

## RFLP Report

# Chromosomal location and RFLP utility in wheat and barley of a wheat gene with homology to a 7S storage-globulin sequence

Teresa Millan<sup>2</sup>, Katrien M. Devos<sup>1</sup>, Catherine N. Chinoy<sup>1</sup>, James L. Litts<sup>3</sup>, Ralph S. Quatrano<sup>4</sup>, and Michael D. Gale<sup>1</sup>

<sup>1</sup> Cambridge Laboratory, Colney Lane, Norwich NR4 7UJ, UK

<sup>2</sup> Departamento de Genetica, ETSIA, Apdo 3048, 14080 Cordoba, Spain

<sup>3</sup> Chiron Corporation, 1400 53rd Street, Emeryville, CA 94608, USA

<sup>4</sup> Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, USA

Received June 30, 1992; Accepted July 30, 1992

Communicated by G. E. Hart

### Source of the probe

A cDNA library was constructed using poly(A<sup>+</sup>) RNA extracted from abscisic acid (ABA)-treated immature wheat embryos as described by Williamson et al. (1985). A cDNA clone (PSP511) was isolated which included a 704-bp insert containing sequences that are similar to regions of the legume 7S storage-globulin genes (Quatrano et al. 1986).

### Chromosomal location

Hybridization of PSP511 to genomic DNA from 21 nullisomic-tetrasomic (NT) lines, digested with *EcoRV*, *DraI* and *HindIII*, and ditelosomic (DT) lines, digested with *EcoRV*, of the *Triticum aestivum* cultivar Chinese Spring (CS) revealed homoeologous sequences only on chromosome arms 4AL, 4BS and 4DS. Six hybridizing fragments were detected with all three restriction enzymes (Fig. 1), indicating the probable presence of two homologous copies of the globulin genes (*Glo*) in each of the three wheat genomes. Similarly, evidence was obtained for two gene copies in rye and barley, located respectively on 4H (barley chromosome 4) and 4R as shown by analysis of the single-chromosome disomic addition lines. Both copies of *XGlo* were polymorphic in all five genomes investigated. Segregation of the alleles on 4AL, 4BS and 4DS in wheat, 4H in barley and 4R in rye, showed no recombinants, indicating that both copies are tightly linked in each of the genomes. Each population consisted of 120 F<sub>2</sub> indi-

viduals and thus the upper limit of distance between the two genes, at 95% confidence, can be calculated as 0.25 cM. Hence, *XGlo* can be considered as a single locus for most marker applications. The map locations of *XGlo-4A*, *-4B* and *-4D* have been determined in the cross CS × Synthetic to lie 19 cM, 23 cM and 39 cM from the midpoints of the map intervals (3, 2 and 5 cM respectively) spanning the centromeres on 4AL, 4BS and 4DS. The larger map distance obtained in chromosome 4D is consistent with previous observations that the D genome chromosomes in this cross recombine more frequently than their A and B homoeologues (Devos et al. 1992).

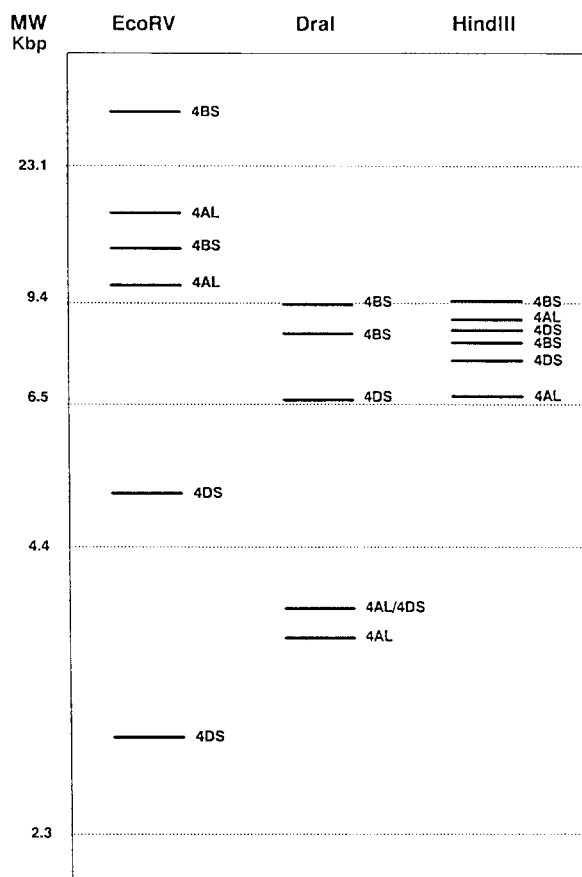
The chromosomal locations of the *XGlo* loci correspond closely with those of the 7S globulin loci on the 4D<sup>t</sup> genetic map constructed by Lagudah et al. (1991) in the wild diploid *T. tauschii*. However, the four copies in the D<sup>t</sup> genome and the dispersal of the three copies which map on 4D<sup>t</sup> are at variance with the results described here. This may be explained by the use of pEG4.6 (P. Chandler, personal communication), a large genomic clone isolated by hybridization with PSP511 which may carry sequences in addition to the globulin genes.

### Locus symbol

*XGlo* (*XGlo-4A*, *-4B*, *-4D*, *-4H*, *-4R*)

### Polymorphisms

Estimates of levels of polymorphism, based on allele frequencies obtained in a panel of 15 wheat varieties



**Fig. 1.** Chromosome assignment of Chinese Spring restriction fragments hybridizing with PSP511

digested with three restriction enzymes, showed that *XGlo-4A* was highly polymorphic with all restriction enzymes ( $H = 51\%$ ), *XGlo-4B* was moderately variable ( $H = 39\%$ ), while at *XGlo-4D* the only variant allele was found in Sears' Synthetic. *XGlo-4H* revealed three alleles in 13 varieties digested with three enzymes, giving a relatively high potential heterozygosity index of 57%.

#### Presence of related sequences in alien relatives of wheat

Hybridization of PSP511 to *DraI*-restricted genomic DNA of accessions of 21 alien relatives of wheat, including *Triticum*, *Hordeum*, *Secale*, *Aegilops*, *Agropyron*

and *Dasypyrum* species, and of the Gramineae species *Lolium perenne*, *Zea mays* and *Pennisetum glaucum*, revealed the presence of at least one, but mostly two, hybridizing fragments in the species analysed, indicating that the globulin gene sequence is highly conserved among the Gramineae.

#### Other studies of wheat *Glo* genes

Expression of the wheat globulin genes is characterized by the appearance of mRNA transcripts of 2.5 kb in RNA isolated from developing wheat grains (Quatrano et al. 1986), ABA-treated wheat embryos (Williamson and Quatrano 1988), and in drought-stressed wheat leaves (Berge et al. 1989).

#### Probe availability

Contact R. S. Quatrano.

**Acknowledgments.** Supported in part by a research grant from the U.S.D.A. Competitive Grants Program (89-37262-4456).

#### References

- Berge SK, Bartholomew DM, Quatrano RS (1989) Control of the expression of wheat embryo genes by abscisic acid. In: Goldberg RL (ed) Molecular basis of plant development. UCLA Symp Mol Cell Biol, New Series. Liss Inc, New York, pp 193–201
- Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939
- Lagudah ES, Appels R, Brown AHD, McNeil D (1991) The molecular-genetic analysis of *Triticum tauschii*, the D-genome donor to hexaploid wheat. Genome 34:375–386
- Quatrano RS, Litts J, Colwell G, Chakerian R, Hopkins R (1986) Regulation of gene expression in wheat embryos by abscisic acid: characterization of the cDNA clones for the Em and putative globulin proteins and localization of the lectin wheat germ agglutinin. In: Shannon L, Chrispeels M (eds) Molecular biology of seed storage proteins and lectins. Am Soc Plant Physiol, pp 127–136
- Williamson JD, Quatrano RS (1988) ABA-regulation of two classes of embryo-specific sequences in mature wheat embryos. Plant Physiol 86:208–215
- Williamson JD, Quatrano RS, Cuming AQ (1985) Em-poly-peptide and its messenger RNA levels are modulated by abscisic acid during embryogenesis in wheat. Eur J Biochem 152:501–507