Theor Appl Genet (1991) 82:665–667

RFLP Report

Chromosomal location and variability in wheat, barley and rye of a wheat gene encoding a bZIP protein (EmBP-1)

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Received April 30, 1991; Accepted May 29, 1991
Communicated by J.W. Snape

Source of the probe

A cDNA expression library in λgt11 was constructed from poly(A^+)RNA extracted from abscissic-acid-treated immature wheat embryos. A total of 120,000 recombinant phages was screened for proteins capable of binding a 76-bp sequence in the abscissic acid response element from the 5' regulatory region of the wheat *Em* gene (Guiltinan et al. 1990). Two positive clones, λGC12 and λGC19, were identified. The 589-bp insert of λGC19 was subcloned in the *Eco*RI site of pUC118 (Guiltinan et al. 1990) and used as a probe for gene localization.

Chromosomal location

Hybridization of pGC19 against *Eco*RI-, *Eco*RV-, *Dra*I- and *Hin*dIII-restricted genomic DNA of 21 nullisomic-tetrasomic lines of Chinese spring (CS) revealed seven hybridizing sequences in the wheat cv CS, and showed that the gene copies were located on 5A, 5B and 5D, 3B, 6A, 6B and 7D. Ditelosomic analysis assigned all copies to the long arms of their respective chromosomes, with the exception of 6B, where a short arm location was established. Chromosomal locations in barley and rye were obtained using *Hin*dIII-restricted DNA of the CS/*Hordeum vulgare* cv "Betzes" and CS/*Secale cereale* cv "Imperial" single chromosome addition lines. Remarkably, only a single band was detected in *Eco*RI and *Eco*RV restriction digests of genomic DNA from 13 barley cultivars. Hybridization of pGC19 against *Hin*dIII-restricted genomic DNA revealed two hybridizing bands in genotypes such as Betzes (Fig. 1), while one hybridizing band was found in other genotypes. Since both Betzes bands were shown to be located on 5H (barley chromosome 7), they are likely to have been drived from the same gene, with an internal cutting site for *Hin*dIII. Four gene copies, located on 2R, 4R, 5R and 7R, were present in rye.

Locus symbol

XEmbp (*XEmbp-3B, -5A, -5B, -5D, -6A, -6B, -7D, -5H, -2R, -4R, -5R, -7R*).

Polymorphisms

The frequency of RFLP detected by pGC19 was determined in 15 wheat varieties using four enzymes, and in 13 barley varieties using three enzymes. Potential heterozygosity values (H), fragment sizes and number of alleles, obtained for each locus with different restriction enzymes, are summarized in Table 1. *XEmbp-5B* and *XEmbp-6B* are highly polymorphic, *XEmbp-3B* and *XEmbp-7D* are moderately polymorphic and the 5A, 5D and 6A loci are monomorphic, as shown with *Hin*dIII digests in Fig. 1. A high overall value of H of ca. 50% is found for barley. Most polymorphisms were detected with all enzymes.

Presence of Embp-related sequences in alien relatives of wheat

pGC19 was hybridized to *Eco*RI-restricted DNA of accessions of 21 alien relatives of wheat, including *Triticum, Hordeum, Secale, Aegilops, Agropyron* and *Dasypyrum* species (not shown). Depending on the species, one to eight gene copies were detected. The wheat *Embp* gene also cross-hybridizes to sequences present in maize, mil-

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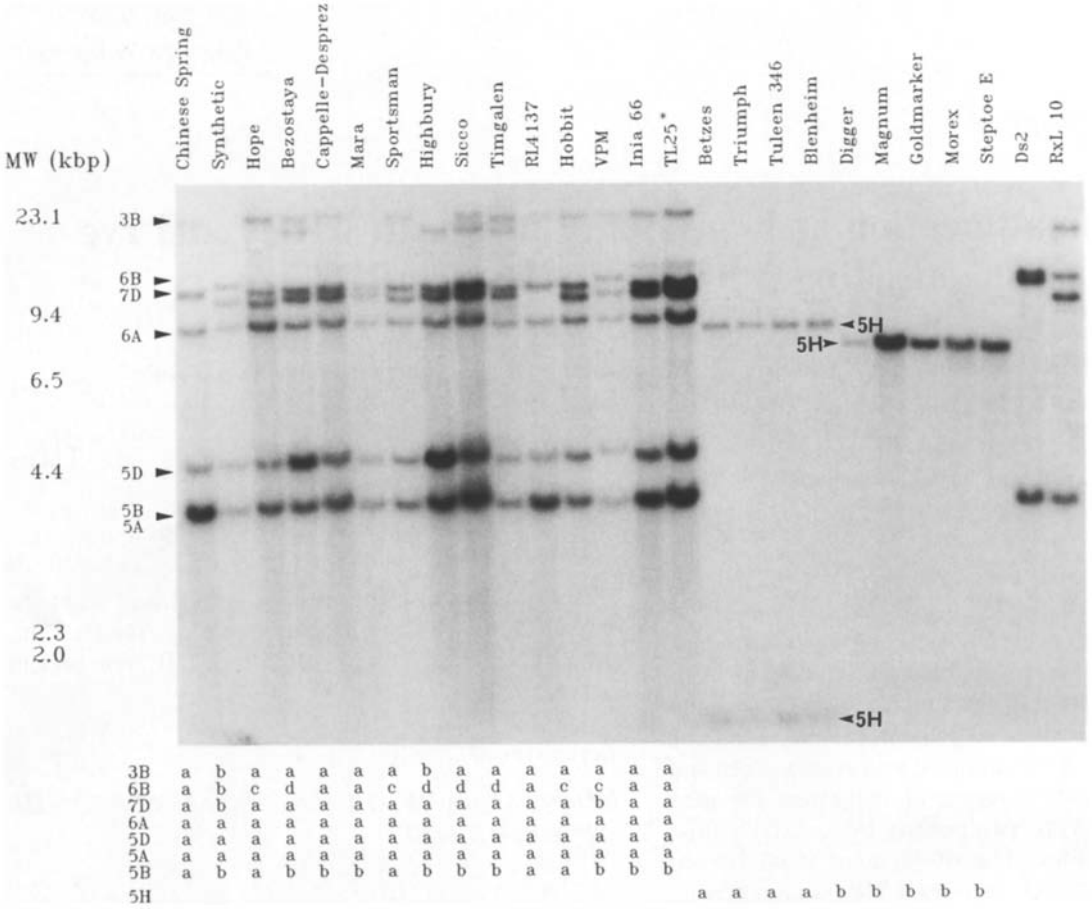

Fig. 1. Hybridization patterns obtained with *Hin*dIII digestion of genomic DNA of 15 wheat varieties, nine barley varieties and two rye varieties. For each locus, the alleles corresponding with each variety are given in the table at the bottom (*TL25 is a breeding line developed at PBI, Cambridge)

Table 1. Potential heterozygosity values in % (H); fragment sizes of the hybridizing sequences in kbp in the A, B and D genomes of the wheat cultivar "Chinese Spring", and the H genome of the barley cultivar "Betzes" (MW), together with the number of alleles (*N*) detected among 15 wheat varieties and 13 barley varieties

	*Eco*RI			*Eco*RV			*Dra*I			*Hin*dIII		
	H	MW	*N*	H	MW	*N*	H	MW	*N*	H	MW	*N*
5AL	0	5.2 3.8	1	0	4.5	1	0	6.7	1	0	3.7	1
5BL	50	15.3	3	43	15.1	2	–	35.3	–	48	3.8	2
5DL	0	12.7	1	0	6.8	1	0	8.7	1	0	4.4	1
3BL	23	4.0	2	26	12.2	2	15	4.6	2	23	18.4	2
6AL	0	6.4 2.5	1	–[a]	23.1	–	0	3.8	1	0	7.9	1
6BS	67	2.2	4	–	25.1	–	72	2.2	4	69	11.4	4
7DL	0	2.8	1	23	8.0	2	28	6.2	2	23	9.7	2
5H	52	6.8	3	50	7.8	2				50	7.4 1.4	2

[a] No data available

let and rice. Among the species tested only *Lolium perenne* gave no signal. Thus, the *Embp* gene sequence must be highly conserved in the Graminae.

Other studies of the Embp gene

The cloning and characterization of the wheat *Embp* gene is described in Guiltinan et al. (1990). The *Embp* product is a DNA binding protein of the basic-leucine zipper class, b-ZIP (Johnson and McKnight 1989). Other members of this class have been cloned from wheat (Tabata et al. 1989) and from other plants (reviewed by Gruissem 1990). EmBP-1 binds to an abscissic acid response element from the wheat *Em* gene, as functionally defined by Marcotte et al. (1989).

Probe availability

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Acknowledgements. Supported in part by a research grant from the National Institutes of Health to RSQ (GM 44288).

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