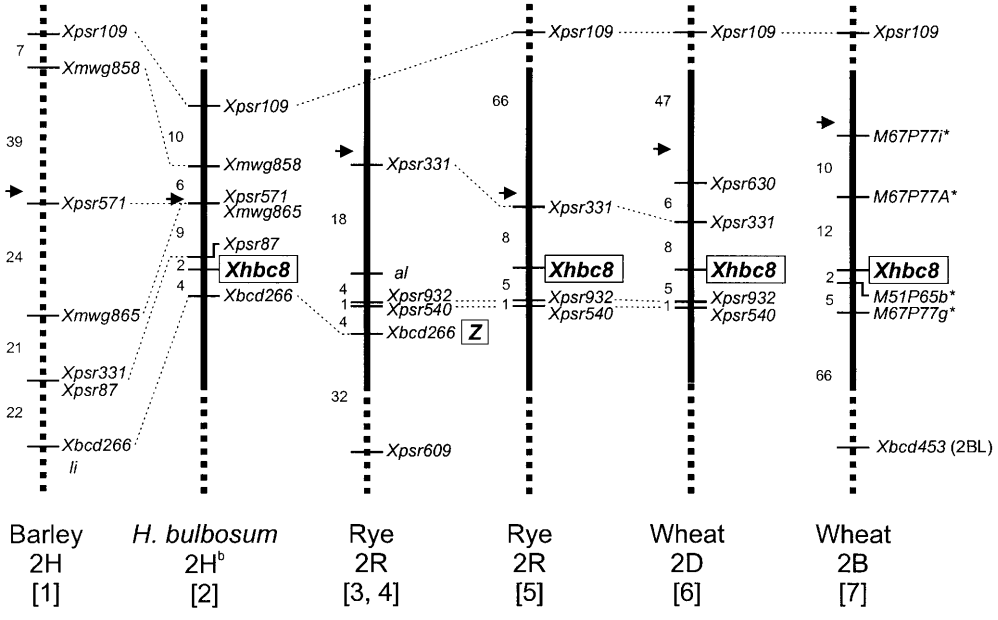


**Fig. 4** Partial RFLP maps of group 2 chromosomes of *H. bulbosum*, barley, rye and wheat showing the location of Hbc8-2 sequences. AFLP markers (asterisks) have been included on chromosome 2B of wheat to indicate the linkage to the anchoring RFLP markers. References: [*1*] Laurie et al. 1993, [*2*] Salvo-Garrido (2001), [*3*] Voylokov et al. (1997), [*4*] Börner and Korzun (1998), [*5*] Devos et al. (1993), [*6*] Devos et al. (1993), [*7*] Quarrie et al. (1994)

the species listed in Table 1 including wheat, rye, barley, oats, maize and rice. As would be expected, Hbc8-2 showed stronger hybridisation to DNA from species of the Triticeae than to other species such as maize, rice, millet and sorghum. In general, a small number of bands were detected, indicative of a low-copy sequence. In cultivated barley, a single fragment was invariably detected in a range of genotypes previously used as parents of mapping populations.

Hbc8-2 was located on chromosome 2H of barley and the group 2 chromosomes of wheat using Chinese Spring/Betzes addition lines and Chinese Spring nullisomic–tetrasomic lines, respectively. In the latter, Hbc8-2 identified two fragments each on 2B and 2D, probably because of internal restriction sites, and a single fragment on 2A. Taken together, these observations clearly indicate that Hbc8-2 was exclusively located on the homoeologous group 2 chromosomes of wheat and barley and was therefore unlikely to be related to the *S* locus which was previously mapped to the short arm of rye chromosome 1 (Voylokov et al. 1997).

The position of Hbc8-2 on the group 2 chromosomes was established using four crosses previously characterised for other markers (Table 1). In the *H. bulbosum*

PB1×PB11 $F_1$ intercross population, Hbc8-2 detected a single-copy sequence with an ab×ac segregation pattern. The *Xhbc8-2* locus was located to the long arm of chromosome 2H$^b$. In relation to markers from the PB1 parent, *Xhbc8-2* was located in a 5-cM interval between *Xbcd880* and *Xwg644b*, 3 cM distal to *Xbcd880*. In the PB11 linkage map, where more markers were available (Salvo-Garrido et al. 2001), *Xhbc8-2* was located between *Xbcd266* and *Xpsr87*, 4 cM proximal to *Xbcd266*. The positions on the PB1 and PB11 maps were equivalent, and only the PB11 map is shown (Fig. 4).

Using the rye Ds2×RxL10 $F_2$ population, we located *Xhbc8-2* on the long arm of chromosome 2R in a 13.2-cM region between *Xpsr331* and *Xpsr932*, 5 cM proximal to the latter. In the wheat Chinese Spring×Synthetic $F_2$ population, *Xhbc8-2* had a similar location on the long arm of chromosome 2D. *Xhbc8-2* could also be mapped on chromosome 2B in the Chinese Spring×SQ1 mapping population, although here there were only two RFLP markers. Hbc8-2 was monomorphic in the barley mapping populations tested, but because of the 2H location established from the addition lines and the known collinearity of the wheat, rye and barley group 2 chromosomes, it is likely to have an equivalent position on 2HL. Voylokov et al. (1997) previously showed that the *Z* self-incompatibility locus of rye was closely linked to *Xbcd266*. Similar results have recently been obtained in *H. bulbosum* (Kakeda et al. 2000). This suggests that the *Z* locus is distal to *Xpsr540*, while *Xhbc8-2* is proximal to the same marker (Fig. 4). If this is correct then Hbc8-2 is not a candidate for the *Z* locus itself.

## Discussion

Hbc8-2 became strongly expressed just prior to anthesis and was maintained at a high level throughout anthesis. This pattern is similar to that of SI genes in species such as *Nicotiana alata* (Anderson et al. 1986) and *Brassica oleraceae* (Nasrallah et al. 1988) and to other pistil-specific genes (Goldman et al. 1992). There was also a clear difference in Hbc8-2 expression between SI and SC cereals. The difference was probably quantitative rather than qualitative as EST searches showed that wheat (SC) contained similar expressed genes.

Although the difference in expression between SI and SC cereals is intriguing, it is unclear what role Hbc8-2-like genes might play in self-incompatibility as the nucleotide and predicted peptide sequences does not resemble any known function gene. Furthermore, comparative mapping showed that Hbc8-2 was unlikely to correspond to the known loci that regulate compatibility. Recent work mapping the *S* and *Z* loci in *H. bulbosum* suggests that they are collinear with rye (Kakeda et al. 2000). Therefore, Hbc8-2 was unrelated to the *S* locus and is probably proximal to the *Z* locus on chromosome 2H$^b$L. However, although Voylokov et al. (1997) and Kakeda et al. (2000) report close linkage between *Xbcd266* and the *Z* locus it would be desirable to confirm the location of *Xhbc8-2* directly in a cross segregating for *Z*.

Hbc8 was highly polymorphic and useful as an anchor probe for comparative mapping. This, together with the results of the EST analysis, shows that Hbc8-2-like genes exist and are expressed in SI and SC species. To characterise Hbc8-2 like genes further, it will be necessary to compare gene sequences and expression patterns from wider range of SI and SC genotypes. Comparison of different *H. bulbosum* accessions and of SI and SC rye might be particularly informative. It would also be interesting to determine if any alteration in Hbc8-2 expression level has a direct effect on incompatibility.

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## References

Anderson MA, Cornish EC, Mau SL, Williams EG, Haggart R, Atkinson A, Bornig I, Grego B, Simpson R, Roche PJ, Haley JD, Penschow JD, Nial HD, Tregear GW, Coghlan JP, Crawford RJ, Clarke AE (1986) Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. Nature 321:38–44

Barclay IR (1975) High frequency haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256:410–411

Baumann U, Juttner J, Bian X, Langridge P (2000) Self-incompatibility in the grasses. Ann Bot 85 [Suppl A]:203–209

Börner A, Korzun V (1998) A consensus linkage map of rye (*Secale cereale* L.) including 374 RFLPs, 24 isozymes and 15 gene loci. Theor Appl Genet 97:1279–1288

Devos KM, Millan T, Gale MD (1993) Comparative RFLP maps of the homeologous group 2 chromosomes of wheat, rye and barley. Theor Appl Genet 85:784–792

De Vries S, Hoge H, Bisseling T (1988) Isolation of total and polysomal RNA from plant tissues. Plant Mol Biol Manual B6:1–13

Goldman MH, Pezzotti M, Seurink J, Mariani C (1992) Developmental expression of tobacco pistil-specific genes encoding extensin-like proteins. Plant Cell 4:1041–1051

Gudu S, Procunier DJ, Ziauddin A, Kasha KJ (1993) Anther culture derived homozygous lines in *Hordeum bulbosum*. Plant Breed 110:109–115

Huynh T, Young RA, Davis R (1988) Constructing and screening cDNA libraries in agtio and agtii. In: Glover D (ed) DNA cloning, vol 1: a practical approach. IRL Press, Oxford, pp 49

Islam AKMR, Shephard KW, Sparrow DHB (1981) Isolation and addition of euplasmic wheat-barley addition lines. Heredity 46:161–174

JafféB, Caligari PDS, Snape JW (2000) A skeletal linkage map of *Hordeum bulbosum* and comparative mapping with barley (*H. vulgare* L.). Euphytica 115:115–120

Kakeda K, Ibuki T, Kowyama Y (2000) Molecular genetics analysis of self-incompatibility in *Hordeum bulbosum*. Barley Genet 8:157–159

Kasha KJ, Kao KN (1970) High frequency haploid production in barley (*Hordeum bulbosum* L). Nature 225:874–876

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburgh I (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genome 1:174–181

Laurie DA, Pratchett N, Devos KM, Leitch IJ, Gale MD (1993) The distribution of RFLP markers on chromosome 2 (2H) of barley in relation to the genetical and physical location of 5S rDNA. Theor Appl Genet 37:177–183

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter ×spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Li XM, Nield J, Hayman D, Langridge P (1994) Cloning a putative self-incompatibility gene from the pollen of the grass *Phalaris coerulescecs*. Plant Cell 6:1923–1932

Li XM, Paech N, Nield J, Hayman D, Langridge P (1997) Self-incompatibility in the grasses: evolutionary relationship of the S gene from *Phalaris coerulescence* to homologous sequences in other grasses. Plant Mol Biol 34:223–232

Lundqvist A (1962) Self-incompatibility in diploid *Hordeum bulbosum* L. Hereditas 48:138–152

Nasrallah JB, Yu SM, Nasrallah ME (1988) Self-incompatiblity in *Brassica oleraceae*: expression, isolation and structure. Proc Natl Acad Sci USA 85:5551–5155

Pickering RA, Hill AM, Michel M, Timmerman-Vaughan GM (1995) The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L to barley (*H. vulgare*) chromosome 2(2H)L. Theor Appl Genet 91:1288–1292

Pickering RA, Steffenson BJ, Hill AM, Borovkova I (1998) Association of leaf rust and powdery mildew resistance in recombinant derived from a Hordem vulgare×Hordeum bulbosum hybrid. Plant Breed 117:83–84

Pickering RA, Malyshev S, Kunzel G, Johnson PA, Korzun V, Menke M, Schubert I (2000) Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. Theor Appl Genet 100:27–31

Quarrie SA, Gulli M, Calestani C, Steed A, Marmiroli N (1994) Location of a gene regulating drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. Theor Appl Genet 89:794–800

Salvo-Garrido H, Laurie DA, Snape JW (2001) An RFLP map of diploid *Hordeum bulbosum* L. and comparison with barley (*H. vulgare* L.) and wheat (*Triticum aestivum* L.). Theor Appl Genet 103:869–880

Sears ER (1954) The aneuploids of common wheat. Mo Agric Exp Stn Res Bull 572:1–59

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Localization of α-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Stam P, Van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Voylokov AV, Korzun V, Bömer A (1997) Mapping of three self-fertility mutations in rye (*Secale cereale* L.) using RFLP, isozyme and morphological markers. Theor Appl Genet 97:147–153

Wehling P, Hackauf B, Wricke G (1994) Phosphorylation of pollen proteins in relation to self-incompatibility in rye (*Secale cereale* L.). Sex Plant Reprod 7:67–75

Xu J, Kasha KJ (1992) Transfer of a dominant gene for powdery mildew resistance and DNA from *Hordeum bulbosum* into cultivated barley (*H. vulgare*). Theor Appl Genet 84:771–777

Xu J, Snape JW (1989) The resistance of *Hordeum bulbosum* and its hybrids with *Hordeum vulgare* to common fungal pathogens. Euphytica 41:273–276