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*RFLP Report*

# The coding sequence for sedoheptulose-1,7-bisphosphatase detects multiple homologues in wheat genomic DNA

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*Source of the probe*

The clone S9.2 was isolated from a wheat cDNA expression library in λgt11 (Raines et al. 1988; Chinoy et al. 1991) by screening with polyclonal antibodies which had been raised to maize sedoheptulose-1,7-bisphosphatase (SBPase) (Nishizawa and Buchanan 1981). The insert from a plaque which gave a positive reaction with the antibodies was subcloned into a plasmid for further analysis. A detailed description of the characterization of S9.2 and of cDNA and genomic clones encompassing the entire coding sequence of SBPase is given by Raines et al. (1992).

*Chromosomal location in wheat*

In Chinese Spring (CS), nullisomic-tetrasomic (NT) and ditelosomic (DT) analysis showed the presence of five sequences homologous to the SBPase sequence, located on chromosome arms 2BS, 3AL, 3BL, 3DL and 7BL (Fig. 1). A minimum number of five hybridizing fragments, located on the same chromosomes, were observed when the NT lines were digested with either *Eco*RI, *Eco*RV, *Dra*I or *Hin*dIII, indicating that only one gene copy was present on any chromosome arm.

Hybridization of S9.2 with DNA from 13 wheat varieties, digested with the same restriction enzymes, revealed two fragments additional to those present in CS (Fig. 2). One of these, subsequently located on 2BL by linkage analysis (see below), was observed in nine varieties including Synthetic (IPSR1190903, McFadden and Sears 1946; Sears 1976). The chromosomal location of the other fragment (labelled X in Fig. 2), present in Hope, RL4137 and Sicco, could not be ascertained.

Precise locations were obtained for the loci on 2BS, 2BL and 3DL from linkage analysis using 120 $F_2$ lines of a CS × Synthetic population which had been extensively mapped for homoeologous group 2 and group 3 loci (Devos et al. 1992a, b). *XSbp-2B(1)* mapped 19 cM from the centromere on 2BS, *XSbp-2B(2)* 12 cM from the centromere on 2BL, and *XSbp-3D* 20 cM from the centromere on 3DL (Devos et al. 1992a, b).

*Locus symbols*

*XSbp [XSbp-2B(1)*(short arm), *-2B(2)*(long arm), *-3A, -3B, -3D, -7B]*

*Polymorphism in wheat*

The level of polymorphism, expressed as potential heterozygosity (H), was determined in 13 wheat varieties, using four restriction enzymes. For each locus/enzyme combination, the size of the hybridizing fragment, the H value, and the number of alleles are given in Table 1. *XSbp-3A, -3B* and *-7B* were monomorphic in the varieties sampled, while for *XSbp-3D* only the synthetic hexaploid, "Synthetic", displayed a different allele with three out of the four restriction enzymes (Fig. 2). *XSbp-2B(1)* is highly polymorphic with *Eco*RV and *Hin*dIII (Fig. 2), but monomorphic with *Dra*I. *XSbp-2B(2)* and the unlocated band (X in Fig. 2) gave H values of 36% on the presence or absence of the single fragments.

*XSbp in related species*

Hybridization of S9.2 with DNA from 13 barley and two rye varieties digested with three enzymes revealed only one fragment in each genotype. The single gene copy in each species was assigned to chromosomes 3R and 3H by hybridization to the wheat/alien chromosome addition lines of CS/*Secale cereale* cv. Imperial and CS/*Hordeum vulgare* cv. Betzes (data not shown). *XSbp-3R* was incorporated in the genetic map of chromosome 3R, constructed in an $F_2$ population from a cross between the

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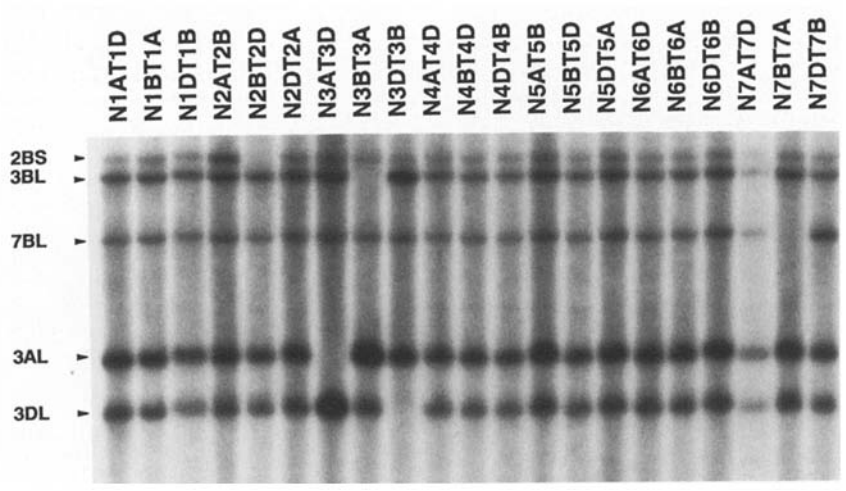


**Fig. 1.** Autoradiograph of *Eco*RV-digested genomic DNA of 21 nullisomic-tetrasomic lines of CS probed with S9.2

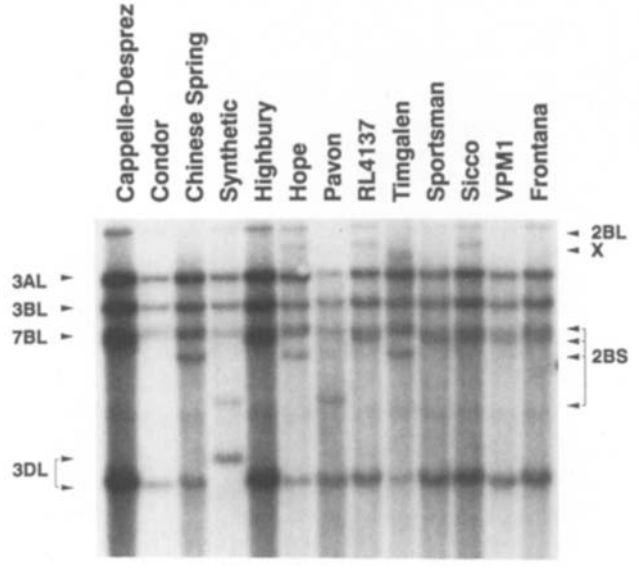


**Fig. 2.** Autoradiograph of *Hind*III-digested genomic DNA of 13 wheat varieties probed with S9.2. The alleles for the 2B and 3D loci are indicated in the different varieties

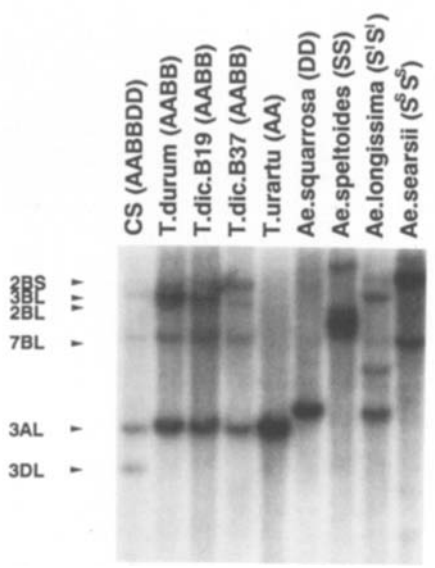


**Fig. 3.** Autoradiograph showing *Eco*RV hybridization patterns obtained in hexaploid, tetraploid (*T.dic.* = T. dicoccoides) and diploid Triticeae species with S9.2

inbred lines Ds2 × R×L10, and mapped 9 cM from the centromere on the long arm. The *XSbp-3H* locus was monomorphic in the 13 varieties examined (Table 1).

Single gene copies were also observed in the *Triticum urartu* accession (AA genome) and *Aegilops squarrosa* accession (DD), but additional fragments were detected in the tetraploid (AABB) wheats examined, and in the accessions of the diploids *Ae. speltoides, Ae. longissima* and *Ae. searsii* (SS) (Fig. 3) of the Sitopsis group, which is favoured as the source of the wheat B genome (Miller 1987).

Hybridization of S9.2 (data not shown) to *Eco*RV genomic digests of a sample of related and other graminaceous crop species revealed strong signals in all genotypes examined, including *Pennisetum glaucum, Zea mays, Sorghum bicolor, Oryza sativa* and *Lolium perenne*, thus further demonstrating the conservation of the SBPase sequence.

### *The origin of the Sbp sequences*

The presence of additional loci, over and above *XSbp-3*, in the B genome of wheat, but neither in the diploid A or D nor in the H and R genomes, raises a question as to their origin. A comparison of the wheat hybridization patterns with those of the single diploid Triticeae accessions examined (Fig. 3), shows that the *T. urartu* (AA genome) *Eco*RV hybridizing fragments correspond precisely to the conserved *Sbp-3A* allele found in all tetraploid and hexaploid genotypes analyzed. Similarly, the *Ae. squarrosa* (DD genome) allele was identical to the *Sbp-3D* allele observed in Synthetic. Although internal

**Table 1.** Potential heterozygosity (H) in %; fragment sizes of the hybridizing sequences in kbp in the A, B and D genomes of the wheat cultivars "Chinese Spring" and "RL4137", and the H genome of the barley cultivar "Betzes" (MW); together with the number of alleles (N) detected among 13 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 |
| 2BS | –[a] | – | – | 56 | 15.8 | 3 | 0 | 2.2 | 1 | 63 | 9.4 | 4 |
| 3AL | 0 | 6.1 | 1 | 0 | 4.1 | 1 | 0 | 4.2 | 1 | 0 | 15.5 | 1 |
| 3BL | 0 | 1.6 | 1 | 0 | 12.8 | 1 | 0 | 7.1 | 1 | 0 | 12.0 | 1 |
| 3DL | 0 | 6.6 | 1 | 15 | 3.3 | 2 | 15 | 15.9 | 2 | 15 | 4.3 | 2 |
| 7BL | – | – | – | 0 | 7.9 | 1 | 0 | 2.5 | 1 | 0 | 7.7 | 1 |
| 3H | 0 | 3.0 | 1 | 0 | 17.0 | 1 | 0 | 2.7 | 1 | 0 | 8.0 | 1 |
| 2BL | 36 | 3.8[b] | 2 | 36 | 11.9[b] | 2 | 36 | 6.1[b] | 2 | 36 | 21.7[b] | 2 |
| | | 2.0 | | | | | | | | | | |
| X | – | – | – | 36 | 20.2[b] | 2 | 36 | 1.5[b] | 2 | 36 | 20.4[b] | 2 |

[a] No data available
[b] Fragment sizes were measured in the wheat variety RL4137

*Eco*RV restriction sites cannot be excluded as a source of the additional hybridizing fragments in the Sitopsis genomes, the results do rather suggest that extra copies of the SBPase sequence were accumulated in the B genome donor species and passed on, first to tetraploid and later to hexaploid wheats.

Null alleles such as described for *XSbp-2B(2)* and a few other RFLP systems, e.g., *XAcl3-5B* (Devos et al. 1991) and *XEmbp-3B* (Devos et al. 1991), are rare in wheat. The loss of loci altogether is, however, likely to occur most readily with supernumerary sequences. All null alleles in wheat to-date have been detected at B genome loci, which raises the question as to whether sequences on the B genome are more vulnerable to duplication and deletion than those on the A and D genomes.

*Probe availability*

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