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Location of β-amylase sequences in wheat and its relatives

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Summary. A β-amylase cDNA clone isolated from barley has been used to locate β-amylase encoding sequences on wheat, rye, and *Aegilops umbellulata* chromosomes by hybridisation to restriction endonuclease digested DNA obtained from wheat aneuploid and wheat-alien addition lines. Structural genes were identified on homoeologous group 4 and 5 chromosomes, confirming the results of isozyme studies. In addition, a further set of structural genes was found on homoeologous group 2 chromosomes. It is proposed that there are two homoeoallelic series, *β-Amy-1* on group 4 or 5 chromosomes, and *β-Amy-2* on group 2 chromosomes. Evidence is presented that each locus contains one or two β-amylase structural genes, and it is suggested that the large number of isozymes seen upon IEF are due to post-translational modifications.

Key words: RFLP – Wheat – *Aegilops* – Rye – β-Amylase

Introduction

The genetics of mature grain β-amylase (α-1, 4-glucan maltohydrolase, E.C. 3.2.1.2) in hexaploid bread wheat *Triticum aestivum* (2n = 6x = 42, AABBDD) and many of its relatives has been well studied at the protein level using isoelectric focusing separations (Ainsworth et al. 1983, 1987). These studies have indicated that the genetic control of mature seed β-amylase isozymes present in 'Chinese Spring' (CS) wheat resides on the 4Aβ, 4DL (*β-Amy-1* loci) and 5AL (*β-Amy-2* locus) chromosome arms, and that related diploid species such as barley (*Hordeum vulgare*, HH), rye (*Secale cereale*, RR) and *Aegilops umbellulata* (UU) have single genes at either the *β-Amy-1* or *β-Amy-2* homoeoloci.

The isolation of a barley β-amylase cDNA clone (Kreis et al. 1987a) has enabled us to determine the locations of β-amylase coding sequences in barley. Kreis et al. (1987b) found β-amylase sequences on chromosomes 2 and 4, (equivalent to 2H and 4H, respectively), contrasting with previous studies which showed only chromosome 4H to be involved in the genetic control of barley grain β-amylase isozymes (Powling et al. 1981; Ainsworth et al. 1987). This paper reports the application of this approach to wheat, rye, and *Aegilops umbellulata*, and shows that β-amylase genes are also present on the group 2 chromosomes of these species.

Materials and methods

Genotypes

The relevant nullisomic-tetrasomic and ditelosomic genotypes of 'CS' (Sears 1954, 1966a, b), the amphiploids and addition line series of both *S. cereale* cv 'Imperial' with 'CS' (Driscoll and Sears 1971), and *Ae. umbellulata* with 'CS' (Kimber 1967) were examined. The lines are maintained at the Plant Breeding Institute, Cambridge, UK.

DNA analysis

General methods were as in Davis et al. (1986). DNA was extracted from powdered freeze-dried leaves of the above genotypes by digestion for 1–2 h in 100 mMTris · Cl pH 8.5, 100 mM NaCl, 50 mM EDTA, 2% SDS, 0.05 mg/ml proteinase K at 37 °C, followed by a phenol/chloroform extraction, and precipitation with 0.6 vol isopropanol. The precipitate was dissolved in TE (10 mMTris · Cl pH 8, 1 mM EDTA), and RNA was removed by digestion with boiled RNase A at 1 μg/ml for 30 m at 37 °C, and the DNA was again isopropanol precipitated and dissolved in TE.

Following digestions of 5 μg aliquots of the DNAs with various restriction endonucleases in accordance with the supplier's recommendations for 16 h and fractionation by electrophoresis through 0.8% agarose/TAE gels, the resulting DNA fragments were transferred to 'Zetapobe' (BIORAD) nylon membranes by alkaline blotting (Reed and Mann 1985).

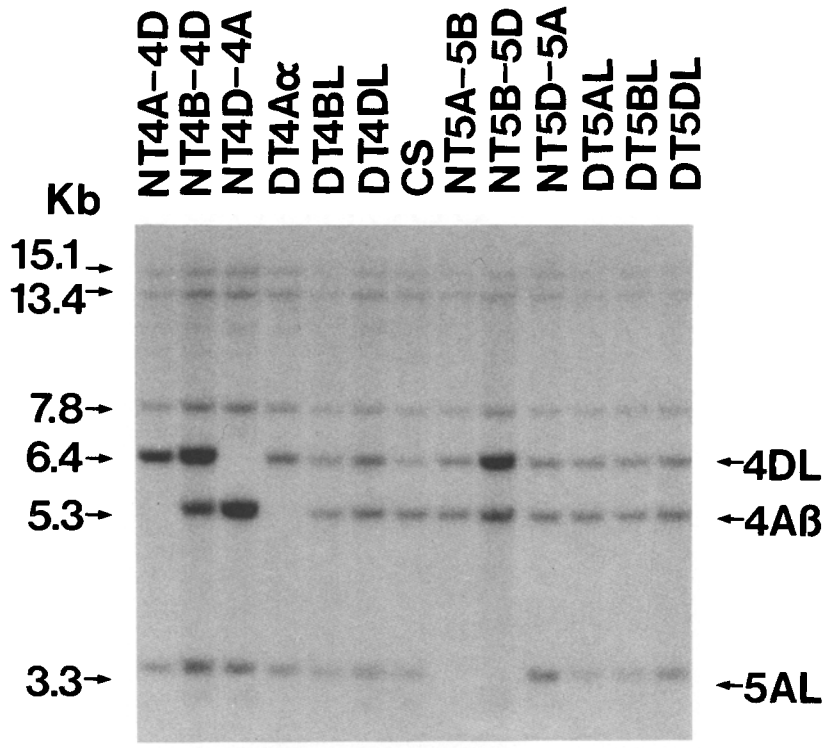

Fig. 1. Autoradiograph from hybridisation of the β-amylase probe to *Bam*H1 digested DNA of 'CS' and the indicated chromosome group 4 and 5 'CS' nullisomic-tetrasomic and ditelosomic stocks. The fragment sizes in kb are shown on the *left* and the assigned chromosomal locations on the *right*

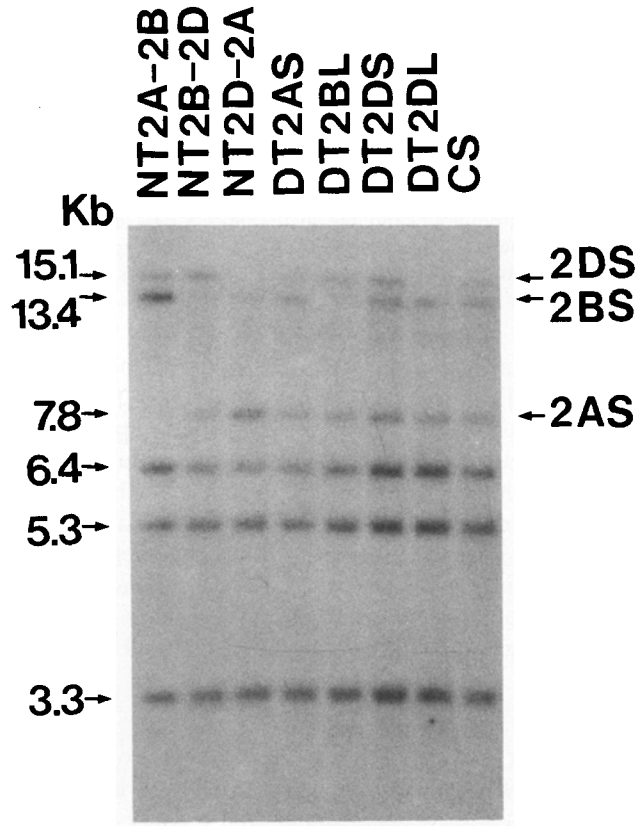

Fig. 2. Autoradiograph from hybridisation of the β-amylase probe to *Bam*H1 digested DNA of 'CS' and the indicated chromosome group 2 'CS' nullisomic-tetrasomic and ditelosomic lines. The fragment sizes in kb are shown on the *left*, and the assigned chromosomal locations on the *right*

Following pre-hybridisation in 0.6 M NaCl, 20 mM Pipes pH 6.8, 4 mM EDTA, 0.2% gelatin, 0.2% Ficoll 400, 0.2% PVP, 1% SDS, 0.5% sodium pyrophosphate containing 500 µg/ml autoclaved salmon sperm DNA for 5 hrs at 65 °C, the membranes were hybridised with ^{32}P-labelled probe in the same buffer at 65 °C for 16 h. The probe used was the 789 bp internal *Acc*I fragment of the barley β-amylase cDNA clone pc βc 51 (Kreis et al. 1987a) which was isolated by electrophoresis through low melting point agarose and labelled with ^{32}P-dCTP to high specific activity by 'oligo-labelling' (Feinberg and Volgestein 1984). After hybridisation the membranes were washed sequentially with 2×SSC, 0.1% SDS and 0.5× SSC, 0.1% SDS for 30 m each at 65 °C, and then exposed to KODAK XAR-5 film at −70 °C between two intensifying screens for 3–5 days. Sizes of the resulting hybridisation bands were calculated by reference to a 1 kb ladder (BRL).

Results

Location of β-amylase sequences in 'CS'

Hybridisation of *Bam*H1 digested 'CS' DNA with the barley β-amylase probe produces a pattern of six bands, three more heavily labelled than the others (Fig. 1). Analysis of some of the available homoeologous group 4 and 5 'CS' aneuploids allows the location to chromosome arm of the three more intensely hybridising bands (Fig. 1). The 5.3 kb fragment is located on 4Aβ by its absence from CSN4A-T4D and absence in CSDT4Aα. The 6.4 kb fragment is located on 4DL by a similar analysis of the *Bam*H1 fragment patterns of CSN4D-T4A and CSDT4DL. DNA from CSN4B-T4D has the same fragments as 'CS'. The 3.3 kb fragment is located on 5AL since it is absent from CSN5A-T5B and

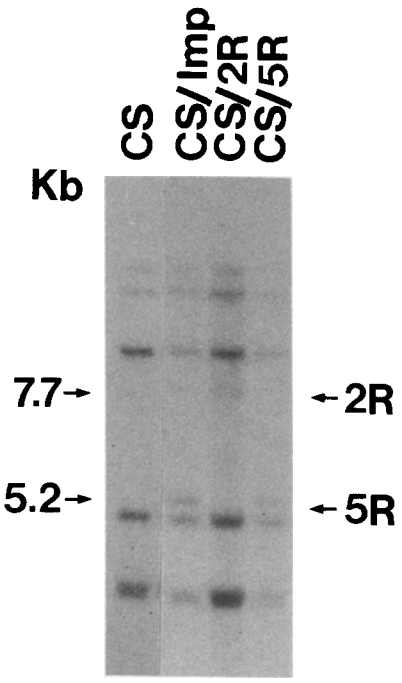

Fig. 3. Autoradiograph from hybridisation of the β-amylase probe to *Apa*I digested DNA of 'CS', the 'CS'/'Imperial' rye amphiploid and 2R and 5R 'CS'/'Imperial' addition lines. The 'CS'/'Imperial' 2R plant was a monosomic addition ($2n=43$). The sizes of the 'Imperial' fragments are shown on the *left* and the chromosomal assignments are on the *right*

present in CSDT5AL. This fragment is also absent from CSN5B-T5D. However, this line is known to be deficient for the 5AL β-amylase isozymes (Ainsworth et al. 1983), presumably because homoeologous chromosome pairing, induced by the removal of the *Ph1* locus on 5B (Riley and Chapman 1958), has resulted in loss of the locus. The three longer, less intensely hybridising fragments of 'CS' were not removed by nullisomy of any homoeologous group 4 or 5 chromosome.

Examination of aneuploids of homoeologous group 2 chromosomes reveals the location of these fragments (Fig. 2). The 7.8 kb fragment is located on 2AS since it is absent from CSN2A-T2B and present in CSDT2AS, and similar analyses located the 13.4 kb fragment to 2BS and the 15.5 kb fragment to 2DS.

Location of β-amylase sequences in rye and Ae. umbellulata

The 'CS' × 'Imperial' rye amphiploid has two additional DNA fragments hybridising to the β-amylase probe when compared to the 'CS' pattern after digestion with *Apa*I, one of 7.7 kb and another more intensely hybridising fragment of 5.2 kb (Fig. 3). When digests of DNA from the 2R and 5R 'CS' 'Imperial' addition lines are examined, the 7.7 kb fragment can be located on

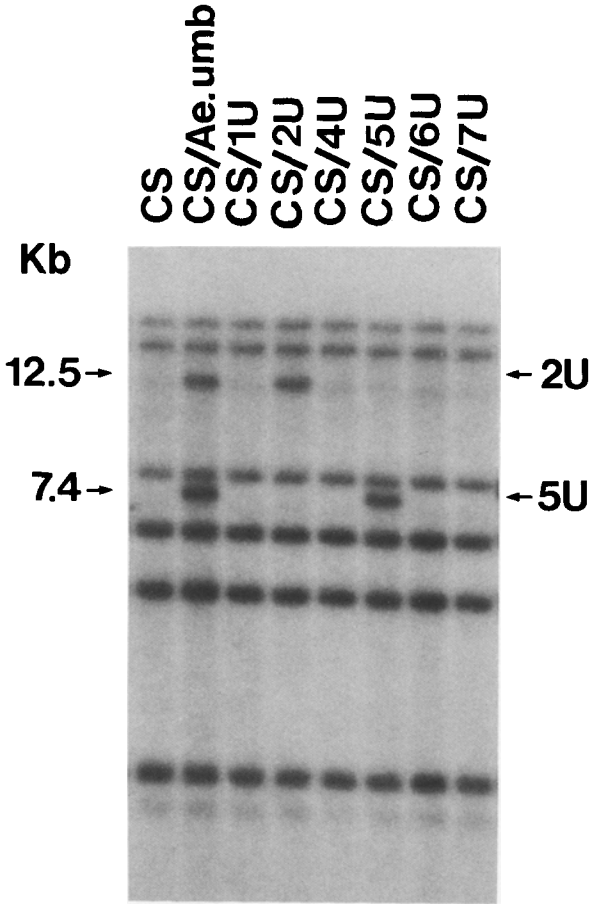

Fig. 4. Autoradiograph from hybridisation of the β-amylase probe to *Bam*H1 digested DNA of 'CS', the 'CS'/*Ae. umbellulata* amphiploid, and the available 'CS'/*Ae. umbellulata* addition lines. The sizes of the *Ae. umbellulata* fragments are shown on the *left* and the chromosomal assignments are on the *right*

chromosome 2R, and the 5.2 kb fragment to chromosome 5R (Fig. 3), since the 7.7 kb and 5.2 kb fragments are present in the 2R and 5R addition lines, respectively.

Similarly, the 'CS' × *Ae. umbellulata* amphiploid has two additional fragments (12.5 and 7.4 kb) superimposed on the 'CS' pattern in *Bam*H1 digested DNA, and analysis of the incomplete addition series (the 3U addition line was not available) locates the 12.5 kb fragment on chromosome 2U, and the more intensely hybridising 7.4 kb fragment to chromosome 5U (Fig. 4).

Discussion

It is clear from this analysis of the chromosomal location of β-amylase sequences in 'CS' hexaploid wheat, rye, and *Ae. umbellulata,* and a study of barley (Kreis et al. 1987 b) that there are two classes of β-amylase genes in these species. The first is located on either homoeologous group 4 or group 5 chromosomes, and corresponds to the β-amylase isozymes of mature

grains described by Ainsworth et al. (1983, 1987). The second class is located on homoeologous group 2 chromosomes in all the Triticeae genomes examined so far (A, B, D, U, R, and H). No evidence of group 2 involvement in the control of seed β-amylase isozymes has been found (Joudrier 1980; Ainsworth et al. 1983, 1987), so that it is evident that this set of genes is not highly expressed in mature grain. This class of genes may control 'house-keeping' β-amylase present at low levels in other tissues such as leaves and roots. There is some evidence for this type of activity in soybean (Hymowitz 1983). Further biochemical and genetical work is needed to examine this possibility in the Triticeae.

β-Amylase gene sets in the Triticeae

The β-amylase sequences on group 2 chromosomes clearly constitute a homoeologous series, which may have diverged in nucleotide sequence from the group 4/5 class since the group 2 DNA fragments hybridise less intensely to the probe than the group 4/5 fragments in all the genomes analysed (Figs. 1–4). Alternatively, this difference may reflect a smaller number of gene copies on the group 2 chromosomes. The presence of this homoeoallelic series on the 2AS, 2BS and 2DS chromosome arms provides evidence that these arms are homoeologous. Together with the location of loci controlling superoxide dismutase on the long arms of group 2 chromosomes (Neuman and Hart 1986), the available evidence from biochemical loci suggests that in the wheat group 2 chromosomes the short arms are homoeologous to each other, as are the long arms. However, there is contrary evidence from chromosome pairing studies between certain of these arms (Sears and Sears 1979), and also the location of genes controlling photoperiodic response (*Ppd*) to 2AL, 2BS and 2DL (Scarth and Law 1984). According to the guidelines for nomenclature of biochemical loci in wheat (Hart and Gale 1986), the group 2 series should be designated as the *β-Amy-3* homoeoallelic series since the group 4 and 5 β-amylase homoeoloci have been designated *β-Amy-1* and *β-Amy-2*, respectively (Ainsworth et al. 1983, 1987). However, we wish to propose that the group 2 series be designated *β-Amy-2*, and that the genetic control of mature seed β-amylase residing on group 4 and 5 chromosomes represents a single homoeoallelic series, *β-Amy-1*.

Our reasons for combining the group 4/5 loci are: First, all genomes in diploid species examined so far have only a single chromosome of either groups 4 or 5 involved in the genetic control of the major seed β-amylase isozymes (Ainsworth et al. 1987). Hybridisation analyses confirm these gene locations, and indicate that the other group 4 or 5 chromosomes in these species and in barley (Kreis et al. 1987b) do not carry β-amylase sequences. Secondly, it is only in hexaploid wheat that there is a suggestion of more than one locus encoding major seed isozymes of β-amylase per genome. Ainsworth et al. (1983) found loci on chromosomes 4A, 5A, and 4D in 'CS' and these locations are confirmed by the hybridisation results (Fig. 1). However, Dvorak (1983) has provided evidence that the chromosome presently designated 4A is actually chromosome 4B. Thus, this leaves only single seed β-amylase loci in the A and D genomes, and the locus on '4A' represents a locus in the B genome. Third, the identification of a locus on 5B in the amphiploid 'Synthetic' (derived from the cross *T. dicoccum* × *Ae. squarrosa* by McFadden and Sears 1946; Sears 1976) poses a further apparent problem to a single group 4/5 locus per genome hypothesis. This locus was demonstrated by Ainsworth et al. (1983) in an analysis of 'Chinese Spring' ('Synthetic') chromosome substitution lines. However, subsequent isozyme analyses have indicated that the 'CS' ('Syn' 5B) substitution was incorrectly assigned (C. Liu, pers comm.). Furthermore, analysis of the hybridisation pattern of 'Synthetic' *Bam*H1 digests shows only six fragments (result not shown). Thus, the available isozyme evidence indicates that loci controlling mature seed β-amylase isozymes are located exclusively on chromosomes 4A, 4D, and 5A.

In conclusion, the evidence from both protein and DNA analyses indicates that, in diploid species and all varieties of hexaploid wheat examined only a single β-amylase locus per genome exists on the group 4/5 chromosomes. The 5A location in hexaploid wheat almost certainly results from a reciprocal group 4/5 translocation, possibly the same chromosomal rearrangement responsible for the group 5 location of β-amylase loci in rye and *Ae. umbellulata*.

Structure of β-amylase loci

Ainsworth et al. (1983) noted 33 zones of activity upon isoelectric focusing of 'CS' mature grain β-amylase. They pointed out that this is a minimum estimate of the number of isozymes and that, because only 16 could be assigned to chromosomes by aneuploid analysis, the remaining bands may be composed of more than one isozyme of the same pI encoded by structural genes on different chromosomes. Because of the small number of loci involved and the large number of isozymes observed, Ainsworth et al. (1983) suggested that each locus is complex, comprising a number of closely grouped structural genes which have diverged from each other, and that the alleles at each complex locus are due to both mutation of the component structural genes and recombination within each locus giving different combinations of several structural genes. Such a system has been found in the loci controlling wheat α-amylase production (Lazarus et al. 1985). However,

the results presented here indicate that this may not be the situation in the β-amylase loci. The hybridisation signals obtained suggest that each *Bam*H1 fragment in 'CS' is present as only one or two copies/haploid genome as in barley (Kreis et al. 1987a), although reconstruction experiments to demonstrate this would be inconclusive because the probe is a cDNA derived from barley and is almost certainly from the *β-Amy-1* homoeolocus. Further evidence for a low copy number comes from comparison of RFLPs revealed by the β-amylase probe in DNA digested by three restriction endonucleases in various hexaploid wheat cultivars (Sharp et al., in preparation), where variation in fragment length relative to 'CS' was found for each of the group 4 and 5 loci, and some of the group 2 loci. If the fragments in 'CS' represented multiple co-migrating fragments, it is unlikely that DNA polymorphisms would exclude entirely the 'CS' forms, as was found to be the case. Finally, consideration of the fragment sizes and the intenity of hybridisation found in *Bam*H1 digests of 'CS' DNA make it unlikely that these fragments contain one gene copy per isozyme band. For example, the barley β-amylase cDNA clone has an insert of 1.8 kb (Kreis et al. 1987a), which represents a very conservative minimum gene length, so that the 7.8 kb 4DL fragment cannot contain 12 structural genes ('CS' 4DL controls at least 12 isozymes). In addition, the 4DL fragment gives a similar hybridisation signal to the 4Aβ fragment, which controls only 2 'CS' isozyme bands. It is likely that the chromosomes involved (2A, 2B, 2D, 4A, 4D, and 5A in 'CS') each contain one or two structural genes, and that the multitude of isozymes observed by Ainsworth et al. (1983) are the result of post-translational modifications [for which some immunochemical evidence exists, Daussant and Corvazier (1970)], and/or are artefacts of the isoelectric focusing technique. The alleles described by Ainsworth et al. (1983) would thus represent structural gene products which respond differently to post-translational modification systems. This does not, however, detract from the use of these highly polymorphic isozymes in genetic studies.

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