Genet. Res., Camb. (1988), **51**, *pp.* 13–16 *With 2 text-figures Printed in Great Britain*

Identification of a second locus encoding β-amylase on chromosome 2 of barley

M. KREIS[1],* M. S. WILLIAMSON[1], P. R. SHEWRY[1], P. SHARP[2] AND M. GALE[2]

[1] *AFRC Institute of Arable Crop Research, Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, U.K.*

[2] *AFRC Institute of Plant Science Research, Plant Breeding Institute. Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.*

(*Received 22 August 1987*)

Summary

A barley endosperm cDNA clone was used to study the polymorphism and chromosomal location of β-amylase genes in barley. Analysis of DNA from seven cultivars digested with three restriction endonucleases showed two types of pattern, one present in Sultan and the other in the remaining six cultivars. A copy-number reconstruction indicated the presence of about three gene copies per haploid genome. Analysis of the six available whole chromosome addition lines and selected telocentric chromosome additions of barley into wheat showed the location of genes on the short arm of chromosome 2 (probably one copy) and the long arm of chromosome 4 (probably two copies).

1. Introduction

β-amylase (1,4-α-D-glucanmaltohydrolase, E.C. 3 . 2 . 1 . 2) is a key enzyme in the mobilization of starch in germinating cereal seeds. It is synthesized during grain development, and is stored prior to release during germination (Hardie, 1975). This contrasts with the other major carbohydrase, α-amylase, which is absent from the mature seed and synthesized *de novo* in the germinating embryo (Briggs, 1973). The β-amylase present in mature barley grain has an M_r of about 57200 (Visuri & Nummi, 1972) and can be separated into at least 8 isoforms by isoelectric focusing (Ainsworth, Miller & Gale, 1987). The structural gene(s) have been assigned to chromosome 4 using the wheat/barley chromosome addition lines (Powling, Islam & Shepherd, 1981), and Nielsen, Johansen Jensen & Hejgaard (1983) reported that the locus (*Bmy1*) was located distally on one arm and closely linked to *yh* (yellow head). These results are consistent with the previous report of Allison & Ellis (1973) that β-amylase isozymes present in developing endosperms of barley are controlled by co-dominant alleles at a single locus.

In the present paper we use a recently characterized β-amylase cDNA clone (Kreis *et al.* 1987) to demonstrate, by DNA restriction-fragment-length polymorphism (RFLP) analysis, that β-amylase genes are present, not only on the long arm of chromosome 4, but also on the short arm of chromosome 2. We speculate that the product of the locus on chromosome 2 (designated *Bmy2*) was not detected by previous analyses due to specific expression in non-seed tissues.

* Corresponding author

2. Materials and Methods

The whole-chromosome addition lines (Islam *et al.* 1981) and telocentric chromosome additions (Islam, 1983) of barley cv. Betzes into wheat, cv. Chinese Spring, were supplied by Dr A. M. K. R. Islam and Dr K. W. Shepherd (Waite Agricultural Research Institute, Adelaide, Australia) and grown in the glasshouse. All barley chromosomes are represented in these lines except chromosome 5. All plants used were checked for chromosome number by analysis of root-tip karyotype.

The methods for DNA RFLP analysis were essentially as described previously (Kreis *et al.* 1987; Sharp *et al.* 1987), using the large (789 bp) *Acc*I fragment of pcβC51 as a probe.

3. Results and Discussion

Total genomic DNA from seven cultivars (Bomi, Magnum, Sultan, Carlsberg II, Vada, Pirrka and Betzes) was digested with three restriction endonucleases (*Eco*RI, *Bam*HI, *Hind*III) and probed with a β-amylase cDNA. Only two types of pattern were observed with each enzyme, one being present in

Sultan and the second in the other six cultivars. These are shown in Fig. 1, tracks a–f. Two *Hind*III fragments were present, one of about 4·7 kb in all cultivars and the second of 6·1 kb in Sultan and 6·9 kb in the others (Fig. 1, tracks a, b). A reconstruction experiment (Fig. 1, tracks g, h) indicated the presence of about three β-amylase gene copies per haploid genome, two in the larger fragment and one in the smaller. Invariant single-copy fragments (in this case 10·5 kb) were also present in the *Bam*HI digests of all cultivars, with more intensely hybridizing second fragments of 7·7 kb in Sultan and of 13·8 kb in the other cultivars. Finally, single hybridizing fragments of 6·3 kb were present in the *Eco*RI digests of all cultivars except Sultan, which also had a second fragment of about 13·5 kb. Previously reported analyses of Hiproly showed identical patterns to Bomi with *Eco*RI and *Hind*III, but the replacement of the 10·5 kb *Bam*HI band with one of 12·3 kb (Kreis *et al.* 1987).

To determine the chromosomal locations of the β-amylase-related restriction fragments, *Bam*HI digested total genomic DNA from the six available addition lines (except chromosome 5), and the relevant ditelocentric addition lines were probed with the β-amylase cDNA. The smaller (10·5 kb) fragment was present in the chromosome 2 addition (Fig. 2, track b) and the chromosome 2S ditelocentric addition (Fig. 2, track h), and the larger (13·8 kb) fragment in the chromosome 4 (Fig. 2, track d) and chromosome 4L ditelocentric addition (Fig. 2, track k) lines. Further confirmation of the locations of β-amylase genes on chromosomes 2 and 4 was obtained by probing *Hind*III digested genomic DNA from the whole-chromosome addition lines (results not shown). The 4·7 kb fragment was clearly present in the chromosome 2 addition lines, while co-migration of the 6·9 kb fragment with a hybridizing band derived from Chinese Spring in the chromosome 4 addition line was inferred from an increased intensity of hybridization.

The results reported above provide clear evidence for a second β-amylase locus, which we propose to designate *Bmy2*, on chromosome 2 of barley. The failure of other workers to detect this locus or related loci on the homoeologous group 2 chromosomes of

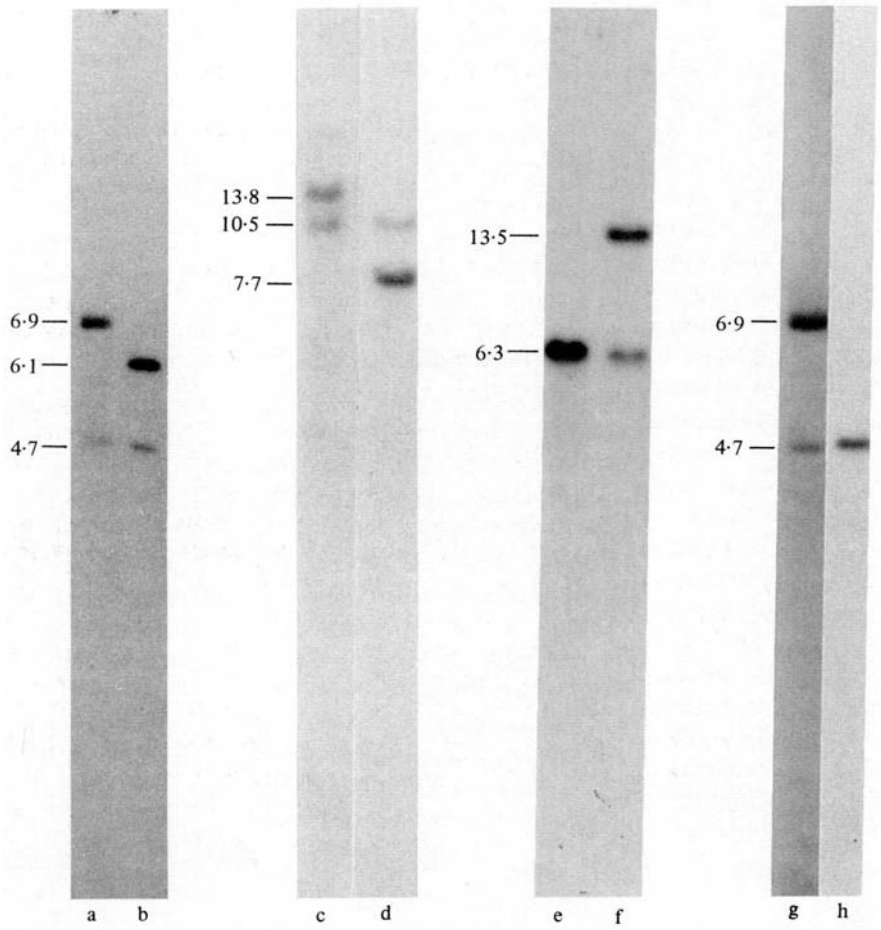

Fig. 1. Allelic variation in the hydridization of a β-amylase cDNA clone (pcβc51) to total genomic DNA of barley. Tracks a, c and e show the pattern present in cvs. Betzes, Pirrka, Vada, Carlsberg II, Magnum and Bomi. Tracks b, d and f show the pattern present in cv. Sultan. Tracks a and b were digested with *Hind*III, tracks c and d with *Bam*HI and tracks e and f with *Eco*RI. Track g is DNA from cv. Betzes digested with *Hind*III, and track h a reconstruction to show the intensity of hydridization expected from one gene copy per haploid genome. Sizes of fragments (in kb) are indicated.

https://doi.org/10.1017/S0016672300023880 Published online by Cambridge University Press

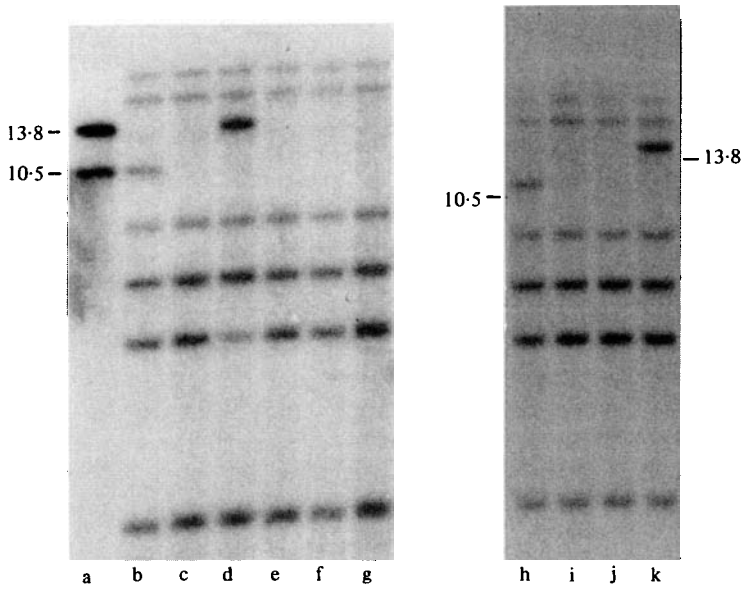

Fig. 2. Hydridization of a β-amylase cDNA clone (pcβC51) to *Bam*HI digests of total genomic DNA from Betzes barley (track a), the addition lines with chromosomes 2, 3, 4, 7, 6 and 1 of Betzes in Chinese Spring wheat (b–g respectively) and the chromosome 2S, 2L, 4S and 4L ditelocentric addition lines (tracks h–k respectively). Sizes of fragments (in kb) are indicated.

wheat (Ainsworth, Gale & Baird, 1983) may be due to tissue-specific expression. Our preliminary studies have shown variation in the molecular weights and pIs of β-amylase isoenzymes present in different tissues of the barley plant, and it is possible that the chromosome 2 genes encode a form present in the leaves and/or roots but not the grains, the only tissue analysed in reports by other workers. Alternatively, the chromosome 2-encoded proteins may correspond to quantitatively minor isoenzymes, which are present in the developing grain but whose chromosomal control has not yet been determined. Further genetic and chemical analyses are clearly required.

The RFLP analyses of the barley cultivars showed limited polymorphism. In the case of Sultan the fragments derived from chromosome 4 varied in size with all three enzymes, which was presumably due to an insertion or deletion event. In Hiproly the size of the fragment derived from chromosome 2 varied with only one of the three enzymes, which may have resulted from a single base change affecting a *Bam*HI recognition site.

Although the reconstruction experiments indicated the presence of at least three β-amylase genes per haploid genome of barley (two derived from chromosome 4 and one from chromosome 2), this is only an approximate estimate, as the intensity of hydridization could also be affected by variation in the degree of homology between the fragment and probe. Despite this reservation, the copy number is likely to be considerably less than the number of isoenzymes revealed by isoelectric focusing of grain extracts (Ainsworth *et al.* 1987). At least some of the latter must arise from post-translational modification or be artifacts produced by the extraction and separation procedures. Further studies at the protein level are again required.

We are grateful to Dr Angela Karp and Mr T. E. Miller for karyotype analyses, and to the EEC for financial support (Plant Productivity Grant no. 4712).

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